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FLYLEAF

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EDITORIAL

Post-traumatic stress disorder risk and brain-derived neurotrophic factor Val66Met

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Abstract

Brain-derived neurotrophic factor (BDNF), which regulates neuronal survival, growth differentiation, and synapse formation, is known to be associated with depression and post-traumatic stress disorder (PTSD). However, the molecular mechanism for those mental disorders remains unknown. Studies have shown that BDNF is associated with PTSD risk and exaggerated startle reaction (a major arousal manifestation of PTSD) in United States military service members who were deployed during the wars in Iraq and Afghanistan. The frequency of the Met/Met in BDNF gene was greater among those with PTSD than those without PTSD. Among individuals who experienced fewer lifetime stressful events, the Met carriers have significantly higher total and startle scores on the PTSD Checklist than the Val/Val carriers. In addition, subjects with PTSD showed higher levels of BDNF in their peripheral blood plasma than the non-probable-PTSD controls. Increased BDNF levels and startle response were observed in both blood plasma and brain hippocampus by inescapable tail shock in rats. In this paper, we reviewed these data to discuss BDNF as a potential biomarker for PTSD risk and its possible roles in the onset of PTSD.

Core tip: Brain-derived neurotrophic factor (BDNF), which regulates neuronal survival, growth differentiation, and synapse formation, is known to be associated with depression and post-traumatic stress disorder (PTSD). However, the molecular mechanism for those mental disorders remains unknown. In this paper, we reviewed these data to discuss BDNF as a potential biomarker for PTSD risk and its possible roles in the onset of PTSD.

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INTRODUCTION

Brain-derived neurotrophic factor (BDNF), first discovered in the early 1980s, is considered a member of the nerve growth factor family of neurotrophins[1], which have important roles in the development, physiology, and pathology of mental disorders[2,3]. BDNF is expressed in a number of tissues and cell types, including the brain and blood[4]. In recent years, BDNF has been implicated in a number of psychiatric disorders, such as depression, anxiety, eating disorders, and posttraumatic stress disorder (PTSD) (Table 1). In this review, we will focus on the findings of the association between BDNF and PTSD and expand upon our recent works to provide an argument for the potential role of BDNF in additional psychiatric disorders with their roots in emotional dysregulation, specifically PTSD.

BDNF is a precursor protein (proBDNF) that is proteolytically cleaved to generate mature BDNF[5] *via* tissue-type plasminogen activator (tPA)/plasminogen[6]. P11 (S100A10), a component of the Annexin Ⅱ and PTSD associated gene[7,8], greatly enhances the activation of plasmin by tPA[6]. It is suggested that p11 may act through the tPA/plasminogen/BDNF pathway to achieve its antidepressant effect[6]. BDNF binds to either of two functionally different classes of cell surface receptors, the TrkB receptor tyrosine kinase or the p75 neurotrophin receptor (p75NTR), a member of the tumor necrosis factor receptor super family[9]. ProBDNF and mature BDNF differentially interact with the TrkB receptor tyrosine kinase or the p75NTR, respectively[9,10]. ProBDNF induces neuronal apoptosis *via* activation of a receptor complex of p75NTR and sortilin[11]. ProBDNF induced p75NTR signaling gives rise to an increase in c-Jun N-terminal kinase and nuclear factor κB, regulating apoptosis, axonal retraction, or the pruning of dendritic spines[12]. BDNF-induced TrkB receptor signaling regulates neurotrophic responses *via* rapid activation of the PI-3 kinase, Ras/MAPK, and Phospholipase C-γ pathways; therefore influencing transcriptional events that affect the cell-cycle, neurite outgrowth, and synaptic plasticity (Figure 1), suggesting that the BDNF plays a key role in stress response and stress-related behaviors[13].

BDNF AND PTSD

In the animal model, it is found that BDNF protein was over-expressed in the plasma and hippocampus of stressed rats compared with non-stressed controls. These data are in agreement with others showing that stress results in BDNF over-expression in the hippocampus, leading to the hypotheses of a BDNF-related compensatory mechanism[14] and the blood levels of BDNF mirror the hippocampus levels induced by traumatic stress[15]. The rats with up-regulated BDNF in both blood and hippocampus induced by inescapable tail shock demonstrated increases of startle response[15]. Acoustic challenge is known to trigger a range of physiological responses, including startle. The startle reaction (also known as the startle response, the startle reflex, or the alarm reaction) is the psychological and physiological response to a sudden unexpected stimulus, such as a flash of light, a loud noise (acoustic startle reflex), or a quick movement near the face. Abnormality of the startle response, which results from an elevated activation of the autonomic nervous system, is a core symptom of PTSD-hyperarousal[16,17]. These data from the studies in animal model suggests that both BDNF and stress play important roles in startle response, although the mechanism needs to be further analyzed.

Within the last several years, the biological basis of PTSD has been an important focus of research in psychiatry due to the Iraq and Afghanistan wars. There are data showing that the blood BDNF is a potential biomarker for PTSD, the traumatic stress-related disorders, and debilitating psychiatric disorders[4,15,18-21]. A common single nucleotide polymorphism (SNP) in the BDNF gene leading a valine to methionine substitution at position 66 (Val66Met) influences human hippocampal volume[22], memory[23] and susceptibility to PTSD[23]. The BDNF Val66Met polymorphism is associated with sense of coherence, a presumed stress-related protective cognition in a non-clinical community sample[24]. Individuals carrying the Met had decreased activity-dependent BDNF secretion from neurons, leading to impairment of learning[22]. Recently, a study demonstrated that the frequency distribution of Val66Met polymorphism was different between subjects with and without PTSD[15]. The frequencies of Met/Met genotype and Met carriers are significantly higher in individuals with PTSD than those without PTSD. The allelic frequency of Met was two-fold higher (33.3% *vs* 17.5%) in individuals with PTSD than in non-PTSD controls, supporting the notion that Met carriers have a smaller hippocampal volume relative to Val/Val homozygous[25-27] and decreased volumes in the temporal and occipital lobe grey matter[28].

These data suggest a role of BDNF in the plasticity of the brain, which might be associated with PTSD. It was found that Met carriers performed more poorly than control subjects (Val/Val carrier) on the memory tasks[29]. The interaction of Met-allele and stress can result in depression, anxiety and arousal[30]. There is a significant three-way interaction between Val66Met, serotonin transporter linked promoter region (5-HTTLPR) and maltreatment history in predicting depression[31]. Children with the Met allele and two short alleles of 5-HTTLPR demonstrated the highest depression scores. However, the vulnerability associated with these two genotypes was only evident in the maltreated children[31]. There is a report showing that veterans with psychotic PTSD carried more Met alleles of the BDNF

Table 1 Brain derived neurotrophic factor and common psychiatric disorders

Mental disorders	Results and references
Schizophrenia	Polymorphisms and BDNF[39]
	The Val66Met allele association[40]
	The TrkB receptor decreased in the hippocampus[41]
	Up-[41] and down[42] regulated BDNF in the frontal cortices
Major depressive disorder	Antidepressant increases BDNF levels[43]
	BDNF protein increased in the NAc[44]
	The decreased prefrontal cortex is correlated with decreased BDNF and TrkB levels[45,46]
	BDNF dose-dependently decreases 5HT uptake[47]
	Unclear whether BDNF polymorphisms contribute to expression of MDD symptoms or antidepressant efficacy[48,49]
Bipolar	Both lithium and valproic acid increase BDNF expression in corticolimbic brain[40]
	BDNF protein levels decreased in post mortem hippocampal tissue[40]
	Serum BDNF levels decreased[49]
	The V66M BDNF allele strongly correlated to BD[35,50]
PTSD	PTSD risk associated with BDNF Val66Met and BDNF overexpression[15]
	Blood BDNF levels and PTSD[4,15,18-21]

NAc: Nucleus accumbens; BDNF: Brain derived neurotrophic factor; MDD: Microgram per square decimeter per day; PTSD: Post-traumatic stress disorder.
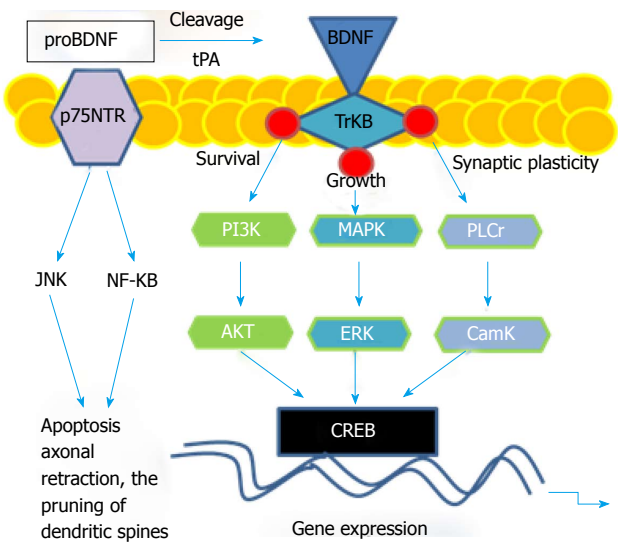
Figure 1 Brain-derived neurotrophic factor signaling pathway. The binding of proBDNF to the p75NTR up-regulates c-Jun N-terminal kinase and nuclear factor κB, triggering apoptosis, axonal retraction, or the pruning of dendritic spines. The proBDNF is cleaved to generate mature BDNF *via* tPA/plasminogen. When BDNF binding to its receptors, the receptor tyrosine kinase TrkB becomes phosphorylated, leading to phosphorylation at various sites or activation of downstream pathways. Such activation shows at PI3K sites, which activates AKT, regulating cell survival. The activation of MAPK/ERK leads to cell growth and differentiation. The activation of PLC-γ pathway regulates IP3 receptor to release intracellular calcium stores, in turn to enhance CamK activity, regulating synaptic plasticity. All three pathways converge on transcription factor CREB, binging to certain DNA sequences called CRE, playing a key role in BDNF-induced gene expression. BDNF: Brain derived neurotrophic factor; PTSD: Post-traumatic stress disorder; tPA: Tissue-type plasminogen activator; CamK: Calmodulin kinase; CAMP: Cyclic adenosine monophosphate; CREB: CAMP response elements; CRE: CAMP response elements; AKT: Protein kinase B; IP3: Inositol trisphosphate.

Val66Met than non-psychotic veterans with PTSD or veterans without PTSD[32]. This further supports the linkage between BDNF and PTSD, especially in a military population at war[15]. However, not all studies show the same results. There is a case-control genetic association study showing no relationship between BDNF Val66Met

and PTSD diagnosis[23]. It is possible the difference in frequency of trauma exposure, age, and other study conditions among the participants in the studies can explain these divergent results[33]; however, this remains to be determined.

In an association study between BDNF Val66Met and the startle score of PTSD Checklist (PCL), a core symptom of hyperarousal in PTSD is observed to be associated with the polymorphism. The distribution of the Met/Met frequency was significantly different between those with and without exaggerated startle[15]. The frequency of the Met/Met genotype was almost four-fold (12.2% *vs* 3.3%) higher in subjects with exaggerated startle than in those without exaggerated startle. In addition, the frequency of the Met allele was higher in subjects with exaggerated startle than in those without exaggerated startle (24.4% *vs* 15.3%), indicating that Met/Met is associated with hyperarousal vulnerability[15]. Since the frequency of Val/Val genotype is higher in the non-startle group than in those endorsing startle reactions, it suggests that Val/Val is related to protection from exaggerated startle reactions and perhaps PTSD. Subjects with fewer stressful life events and carrying the Met/Met homozygote have significantly higher startle scores than those Val carries, indicating that less exposure to stressful life events is associated with higher risk of hyperarousal in Met/Met carriers, but lower risk of hyperarousal in Val carriers[15].

It is found that the BDNF Val66Met does not significantly effect on the PCL total score in the subjects who experienced higher (four or more) stressful life events. However, among those subjects who reported fewer exposures of stressful life events, the Met carriers show higher PCL total scores (*i.e.*, reported greater PTSD symptoms) than Val carriers. Therefore, at a lower exposure of stressful life events, Met carriers had a higher risk of PTSD symptoms, and the presence of Val led to a lower risk of PTSD symptoms[15]. This indicates that there are protective effects at higher levels of

stress exposure. A similar phenomenon is observed in a catechol-O-methyltransferase gene association study, which showed that those homozygous for the Met allele demonstrated a high risk for PTSD, independent of the severity of traumatic load[34]. Alternatively, different neuronal mechanisms[35] may be active in minimally exposed and highly exposed individuals who develop PTSD, supporting different underlying trajectories of this disorder and perhaps different treatments[15].

It is also found that at protein levels, subjects with PTSD had significantly higher serum levels of BDNF than the non-PTSD controls[20]. In addition, the BDNF levels in Met carriers are higher than in Val/Val homozygotes[36]. The findings are consistent with other results showing that serum BDNF levels in individuals with PTSD are higher than in age and sex matched controls right after traumatic events[19,37]. However, some studies have shown either significantly lower levels of BDNF among those with PTSD[4] or significant difference of BDNF levels in serum between PTSD and non-PTSD controls[21]. These contradictory results may be due to the different methods used in the various studies. The samples may have been collected from dissimilar population[18,20,21,37], at different time points during the course of the disease[18,20,21,37], or from different animal models[38]. These assumptions need to be further analyzed. Nevertheless, these data suggest that BDNF is associated with PTSD risk at both translational and genomic levels[15]. Therefore, blood levels of BDNF may be of benefit in developing non-invasive diagnostics for PTSD[15].

CONCLUSION

The association between BDNF and PTSD has been suggested. The frequency of the Met/Met was greater among those with PTSD than non-PTSD controls. In addition, this SNP is associated with exaggerated startle, but not with other items on the PCL. Among individuals who experienced fewer lifetime stressful events, Met carriers have significantly higher total and startle scores on the PCL than Val/Val carries. At protein levels, subjects with PTSD had higher levels of BDNF in their peripheral blood plasma than the non-PTSD controls. In a rodent model, complementing the data from the human subjects, increased BDNF protein levels accompanied by an obvious elevation of the startle response were obtained in both blood plasma and brain hippocampus by inescapable tail shock. Therefore, protein BNDF in the blood and startle test, aside from genotype, and neuroimaging could also serve as biomarkers to direct more personalized PTSD treatment. Future studies on patient cohorts will elucidate whether these biomarkers, particular BDNF for PTSD prove to be useful in a clinical setting.

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Gender differences in caregiving among family - caregivers of people with mental illnesses

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Abstract

All over the world women are the predominant providers of informal care for family members with chronic medical conditions or disabilities, including the elderly and adults with mental illnesses. It has been suggested that there are several societal and cultural demands on women to adopt the role of a family-caregiver. Stress-coping theories propose that women are more likely to be exposed to caregiving stressors, and are likely to perceive, report and cope with these stressors differently from men. Many studies, which have examined gender differences among family-caregivers of people with mental illnesses, have concluded that women spend more time in providing care and carry out personal-care tasks more often than men. These studies have also found that women experience greater mental and physical strain, greater caregiver-burden, and higher levels of psychological distress while providing care. However, almost an equal number of studies have not found any differences between men and women on these aspects. This has led to the view that though there may be certain differences between male and female caregivers, most of these are small in magnitude and of doubtful clinical significance. Accordingly, caregiver-gender is thought to explain only a minor proportion of the variance in negative caregiving outcomes. A similar inconsistency characterizes the explanations provided for gender differences in caregiving such as role expectations, differences in stress, coping and social support, and response biases in reporting distress. Apart from the equivocal and inconsistent evidence, there are other problems in the literature on gender differences in caregiving. Most of the evidence has been derived from studies on caregivers of elderly people who either suffer from dementia or other physical conditions. Similar research on other mental illnesses such as schizophrenia or mood disorders is relatively scarce. With changing demographics and social norms men are increasingly assuming roles as caregivers. However, the experience of men while providing care has not been explored adequately. The impact of gender on caregiving outcomes may be mediated by several other variables including patient-related factors, socio-demographic variables, and effects of kinship status, culture and ethnicity, but these have seldom been considered in the research on gender differences. Finally, it is apparent that methodological variations in samples, designs and

assessments between studies contribute a great deal to the observed gender differences. This review highlights all these issues and concludes that there is much need for further research in this area if the true nature of gender differences in family-caregiving of mental illnesses is to be discerned.

Core tip: Women form the bulk of those who provide care for people with mental illnesses. Many studies have found that they are more exposed to caregiving stressors and report greater strain, burden and distress than men. However, the evidence for such gender differences in caregiving is equivocal and inconsistent leading to the view that caregiver-gender explains only a minor proportion of the variance in negative caregiving outcomes. Moreover, the evidence is not representative and often methodologically flawed. There is, thus, much scope for further research to understand the true nature of gender differences in family-caregiving of mental illnesses.

INTRODUCTION

Caring for someone with a mental illness has always been a family endeavour. This is true for developed as well as developing countries. Despite their relatively greater mental health-care resources, changing demographics and health-care norms in developed countries have shifted the locus of care from institutions to communities[1,2]. Social and health-policy changes have also placed greater emphasis on home and family-care for the chronically mentally ill in these countries. In contrast, families have always been the mainstays of care for the mentally ill in developing countries[3,4].

Family-caregiving is a term used for unpaid care provided by family members or friends to chronically ill or functionally impaired persons[1,5]. The amount of assistance provided by the family-caregiver usually exceeds the level of help provided under ordinary circumstances. Not only is the majority of informal care provided by family members, but the majority of family-caregiving is also carried out by women[6,7]. All over the world, women are the predominant providers of informal care for family members with chronic medical conditions or disabilities, including the elderly and those with other

mental illnesses[6-14]. Family-caregiving still remains a predominantly feminine activity despite the fact that with changing demographics and changes in social structures and norms, men are increasingly assuming roles as caregivers[15]. While providing care may have its rewards for family-caregivers, it often entails bearing emotional, physical, social and financial burden, which makes the experience stressful. Despite the voluminous amount of literature on family-caregiving, there is much that remains to be understood about why people take on strenuous caregiving duties, how they approach their caregiving responsibilities, and the consequences of taking up the role of a caregiver[16,17]. For an improved grasp of the experience of caregiving, a more accurate understanding of the caregiving-context which includes gender, familial relationship and cultural background of the caregiver is required[16]. Among these contextual factors, the impact of gender on caregiving has attracted the maximum research attention. The bulk of this research has been carried out among family-caregivers of the elderly with dementia or physical conditions, while gender differences among caregivers of other mental illnesses have been relatively neglected. However, even in this body of research there is considerable disagreement about the exact nature of gender differences in caregiving, and no consistent explanations about how gender influences caregiving.

GENDER DIFFERENCES IN FAMILY-CAREGIVING AMONG THE ELDERLY

Research on gender differences among caregivers of the elderly with dementia and physical illnesses have brought to the fore several themes of interest.

Women predominate among caregivers of the elderly

Worldwide, nearly 70% to 80% of the impaired elderly are cared for at home by their family members[5-7,9,11,17-20]. Varying estimates across different countries indicate that 57% to 81% of all caregivers of the elderly are women[1,6-8,10,12-14,17-26]. In most cases female caregivers are wives or adult daughters of the elderly person. They are usually middle-aged, with a considerable proportion of them being over 65 years themselves. They are also more likely to be employed outside home than in the past[7,17,19,27]. The elderly recipients of care are either frail or chronically physically ill; the majority, however, have dementia or other forms of mental illnesses[7]. Despite the preponderance of women, increased life expectancy, more women working outside home, and smaller families have all increased the pressures on men to assume roles as caregivers of the elderly. Studies in the eighties in the United States suggested that though women predominated as caregivers, somewhere between 20% and 33% of the caregivers of the elderly were men[6,18,19,26]. More recently, it has been reported that the proportion of

men providing care for the elderly has been steadily increasing, so much so that men may constitute nearly half of the primary caregivers of the elderly[8,15,16,19,23,28-35]. Despite the increasing emergence of men as caregivers, research has not taken into account this trend and continues to maintain its traditional focus on female caregivers. Although it appears that men approach caregiving differently, the experience of caregiving among men has not been examined as often as it has been among women[8,15,23,26,29-34].

Gender differences in the experience of caregiving

A number of studies have suggested that the experience of caregiving differs among men and women. Gender-specific differences in the provision of care for those with dementia or physical illnesses have been found to exist in several areas.

Time spent on caregiving and the duration of caregiving: Gender-differences in the time spent on caregiving have been considered in several reviews and studies on the subject. Some of them have concluded that despite conflicting reports, the bulk of the evidence indicates that women devote greater time to caregiving for the elderly, compared to men[1,16,20,25,27-29,34,36,37]. In a comprehensive narrative-review of 30 research-reports, Yee *et al*[22] concluded that the majority of studies which had examined gender differences in the time spent on caregiving had found that women spend more time on caregiving than men. Explanations based on the gendered nature of paid work have argued that women are more likely to care for the elderly because they are less likely to be employed outside home[38]. Women's work roles are viewed as being centred in the home and may reflect a greater sense of family obligation among them[11,16,17,23,38,39]. This increases the likelihood of women spending more time providing care. Time-intensive care among women is also more likely in those societies and cultures, which endorse the traditional value of the woman as the natural caregiver[8,11,23]. However, research findings about gender differences in the time spent on caregiving have not always been consis-tent. A number of reviews and studies have not found gender to be a significant predictor of the time spent on caregiving[8,10,18,26,38,40-42]. In particular, two meta-analytic reviews on the subject, one of which included 229 studies, have concluded that though women spent more time on caregiving, differences between men and women in this regard were small and of doubtful practical significance[24,43]. There is also considerable agreement that gender differences in the time spent on caregiving are confounded by several other variables such as kinship (spouses *vs* children), and cultural or ethnic influences[10,22,24,26,38,42,43]. Regarding the duration of caregiving, there is far greater consensus that gender does not have an impact on total duration of caregi-ving[8,10,24,43].

Types of tasks: The literature on gender differences in the type of caregiving tasks has also yielded conflicting findings. A distinction has been made in this literature between tasks associated with personal care such as bathing, dressing and managing incontinence, and tasks associated with management of everyday living. Some studies have found that women are more likely than men to provide assistance with tasks related to personal care[18,44], while others have not reported similar gender differences[9,10,38]. Reviews on the subject have also concluded that gender differences in the types of tasks have only been reported in some but not all studies, and only for tasks related to personal-care. Female caregivers are more likely than men to carry out these tasks[16,17,22,27-29]. Gender differences have not been found in tasks associated with everyday living[38]. These conclusions were endorsed by two meta-analytic reviews[24,43], but these further concluded that gender differences in personal-care tasks were small in magnitude. Gender differences in the types of tasks also appear to be influenced by several mediating variables such as the patient's gender and disability levels, kinship, caregivers' marital and employment status, family composition, social class, and race or ethnicity[10,22,27,38,45].

Role-strain and role-conflict: Caregiver role-conflicts refer to the perceived difficulties in fulfilling the caregiver-role, and the negative consequences emanating from this role[36]. Female caregivers often have to play multiple roles such as wives, daughters, mothers, or employees[16,17,38]. The pressures of enacting these conflicting roles may create difficulties for women. Role-conflicts and role-strains may manifest in many ways[16,17,38,46]. Role-conflicts arise when conflicting and incompatible demands are made of the caregiver himself/herself[16,17]. Role-strain occurs when one is unable to meet the expectations and obligations of multiple roles. Role-overload sets in when these competing demands overwhelm the person's ability to carry out his/her role[16,17]. This might lead to role-captivity, which refers to the caregivers' feelings of being trapped in their roles[17,46]. Role-conflicts give rise to several adverse consequences for caregivers such as physical problems, fatigue, burnout, depression and other emotional disturbances, and feelings of resen-tment towards the patient[17,46]. Many studies have found that female caregivers of the elderly with physical problems or dementia experience greater role-strain and role-conflict than male caregivers[1,13,14,16,20,27-29,36,39,44,47-52]. Women appear to experience greater interference and limitations in their work and social life because of their role as caregivers. They are generally believed to experience greater role-strain due to the more intense care they provide. Greater role-strain in women pro-duces more frequent health problems, a less positive outlook on life, and a greater need for external support. From their review of nine studies on gender differences in caregiving role-strain, Yee *et al*[22] concluded that

female caregivers report that their caregiving-roles interfered with their work and social life to a greater extent than men. However, such findings have not always been consistent, with several studies finding that caregivers' gender has no impact on their evaluations of role-strain[20,32,42,53]. It has been suggested that differences between studies arise more from the fact that perceptions of role-strain may vary depending on whether the caregivers are spouses or children of the elderly[36,44,47,50,54].

Satisfaction with caregiving: There is a relatively small amount of research-data on gender differences in other aspects of caregiving such as satisfaction with caregiving. The findings are equivocal, with some studies reporting that women are less satisfied[55-58], while a similar number of studies have found no differences in satisfaction between male and female caregivers[10,33,59,60].

Reasons for providing care: Several authors have identified emotional and social connectedness of women towards their patients, as well as their sense of family obligation as the basis for their nurturing approach to caregiving[16,61,62]. Women appear to be more concerned about the emotional well-being of the people they provide care for. This attachment often motivates them to engage in caregiving[20,38,61-63]. A greater sense of responsibility towards the patient, altruism, and self-sacrifice has also been found to characterize women's attitudes to providing care[10,25,27,33,38,45,61-63]. However, studies of male caregivers have suggested that caregiving among men is also driven by a similar sense of affection, commitment, and family responsibility[27,30].

Gender differences in caregiver-burden
Caregiver-burden has been defined as "a multidimensional response to physical, psychological, emotional, social, and financial stressors associated with the caregiving experience"[5,64,65]. Caregiver-burden is often the final outcome of a stressful and negatively perceived experience of providing care[66]. Not surprisingly, the greater part of the literature on gender differences in caregiving has been devoted to the subject of caregiver-burden. However, the results have been far from conclusive. Though a number of studies have found that female caregivers report greater levels of both objective and subjective burden[8,10,11,17,23,33,34,39,44,46-48,51,65,67-73], a similar number have been unable to find any gender differences in caregiver-burden[9,12,30-32,36,50,53,54,74-83]. Moreover, some studies have found differences in only certain aspects of burden, *e.g.,* subjective burden, and not in others[8,10,17]. Narrative reviews on the subject have been similarly uncertain in their conclusions. While some of them have concluded that caregiver-burden is higher among female caregivers[5,22,25,27,29,84-86], others have not found evidence in favour of greater levels of burden among women[12,15,30]. In their seminal

narrative-review on gender differences in caregiving, Yee *et al*[22] extracted data on caregiver-burden from 17 of the 30 studies they had included in their review. The vast majority of these studies reported that women experienced higher levels of caregiver-burden than men. However, meta-analytic studies have come to somewhat differing conclusions. An early meta-analysis included 14 studies on caregiver stressors and burden among the frail elderly[43]. It found that though female caregivers were more likely to report greater caregiver-burden, differences between the genders were small. In another meta-analysis of 4 studies, female caregivers of patients with dementia reported poorer global self-health, but did not differ from male caregivers on other risk factors[52]. In a meta-analysis of 84 studies of caregivers of the frail elderly, Pinquart *et al*[87] found that higher stress and poorer well-being among caregivers were more common among older women who were spouses of the patients. However, a later meta-analysis of 176 studies of caregivers of the elderly by the same authors found that associations of caregiving stressors with health were stronger among older men proving care for those with dementia[88]. The same authors have also carried out the most comprehensive meta-analysis till date of 229 studies on gender differences in caregiving of the elderly[24]. In this meta-analysis, the authors found that female caregivers had higher levels of burden and lower levels of subjective well-being and physical health compared with men, but these differences were small and barely reached the threshold of practical significance. Thus, they concluded that the available evidence indicated that there are more similarities than differences between male and female caregivers in this regard, and that some of the apparent gender differences could have arisen from methodological variations, or the effect of other confounding factors on caregiver-burden[24].

Gender differences in psychological morbidity
In their review, Yee *et al*[22] found nine studies which had examined gender differences in depression among caregivers of the frail elderly, and three studies which had reported gender differences in general psychiatric symptomatology. Overall, in 10 out of these 12 studies higher levels of depression and psychological morbidity was reported among female caregivers. Other reviewers have also reported greater psychological morbidity, principally depression, among female caregivers of the elderly[20,27,34,85,89,90]. Additionally, gender differences in psychological morbidity have been found in other studies[35,48,67,69,71-73,91]. In contrast, several studies have not been able to find significant differences among male and female caregivers in depression or psychiatric symptom-scores[23,33,75-78,82,92]. Meta-analytic reviews, though finding a higher prevalence of depression among female caregivers of the elderly, have reported that these gender differences were of much smaller magnitude than expected[10,24,43,52].

GENDER DIFFERENCES IN FAMILY-CAREGIVING AMONG SCHIZOPHRENIA AND MOOD DISORDERS

The issue of gender differences in family-caregiving in schizophrenia and mood disorders, or other psychiatric conditions has not been examined as comprehensively as among the elderly. Studies, which have evaluated burden among caregivers of such illnesses, have only occasionally considered gender of the caregiver when examining the numerous correlates of caregiver-burden. Nevertheless, certain trends similar to the literature on gender differences in the elderly are still evident.

Gender and type of caregivers of patients with schizophrenia and mood disorders

In a recent review of 42 studies on caregiver-burden in schizophrenia, the majority of caregivers were mainly the parents (usually mothers), followed by spouses and siblings of patients[93]. In an earlier review, Awad *et al*[94] had reported that women, either wives or sisters formed the greater part of caregivers of those with schizophrenia. They quoted a United States community survey, in which women constituted 82% of caregivers, with 90% being mothers of patients; 70% of them were over 60 years of age. This trend has been endorsed by a number of other reviews, which show that most family-caregivers of those with schizophrenia are their parents, mostly mothers of patients, and they are usually elderly[95-101]. However, the number of male caregivers seems to be on the increase[94,98,102], while in certain cultures men often predominate as caregivers[93,98,103,104].

Gender differences in caregiver-burden and psychological distress

Not only is there limited research on gender differences in caregiver-burden among schizophrenia and mood disorders, but the evidence for such differences is also less obvious. In their review of caregiver-burden in schizophrenia, Caqueo-Urízar *et al*[93] noted that female gender, unemployment and time spent in caregiving were all associated with higher burden. In contrast, in an earlier review of patients with severe mental illnesses other than dementia, Baronet[101] had identified 10 studies, which had evaluated the relationship between burden and caregivers' gender. None of them had found gender differences in overall burden, objective burden, subjective burden, worry, fear, or stigma. The results of individual studies conducted among family-caregivers of those with schizophrenia have also varied considerably. A number of these studies have reported higher levels of caregiver-burden, stress, burnout, psychological morbidity and poorer quality of life among female caregivers of those with schizophrenia[97,100,102-114]. However, several other studies have not found any differences in caregiver-burden between the genders[98,115-124]. Then again, very few of

these studies on schizophrenia have actually conducted comprehensive examinations of gender differences among caregivers. In an Indian study, caregiver-burden was examined in 70 spousal caregivers of patients with schizophrenia. Results showed that female spouses experienced significantly greater total burden and burden in the areas of external support, caregivers' routine, patients' support, patients' behaviour, and caregivers' coping strategies[106]. Female spouses also felt more anxious, tired, frustrated or isolated, and had to face a greater work load. Another study examined differences in caregiving between mothers and fathers who had a son or daughter with schizophrenia, in 100 such caregiver-couples[115]. The results showed that men and women were equally vulnerable to caregiving stressors. Studies among caregivers of patients with bipolar disorder are fewer. Perlick *et al*[125] examined gender differences among 150 primary caregivers of patients enrolled in the Systematic Treatment Enhancement Program for Bipolar Disorder. They found that men and women did not differ on depression or caregiver-strain. Some of the other studies of bipolar disorder have found higher levels of caregiver-burden or poorer quality of life among female caregivers[111,126,127], while others have not[128]. In a study of depressed patients with both unipolar and bipolar depression, wives were found to be more isolated and upset compared to their husbands[129]. However, results of other studies on depressive disorders have been mixed, with some reporting higher caregiver-burden or greater levels of depression among female caregivers[97,130,131], while others have not found significant gender differences in either burden or psychological morbidity[128,132]. In a recent study, a comprehensive examination of burden, psychological morbidity and other caregiving-indices was undertaken among male and female caregivers of 100 Indian patients with schizophrenia and recurrent mood disorders[133]. The majority of female caregivers were housewives. Male caregivers were more likely to be in paid employment than the female caregivers, had significantly higher income and were more likely to belong to the upper socio-economic strata than female caregivers. A significant gender difference emerged in the time spent on caregiving, with female caregivers spending more time providing care for their patients. Female caregivers also scored significantly higher in one domain of negative appraisal, while male caregivers had significantly higher scores on family-cohesion. Men cited family tradition, familial obligation, and concern about the patient's ill health as their reasons for providing care more often than women. Women, on the other hand, were more likely than men to report dependence, especially socio-economic dependence on their male patients, feelings of affection and sympathy for them, and a greater concern about the patient's future as their reasons for providing care. However, there were no significant differences between male and female caregivers in any of the areas of objective or subjective burden, or psychological morbidity. Moreover, there

were no differences in coping strategies, availability of social support, or personality traits such as neuroticism and rumination between men and women. The correlates of caregiver-burden and distress were largely similar among male and female caregivers. Finally, multivariate analyses showed that caregiver-gender explained only a minor proportion of the variance in caregiver-burden and distress. The results of this study thus endorsed what appears to be the consensus view in literature, largely derived from research among the elderly, that gender differences in caregiving though present are minor in nature, and caregiver-gender explains only a very small proportion of the variance in caregiver-burden and distress.

GENDER DIFFERENCES IN FAMILY-CAREGIVING: PROBABLE EXPLANATIONS

Several theories have been advanced to explain gender differences among caregivers of the elderly. Sociological explanations have emphasized expectations of traditional gender roles, in which women are expected to adopt the role of a caregiver. This is ingrained in females through their social and cultural experiences starting from childhood, and leads to a different approach to caregiving compared to men. Additionally, theories of segregation of labour indicate that since women are more likely to stay at home it is natural for them to take up the caregiver role[11,16,17,24,36, 38,39,45]. Men are not traditionally expected to become caregivers, which leads to a dissimilar approach towards caregiving among them. Due to their role-socialization, men may also be less adept at expressing their difficulties or emotions. This could result in a response-bias, in which men may be less likely to report difficulties in providing care than women[8-11,23,27,72]. However, it has been noted that empirical support for these theories is lacking[10,17,24,43,52]. Therefore, a second set of explanations based on the "stress-coping" theory has been proposed. It has been argued that gender differences arise because female caregivers have greater exposure to caregiving stressors, and differ in their appraisal, coping and availability of social support while managing these demands[10,11,17,22,24,65]. Though this theory has found more support from different studies, but unequivocal evidence of gender differences in appraisal, coping and social support is also lacking[8-11,39,22-24,27,48,68,72,73,134]. Some studies have indicated that gender differences in caregiver-burden and distress could be due to a differentiated appraisal of the caregiving situation among men and women[10,73], but the evidence for such gender differences is limited. Gender differences in coping strategies have been examined more extensively. Among family-caregivers of the elderly, a number of studies have found that women use emotion-focused coping and other ineffective coping styles such as fantasy, wishful thinking denial, escape,

or avoidance, more frequently than men. In contrast, men have a wider coping repertoire than women, and use more effective coping strategies such as problem-solving, acceptance, detachment or distancing more frequently[11,25,48,72,77,134]. These differences in coping strategies could potentially explain the higher levels of caregiver-burden and psychological morbidity among women[8,11,22,23,48]. Similar gender differences in coping have occasionally been reported among caregivers of patients with schizophrenia and mood disorders[106,135,136]. However, the number of such studies is limited, both among the elderly, as well as in schizophrenia and mood disorders. Contrary findings of lack of differences in coping between male and female caregivers have also been reported[22,73]. Some authors have proposed that female caregivers experience higher burden and distress because of lack of available social support[8,16,22,67]. According to them, women who care for the elderly are less likely to seek or receive support, because of the restrictions imposed on them by their caregiving roles. Men, on the other hand, are more inclined to seek and receive outside help for caregiving from formal and informal sources. Women seem to have larger social networks and more available sources of informal support, while men who have less access to formal and informal support, may be more motivated to seek help from these sources[22,24,137]. A greater lack of social support among women has also been found in spouses of patients with schizophrenia or mood disorders[106,129]. Again, the available results evidence are inconsistent in this regard, and gender differences in social support are not as pronounced as expected[10,22,24], either among caregivers of elderly persons or those with other psychiatric illnesses. Neuroticism, the greater propensity to break down under stress has been shown to have a significant influence on burden and psychological morbidity among caregivers. Some of the evidence indicates that the higher levels of depression and psychiatric symptoms among female caregivers could be partly accounted for by their higher neuroticism and greater use of escape-avoidance coping, but the number of such studies is small[22,46,72].

Since gender differences in appraisal, coping, social support and personality traits have been minimal and inconsistent, other explanations have been sought to account for differences in family-caregiving between men and women. It has been argued that the impact of gender is mediated by several other variables. These include characteristics of the patients, the severity of their illnesses including behavioural problems and associated disabilities, composition of the family, caregivers' demographics such as age, marital status, education, employment and socioeconomic status, their relationship with the patient, and the effects of culture, and ethnicity[5,8-11,22-28,34,38,39,44,46,50,67,68,73,79,86]. The influence of culture and ethnicity and kinship with the patient and has been explored in a number of studies. It is an undisputed fact that culture and ethnicity have a seminal influence on caregiving[138]. However, whether

cultural and ethnic factors impact gender differences in caregiving is a matter of some dispute. Certain authors have stated that studies from various cultures generally find that female caregivers are at greatest risk for caregiver-burden[5,93]. Others have proposed that gender differences in caregiving are less likely in cultural and ethnic groups with more positive attitudes towards the elderly, a traditional emphasis on women as caregivers, and the relative unavailability of formal sources of care[8-11,13,14,38,45,50,73,79,139]. It has also been suggested that among certain cultural or ethnic groups, familial-cultural variables such as familism, family-support, filial responsibility and family-cohesion may contribute to the gender differences in caregiving[5,8,10,11,73]. Familism refers to the precedence given to the family needs over the needs of the individual, while family-cohesion refers to the emotional bonding that family members have towards one another, and filial responsibility or piety refers to the tradition of caring for one's elders[5,140]. However, the exact direction of gender differences due to familial-cultural variables is unclear, because both higher burden among female caregivers[5,8,10,11,73], or similar levels of burden between the two genders[12,79,133,141] has been reported among cultural or ethnic groups with these familial-cultural values. Kinship status of the primary caregiver is another factor, which is thought to have a significant bearing on gender differences in caregiving[5,20,22,24,26,27,34,39,45,46,54,65,86]. Many studies have found greater burden or strain among spouses (usually wives) than children[9,11,23,38,79]; others have found the obverse[39,47,67], while some have found no effect of kinship ties on gender differences among caregivers[50,74].

GENDER DIFFERENCES IN CAREGIVING: METHODOLOGICAL VARIATIONS

Methodological variables contribute a great deal to the observed gender differences[15,24,29,86,101]. It has been repeatedly pointed out that there is a great deal of difference across studies in their samples, designs, assessment-procedures, data analyses and theoretical frameworks. These methodological variations could account for a large proportion of the variance in findings, and may give rise apparent rather actual differences between male and female caregivers[1,15,22-24,27,29,36, 43,52,85,86,89,101].

GENDER DIFFERENCES IN CAREGIVING: CONCLUSIONS AND FUTURE DIRECTIONS

Across the world women still constitute the majority of caregivers either of the elderly, or of those with other psychiatric disorders. However, the proportion of men taking up the caregiver's role is steadily increasing. Although a large body of the evidence seems to indicate

that women suffer more from the negative consequences of providing care, several other trends apparent in research need to be noted. Despite extensive examination of the area, gender differences in caregiving have not been consistently or conclusively documented. The magnitude and significance of the gender differences, which have been found is also uncertain. The majority of studies have been carried out among women; the experience of male caregivers has been relatively neglected. The bulk of the evidence comes from studies conducted among the elderly; gender differences in conditions such as schizophrenia or mood disorders have not been examined as comprehensively. Many explanations have been provided for greater burden and distress among female caregivers, but most of them are not supported by data. The effect of several variables, which mediate the influence of gender on outcomes of caregiving is uncertain. Finally, methodological variations between studies may conceal the true nature and extent of gender differences. Future research will need to address all these deficiencies before a better understanding of the subject can be obtained. If significant gender differences are indeed found, these will have major implications for development of gender-specific caregiver interventions, and social policy recommendations to improve the plight of female caregivers. It is for this very reason that there is much scope for further research in this area.

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Neuronal networks in mental diseases and neuropathic pain: Beyond brain derived neurotrophic factor and collapsin response mediator proteins

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Abstract

The brain is a complex network system that has the capacity to support emotion, thought, action, learning and memory, and is characterized by constant activity, constant structural remodeling, and constant attempt to compensate for this remodeling. The basic insight that emerges from complex network organization is that substantively different networks can share common key organizational principles. Moreover, the interdependence of network organization and behavior has been successfully demonstrated for several specific tasks. From this viewpoint, increasing experimental/clinical observations suggest that mental disorders are neural network disorders. On one hand, single psychiatric disorders arise from multiple, multifactorial molecular and cellular structural/functional alterations spreading throughout local/global circuits leading to multifaceted and heterogeneous clinical symptoms. On the other hand, various mental diseases may share functional deficits across the same neural circuit as reflected in the overlap of symptoms throughout clinical diagnoses. An integrated framework including experimental measures and clinical observations will be necessary to formulate a coherent and comprehensive understanding of how neural connectivity mediates and constraints the phenotypic expression of psychiatric disorders.

Core tip: Increasing evidences suggest that mental diseases are neural network disorders. Neurites and synapses represent the sub-cellular elements organizing these networks, and the molecules that regulate their formation, retraction and adaptive remodeling may contribute to the pathology of mental disorders. Various syndromes may share alterations of functional network leading to symptoms overlapping through clinical diagnoses.

INTRODUCTION

Despite their great diversity of morphology, most vertebrate nerve cells exhibit distinctive polarized structures with a single long axon and a specific dendritic arbor depending on their location. The axon/dendrite identity that influences the synaptic genesis and inputs that each neuron can integrate emerges as a convergent product of specific pattern of growth, branching and retraction and is differentially regulated at multiple points, including the control of the number of primary branches and their mode and frequency of branching by inhibitory (*e.g.,* Sema3, Nogo-A) and permissive [*e.g.,* brain derived neurotrophic factor (BDNF); fibroblast growth factor (FGF)][1-4] factors. Moreover, neurons do not connect indiscriminately between themselves but form an intricate non-random highly selective short [the hippocampal-prefrontal cortex (HIP-PFC) or hippocampal-amygdala-prefrontal cortex circuits][5,6] to long (the corpus callosum that connects the two cerebral hemispheres -facilitating an array of connective function- and influences higher cognition as well) range white matter axon fibers connections[7] producing characteristic networks capable of ensuring proper healthy behavior. The neuronal structure and neurotransmission in these networks, developed by interactions with the environment, are constantly remodeled *via* "activity-dependent synaptic plasticity"[8] to process, store and transmit relevant information. Accordingly, abnormal changes in the production of neurotrophic factors and permissive or inhibitory guidance cues may induce structural and/or functional abnormalities which may alter information processing and consequently link

neuronal network formation/maintenance to mental disease(s). Since psychiatric disorders have overlapping symptoms - *e.g.,* cognitive impairment and emotional dysregulation that can be found in schizophrenia, depression and anxiety disorders it is likely that this similarity is the consequence of disruption in common brain circuits (*e.g.,* hippocampus-prefrontal cortex) over time. In this review, we provide new molecular and cellular insights detailed from gene targeting/genome-wide association data (GWAS)[9,10] that extend the understanding of mental diseases and improve their treatment, to network theories that highlight structural and functional brain connectivity. Structural connectivity corresponds to the anatomical neurites, synapses-connections between neural elements whereas the functional and effective connectivity refers to a statistical dependence between the physiological signals measured in each region and the influence that one region exerts over another respectively. We focus on two brain areas essential in the emotional and cognitive domains: The hippocampal formation (hipF) and the prefrontal cortex. Their functional coupling support multiple functions, including emotion, mood, memory, thinking about the past and future, self and others, which are altered in mental diseases.

MOLECULAR BASIS OF NEURONAL NETWORK GENESIS AND MAINTENANCE

Neurogenesis and neuritogenesis

The generation of neurons (neurogenesis) in various regions of the central nervous system depends on a carefully regulated process of neural progenitor cells proliferation and differentiation. During early development, the neural tube wall constitutes a pseudo-stratified epithelium made of highly polarized neuroepithelial cells. The proper amount of neurons is spatially and temporally controlled by accumulative activities of numerous extra-/intra-cellular factors. At the onset of neurogenesis and initiated by extracellular regulators (Notch, bone morphogenetic proteins, BDNF/TrKB/p75, Wnt/β-catenin/Sonic hedgehog)[11-13] and intracytoplasmic transcription factors (bHLH, Insm1, AP2γ, TRIM32)[14-16], these cells switch their identity and turn into radial glial cells (RG) expressing glutamate-aspartate transporter and brain-lipid binding protein[17]. They generate all diverse intermediate progenitor neurons and glial cells through orchestrations of numerous molecules (Nrg1, Foxg1, Retinoid acid)[18-20] and signaling mechanisms (Jack/Stat, Notch, BMP, FGF)[21-23]. Shortly after, the pro-neural factors (Ngn1/Ngn2/Ascl1, also known as Mash1)[24,25] and transcription factors (Salb2, Sima1Am2, Lhx2)[26-28] activate a generic program of neurogenesis, arrest the division of progenitor cells, suppress the alternative astroglial fate and select the neuronal fate. Subsequently, the newborn neurons extend neurites (neuritogenesis which begins with the concerted accumulation and organization of actin/microtubules)

and migrate to appropriate locations. However, due to heterogeneous neuronal phenotypes generated at different times, in different layers and locations, there is considerable heterogeneity in neuritogenesis mechanisms. This makes it difficult to observe *in vivo* although we found recently that collapsin-response-mediator-protein (CRMP)3[29] has a profound influence on lamellopodia formation, neuritogenesis and dendritic arborization in vertebrate hippocampal neurons: The CRMP3^{-/-} knock-out mice[30] display abnormal functional and structural neural networks associated with a delay in neurite outgrowth and alterations of dendrites and spines but not axon-morphology in hippocampal neurons during development that persist in adults. These alterations affect a subset of hippocampal circuits and hippocampal function: CRMP3^{-/-} mice have a deficit in prepulse inhibition found in several mental diseases and abnormal long term potentiation (LTP). Such a bona fide mouse model is a critical first step towards exploring pathogenic mechanisms. There is increasing evidence that other CRMPs are involved in neurogenesis in the adult dentate gyrus and the olfactory system, and also regulate dendritic/axonal outgrowth[31-33]. Specifically, required for NT3-induced axon outgrowth/branching and linking kinesin to the Sra-1/WAVE cargo complex in axons, CRMP2 can convert established dendrites to axon or induce supernumerary axons[34] while mice lacking individual CRMP1, 4 or 5 present alterations in neuronal differentiation of specific brain areas and the behavior they control[35-37].

Another way to investigate neuritogenesis is using primary hippocampal neurons in culture. Few hours after plating, neurons start to extend minor neurites; then one of the multiple neurites extends rapidly and by morphological transformation generates the axon through stochastic selection; the other neurites mature into dendrites, leading to neuronal polarity. Live cell imaging using time-lapse video microscopy shows that the first two neurites have the highest potential to become axon. Because cultured neurons develop polarity without any presence of exogenous extracellular guidance/neurotropic cues, it has been suggested that an internal polarization program exists involving several intra-neuronal organelles and distinct repertoires of signaling molecules intrinsic to the neuron. Using that model, we reported that whereas neurons from heterozygous CRMP3^{+/-} mice polarized and grew similarly to control wild type (WT), all CRMP3^{-/-} neurons from homozygous CRMP3^{-/-} littermates did not establish neuronal polarity. Such impairment in neurites to progress from stage 1 to stage 2 represents a failure of neurite initiation. Moreover, the correlation between the levels of CRMP3 expression and its activation of L- and N-type of voltage-gated calcium channels suggests that the facilitatory role of CRMP3 on neurite initiation, dendritic development and plasticity may be mediated *via* Ca^{2+} influx[38]. Other factors such as neurotrophins, extracellular matrix proteins, attractant/repellent guidance cues and guide-post proteins are considered extrinsic signals. Interactions between these molecules can provide short/long range guidance information or stably change the intrinsic ability of a neuron to extend/retract neurites during development or to engage them into an axono/dendritic differentiation path. It is tempting to suggest that similar players are required to refine neuritogenesis and to make synaptic connections (synaptogenesis) *in vivo* within the developing brain.

Synaptogenesis and neuronal network genesis

Brain complexity comes from the large diversity and number of neurons and the variety and number of synapses where neurons transfer their electrical and/or chemical signals to other neurons/cells. Electrical and chemical synapses differ in the molecular mechanisms supporting the transmission of information and in their morphological organization[39,40]. Their structure and composition vary across brain regions and their disruption in function and morphology may be involved in many neurological/mental diseases and after trauma. Electrical synapses are also a prerequisite for the chemical synapses formation in mammal brain during development. Within electrical synapses the gap junction channels processed by connexins and pannexins serve as conduits allowing a direct bidirectional communication and passage/exchange of metabolites, intracytoplasmic messengers, and ions between the cytoplasm of two cells (Figure 1B). Within neuronal networks, these electrical synapses provide synchronous electrical activity and field potential oscillations. They mediate an important form of direct intercellular communication and allow rapid transfers of pre-synaptic excitatory electrical impulses to post-synaptic potentials throughout the intercellular gap by generating synchronous oscillations of gamma-frequency (30-70 Hz) rhythms important for field potential oscillation within neuronal networks and necessary for the interplay of neural populations involved in memory processes.

In contrast, there is no cytoplasmic junction between two cells at the two chemical synaptic subtypes: The excitatory asymmetric (mainly glutamatergic; Figure 1A) type I synapse has marked postsynaptic densities (PSD) while the inhibitory symmetric (mainly GABAergic) type II synapse has no thickened PSD. The genesis of chemical synapses is characterized by an enormous degree of complexity and diversity of protein-protein interactions. Axonal presynaptic boutons of excitatory synapses contain round clear vesicles loaded with the neurotransmitter glutamate and connected with dendritic spines while inhibitory presynaptic boutons contain slightly smaller vesicles and are most abundant at the neuronal soma. In CNS, maturation and stabilization of synaptic structures depend on neurexins and neuroligins, the molecule pairs in the CAM family[41-43], while plasticity which allows an individual to adapt to a rapid changing environment through strengthening, weakening, pruning or adding synaptic connection- is partially dependent on BDNF, ephrin, Wnts, NgR1, semaphorins class 3 and non-coding RNAs[44-48]. The
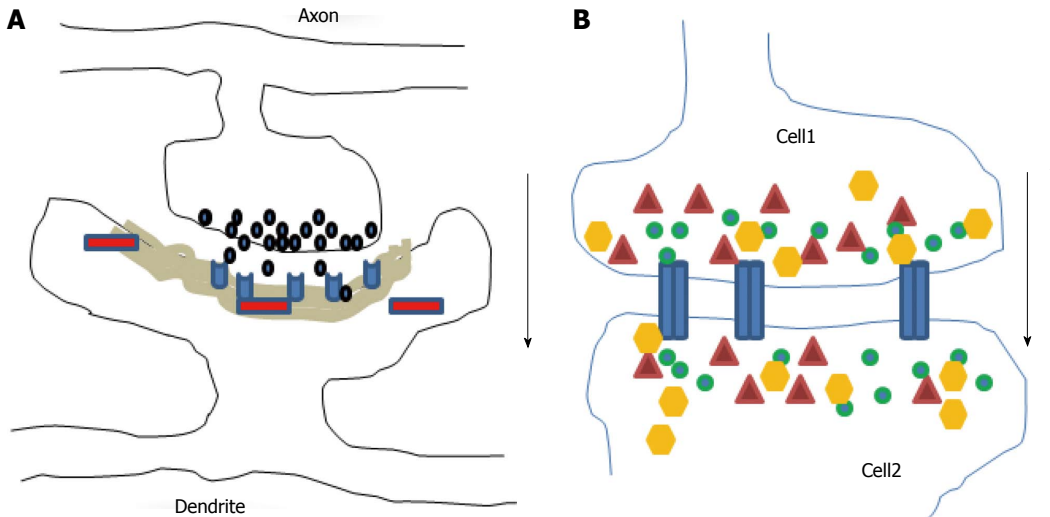
Figure 1 Diagrams illustrate chemical (A) and electrical (B) synapses. At chemical synapses, neurotransmitters (black) released from axonal boutons bind to postsynaptic receptors (light blue) and trigger specific signaling pathways *via* activation of proteins (red) in postsynaptic cells with prominent postsynaptic densities component (grey area). The information transmission is unidirectional (black arrow). At electrical synapses, gap junction channels (blue) directly connect the two adjacent cells, thus enable the bidirectional passage of electrical currents (black arrows) carried by ions (green), and of small peptides (yellow) or second messengers (dark red).

mechanisms contributing to the synaptic plasticity include structural remodeling of the synapse, structural reorganization of presynaptic active zone, postsynaptic density area, protein synthesis, signal transduction pathways, Ca^{2+} fluxes, kinases activities, neuronal activities, change in transmitters release and receptors trafficking. However, plasticity creates a significant challenge to the intrinsic architecture and integrity of neural networks which is counterbalanced by compensatory regulatory mechanisms for maintaining neuronal homeostasis, leading to a balance between wiring plasticity and stability.

Synapses, the sites where two neurons connect and pass information, are the building blocks of the neuronal networks defined by "a set of elements with time-variant properties that interact with each other" and network dynamics defined by the real-time changes in response to internal and external stimuli. It should be noted however that information is not stored in a chemical form but is processed and retrieved by neuronal networks. The global neuronal network complexity can be defined as a dynamic interconnected functional system characterized by a series of simpler networks organized into increasingly effective local or global complex networks constrained however by the intrinsic structural brain architecture[49]. It has the capacity to support complex thought, action and learned behavior that any single neuron/element of the system would not be able to support alone and its resting-state reflects the stable and intrinsic functional architecture of the brain. Importantly, dysfunction of local networks may spread easily between linked elements, leading to pathological cascades that cause dedifferentiation or trans-neuronal degeneration and encompass large areas of the system. It may also lead to dynamic adjustments and reorganization of the other networks to compensate

these changes. Consistent with the view that network organization fundamentally influences brain diseases, many studies including connectomic approach, address the behavioral impairments that arise from network insults or dysfunction and challenge to predict patterns of disease spread and targets of intervention[50,51]. In various mental diseases, neuroimaging observations, diffusion tensor imaging, electroencephalography (EEG) and magnetoencephalography report altered structures and functions of PFC and hipF and deficits in functional integration between these two elements (Figure 2) suggesting overlapping pathogenic mechanisms.

The PFC, which matures later in development than more caudal cortical regions, exerts "top-down" control of many cortical and sub-cortical areas; moreover some of its neuronal subpopulations exhibit complex dendritic arbor. Its development is characterized by growth in early childhood, decrease in adolescence and continued maturation in adulthood. It is well established in human that PFC is involved in language, maintenance of attention, executive functioning, organization of inputs from diverse sensory sources, coordination of goal-directed behavior, socialization and moral decisions. HipF is structurally and functionally heterogeneous. The anterior and posterior parts receive/extend different afferent and efferent connections and play a role in various functions such as learning and memory, stress, spatial and emotional processing. The neuronal projection from the HIP either directly -monosynaptic- or indirectly polysynaptic to the PFC is referred to as the hipocampal-PFC pathways (HIP-PFC). In rats, the direct monosynaptic HIP-PFC pathway originating from the CA1 and the subiculum projects to the anterior cingulated areas of the PFC through fimbria/fornix system. It exhibits activity-dependent synaptic plasticity such as LTP/LTD or depotentiation. Treatment with
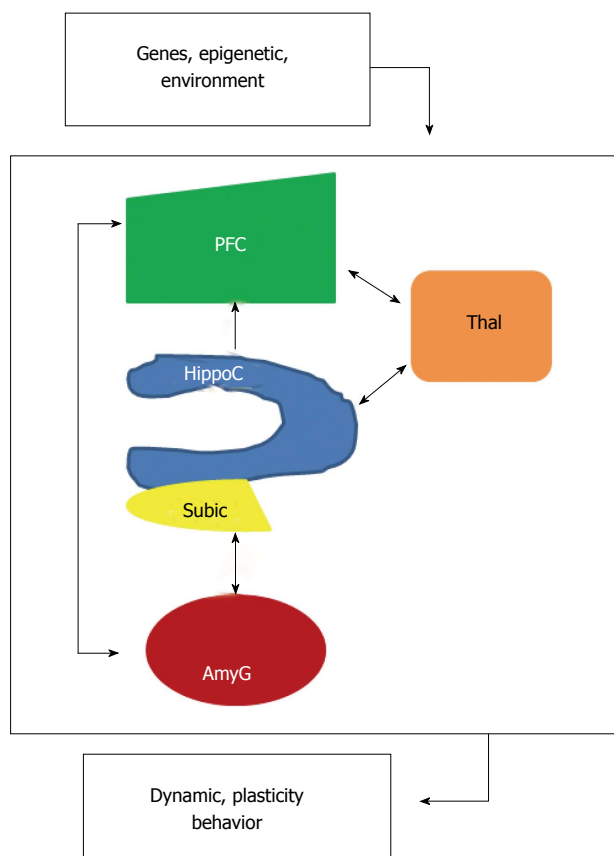
Figure 2 Localized interregional connectivity. Diagram shows pathway connections of hippocampus (blue), amygdala (dark red), thalamus (orange) and prefrontal cortex (green). HippoC-PFC pathway originating from the subiculum and the CA1 of the hippocampus to the PFC is unidirectional, direct in a monosynaptic manner in rodents and primates. HippoC-AmyG pathway shows bidirectional connection between ventral HippoC with AmyG and the pathway AmyG-PFC has also bidirectional connections. Together, both the HippoC and PFC are reciprocally connected with the AmyG and disruption of these pathways, anatomically or functionally may be a common origin of mental diseases. Moreover, there are bidirectional connections between PFC/HippoC with Thalamus. PFC: Prefrontal cortex.

lidocaine disrupts its performance. However, fine details of the human HIP-PFC are lacking because direct powerful tract-tracing techniques cannot be applied. Within the hipF, neuroanatomical studies show that the ventral CA1 and subiculum also project to the basolateral amygdala (AmyG) which has a critical role in expression of fear and autonomic defense responses whereas recent works highlight the importance of distinct AmyG projections to other structures and their importance in controlling reward/learned behavior. Functional imaging in patients with major depressive disorder (MDD) and bipolar disorder (BP) shows that the rate of resting cerebral blood flow and glucose metabolism in AmyG is elevated and positively correlates with depression severity and relapse. In addition, the dual-projection of hippocampal neurons is crucial for coordinating PFC/AmyG activity during memory retrieval. Meanwhile, AmyG neurons densely arborize within superficial layers of PFC and form synapses with layer Ⅱ pyramidal neurons. Disruption of the AmyG-PFC pathway increases

choice of risky rewards suggesting that it is important for top-down control of emotion, anxiety and fear. Presently, it is well-established that patients suffering from a number of mental diseases, such as schizophrenia, major depression, bipolar and PTSD, display cognitive impairment, have structural abnormalities, disorganized neural networks and aberrant functional coupling within HIP/PFC/AmyG and their pathways[52-56].

NEURONAL NETWORK ABNORMALITIES IN MENTAL DISEASES

Schizophrenia

Schizophrenia is a complex psychiatric disorder with variable symptomatology characterized by hallucination, delusion, anhedonia and cognitive dysfunction. Studies of post-mortem brains provide patterns of abnormalities that reflect failures in early brain development and maturation which can be attributed to alterations in gene expression (NRG1/Akt/Dysbindin-1/Reelin/COMT/DISC1) affecting neuronal differentiation[57-60], gene duplication (25q11-13, 16p11.2, 16p13.1)[61,62] or deletions (2p53, 3q29, 15q13-q14, 15p11.322q11.2)[63,64] . There is also evidence for contribution of multiple different epigenetic events [stress that activates the hypothalamic-pituitary-adrenal (HPA) axis and increases dopamine brain function, viral infection, DNA methylation, histone modification, non-coding RNAs]. Of note, recent proteomic, gene targeting, post-mortem and SNP linkage studies include CRMP1 and 2 in the list of schizophrenia susceptibility genes[65,66]. As schizophrenia typically starts in the late adolescence and as brain development is a continuous process, the remodeling of cortical and hippocampal structures and synaptic connections is thought to be critical. Indeed, patients with schizophrenia show structural anomalies in the HIP and cortical thinning in the PFC associated with aberrant functional coupling -*i.e.,* reduced fractional anisotropy values in the superior longitudinal fasciculus white matter bundle between two areas during resting state or working memory present in both first-episode patients and persons at risk[67]. This scheme suggests that the dysfunction is not a consequence of the disease or treatment but is consistent with abnormal HIP/PFC interaction. Precisely, it has been proposed that deficit in emotional regulation is likely dependent on the dysfunction of HIP, which contributes to aberrant dopaminergic synaptic activities in nucleus accumbens, which in turn influences PFC maladaptive processes leading to delusion and psychosis. To this extent, structural and functional vulnerability and abnormalities of GABAergic, glutamatergic serotoninergic and cholinergic synapses have been reported by several groups[68,69]. As synapses are ultimately linked to neurotransmitter release and their signal transduction and as spine morphology is closely linked to synaptic function, altered spine shape, size and density have multiple functional effects on neuronal networks, and

dendritic spine dysfunction may have an etiological role in schizophrenia. Several post-mortem studies reveal altered white matter myelination/projection and a profound reduction in spine density in the PFC of patients while the reduction in spine density found in the auditory cortex could potentially be associated with auditory hallucinations. Other evidence suggest that the heterogeneity of schizophrenia may originate from larger disturbances in neural elements of several interconnected brain circuits and structures[70] including HIP, parahippocampal gyrus, entorhinal cortex, AmyG, superior and transverse temporal gyri, prefrontal and anterior cingulate cortex, and several nuclei of the thalamus. Indeed normal neural structure in morpho-metric imaging may not guarantee normal function.

Bipolar disorder

Studies of physiopathology in BP have identified brain structural/functional and connectivity alterations associated with the prominent mood swings -*i.e.,* alternating recurrent episodes of mania and depression with psychotic symptoms in some cases. Neuroimaging has convincingly showed that bipolar disorder (BP) is a brain disease involving multiple abnormal brain structures -AmyG, HipF, PFC, thalamus and basal ganglia- and neuronal circuits, particularly the limbic-AmyG-thala-mic-cortical pathways interconnected by excitatory glutaminergic projections and the limbic-cortical-striatal-pallidal-thalamic circuits[71]. These two networks share components and regulate AmyG response in complex emotions such as melancholic feeling and neuroendocrine/diurnal rhythms. Functional imaging, which permits direct examination of functional brain structures, find decreased blood flow and metabolism in PFC during depression and a reverse increased metabolism during mania[72]. Additionally, postmortem histopathology shows reductions in cortex volume, glial cell counts, and neuronal size in PFC, AmyG, basal ganglia and dorsal raphe nuclei[73,74]. The reduction of glial cells oligodendroglia and microglia that a play critical role in modulating neurotransmission- provides new insights into possible key CNS cellular abnormalities in BP. Altogether, the altered brain structure/network characteristics suggesting that BP is a developmental disease and the identical-twin concordance rates/adoption studies/family history confirm that it has a strong genetic component. The involvement of multiple genes and their epigenetic (psychosocial and environ-mental interaction)/epistasis (genes interaction) effects make the clear-cut elucidation of altered risk gene expression particularly challenging, although recently the Human Genome Project has helped to overcome some of these difficulties. Linkage and GWAS have reported many BP risk genes, including *ANK3*, *ZNF804A* (important for white matter integrity)[75], *NCAN* (cortical thickness)[76], *TCF4* (ventricular volume)[77], *CACNA1C* (functional connectivity during executive task. *CACNA1C* encodes a subunit of the L-type voltage dependent calcium channel[78] that seems to be involved in the

dendritic arborization activity of CRMP3 in HIP)[38], *BDNF* (executive function deficit, neurotrophic factor, plasticity)[79]. Importantly, meta-analyses of the poly-genic score profile indicate a large molecular overlap in vulnerability alleles for BP and schizophrenia. In BP, CRMP2 protein levels are decreased in the CA2/CA3 areas and the frontal cortex whereas CRMP4 is decreased in HIP[80].

MDD

MDD is often a recurrent and severe psychiatric disease characterized by decreased density in dendrites and dendritic spines in hippocampus that can be reversed by antidepressant treatments[81]. In the past, most studies have focused on mono-amine system. Another etiolo-gical hypothesis proposes that deficiency of neurotrophic factors may mediate depressive symptoms. BDNF is one such factor and there are numerous reports of reduced BDNF in MDD[82]. It has been suggested, in a gene-environment interaction network analysis, that BDNF polymorphism may be involved in MDD. Other evidence which strengthens this hypothesis is provided by studies in BDNF mutant mice[83]. However there are enormous gaps in our understanding of MDD and looking beyond mono-amine and neurotrophic mechanisms to explore the complex neuronal network topologies influence[84] may bring new effective treatment. This notion has received considerable experimental support: (1) it has been shown that the level of CRMP2 important for growth cones formation and neurite arborization is decreased in the brain of patients with depression[85]; (2) recent neuroimaging studies highlight structural alterations in various brain areas of MDD patients[86] -predominately in the HIP and PFC, suggesting overlapping brain abnormalities between the main mental disorders-while the resting-state functional magnetic resonance imaging provides evidence of major change in HIP-PFC circuits[87] responsible for action responses, emotion, sleep, EEG synchronization, attention and memory; (3) the structural/functional abnormalities may contribute to disturbance in mood and cognition in MDD patients and strengthen the hypothesis that MDD is associated with the breakdown of the healthy neuronal networks circuitries; and (4) animal models of depression present similar neuronal dystrophy, reduced synaptic density in PFC and pyramidal cells of the HIP[83].

Post-traumatic stress disorders

After exposure to a traumatic event, *e.g.,* war-related events, physical assault, violence, a small percentage of individuals develop post-traumatic stress disorders (PTSD), characterized by re-experiencing the event with emotional numbing and hyper-arousal symptoms. The identification of structural brain abnormalities, biological and genetic risk in PTSD is required to identify the causal pathways, and inform treatment. Neuroimaging studies of PTSD patients reveal structural and functional alterations within HIP, AmyG, medial frontal cortex and bilateral orbito-frontal cortex[88]. The functional

connectivity studies show that PTSD patients exhibit diminished levels of connectivity between the posterior cingulated cortex region and the right superior frontal cortex and the left thalamus during the resting state[89]. Other biological findings detect the dysregulation of HPA axis and release of corticosteroids -critically involved in mediating the deleterious effect of stress including the decrease of dendritic spines density in HIP in line with the decrease of BDNF[90].

Neuropathic pain

Neuropathic pain (NP)[91] is linked either to peripheral nervous system lesions with drastic changes in gene expression pattern, protein interaction network and non-coding RNAs (*i.e.,* likely induced by inflammatory molecules such as histamine, prostaglandins or bradikinin)[92,93] or in relay structures of CNS (*i.e.,* arisen from metabolic disorders, traumatic injury or neurotoxicity)[94]. These changes can persist long after the initial injury (nerve loss, phantom limb). Common causes of NP are acute or chronic trauma, neurotoxins, diabetes, tumor compression, viral infections or side effect of chemotherapy. A systematic approach of NP is based on the characterization of all pain aspects, including emotional, behavioral, psycho-social, anatomical, genetic and molecular genetic factors[95,96]. Neuroimaging studies provide evidence suggesting that NP is associated with structural, functional and neurochemical alterations distributed across multiple brain structures and networks[97]. However, because many environmental factors may interact with genetic polymorphism to influence pain perception, data from proteomic studies to elucidate genetic contribution to NP remain limited and inconsistent although diverse molecules (NMDA/AMPA/ P2X3 ion channel receptors, G-protein-coupled receptors, AnnexinV/CaM/CRMP2 calcium signaling protein, N-type voltage-gated calcium channels CaV2.2, receptor tyrosine kinases, trkB for BDNF, non-coding RNAs)[98-101] can account for changes that arise in pathological pain states.

TREATMENT OF NEURAL NETWORK DEFICITS AND NP

Remodeling neuronal connectivity by transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS) consists of promoting a localized electric current through a localized magnetic field produced by a TMS-coil[102]. It is assumed that TMS and repetitive TMS have an inhibitory or facilitatory effect on neurons and neuronal networks. They can induce plasticity, modulate neurotransmission and increase neuroprotection against oxidative effect via BNDF/TrkB signaling system in the stimulated site and in other structures functionally connected with it[103-105]. These effects associated with magnetic stimulation -low intensity stimulation results in neurite sprouting and increase in synaptic contacts while high intensity

stimulation has devastating effects- can be maintained as long as 6 mo after treatment[106]. Furthermore, it has been shown that TMS reduces structural, functional and behavioral abnormalities in ephrin-A2A5^{-/-} mice but do not adversely affect the control WT[107]. Altogether, these observations suggest that TMS treatment may modulate synaptic strength not only locally but at distant sites, modulating the connectivity networks and offering the hope of a focal intervention capable of ameliorating the altered circuitries underlying psychiatric disorders. Indeed clinical trials directed to the treatment of major depression, schizophrenia, bipolar disorders, anxiety disorders, PTSD and neuropathic pain have supported a possible therapeutic effect of TMS alone or in combination with drugs[108]. Although the mechanisms of TMS activity are hypothesized to be based on induction of neuronal firing, certain effects may be due to the cryptochrome (CRY)/photolyase family: Present in all cell nuclei, CRYs that contain magnetosensitive radical pairs may provide the abilities of cells to specifically respond to magnetic field[109,110].

Remodeling neuronal connectivity with selective modulation of BDNF/CRMPs expression

One of the most studied and best characterized neurotrophins in CNS is BDNF. It has received remarkable attention from scientists because it is essential for neurogenesis, neuronal differentiation (including neuritogenesis/dendritic arborization, spine formation and axonogenesis *via* nonphosphorylated CRMP2), survival, migration, apoptosis, synaptic plasticity (*via* TrkB and p75NTR activation) and neuronal network formation, and from clinicians because it is required for normal development/functions of the brain and its expression is found decreased in several brain regions in post-mortem studies of patients with neurodegenerative and psychiatric diseases[60,79,81-83,111,112]. In physiological condition, BDNF activity depends on the activation of its downstream intracellular signaling cascades -Ras/MAPK, PLC-γ, PI3K/AKT. An additional level of regulation is provided by the balance between neurotrophic signaling through mature-BDNF/TrkB and apoptotic signaling through pro-BDNF/p75, which determines which connections are maintained within the neuronal network and which neurons are eliminated. Importantly, altered BDNF activity has been reported in brain pathology, particularly in limbic structure, AmyG, orbital and medial PFC and related keys circuits. Antidepressant activity seems to be linked to increasing levels of BDNF. Similar to BDNF, CRMPs express abundantly in the nervous system and while manipulations of their expression by RNAi, gene targeting or overexpression has confirmed their critical role in axono-dendritic growth and collapse, neuronal migration/survival and spinogenesis[29,30,33,34,36], other studies reveal that their expression is also altered in human pathologies including mental disorders[33,85,113-115]. Restoration of altered BDNF/CRMPs activities in these affected areas and/or affective circuits with newer and more refined/targeted immuno-
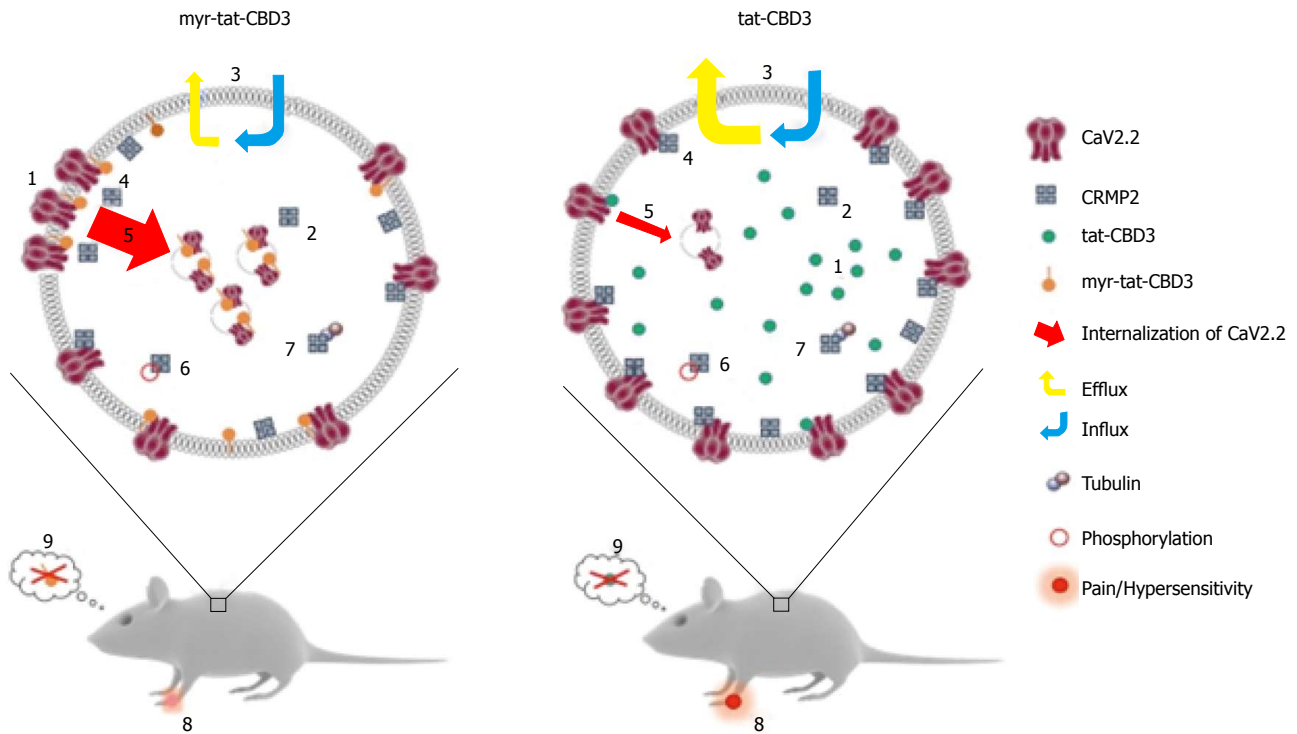
Figure 3 A possible model for N-myristoylated collapsin response mediator protein 2 peptide's actions on CaV2.2 trafficking and efficacy in neuropathic pain model. Application of an N-myristoylated tat-conjugated CRMP2 peptide (myr-tat-CBD3) results in membrane-delimited "rimming" of the peptide whereas the non-myristoylated version (tat-CBD3) appears to be spatially diffusely distributed in the cell cytoplasm. Analysis of penetration of peptides into GPMVs, which are "blebs" of membrane devoid of organelles and actin cytoskeleton, reveals an unrestricted distribution of the membrane sensitive dye (di-4-ANNEPDHQ) with tat-CBD3, whereas the myristoylated peptide induces a lateral heterogeneity of the fluorescent signal resulting in dye aggregation into micro domains within these model membranes[122]. While CRMP2 has been demonstrated to exist as a tetramer, the oligomeric state of membrane proximal CRMP2 is as yet unknown; however, neither peptide appears to affect CRMP2 oligomerization[123]. Whereas the cells take up both forms of the peptide with similar efficiency, the myristoylated peptide demonstrates a lesser degree of efflux[124]. The apparent increase in retention of myr-tat-CBD3 translates into a superior potency and efficacy in inhibition of evoked calcium influx in sensory neurons presumably *via* greater uncoupling of CRMP2-CaV2.2 interactions at or juxta-membrane[125]. Increased inhibition of CaV2.2 surface trafficking induced by myr-tat-CBD3 compared with tat-CBD3 may account[126] for the more pronounced restriction of calcium influx imposed by the myristoylated peptide. Cdk5-phosphorylated CRMP2 has been demonstrated to have an enhanced interaction with CaV2.2[129]; however myr-tat-CBD3 does not affect the levels of Cdk5-phosphorylated CRMP2[127], thereby ruling out a role of phosphorylated CRMP2 in regulating calcium influx. CRMP2 binding to tubulin is strengthened by the peptides[130]; the consequences of this are currently unknown. Importantly, where tat-CBD3 is completely ineffective in reversing mechanical hypersensitivity in a rat neuropathic pain model (tibial nerve injury), the myristoylated peptide reverses this hypersensitivity when administered *in vivo*[122]. Neither peptide elicits any reward-like addictive behaviors. GPMVs: Giant plasma membrane vesicles; CRMP: Collapsin response mediator proteins.

pharmacological agents will likely yield more effective treatments, particularly in treatment-refractory cases, and greater understanding of the mechanisms underlying mental disorders.

NP treatment with CBD3, a peptide derived from CRMP2
Presently, the understanding of the molecular and cellular mechanisms of NP is incomplete, and new concepts are needed to improve its treatment. Genetic and clinical studies have validated N-type voltage-gated calcium channels (CaV2.2) as targets for NP treatment[116-118] but selective blockers have potential serious side effects. Targeting protein interactions to direct channel block has been proposed[119]. This approach has yielded a novel peptide-derived therapeutic prototype for NP relief: A CRMP2-derived peptide (tat-CBD3; Figure 3) that disrupts the CaV2.2/CRMP2 interaction shows anti-nociceptive activity in animal models of neuropathic pain[120]. The relative lack of toxicity provides evidence that CBD3 has therapeutic promise[121]. Recent efforts to optimize the peptide's efficacy resulted in the generation

of a myristoylated version of the peptide; myr-tat-CBD3 -a myristoylated CBD3 peptide harboring a 14-carbon fatty acid, myristate, onto an N-terminal glycine[122]. *N*-myristoylation is a lipid anchor modification of eukaryotic and viral proteins targeting them irreversibly to locate to the membrane[123-125]. Tethering the CBD3 peptide to the membrane through myristoylation confines its action(s) to the uncoupling of membrane CaV2.2-CRMP2 and blocks Ca^{2+} influx without affecting the CRMP2 functions mediated by interactions with other cytoplasmic proteins[126].

As summarized in Figure 3, the myr-tat-CBD3 peptide is equal or better in its efficacy to tat-CBD3 on reducing pain-related behaviors with pronounced reversal of mechanical hypersensitivity in a postoperative incisional pain model[127] at dose of 0.1 mg/kg in contrast to the lack of effect observed even at 20 mg/kg of tat-CBD3[122]. Moreover sustained relief (> 6 wk) of NP was obtained with an AAV-targeted expression of CBD3 peptide in rat DRG[128]. Both tat-CBD3 and myr-tat-CB3 reduce pain-like behaviors without demonstrating any

reward-like potential. These experiments demonstrate the possibility of tailoring molecules that affect membrane targets for specific inhibition of CaV2.2-CRMP2 interactions (Figure 3).

CONCLUSION

Data from various basic science studies together with experimental/clinical studies suggest a correlative causal link between specific neural networks dysfunctions and mental disorders. However, one could argue that (1) a correlation between experimental and clinical observations is a correlative statement but not a causal demonstration; (2) an observed electrical current dysfunction may not support this common view of correlative network specificity because it can result from different network stimulations with many unknown parameters and consequently underspecifying any structural function; and (3) more direct experiments will be needed to consolidate these observations. This approach is critical since it may raise further technical inventions/interventions and challenges such as generating new tools more powerful optogenetic/acousto-optical deflectors approaches in animal models[131,132] to visualize, control and compute simultaneously single neuronal activity and neuronal circuitries, to integrate in parallel behavioral measures for a better treatment of mental diseases, less side-effects associated to newer psychotropic drugs and gene therapy[133-137], and more importantly a better understanding of the physiology and pathology of the human brain in healthy activity and abnormal behavior respectively.

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REVIEW

Role of astrocytic glutamate transporter in alcohol use disorder

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Abstract

Alcohol use disorder (AUD) is one of the most widespread neuropsychiatric conditions, having a significant health and socioeconomic impact. According to the 2014 World Health Organization global status report on alcohol and health, the harmful use of alcohol is responsible for 5.9% of all deaths worldwide. Additionally, 5.1% of the global burden of disease and injury is ascribed to alcohol (measured in disability adjusted life years, or disability adjusted life years). Although the neurobiological basis of AUD is highly complex, the corticostriatal circuit contributes significantly to the development of addictive behaviors. In-depth investigation into the changes of the neurotransmitters in this circuit, dopamine, gamma-aminobutyricacid, and glutamate, and their corresponding neuronal receptors in AUD and other addictions enable us to understand the molecular basis of AUD. However, these discoveries have also revealed a dearth of knowledge regarding contributions from non-neuronal sources. Astrocytes, though intimately involved in synaptic function, had until recently been noticeably overlooked in their potential role in AUD. One major function of the astrocyte is protecting neurons from excitotoxicity by removing glutamate from the synapse *via* excitatory amino acid transporter type 2. The importance of this key transporter in addiction, as well as ethanol withdrawal, has recently become evident, though its regulation is still under investigation. Historically, pharmacotherapy for AUD has been focused on altering the activity of neuronal glutamate receptors. However, recent clinical evidence has supported the animal-based findings, showing that regulating glutamate homeostasis contributes to successful management of recovery from AUD.

Key words: Alcohol; Addiction; Glutamate; Astrocyte; Excitatory amino acid transporter type 2; Glia; Striatum

Core tip: Review of astroglial involvement in alcohol use disorder and potential for astrocyte-specific glutamate transporter excitatory amino acid transporter type 2 (EAAT2, Slc1a2)/GLT-1 in pharmacological treatment, including alcohol withdrawal symptoms.

INTRODUCTION

Substance abuse and addiction is a pervasive problem at all socioeconomic levels worldwide. Alcohol, though one of the most common addictive substances, is recreationally used by many people without incident. Alcohol use disorder (AUD) or alcohol addiction often results in one of the most lethal sets of withdrawal symptoms, including seizures, hallucinations, and delirium tremens, termed alcohol withdrawal syndrome (AWS). As with most addictive substances, craving and relapse rates are high, and predictability, and even recognition, of addiction is difficult to ascertain. This makes alleviation of withdrawal symptoms the primary focus on treating AUD.

Like other neuropsychiatric conditions, AUD involves dysfunction of normal signaling pathways. Importantly, neurons are not the only cells involved in neural signaling. The majority of cells in the central nervous system (CNS) are of the glial lineage[1]. Two of the major glial cell types, oligodendrocytes and microglia, have been well characterized, as they are relatively monofunctional: Oligodendrocytes myelinate neurons, and microglia regulate the CNS immune response[2]. Ironically, the most abundant glia, the astrocyte, is not as well understood. Its diverse role in maintaining vascular integrity and metabolic homeostasis, and regulating synaptogenesis and immune response[1,3], means that its morphology and gene expression vary greatly depending on its location, local contacts, and microenvironment[4,5].

AWS is attributed to elevated extracellular glutamate levels in certain brain regions, such as the striatum and hippocampus[6-8]. These regions are part of the mesocorticolimbic circuit, which is highly implicated in addiction disorders[9,10]. Astrocytes provide critical regulation of synaptic glutamate concentrations in these regions through bi- and uni-directional transporters. Glutamate, the most important excitatory neurotransmitter in the CNS, serves to stimulate action potentials by activating its cognate ionotropic receptors on the postsynaptic neurons, and modulate synaptic connectivity through its cognate metabotropic receptors. Astrocytes are responsible for removing glutamate from the extracellular space, thereby limiting its effects. Within the astrocyte, glutamate is converted to glutamine *via* glutamine synthetase, and released back out to the extracellular environment for uptake by presynaptic neurons, which convert it back to glutamate to be packaged into vesicles for synaptic release. In AWS, this glutamatergic tone is dysregulated, resulting in a hyperglutamatergic state that can lead to neuronal excitotoxicity. Clinically, benzodiazepines are often used to alleviate withdrawal symptoms, but patients can become physically dependent on these, as well[11].

Animal models have been extremely useful in studying addiction. The rodent corticolimbic circuitry is very similar to human, and thus provides an effective method for studying various aspects of the addiction and withdrawal processes. Certain species are useful for studying behaviors of different points and aspects of the addiction process, including consumption, preference, learning, tolerance, and withdrawal[12]. Additionally, through genetic manipulation of specific components of glutamatergic signaling, we have come to understand ethanol's effects on involved neuronal glutamate receptors, such as NMDA and AMPA receptors[13-17]. This review will focus on the role of glia in neuronal function, both normal and pathological, and illuminate the importance of understanding astrocyte function in neuropsychiatric disorders, like AUD.

GLIAL FUNCTION IN NEUROPSYCHIATRIC DISORDERS

Glial cells are mostly known for their contributions to physiological processes and pathological involvement. There are many more glial cells than neurons (glia to neuron ratio = 4:1), and they are generally smaller[18]. Three subtypes have been identified in the CNS: Astrocytes, microglia and oligodendrocytes. The major function of glial cells is to maintain homeostasis in the CNS, including supporting blood vessels, providing nutrition and oxygen to neurons, regulating neurotransmitter metabolism and modulating the immune response. Therefore, balance disruption derived from any of these cell types could be associated with multiple kinds of psychiatric disorders.

Oligodendrocytes are one of the more numerous than the types of glial cells. Their primary role is to myelinate axons, which increases the resistance and reduces the effective capacitance of axonal membranes, accelerating the transmission of electrical signals. They arise from a large population of oligodendrocyte precursor cells (OPCs), a source of replacement cells for damaged oligodendrocytes after injury or in disease. There are multiple trophic and growth factors that maintain oligodendrocyte survival and myelination[19]. One therapeutic option is to increase and amplify this OPC pool once the pathologically activated signa-

ling pathways are identified[20]. Moreover, mounting evidence shows that oligodendrocytes may also play a critical role in neuropsychiatric disorders[21].

Astrocytes represent highly heterogeneous mediators that interact directly with neurons, which is crucial for maintaining brain function. Astrocytes play an essential role in neurogenesis and the development of the CNS, energy metabolism, synaptic signaling, immune defense, amino acid neurotransmitter clearance, neurotrophin production and ionic homeostasis[22]. More recently, many studies have suggested that astrocyte dysregulation is associated with neuropsychiatric disorders, such as Wernicke's encephalopathy[23] and Korsakoff psychosis[24], schizophrenia[25], depression and addiction[26].

Microglia originates from hematopoietic progenitors and have been considered the resident immune cells in CNS. However, recent evidence has provided a notion that microglia are not only activated by inflammatory challenge or neuronal damage, but that they extend an array of physiological functions through synaptic maturation[27], maintenance of CNS homeostasis, memory processes and neurogenesis[28]. Additionally, disturbance of microglia responding to minor pathological intrusion is implicated in multiple neuropathological and neuropsychiatric disorders.

Pathological potential of neuroglia in AUD
Astrocytes play critical roles in CNS abnormalities associated with ethanol-induced neurotoxicity. Chronic ethanol exposure profoundly affects the expression of GFAP, an astroglial stress fiber, as well as the function of astrocytes. One interesting study found that by increasing astrocyte number by injecting purified astrocytes into rat forebrain learning and memory abilities associated with cholinergic signal recovery were improved[29]. It is well known that alcohol and its metabolite acetaldehyde show direct neurotoxicity by disturbing thiamine-related enzymes, resulting in thiamine deficiency and damage to astrocytes[30]. Our laboratory demonstrated the importance of adenosine signaling in regulating alcohol-related behaviors. Mice lacking type 1 equilibrative nucleoside transporter (ENT1), the glial transporter responsible for maintaining adenosine tone in the synapse, consume more ethanol in two bottle choice experiments when compared to wild-type littermates[31]. In addition, antagonists and agonists acting on the adenosine A_{2A} receptor ($A_{2A}R$) modulate ethanol preference and withdrawal symptoms. Moreover, inhibition of $A_{2A}R$ in the dorsomedial striatum promoted goal-directed behavior and increased sucrose or ethanol drinking[32]. We revealed that deletion of ENT1 decreased the expression of astrocytic glutamate transporter GLT-1, the protein responsible for regulating extracellular glutamate concentration in the striatum. Adenosine-mediated glutamate signaling in neuroglial interaction in ethanol intoxication and preference has been thoroughly described elsewhere[33,34].

Microglia are the resident macrophages and are distributed ubiquitously throughout the CNS. Alterations in microglia have even been noted in AUD models. After intensive chronic ethanol intoxication in rats, there were structural lesions in the CNS, including infiltration of mononuclear cells and lymphocytic-microglial nodules[35]. Changes in immune-related genes have been found in human alcoholic brain, including chemokines (Ccl2, Ccl3) and chemokine receptors (Ccr5, Ccr1 motif)[36]. Consumption and preference of ethanol decreased in mice after deleting either chemokines (Ccl2 or Ccl3) or chemokine receptors (Ccr2). Particularly the proinflammatory cytokine, CCL2, was extensively higher in alcoholics[37]. Also, consecutive 10-d injection of ethanol increased pro-inflammatory cytokine TNFα in the brain. Meanwhile, ethanol enhanced LPS-induced increases in TNFα, MCP-1, and IL-1β in brain[38]. Previous studies indicated that TLR4 response might be a vital mechanism of chronic ethanol-induced neuroinflammation. The signaling pathways related to IL-1RI/TLR4 receptors were facilitated by chronic ethanol treatment[39], while the deletion of TLR4 prevented ethanol-induced glial activation and production of inflammatory mediators[40].

Regarding oligodendrocytes, alcohol appears to have different effects, but much of the evidence points toward reduced oligodendrocyte function. Prenatal alcohol exposure decreased the level of myelin basic protein (MBP) expression[41], consequently leading to a reduction in brain myelination that may contribute to the development of fetal alcohol syndrome[42]. Protein kinase C was activated after ethanol exposure, which delays MBP expression in differentiating CG-4 oligodendrocytes. Oligodendrocyte myelin glycoprotein was significantly decreased in rat hippocampus by chronic ethanol exposure[43]. This deficit may explain the demyelination observed in human alcoholics[44]. Recent studies showed that premyelinate oligodendrocytes and myelin-associated protein were decreased in medial prefrontal cortex (mPFC) during chronic intermittent ethanol vapor exposure (CIE). However, protracted abstinence increased MBP levels in the mPFC, possibly a compensatory effect after CIE[45]. In contrast, the activity of enzyme 2', 3'-cyclic nucleotide 3'-phosphodiesterase, a maker for oligodendrocytes, was increased in ethanol-treated cultures[46].

Neuroglia pathology in other psychiatric conditions
Glial pathology is also implicated in other neuropsychiatric conditions, like schizophrenia and bipolar disorder (BD). One study demonstrated decreased astrocytic gene expression in the deep layers of the anterior cingulate gyrus, including excitatory amino acid transporter 2 (EAAT2)[47]. In this regard, animal models have been somewhat helpful in investigating structural changes, glycogen metabolism and glutamate signaling[48].

BD, like AUD, involves the corticostriatal circuit. Morphometric postmortem and neuroimaging studies found decreased glial cell density in the mPFC of

patients with BD[49]. Recent studies indicate that chronic treatment with anti-bipolar drugs could inhibit astroglial glutamate release[50]. Additionally, S100β, a neurotrophic factor mainly released by astrocytes, was increased after chronic lithium treatment, although astrocytes or interaction between neurons and astrocytes were unaffected[51].

Regarding microglia, studies in schizophrenia patients have yielded more consistent results. Microglia are activated along with increased cell population in the postmortem brains of schizophrenic patients[52]. Consistently, the level of cytokines, IL-1β, IL-6, and TGF-β, were elevated in schizophrenia. Interestingly, these alterations were reversed by antipsychotic treatment[53]. The dysfunction of microglia is associated with impairment of nervous system development, synaptic formation and pruning[27,54], processes that may also affect neural circuitry in AUD.

Neuroinflammation has also been linked to BD. The level of IL-1β was increased in cerebrospinal fluid of euthymic BD patients when compared with normal controls, and even higher in those who had recently experienced one or more manic/hypomanic episodes[55]. In addition, microglia activation induced higher levels of IL-1β, which was associated with increased suicide behavior in BD patients[56]. Moreover, concentrations of pro-inflammatory cytokines, which are associated with increased activation of MAPK and NF-κB pathways in BD patients, were higher, as well. These results suggest that microglia play a potentially crucial role in BD. Interestingly, there is an increased incidence of BD in patients with the autoimmune disorder, multiple sclerosis, where oligodendrocytes are targeted by the immune system. And, oligodendrocytic markers are down regulated in patients with BD[57].

Multiple studies have confirmed oligodendrocytes and myelination are impaired in schizophrenia[58-60]. Oligodendrocytic and myelination abnormalities damage saltatory conduction and information transfer between neurons in schizophrenia[61]. White matter anomalies were found in schizophrenia patients from maternal and early postnatal immune challenge[62], perinatal hypoxia[63], and during childhood and adolescence[58,64], suggesting that damage to axonal integrity and conduction velocity may play a causal role in schizophrenia.

Taken together, studying glial cells is warranted for understanding neuropsychiatric disorders in regards to glutamatergic abnormalities.

SYNAPTIC, PERISYNAPTIC AND EXTRASYNAPTIC ASTROCYTIC PROCESSES IN REGULATING GLUTAMATE SIGNALING

Neurons connect to each other with a kind of chemical junction called a synapse, which consists of the pre-synapse, post-synapse, and synaptic cleft as a key information communication relay and central functional element of the nervous system[65,66]. Each neuron can receive a number of synaptic inputs and these synapses have diverse properties, including the type of neurotransmitter and the number of postsynaptic receptors. Neurotransmitter vesicles accumulate in the presynaptic terminal, while the neurotransmitter receptors located in the postsynaptic neurons are recruited to the postsynaptic.

Synaptic astrocytic processes

Astrocytes were widely thought to provide only metabolic and physical support for neurons, but now they are demonstrated to directly participate in neuronal signaling, even locally at synapses[67,68]. Many investigations have shown that astrocytes exhibit unique biophysical and functional electrical properties, are sensitive to neuronal activity and are actually involved in the control of synaptic transmission. Astrocytes are indeed equipped to sense and integrate neuronal information through ionic channels, neurotransmitter receptors and transporters, and intracellular signaling pathways[69]. Astrocytes are also known to influence synaptic activity *via* synthesizing and recycling glutamate[70] and responding to synaptic release of neurotransmitters[68,71,72]. Glutamate, as a major neurotransmitter in the brain, exerts a critical role in mediating synaptic activity and also causing a response in astrocytes[73,74]. Importantly, the involvement of astrocytes in glutamatergic regulation is very widespread, as most glutamate is taken up by transporters expressed on astrocytic membranes when it is released into the extracellular space between neurons[75,76], and it is estimated that only 20% of synaptic glutamate is taken up by transporters on the postsynaptic neurons[77]. Astrocytes can synchronize with neuronal activity, and subsequently regulate glutamate transmission between neurons[78]. Astrocyte-to-neuron glutamate signaling may mediate activity-dependent modulation of inhibitory synapses in the hippocampus, with glial glutamate release taking place at some distance from the synapse[79]. The increases in calcium (Ca^{2+}) evoked by neuronal activity results in a release of glutamate from astrocytes[80,81]. This release of glutamate process is known to be a Ca^{2+}-dependent exocytic pathway[82]. In addition, during synaptic activity neurons or astrocytes release ATP, which cause an increase in intracellular (Ca^{2+}), mediated by the P2Y1 type purinergic receptors (P2Y1Rs) in hippocampal astrocytes[83]. As a G protein coupled receptor, P2Y1R signaling in astrocytes is coupled to Ca^{2+}-dependent glutamate exocytosis[84,85].

Glutamate is able to induce a wide range of effects in astrocytes *via* metabotropic glutamate receptors (mGluR), NMDA receptors, and AMPA receptors[86]. Hippocampal astrocytes express functional AMPA receptors[87], the properties of which can be changed by astrocytes during postnatal development. It has been reported that immature astrocytes had a prolonged activation of the AMPA receptors, while glutamate

- Glutamate
- Glutamine
- Ca²⁺
- EAAT2
- Cysteine-glutamate transporter, Xc-
- Equilibrative nucleoside transporter 1, ENT1
- VGLUT1
- Glutamine synthetase
- Connexin hemichannel
- NMDA receptor (GluN2A subunit)
- NMDA receptor (GluN2A subunit)
- AMPA receptor
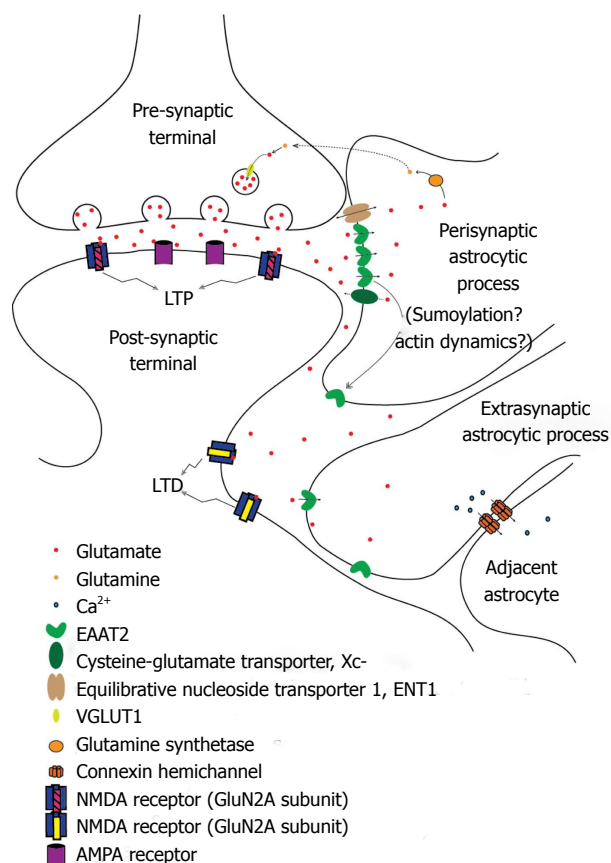
Figure 1 Fine regulation of glutamate levels and excitatory amino acid transporter 2 localization in neuron-glial interaction. The perisynaptic astrocytic process contains a higher concentration of EAAT2 immediately adjacent to the synapse. The EAP also contains EAAT2, but in lower concentrations. Glutamate spillover results in activation of GluN2B-containing NMDA receptors (increasing LTD response in the post-synaptic neuron), increased EAAT2 surface expression on and migration of EAPs, and activation of adjacent astrocytes via calcium waves propagated through connexin channels. LTP: Long term potentiation; LTD: Long term depression; EAAT2: Excitatory amino acid transporter 2; Xc-: Cysteine-glutamate transporter; ENT1: Equilibrative nucleoside transporter 1; VGLUT1: Vesicular glutamate transporter 1; NMDA: N-Nitrosodimethylamine; AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; EAP: Extrasynaptic astrocytic process.

responses were greatly increased as astrocytes matured. In addition, the presynaptic NMDA receptors are activated and the excitatory communication between neurons is increased once astrocytic glutamate is released[85]. Additionally, astrocytes also use the mGluRs to modulate neuronal activity. However, previous results suggest developmental changes in the expression of mGluRs in astrocytes[88], predicting its important role in development. Reports reveal that synaptic glutamate stimulates astrocytic mGluRs and subsequently triggers glutamate release from astrocytes[80,89].

Perisynaptic astrocytic processes

It has recently been recognized that astrocytes act as a third partner in synaptic processes, leading to the description of a "tripartite synapse[90]", with the astrocytic processes in close apposition to the synaptic cleft[91]. Perisynaptic astrocytic processes (PAPs), assumed to mediate this communication, may detect glutamate

spillover and other substances from active synapses[76,92]. Glutamate can also cause structural alterations of astrocytes, driving them to extend and modify their own processes[73,93] (Figure 1). It is thought that PAPs position next to the synaptic cleft, as the effectors of glia-neuronal crosstalk at the synapse, to exert their roles in ion buffering and transmitter uptake, thus keeping a low diffusion distance[94]. Glutamine synthetase is mainly expressed in the PAPs around identified glutamatergic synapses[95].

There have been a few approaches that described peripheral astrocyte processes related to synapses using direct or indirect observations. Glial gamma-aminobutyricacid (GABA) receptor channels could be of functional importance in buffering extracellular Cl⁻ in the perisynaptic microenvironment using patch-clamp techniques, and might relate to mechanisms not present in the peripheral processes[96]. Electrically stimulated parallel fibers increased intracellular calcium concentration in peripheral processes of Bergmann glia, astrocytic cells located in the cerebellum[97]. The transmitter release is affected by reduction in glutamate clearance through modulation of presynaptic mGluR. Therefore, astrocytic wrapping of neurons may contribute to the regulation of synaptic efficacy in the CNS[98]. Glia-synaptic interactions can be attributed to the astrocytic peripheral processes in the perisynaptic microenvironment. As the functions of PAPs displayed might not be representative of the astrocyte as a whole, they could be recognized as a specialized astroglial compartment[94].

Extrasynaptic astrocytic processes

In some instances, glutamate may escape from the synaptic cleft, generating extrasynaptic glutamate dynamics[99,100]. The kinetic simulations of extrasynaptic glutamate uptake show that glutamate can bind to several subtypes of receptors, but transporters rapidly reduce the free concentration of transmitter[101]. Extrasynaptic glutamate dynamics is believed to regulate a variety of important neural and glial functions including synaptic transmission[102], synaptic plasticity[103], synaptic crosstalk[104,105], nonsynaptic neurotransmission[106], neuronal survival[107], and gliotransmitter release[108].

Synaptic "spillover", or extracellular diffusion of transmitters and modulators after extrasynaptic release in the local circuit regions, is an important short distance form of volume neurotransmission. The neurotransmitters bind to extrasynaptic neuronal and glial receptors and then activate related signaling pathways in the neuroglial networks of the brain (Figure 1). G protein-coupled receptor heteromers play a major role in the integrative processes of extrasynaptic signaling on glutamatergic synapses. The balance of activity in the different types of projection neurons is determined by the diverse distributions of high affinity receptors on the neurons and glia, where both neuronal extrasynaptic and long distance volume transmission signaling can be involved[109]. Temporal resolution at axon terminals of

fast-spiking neurons might be due to rapid glutamate receptor desensitization. Transmitter diffusion is partly responsible for transmitter clearance from the synaptic cleft[76]. The glutamate transient duration seen at a distance from the release site can be greatly increased if interactions between glutamate and extrasynaptic binding sites reduce the effective diffusion constant, resulting in a greater probability of NMDA receptor activation[110]. Relatively small amounts of glutamate are likely to activate extrasynaptic receptors, especially if the diffusion coefficient in the extracellular medium is low[101]. Extrasynaptic glutamate spillover implies significant cross-talk between neighboring synapses, which could have major repercussions for information processing[111].

GLUTAMATE TRANSPORTERS IN AUD

Glutamate is arguably the most important neuroexcitatory amino acid in the CNS. As such, its concentration, both intra- and extra-cellularly, is tightly regulated by membrane transporters. Within neurons, the vesicular glutamate transporters, of which there are three known subtypes[112], pack glutamate from the cytosol into presynaptic vesicles utilizing the proton electrochemical gradient[113]. In glia, the cysteine-glutamate transporter (Xc-) is the major transporter responsible for releasing glutamate into the extracellular space, while a family of sodium-dependent transporters, called excitatory amino acid transporters (EAAT), removes glutamate from the extracellular environment. The function of these latter transporters is critical, as excessive synaptic glutamate can lead to neuronal excitoxicity and/or death. There are five mammalian EAAT subtypes (EAAT1-EAAT5) that have been identified to date. EAAT1 and EAAT2 (in rodents, designated glutamate and aspartate transporter, GLAST, and glutamate transporter 1, GLT-1, respectively) are the primary subtypes responsible for extracellular glutamate clearance throughout the CNS during neurotransmission, and are predominantly expressed in astrocytes[114,115]. EAAT1 is most abundantly expressed in the cerebellum, while EAAT2 has a more ubiquitous expression pattern, which is strongest in the forebrain[116]. EAAT3 (EAAC1 in rodents) is the most widely distributed, expressed in peripheral tissues, as well as in both neurons and astrocytes[117]. EAAT4 is most strongly expressed in cerebellar Purkinje cells[118], and EAAT5 is almost exclusively expressed in the retina[119].

In recent years, EAAT2 has received increasing attention in addiction research, as it is the primary EAAT subtype expressed in the striatum, a component of the basal ganglia that has been implicated in addiction and other psychiatric disorders, and is responsible for clearance of at least 90% of extracellular glutamate. Conceptually, alterations in EAAT2 surface expression should impact neurotransmission, and indeed, this has been demonstrated[120,121].

Structure, function and localization

Structurally, EAATs contain eight transmembrane segments. The N-terminal structures are involved in intersubunit contacts, while the C-terminal half is implicated in substrate transport[122]. EAATs appear to be oligomeric, and EAAT2 tends to form homotrimers and heterotrimers with its other isoforms[123,124], generating a bowl-shaped complex with the aqueous, concave side facing the extracellular space and the pointed side facing the cytoplasm. Interestingly, between the subunits on the hydrophobic transmembrane sides are distinct crevices that allow lipids to interact with key functional domains of the transporter[122], suggesting a role for lipid modulation of EAAT2 activity.

EAATs are capable of transporting both glutamate and aspartate. With one molecule of glutamate, they co-transport three sodium ions and one proton in exchange for one potassium ion[122]. Mouse EAAT2 (GLT-1) shares 97% sequence homology with humans and 99% with rats[125]. The Km of EAAT2 is fairly small (for D-aspartate, 17 ± 5 μmol/L for mouse[126], 54 ± 9 μmol/L for human[127]). Synaptic levels of glutamate can be as high as 1 mmol/L[128], so transport of glutamate out of the synapse occurs very rapidly. In this way, EAAT2 maintains low extracellular glutamate concentrations, preventing it from reaching excitotoxic levels.

In the striatum, EAAT2 is primarily located on the membrane surface of astrocytes, but can also be found on the outer mitochondrial membrane, on rough endoplasmic reticulum, and on polyribosomes[129]. EAAT2 tends to concentrate at cellular processes adjacent to glutamatergic synapses (PAPs; Figure 1) and in astrocytic endfeet near capillaries in the neurovascular unit[1]. It is tightly associated with cholesterol-rich lipid rafts[130,131], and it has been suggested that it may be part of a macromolecular complex that also contains Kir4.1 and AQP4 at capillary endfeet, implying a combined effort for glutamate and potassium ion homeostasis[132].

Glutamate transporter regulation

EAAT2, like most proteins, can be regulated by multiple methods. It is subject to transcriptional regulation by transcription factors and differential splicing, epigenetically by histone modifications and DNA methylation, post-translationally by various residue modifications, and functionally by certain toxins, membrane lipid composition, and cytoskeletal rearrangements[115,131,133-137] (Table 1 provides a more complete list of modulators).

The EAAT2 promoter contains several transcription factor binding sites, including CREB, KBBP, NF-κB, N-myc, Sp1 and Yin Yang 1[115,134,138]. The most studied of these, NF-κB, has been shown to be an effective regulator of EAAT2 expression, though by different mechanisms depending on the initiation factor[139-145]. For example, EGF-receptor stimulation induces increased EAAT2 transcription, but not *via* IκB degradation[115], whereas Ceftriaxone, a β-lactam antibiotic, does appear to

Table 1 Modulators of excitatory amino acid transporter 2 expression and/or function

Compound	Demonstrated effect on EAAT2	Process/pathway	Ref.
17β-estradiol	Increased expression	Activation of both NF-κB and CREB	Lee *et al*[142]
cAMP/bromo-cAMP/dB-cAMP	Increased expression	PKA pathway, PI-3K and NF-κB	Su *et al*[115]
Ceftriaxone	Increased expression	Conventional NF-κB activation	Lee *et al*[146]; Rothestein *et al*[143]
EGF	Increased expression	Non-canonical NF-κB activation	Zelenaia *et al*[144]
PACAP	Increased expression	PKA and PKC activation	Figiel *et al*[139]
Parawixin1	Increased transport speed	Allosteric enhancement of receptor function	Fontana *et al*[133]
Raloxifene	Increased expression	Activation of both NF-κB and CREB	Karki *et al*[135]
Riluzole	Increased expression and activity	Multiple pathways, mediated by estrogen receptors and HSF1	Karki *et al*[135]; Liu *et al*[137]
Tamoxifen	Increased expression	Activation of both NF-κB and CREB	Karki *et al*[141]
TGF-α	Increased expression	Tyrosine Kinase activation	Lee *et al*[136]; Su *et al*[115]
TNFα	Decreased expression	Co-activation of NF-κB and N-myc	Sitcheran *et al*[145]

Listed in this table are agents that have been demonstrated to affect EAAT2 expression and/or function, and their mechanism of action. EAAT2: Excitatory amino acid transporter 2; cAMP: Cyclic adenosine monophosphate; CREB: cAMP response element-binding protein; NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells; PI-3K: Phosphatidylinositol 3-kinase; PKA: Protein kinase A; EGF: Epidermal growth factor; PACAP: Pituitary adenylate cyclase-activating peptide; TGF-α: Transforming growth factor alpha; TNFα: Tumor necrosis factor alpha.

activate EAAT2 through the canonical NF-κB pathway[146]. However, NF-κB has also been shown to reduce EAAT2 transcription through co-activation of the TNFα-mediated NF-κB activation and N-myc activation, converting NF-κB to a repressor[145] (Table 1). One intriguing method of EAAT2 up regulation is through an as yet unknown mechanism driven by the presence of neurons or neuronal supernatant in cultured astrocytes, involving NF-κB and the kappa B-motif binding phosphoprotein (KBBP)[147]. Interestingly, in cultured astrocytes, transcription factor Ying Yang 1 appears to act as a repressor by recruiting histone deacetylases, coordinating an epigenetic method of silencing the gene[134]. Hypermethylation of EAAT2 promoter DNA is another epigenetic mechanism for EAAT2 down regulation that has been suggested[147,148].

Several post-translation modifications have been suggested to control EAAT2 levels and cellular localization. EAAT2 is internalized and degraded through Nedd-2 dependent ubiquitination at its C-terminus[149]. Sumoylation may also be involved, as non-sumoylated EAAT2 is primarily localized to the plasma membrane while only sumoylated EAAT2 is found in intracellular compartments[150] (Figure 1).

Functionally, EAAT2 has been show to exhibit reduced glutamate uptake when membrane cholesterol levels are reduced[130], as suggested by the inter-subunit crevices revealed by its structure. Additionally, certain exon-skipping EAAT2 splice variants appear to have an effect on EAAT2 function and surface localization[123].

In neuropathology, EAAT1 and EAAT2 have been shown to be reduced in several neurodegenerative disorders, such as amyotrophic lateral sclerosis, Alzheimer's disease, and Huntington's disease, where it was causally related to excitotoxic neuronal cell death[151]. In AUD, one genetic variant of human EAAT2, G603A, has been associated with antisocial[152] and cirrhotic alcoholics[153]. Rodent models, however, have provided an excellent tool for studying EAAT2 in AUD. The mouse EAAT2 gene (*slc1a2*) is located near a quantitative trait loci on chromosome 2 (E2)[125] that modulates seizure frequency and neuro-excitability in mouse models of epilepsy and alcohol withdrawal[154]. Many mouse studies have supported the involvement of EAAT2 in both the acute and chronic effects of ethanol, as well as the symptoms of withdrawal. Mulholland *et al*[155] showed disruption of NMDA and EAAT2 dependent Kv2.1 potassium channels by acute ethanol in the hippocampus. Several studies have demonstrated increased glutamatergic tone in regions of the cortico-striatal circuit[156], but there are only a handful of publications providing strong support for a correlating decrease in EAAT2. Increased EAAT2 expression and/or function alleviates withdrawal symptoms and reduces ethanol consumption[157,158], highlighting the importance of EAAT2 in regulating synaptic glutamate signaling.

CONCLUSION

According to the DSM-5® (diagnostic and statistical manual of mental disorders), alcohol use becomes a disorder when at least two of eleven specific criteria are met, including uncontrolled consumption, alcohol craving, or a development of tolerance. Addiction is a chronic, relapsing disorder characterized by the progression from occasional, controlled use to uncontrolled use and behavior over seeking and consumption, to chronic relapse after protracted abstinence. This process involves neuroplastic changes, primarily in the mesolimbic dopamine system, going from impulsive, positively reinforced behaviors (euphoria, social aspects) to compulsive, negatively reinforced behaviors (removing withdrawal symptoms)[10,159].

Understandably, early research in the field of addiction was focused on the ascending monoamine pathways of the dopaminergic reward system (mesocorticolimbic and extended amygdala), including the involvement of GABA and serotonin, all of which are

important in the early stages of addiction. In the past two decades, increased focus has shifted to glutamatergic signalling, as glutamate receptors in this system are greatly altered, and highly implicated in all stages of addiction, especially the later stages of dependence and withdrawal. The effects of alcohol on all types of glutamate receptors are under intense investigation, and much has been learned to date on how the dysregulated synaptic glutamate alters synaptic plasticity[66,67,86,121] (Figure 1). This shift in focus coincides with the success of clinical pharmacotherapies that alter the glutamatergic system. Acamprosate, a homo-taurine derivative similar in structure to GABA, especially when used with naltrexone, an opioid antagonist, has been one of the most effective drugs in preventing relapse in AUD[160]. However, the mechanistic understanding of these drugs is still not well understood. But, because of these relative successes, we are increasing our efforts in understanding synaptic glutamate modulation. For example, ceftriaxone, a beta-lactam antibiotic, has been shown not only to increase EAAT2 transcript levels, but also to attenuate ethanol consumption and withdrawal symptoms[157,158,161].

Regulation of EAAT2 and glutamate is complex, and understanding how to effectively control astrocytic EAAT2 function and/or expression could be the key to successfully treating AUD. By maintaining stable synaptic glutamatergic tone to ease withdrawal symptoms and allow re-establishment of non-pathological connections in the mesocorticolimbic circuit, recovery from addiction could be greatly ameliorated. In conclusion, a medication targeting an astrocytic glutamate transporter, especially EAAT2, will be an appropriate treatment option for AUD patients with pathologically increased glutamate levels.

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Cortical and subcortical gamma amino acid butyric acid deficits in anxiety and stress disorders: Clinical implications

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Abstract

Anxiety and stress disorders are a major public health issue. However, their pathophysiology is still unclear. The gamma amino acid butyric acid (GABA) neurochemical system has been strongly implicated in their pathogenesis and treatment by numerous preclinical and clinical studies, the most recent of which have been highlighted and critical review in this paper. Changes in cortical GABA appear related to normal personality styles and responses to stress. While there is accumulating animal and human neuroimaging evidence of cortical and subcortical GABA deficits across a number of anxiety conditions, a clear pattern of findings in specific brain regions for a given disorder is yet to emerge. Neuropsychiatric conditions with anxiety as a clinical feature may have GABA deficits as an underlying feature. Different classes of anxiolytic therapies support GABA function, and this may be an area in which newer GABA neuroimaging techniques could soon offer more personalized therapy. Novel GABAergic pharmacotherapies in development offer potential improvements over current therapies in reducing sedative and physiologic dependency effects, while offering rapid anxiolysis.

Key words: Brain imaging; Anxiogenesis; Gamma amino acid butyric acid; Anxiety disorders; Anxiolysis

Core tip: Preclinical and clinical studies strongly support the notion that impairments in gamma amino acid butyric acid (GABA) neurotransmission underpin human stress and anxiety disorders. Measurement of *in-vivo* brain GABA function with modern neuroimaging tools, such as proton magnetic resonance spectroscopy, in healthy and disease populations, has contributed greatly to this literature, and also offers the possibility of monitoring GABAergic anxiolytic therapy.

INTRODUCTION

Anxiety and stress disorders are a major public health problem. They are the most common mental health conditions in the United States with a 12-mo prevalence rate of 18%[1]. Moreover, in their lifetime, over 25% of the United States population is expected to suffer from at least one anxiety disorder[1]. Anxiety disorders are responsible for long-term morbidity, and are now thought to be even more chronic than either substance use or mood disorders[2]. Similar observations have been reported from surveys conducted around the globe[3-5]. Across these studies, another consistent finding was the disproportionate impact of clinical anxiety on women. Finally, the societal and economic impact of anxiety syndromes is remarkable. In 1990, for instance, the direct and indirect cost to the United States economy due to these disorders was $42.3 billion[6].

Over the last three decades, diagnostic assessment and treatment options for morbid anxiety have improved considerably. Despite many theories, however, the pathogenesis of these conditions remains unclear. With a deeper understanding of fear and stress neurocircuitry, and the availability of more sophisticated imaging and genetic analytic tools, progress is being made. Within the field, there has been an emerging emphasis on the role of amino-acid neurochemical systems, such as the amino-butyric acid [The gamma amino acid butyric gamma acid (GABA), the major inbibitory neurotransmitter in the central nervous system (CNS)] and its excitatory counterpart, glutamate, in anxiogenesis and anxiolysis. This review will examine the evidence implicating abnormalities in GABA neurotransmission in the genesis of stress and anxiety in health and disease. Key anxiety and stress disorders such as panic disorder (PD), generalized anxiety disorder (GAD), and posttraumatic stress disorder (PTSD) will be reviewed through the lens of relevant animal models and human imaging studies implicating GABA deficits in anxiogenesis. The potential role of GABA in developmental anxiety will be mentioned, as will the evidence for GABA deficits in other neuropsychiatric syndromes in which anxiety is prominent. Finally, an overview will be provided of anxiolytic agents, which directly or indirectly support GABA neurotransmission, and which can address deficits in GABA functioning in the clinical disorders.

RESEARCH

A literature search was conducted using the PubMed and Thomson Web of Science v5.15 search engines. References were identified that directly related to the search terms, "GABA and clinical anxiety", including several review papers. Preference for inclusion in the current paper was given to articles published after 2009. However, some key/landmark papers published prior to 2009 were also included.

GABA NEUROTRANSMISSION AND NORMAL ANXIETY

Several rodent models have highlighted the role of the GABA synthetic isoenzymes glutamic acid decarboxylase 65 (GAD65) and GAD67 in the expression of normal mammalian fear. For example, knockdown of GAD67 protein in the mouse amygdala impaired normal fear extinction and decreased sensitivity to the benzodiazepine anxiolytic, diazepam[7]. In another experiment, a genetic impairment in GAD65 expression was linked to decreased GABAergic transmission and plasticity in the lateral amygdala (LA), which, in turn, was associated with generalization of conditioned fear responses[8]. A study of male rats additionally demonstrated the importance of the sex hormone, 17-estradiol, as a promoter of GAD65 expression, with pharmacologic inhibition of 17-resulting in increasing expression of anxious behaviors (decreased open field exploration)[9].

Improvements in proton magnetic resonance spectroscopy (1H-MRS) technology and editing have led to the ability to quantify regional brain GABA concentrations, and other related amino acid metabolites, non-invasively. As a result, over the last 5 years, a number of studies applying these techniques in healthy humans have been published. These investigations have begun to define the relationships between normal human stress responses, personality type, and cortical GABA changes. For example, harm avoidance, as a normal human temperament trait, was observed to correlate positively with anterior cingulate cortex (ACC) GABA concentrations, and negatively with glutamate levels[10]. Evaluating extraversion/introversion and neuroticism in healthy subjects, another group reported a negative correlation between frontal GABA/creatine ratios (data acquired at 3 Tesla), and extraversion[11]. In another line of inquiry, acute psychological stress in healthy humans (threat of footshock) was associated with an acute decrease in prefrontal cortical GABA concentrations, similar to acute stress findings in the animal literature[12]. Investigating the impact of wellness interventions, such as yoga and walking, on cortical GABA, one group reported a relationship between improvements in stress level and mood, and increases in thalamic GABA levels, for yoga subjects only[13]. Other investigators using an fMRI/MRS assessment strategy, observed, in healthy humans, that lower insula cortex GABA levels and enhanced insula responses to interoceptive stimuli, together predicted higher levels of reported depressive affect[14]. However, studying female subjects, others did not observe a relationship between low insula GABA and inclination toward fearfulness[15].

Thus, cortical and subcortical GABA concentrations can be informative biological correlates of components of personality function and emotional processing, and appear to be change-sensitive markers of normal responses to acute stress and relaxation. It is foresee-

able that routine assessment of inhibitory brain function in this manner is likely to enhance the effectiveness of early-intervention and prevention protocols designed to interrupt the genesis of chronic anxiety or depressive states.

GABA DEFICITS IN PD

Although the neurobiological mechanisms underlying this common and disabling psychiatric syndrome remain unclear, a range of preclinical and clinical findings have implicated disturbances in GABA function in its pathophysiology. Animal modeling work[16] has demonstrated that biochemically-induced GABA deficits in the dorsomedial hypothalamus (DMH) of rats predispose to sodium lactate-induced panic, also an important clinical feature of human PD[17]. Follow-up work with this particular model has observed that lactate sensitivity and other anxious rodent behaviors could be driven by loss of GABAergic inhibition to a local DMH and perifornical population of peptidergic orexin (ORX) neurons[18]. Thus, impaired GABA function may facilitate ORX neuronal hyperactivity, thereby leading to increased sympathetic activation, and panicogenesis.

Other animal models of chronic anxiety/panic have focused on deficits in functioning of synaptic GABA$_A$ receptors as a risk factor for anxiety-proneness. For example, genetically induced deficits (moderate reductions) in expression of GABA$_A$ receptor 2 subunits (by heterozygous knockdown or knockout), were associated with neophobic behaviors, behavioral inhibition, or exaggerated defensive responses to mild threat[19]. More recently, the same group demonstrated that 2-containing GABA$_A$ receptor subpopulations are also implicated in the defensive response to mild threat, in that mice lacking 2 subunits exhibited anxious phenotype[20]. The animal models above also have parallel human findings, which we will now mention.

Deficits of GABA neuronal functioning have been implicated in the pathophysiology of PD by recent ^{1}H-MRS[21-23], GABA$_A$-benzodiazepine receptor single photon emission computed tomography (SPECT)[24] and positron emission tomography (PET) studies[25,26]. Not unlike the lactate-sensitive animals referred to earlier, humans with PD have been reported to have cortical GABA deficits in occipital, ACC/medial prefrontal cortex (mPFC), and basal ganglia regions of interest, though one MRS-GABA study of the prefrontal cortex was negative[27] (Table 1 for additional details). Similar GABA deficits (also identified by MRS) have been reported in other human anxiety spectrum disorders, such as social anxiety disorder[28] and obsessive compulsive disorder (OCD) (thalamic and mPFC deficits respectively)[29]. If GABA deficits in humans with PD also extend to impairment of GABAergic inhibition of DMH ORX neurons, this could account for spontaneous or lactate-induced panic in PD patients and in other anxiety patients who experience panic. Other domains of PD symptomatology, such as neophobia, anticipatory fear, and phobic avoidance, could conceivably be more related to the cortical deficits in GABA$_A$ receptor status identified by the PET and SPECT investigations above.

Furthermore, low cortical GABA in PD might be a trait-like entity, since neither acute nor chronic administration of anxiolytic pharmacotherapy was associated with reversal of these deficits[30]. Thus, low cortical GABA could be an important ongoing vulnerability factor conferring panic-proneness[31]. Moreover, in a retrospective analysis of one data set, the presence of a mood or anxiety family history appeared related to the magnitude of cortical GABA deficits observed in PD[32]. Low cortical GABA therefore has potential as a biomarker for PD and related stress conditions.

Human genetics studies of GABA and PD

The familiality and heritability of PD have been well established. Based on a heritability estimate of 43%[33], genetic factors are a significant contributor to the pathogenesis of PD. However, despite extensive clinical investigations in a number of anxiety disorders, the question remains open as to which genes are critically involved in anxiogenesis[34]; this is likely due to the fact that the "genetic architecture" of PD, similar to other high-prevalence medical conditions, is complex and attributable to multiple genes of small effect. The GABA neuronal system, though, continues to be a logical candidate system for future genetic studies of PD because of the current clinical neurobiological data (reviewed above) implicating GABA abnormalities in this condition, as well as the effectiveness of established and promising GABAergic therapies (Table 2).

Despite the recent positive GABA$_A$ receptor PET imaging findings in PD, work evaluating the potential role of GABA$_A$ receptor genes (*GABRA2*, *3*, *6*, and *GABRAG2*) in anxiety spectrum disorders has thus far been negative[35]. Other studies have focused on the genes *GAD1* and *GAD2*, which code for the GABA synthetic isoenzymes, GAD$_{67}$ (found throughout the neuron), and GAD$_{65}$ (found more in axon terminals, and related to regulation of short-term demands for GABA), respectively. In the investigation of Hettema et al[36] 2006, involving 589 patients and 539 controls, the data suggested an association of several SNPs of the *GAD1* gene with the personality trait of neuroticism (N), a risk factor for both anxiety disorders and major depression (MDD). A more recent case-control study, in a cohort of $n = 268$ anxiety patients, $n = 542$ MDD patients, and $n = 541$ healthy subjects, identified an association between elevated levels of behavioral inhibition trait (BI) in the patient groups, and several *GAD2* gene SNPs[37]. Finally, an association study in a cohort of $n = 238$ anxiety patients (84% with PD), and $n = 267$ healthy subjects recently linked several SNPs (rs2930152, rs2697153, and rs956053) of the GAT1 transporter gene, SLC6A1, with panic attacks. The odds ratio of the association increased in more severely

Table 1 Neuroimaging studies of gamma amino acid butyric acid function in anxiety disorders

Method	Finding	Design	Clinical significance	Ref.
[123]I-Iomazenil SPECT	Decreased L hippocampus and precuneus GABA$_A$ R binding	Parallel gp, 13 PD patients vs 16 healthy control subjects (HCs)	Chronic stress due to active PD can result in impaired limbic processing via reduced GABA$_A$ receptor density or function	Bremner $et\ al$[24]
^{1}H-MRS	Decreased OC GABA in PD	14 PD vs 14 matched HCs (historical)	Could be related to impaired production of GABA or of GABA-glutamine cycling in PD	Goddard $et\ al$[21]
GABA, 1.5T	No change in OC GABA pre and post-acute and chronic BZD Rx	10 PD vs 9 HCs	Suggests low GABA is a trait-like biomarker for PD	Goddard $et\ al$[30]
^{1}H-MRS GABA, 3T	Decreased ACC and BG GABA in patients	22 PD (medicated) vs 24 matched HCs, single voxel study	Impaired top-down inhibition of limbic activity in PD	Ham $et\ al$[23]
[11]C-flumazenil PET	Reduced GABA$_A$ R binding in L and R insula Cx of PD	11 PD vs 21 HCs	Interoceptive/somaticsensitivity in PD likely could be insula-mediated	Cameron $et\ al$[25]
^{1}H-MRS-GABA, 4T	Reduced thalamic GABA in SAD	10 SAD patients vs 10 matched HCs	Impaired GABA function in thalamus in SAD could affect social cognition via amplification of external threat cues	Pollack $et\ al$[28]
[11]C-flumazenil PET	Reduced frontal, temporal, parietal Cx GABA$_A$ binding pot. in PD	15 BZD-naïve PD vs 18 HCs	Generalized cortical impairment in GABA$_A$ function in PD could be a cause or effect of PD. If endogenous BZD-like ligands overproduced could be evidence of a compensatory response to chronic stress	Hasler $et\ al$[26]
^{1}H-MRS-GABA, 3T	Normal PFC GABA in PD	Parallel gp 17 PD vs 17 sex-matched HCs	In contrast to previous + results in ACC and OC ROIs	Hasler $et\ al$[27]
^{1}H-MRS-GABA, 3T	Reduced mPFC GABA in OCD	24 OCD patients vs 22 matched HCs	Could contribute to cortical-striatal circuit dysfunction in OCD	Simpson $et\ al$[29]
^{1}H-MRS-GABA, 3T	Reduced ACC/mPFCx GABA in PD	Parallel gp 11 PD vs 8 matched HCs	Effect size greater in FHx+ PD ACC GABA negatively correlated with enhanced ACC-precuneus, connectivity, 2 DMN nodes	Long $et\ al$[22] Shin $et\ al$[70]
^{1}H-MRS-GABA, 4T	Low R A^r insula Cx GABA in PTSD	13 PTSD patients vs 13 matched HCs	Relationship btw low insula GABA and higher state-trait anxiety levels	Rosso $et\ al$[50]
^{1}H-MRS-GABA, 4T	Lower GABA in tempo-parietal Cx and occipital-parietal Cx in PTSD	27 PTSD patients vs 18 trauma-exposed controls	Low GABA finding mediated by high levels of insomnia	Meyerhoff $et\ al$[51]
^{1}H-MRS-GABA, 3T	Elevated DLPFC and ACC GABA and glutathione levels	12 PTSD patients vs 17 non-PTSD trauma controls	Oxidative stress implicated in the pathophysiology of PTSD, as well as elevated prefrontal inhibitory neurotransmission	Michel $et\ al$[52]

SAD: Social anxiety disorder; PTSD: Post-traumatic stress disorder; GABA: Gamma amino acid butyric acid; mPFC: Medial prefrontal cortex; PET: Positron emission tomography; FDG: Fluorodeoxyglucose; PGB: Pregabalin; ACC: Anterior cingulate cortex; BZD: Benzodiazepine full agonist; MRS: Magnetic resonance spectroscopy; CNS: China national standards.

ill patients (frequent panickers), reaching a value of 2.5[38]. However, none of these studies assessed cortical GABA as part their study design. One recent investigation, employing this strategy to explore 5-HT/GABA interactions as a risk factor for panic/anxiety disorder, reported an association between (higher) prefrontal GABA concentrations and presence of a tryptophan hydroxylase isoform 2 gene polymorphism, especially in female mood/anxiety patients. This polymorphism had been previously linked to decreased TPH2 mRNA expression in PD. Follow-up studies are needed to confirm this association due to the small subgroup size of patients studied; however, this line of inquiry is exciting given the high rate of women affected by PD. There is also preliminary evidence of *GAD1* gene hypomethylation as a potential epigenetic response to negative life stressors in PD[39]. This is of significance clinically due to the close association of life events and onset of PD illness episodes, and in view of the MRS

data suggesting that low cortical GABA is a risk factor for panic-proneness.

GAD/TRAIT ANXIETY AND GABA

Several animal models have linked perturbations in GABA function to elevated trait anxiety. For example, mice bred for high anxiety behaviors (HAB), compared to control animals, were found to have a complex pattern of intra-amygdala GABA neuronal changes[40]. The amygdala has been identified as a key fear-processing structure within the fear circuit. Levels of GAD65 and GAD67 mRNA and protein were elevated in the basolateral amygdala (BLA) in HAB animals vs controls. In addition, mRNA expression of GABA$_A$ receptor subunits 1, 2, and 2 in the BLA was increased in HAB mice, while transcription of 5 and 1 subunits was reduced in the central and medial amygdala. Also, BLA levels of FosB, a marker for neuronal activation,

Table 2 Gamma amino acid system butyric acid effects of psychotropics with anxiolytic activity

Drug	Effect on GABA system	Test paradigm	Comments	Ref.
Benzodiazepines	Agonists at GABAA receptor complex	Alprazolam blocked ACC activation induced by CCK4 in controls.	fMRI study in 16 healthy male subjects	Leicht et al[71]
		Preclinical study of BZD effects on conditioned and unconditioned fear in mice	α2 subunit-containing GABAA receptors sufficient for BZD anxiolysis of unconditioned fear. α1 and α2 subunits needed to suppress conditioned fear	Smith et al[72]
SSRIs	Increased cortical GABA levels	Clinical MRS-GABA in MDD patients		Sanacora et al[73]
	Increased neuroactive steroid levels in the CNS	CSF ALLO increases in SSRI treated MDD patients (n = 15)		Uzunova et al[74]
TCAs	Increased release of GABA	Preclinical; desipramine effects studied		Korf et al[75]
MAOIs	Increase total brain GABA	Preclinical study of phenelzine effects		Paslawski et al[76]
Gabapentin	Increased cortical GABA	MRS-GABA in seizure patients	Chronic medication admin	Petroff et al[77]
PGB	Increased release of GABA	PGB decreases activation of left insula and amygdala in response to emotional images	fMRI activation study in 16 healthy humans	Aupperle et al[78]
Tiagabine	Blocks GAT-1 and inhibits synaptic GABA reuptake	FDG-PET study pre and post a 6-week tiagabine trial for social phobia	15 social anxiety disorder patients and 10 controls. vmPFC metabolism increased with treatment	Evans K et al[79]
Vigabatrin	Blocks GABA transaminase	Blocks CCK4 panic in healthy humans	Not clinically available due to ocular AEs	Zwanger et al[80]

GABA: Gamma amino acid butyric acid; mPFC: Medial prefrontal cortex; PET: Positron emission tomography; FDG: Fluorodeoxyglucose; PGB: Pregabalin; ACC: Anterior cingulate cortex; BZD: Benzodiazepine full agonist; MRS: Magnetic resonance spectroscopy; CNS: China national standards.

were notably increased. This pattern of findings in HAB animals can be interpreted as evidence of excessive excitation in the BLA due to loss of inhibitory GABA tone from the central and medial nuclei, with compensatory upregulation of BLA GABA synthetic enzymes. In another study, liver X receptor knockout mice were noted to exhibit anxious behaviors and to have reduced expression of GAD65 and 67 enzymes in the ventro-mPFC[41]. Anxiety in this protocol could have been mediated by loss of ventromedial prefrontal inhibitory GABA tone to the amygdala. Chronic anxiety in rodents was induced by inhibition of GABA synthesis in the bed nucleus of the stria terminalis area of the extended amygdala, a model reminiscent of human GAD, since these animals not only had persistent anxiety, but were also lactate-insensitive[42].

Thus far, there have been no clinical studies of cortical GABA levels or GABAA receptor binding in GAD, and, to date, genetic association studies of GABAA receptor subtypes have been negative[35]. Of interest clinically are studies which provide indirect evidence of excessive amygalar excitability in GAD patients. In one fMRI study comparing GAD patients and healthy subjects, neutral and threat cues triggered excessive amygdalar activation responses in GAD subjects[43]. This result is consistent with of an excitation/inhibition imbalance (glutamate/GABA function imbalance) within the amygdala in GAD, and is also consistent with the animal model findings presented above.

PTSD AND GABA FUNCTION

Animal studies

An unpredictable stress paradigm (unpaired odor-shock administration) in neonatal rodents, was associated with anxiety phenotypic behavior in adulthood, together with amygdalar upregulation of genes related to synaptic transmission, such as serotonin (5-HT) and GABA genes[44]. In another rodent study focusing on inescapable stress (inescapable footshock), and examining morphological and neurochemical changes in the prefrontal cortex and hippocampus, post-stress hippocampus cell damage was observed and found to be related to a glutamate/GABA neurochemical imbalance[45]. One group has recently developed a PTSD mouse model by inducing a null mutation in the GAD65 gene. The GAD65 enzyme, as noted previously, is critically involved in activity-dependent regulation of GABA release, and is necessary for proper maturation of the GABA system in adolescence. Mutant mice had hyperexcitability of the amygdala and hippocampus, increased anxiety, and pathological fear memory, all features of human PTSD[46]. In contrast, a GAD65 haplo-deficiency animal model was associated with delayed elevations in cortical and limbic GABA levels, yet was found in these animals to confer resilience to the effects of stress[47]. In another animal model of chronic stress (immobilization/restraint stress), tonic (but not phasic) GABAA receptor currents in the amygdala were observed to be persistently reduced following this type of stress, resulting in excessive amygdalar excitability[48]. Such enduring, stress-related changes could be relevant to the development of human anxiety and stress disorders. Furthermore, chronic restraint stress in high-anxiety animals in one study led to decreased brain (cortex and hippocampus) expression of 2 subunits of the GABAA receptor, suggesting a role for these changes in HPA axis dysfunction (upregulation) and stress

symptomatology[49].

Several MRS-GABA studies have also documented abnormalities in cortical GABA in human PTSD (Table 1). For example, insula cortex GABA levels in PTSD patients were recently found to be decreased[50]. In another protocol, combat PTSD patients *vs* non-combat controls exhibited abnormally low levels of parieto-occipital and temporal cortical GABA, which were accounted for by insomnia severity[51]. Yet another group has reported abnormal increases in prefrontal cortical GABA and glutathione in PTSD, implicating oxidative stress and possibly increased frontal cortical inhibition in PTSD pathophysiology[52].

Clinical significance

From the available data it is unclear whether deficits in GABA neurotransmission are a risk factor for PTSD, or are mainly the result of chronic stress, and the associated symptoms of PTSD. It is also unclear whether GABA deficits/dysfunction in PTSD could be the result of compensatory responses to stress that have become depleted, or whether they are particularly involved in the perpetuation of components of chronic PTSD (*e.g.*, hyperarousal, cue-sensitivity, increased startle).

SEPARATION ANXIETY AND GABA

Animal work has implicated the GABA system in unexpected ways in the behavioral and neurochemical response to maternal separation (MS). In one investigation, MS, in addition to promoting anxiety behaviors, enhanced tonic GABA currents in cortical layer 5 pyramidal neurons (juvenile rats), and promoted subsequent neurogenesis (subventricular zone, cortical layer 1, and dentate gyrus), and differentiation into GABA neurons (adult rats)[53]. These persistent brain changes might, in turn, predispose to later-life behavioral disturbances. Some investigators have noted that MS during breast-feeding produced behavioral changes and GABA$_A$ receptor 1 subunit expression changes that were gender-dependent[54]. In this study, MS male rats *vs* controls tended to exhibit less exploratory behavior, and have less 1 subunit expression in the amygdala, mPFC, and paraventricular nucleus (PVN). Females, however, had more exploratory and head-dipping behaviors *vs* controls, and less subunit staining in the mPFC, PVN, preoptic area, and hippocampus. These results highlight the possibility of gender difference in mechanisms of anxiogenesis. This line of inquiry may well improve our understanding of gender differences in the expression of human anxiety syndromes. A recent review of biological underpinnings of critical periods in fear learning and memory encoding highlighted the potential role of the GABA system on neuroplasticity in the peri-adolescent period. Adolescence is a time when GABA neurotransmission is rapidly improving in efficiency, and disruption in these often nonlinear developmental processes could predispose to anxiety and mood patho-

logy in the adolescence and beyond[55]. To date, there has been little work in humans directly exploring the impact of developmental events/stressors on GABA function, but this could be a fruitful area of investigation.

GABA DEFICITS AND ANXIETY IN OTHER NEUROPSYCHIATRIC DISORDERS

Studies of other pathological conditions with anxiety as a feature [traumatic brain injury (TBI), temporal lobe epilepsy (TLE), and depression (MDD), have also suggested a relationship between impaired GABA function and anxious behavior. Using a rodent model of mild TBI (mild controlled cortical impact), experimenters reported trauma-related increases in anxiety, and reduced BLA GABA function (decreased GABA cell numbers) leading to BLA hyper-excitability[56]. In TLE patients with mood or anxiety syndromes, together with several other GABA system changes, temporal lobe tissue levels of GABA were noted to be lower *vs* autopsy controls[57]. Finally, in a CSF assessment study of un-medicated MDD patients, those with anxious features tended to have abnormally low CSF GABA levels in contrast to patients without anxious features[58]. Thus, abnormal GABA function could be an important mediator of anxiety across multiple neuropsychiatric syndromes, and these preliminary data suggests the potential efficacy of GABAergic pharmacotherapies to stabilize this symptom cluster (Table 2).

DISCUSSION/TREATMENT IMPLICATIONS

Table 1 summarizes the human neuroimaging studies implicating GABA neuronal dysfunction in anxiety and stress disorders. The majority of the studies, utilizing ^{1}H-MRS techniques, have reported cortical or subcortical GABA deficits in structures relevant to the fear circuit across several different diagnoses. Most studies, however, have been conducted with relatively small samples. The PTSD findings should be interpreted with caution as other factors such as state anxiety, and insomnia appear to be mediating some of the GABA changes reported. In the case of PD, there were 3 positive findings (2 in the ACC/mPFC and 1 in the OC), and one negative finding (prefrontal cortex). One of the ACC studies (Ham *et al*[23]), however, was conducted in medicated PD patients, and hence it is impossible, in this instance, to attribute the GABA changes to the PD diagnosis. The ability to look at interrelated amino-acid metabolites (GABA, glutamate, glutamine) can add power to a study, as demonstrated in the OCD report of Simpson *et al*[29] where impaired mPFC GABA inhibition of subcortical structures was associated with elevated glutamate/glutamine levels in the thalamus. The majority of studies provided a baseline GABA assessment, and therefore, while suggestive of an

association with a specific disorder, at this stage of the research, the idea that the findings indicated effects of chronic stress cannot be ruled out totally. One exception was one of the PD studies in which acute and chronic benzodiazepine medication effects were prospectively measured, and which suggested loss of normal acute GABA counter-regulatory mechanisms, and tonically low GABA in PD[30]. A limitation of this study was the selection of the OC ROI, which is not directly related to fear-processing circuitry. While there is more consistency with the GABAA receptor findings in PD, again cause-effect relationship cannot readily be disentangled with the study designs used. Future study designs are likely to benefit power-wise from a careful assessment of family history status[32], and the use of a combination of functional imaging techniques (*e.g.,* fMRI or fcMRI together with MRS-GABA assessments), as well as the use of more dynamic MRS approaches (*e.g.,* 13C-labelled glucose/MRS evaluations assessing neuronal and glial contributions to the total GABA pool). The use of more dimensional approaches to anxiety psychopathology classification, as proposed in the NIMH RDoC project, may improve consistency of results (*e.g.,* studying acute responses to fear *vs* anticipatory fear across a range of DSM-V anxiety conditions). Finally, there are important limitations with the GABA neuroimaging paradigms reviewed. MRS evaluations of GABA offer an integrated assessment of intra-neuronal GABA in a large ROI, while current SPECT and PET methodologies offer the ability to study post-synaptic GABAA receptor status. However, the ability to adapt PET methodology to study the intra-synaptic fraction of GABA, as recently reported[59], now permits a more comprehensive evaluation of GABA neurotransmission across neuropsychiatric disorders.

GABA AND ANXIOLYTIC TREATMENT MECHANISMS

The GABA system has been implicated in the therapeutic mechanism of action of a number of psychotropic agents with anxiolytic activity (Table 2). Benzodiazepine full agonists (BZDs) are the prototypical class of agents in this respect, and their allosteric enhancement effect at the BZD site of GABAA receptor complex is well known[60]. Preclinical work has further defined the role of discrete GABAA receptor subunits in the separate clinical effects of the BZDs such as anxiolysis, sedation, muscle relaxation, and anticonvulsant effects. The 2 subunit for instance, is necessary to the anxiolytic action of BZDs[60]. In contrast, sedative, anticonvulsant, amnestic, and dependency effects in general require the presence of the 1 subunit[61]. Antidepressant agents also have the capacity to facilitate GABA function *via* augmentation of GABA levels, and neurosteroid levels. Also, newer-generation GABAergic anticonvulsant medications have begun to demonstrate anxiolytic effects, in parallel with localized physiological changes within the fear circuit.

From the overview provided in Table 2, enhancement of GABA neurotransmission might be viewed as a final common pathway of anxiolytics in general, or at least a key pathway for anxiolysis. If, as the literature currently suggests, GABA neuronal deficits/abnormalities are present in a range of clinical anxiety conditions, then GABA enhancers of different types might be expected to offset these deficits, thereby promoting anxiolysis, and restoration of function. The future prospect of more predictive and personalized anxiety treatment planning and monitoring is also attainable given the availability of imaging tools that can reliably measure cortical GABA, and other amino-acid metabolites. In the specific case of PD, where cortical GABA level deficits may be trait-like, it would be of interest to know if long-term GABA deficits persist, or whether they resolve at some point during maintenance treatment. "Normalization" of CNS GABA levels might be a more appropriate end point/cue to taper psychotropic treatment when combined with more traditional clinical indices of remission.

NOVEL ANXIOLYTICS TARGETING GABA

The GABA system provides a rich array of molecular targets for ongoing drug development initiatives, offering hope for stress/anxiety conditions in which GABA impairments are implicated. Considerable effort has been devoted to the enterprise of generating non-sedative anxiolytics, based on targeting selective GABAA receptor subunits. While, in this regard, 2/3 subunit-selective compounds have shown much promise in the lab[62], translation to the clinical has been limited by adverse events (*e.g.,* liver toxicity)[63]. More recently, attention has focused on subunit-selective compound development with both preclinical[64] and clinical progress being made[65]. The latter agent, etifoxine, exhibits a dual mechanism, 2/3 subunit selective agonism and neurosteroidogenic stimulation, to enhance GABA neurotransmission. A novel molecular target for ligand development, a mitochondrial Translocator Protein (18 kD), regulates the initial and rate-limiting step in neurosteroidogenesis[66]. Neuroactive steroids synthesized from this pathway, such as allopregnenolone, act as positive allosteric modulators at a specific neurosteroid site within the GABAA receptor complex. Positive synthetic ligands at this site include the agent XBD173, which showed preliminary clinical benefit for panic anxiety, and YL-IPA08, which displayed anxiolysis in a PTSD animal model[67]. In addition, a synthetic neurosteroid analog, ganaxolone, has shown therapeutic potential in a mouse model of PTSD[68]. Within the glutamate system, bilateral intra-amygdala (BLA) administration of the GluK1 kainate receptor agonist ATPA, facilitated GABA neurotransmission to promote anxiolysis in one animal model of stress[69].

CONCLUSION

Anxiety and stress disorders are a major public hea-

lth concern. However, their pathophysiology is still unclear, and requires ongoing investigation. The GABA neurochemical system has been strongly implicated in their pathogenesis and treatment by numerous preclinical and clinical studies, the most recent of which have been highlighted in this review. Changes in cortical GABA appear related to normal personality styles and normal responses to stress. While there is accumulating *in-vivo*, neuroimaging evidence of cortical and subcortical GABA deficits across a number of anxiety conditions, a consistent pattern of findings in specific brain regions for a given disorder is yet to emerge. Neuropsychiatric conditions with anxiety as a clinical feature may have GABA deficits as an underlying feature. Different classes of anxiolytic therapies appear to support GABA function, and this may be an area in which newer GABA neuroimaging techniques could soon offer more personalized therapy. Novel GABAergic pharmacotherapies in development offer potential improvements over current therapies in reducing sedative and physiologic dependency effects, while offering rapid anxiolysis.

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REVIEW

Sex differences in cognitive impairment in Alzheimer's disease

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Abstract

Sex differences in neurocognitive abilities have been extensively explored both in the healthy population and in many disorders. Until recently, however, little work has examined such differences in people with Alzheimer's disease (AD). This is despite clear evidence that AD is more prevalent in women, and converging lines of evidence from brain imaging, post-mortem analyses, hormone therapy and genetics suggesting that AD affects men and women differently. We provide an overview of evidence attesting to the poorer cognitive profiles in women than in men at the same stage of AD. Indeed, men significantly outperform women in several cognitive domains, including: Language and semantic abilities, visuospatial abilities and episodic memory. These differences do not appear to be attributable to any differences in age, education, or dementia severity. Reasons posited for this female disadvantage include a reduction of estrogen in postmenopausal women, greater cognitive reserve in men, and the influence of the apolipoprotein E ε4 allele. Assessment of cognitive abilities contributes to the diagnosis of the condition and thus, it is crucial to identify the role of sex differences if potentially more accurate diagnoses and treatments are to emerge.

Key words: Dementia; Gender; Sex differences; Cognition

Core tip: This review assesses evidence that women with Alzheimer's disease (AD) show greater cognitive impairment than men. The evidence shows that female AD patients are outperformed by males in multiple cognitive domains including visuospatial, verbal processing, semantic and episodic memory. This disadvantage is not attributable to sex differences in age, education level, or dementia severity. Possible explanations include estrogen loss in women or a greater cognitive reserve in men, which may provide protection against the disease process. Such findings have implications for tailoring more specific gender-based treatments.

INTRODUCTION

"Biomedical research in general, and neuroscience in particular, has been built on a false assumption that one may safely ignore potential sex influences"[1].

Alzheimer's disease (AD) is the most common neurodegenerative disease associated with aging, with worldwide estimates of 30 million people with dementia, 4.6 million new cases annually, and one new case every 7 s[2]. The prevalence and the incidence of AD are greater amongst women than men and this discrepancy increases with advanced age[3-5].

A meta-analysis of 13 population studies from across United States, Europe, and Asia indicates that women are at significantly greater risk of developing AD, though not other dementias[6]. The neurocognitive profiles of male and female AD patients, however, are less well-described. These profiles require far greater examination as sex differences in the pattern of cognitive decline may yield vital information about differential risk factors, pathogenesis and most importantly, treatment of AD in men and women. In order to make sense of any sex differences that emerge under AD neuropathology, we first need to have a clear understanding of those sex differences that may already exist in the normal population, and specifically the healthy elderly population.

SEX DIFFERENCES IN THE GENERAL POPULATION

Sex differences in cognitive abilities receive consistent, extensive discussion in the "normal" literature, with the prevailing view being that women tend to have better verbal abilities[7,8], while men display a visuospatial advantage[8,9].

VERBAL ABILITIES

Tasks measuring verbal ability generally rely on rapid access to semantic and phonological information. Category fluency requires a person to name as many exemplars of a category (*e.g.*, tools) as possible, usually within one minute, while lexical fluency involves listing as many words beginning with a given letter (typically, F, A and S). In confrontation naming, the respondent must produce names for a series of visually presented items, usually but not exclusively line drawings. This task is regarded as a measure of core semantic memory function, though performance depends also on visual acuity and retrieval of phonological information.

In their meta-analysis of sex differences in almost 1.5 million participants, Hyde *et al*[7] found a small, but reliable female advantage in verbal ability with a *Cohen's d* = 0.11 (Cohen's *d* is calculated from: Mean of group A - Mean of group B/pooled standard deviation of A and B); and effect sizes are traditionally viewed as small (*d* = 0.2), medium (*d* = 0.5) or large (*d* = 0.7+). The small effect size reflects the mixed findings, with 44 studies (27%) reporting females outperformed males, 109 (66%) found no significant difference, and in 12 (7%) males outperformed females. Recent evidence suggests that sex differences in verbal ability are not universal but task-dependent. For lexical fluency, a female advantage has emerged in some studies[10,11], but not others[12,13]. Category fluency tasks do not elicit reliable sex differences[14] although differences may emerge for specific categories; for example, a female advantage for fruits and a male advantage for tools[15]. Confrontation naming, by contrast, has shown a male advantage in the few published papers examining sex differences[16]. In summary, sex differences in verbal abilities in the general population may be smaller than once believed[17].

VISUOSPATIAL ABILITIES

Linn *et al*[18] partitioned visuospatial tasks into three groups: Mental rotation, spatial perception (spatial working memory) and spatial visualisation (navigation). Mental rotation involves the ability to mentally rotate a two or three-dimensional figure rapidly and accurately. Spatial perception tasks are those where participants are required to determine spatial relationships with respect to the orientation of their own bodies in spite of distracting information. Spatial visualisation involves complicated multi-stage manipulations of spatially presented information (*e.g.*, the embedded figures test).

Sex differences in normative visuospatial processing are more robust than those documented for verbal processing. Voyer *et al*[9] meta-analysed 286 studies spanning 50 years, finding that men significantly outperformed women on most visuospatial tasks. Within Cohen's[19] nomenclature, the effect sizes were large for mental rotation (*d* = 0.73), medium for spatial perception (*d* = 0.44), and small for spatial visualization (*d* = 0.13). A later review by Li *et al*[20] examined 16 studies comparing men and women on spatial rotation and 11 on spatial navigation, and all 27 pointed to a male advantage.

AGE RELATED COGNITIVE DECLINE

As expected, many aspects of cognitive performance do decline with age[21-24]. Although not typically apparent for category fluency[25] and spatial perception[26], age-related deficits are seen in lexical fluency[27] and block design tasks[28] - underscoring how the precise requirements of the verbal and spatial tasks are crucial.

Greater age-related cognitive decline in healthy

men than women has been established not only in cross-sectional studies, but also crucially in longitudinal studies[29-34]. For example, Wiederholt *et al*[33] assessed 1692 participants aged 55-94 years finding that performance on all cognitive tests decreased progressively with age, but that the decline was slower in women. More recently, using internet testing, Maylor *et al*[23] examined sex differences and age-by-sex differences on various cognitive tasks in a very large sample of healthy individuals (109612 men and 88509 women). Importantly, and consistent with other studies, men showed greater age-related decline than women, irrespective of whether the task was one on which they were better. Of course, such large samples are sufficiently powered to detect even the most trivial differences and although the age by gender interactions described in this study are highly significant, they account for just 0.1% of the variance in cognitive performance.

As part of the Whitehall Ⅱ cohort study, Singh-Manoux *et al*[35] assessed 5198 male, and 2192 female, civil servants, aged 45-70, monitoring their cognitive performance (memory, vocabulary, reasoning and verbal fluency) over 10-years. Their results suggest that cognitive performance may decline earlier than previously thought. For example, reasoning scores decreased by 3.6% for men and women aged 45-49, and 9.6% and 7.4% respectively for those aged 65-70. The authors also noted the much larger cross-sectional than longitudinal age-related decline in women, which they attributed to cohort differences in education, with older women tending to be less well educated.

Based on neurocognitive and behavioural data, some have proposed that "age is kinder to women"[36]; however, any such female advantages are quite small and contentious. Meinz and Salthouse[36] meta-analysed 25 studies (5201 individuals aged 18-64) investigating sex differences in the patterns of age-cognition relations across a wide range of tasks. They concluded that "large (12%-25% of the total variance) effects related to age, small (at most 3% of the variance) effects associated with gender, and small to non-existent (less than 1%) effects associated with interactions of age and gender on measures of cognitive performance" (p 63). Only a minority of measures (speed and reasoning) revealed significant age by sex interactions: Men had smaller age-related declines. Although the age-related differences across sex were quite small, favouring women (only spatial abilities demonstrated a large sex gap favouring men), the confidence intervals for estimated effect sizes were small, suggesting the results are reliable.

Other research, however, suggests that men and women decline at similar rates[22,27,37,38]. Gerstorf *et al*[22] examined 368 participants aged 70-100 over a 13-year period, finding that for all cognitive tests men and women declined in parallel. Proust-Lima *et al*[38] however, found that after adjusting for vascular status, sex differences in cognitive decline did emerge, but

only at the oldest age, with women showing a steeper decline than men do. Similarly, in a sample of 647 twin pairs (both dizygotic and monozygotic) aged between 65 and 98 years, Read *et al*[24] described larger sex differences in working memory and perceptual speed deficits at later ages, with women faring worse.

Therefore, although age-related cognitive decline is evident on many tasks, the weight of evidence points to similar levels of impact in men and women until the very oldest age, when women suffer a faster decline. We might then expect to find that sex differences in the younger population persist in the elderly. Furthermore, any sex effects in AD patients that differ from that seen in the healthy population are likely to be due to the disease process rather than aging *per se*.

SEX DIFFERENCES IN THE ELDERLY

Research suggests that typical sex differences in cognitive performance may persist into old age, with better visuospatial and language skills in males and females respectively[11,23].

VERBAL ABILITIES

The pattern of sex differences in category fluency of the healthy elderly is far from consistent, with some finding a female advantage[11,39-44], others no sex differences at all[22,25,45-48] and at least one report of a male advantage[33]. The presence of an effect may depend upon the specific category examined, with reports of both a female advantage and an absence of sex differences[49] or both a female advantage and a male advantage[41] or all three possibilities depending upon the specific semantic category examined[15].

A picture naming advantage in elderly males has been documented[16,50-53] mirroring the advantage often seen in young adults, although not in all studies of the elderly[16,39,43-46,54-59]. In a longitudinal study by Connor *et al*[16], the rate of decline in naming was comparable for men and women.

VISUOSPATIAL ABILITIES

Corresponding to the findings in the healthy young, elderly men outperform elderly women on mental rotation[60] and spatial perception tasks[39]. For spatial visualisation tasks, the findings are variable and task-specific. A male advantage was reported by some[11,24] although equivalent male and female performance was observed by others including on the block design task[11] and figure copying[39], mirroring the conflicting patterns seen in the young.

MEMORY

Little evidence supports an unambiguous sex difference in memory function for the healthy elderly. Some report women being better at immediate word learning[47],

Table 1 Sex differences in studies assessing fluency and naming in the elderly

Ref.	Sample size		MMSE		Tasks	Finding
	M	F	M	F		
Marra *et al*[41]	85	168	19.1	17.6	CF (fu)	NS
					CF (b)	M > F
Monsch *et al*[42]	43	46			LF (F, A, S)	NS
					CF (s, *n*)	NS
					CF (a + f + ve)	F > M
Beinhoff *et al*[45]	26	23	25.6	24.7	CF (a)	NS
					CN	NS
Henderson *et al*[46]	22	24			CN	M > F
Henderson *et al*[46]	270	377	17.5	17.3	CF (a)	M > F
					CN	M > F
Moreno-Martinez *et al*[49]	28	33	21.2	18.9	CF (I, tr, v, t, mi)	M > F
					CF (a, fl, f, fu, k, c, bg, bp)	NS
Randolph *et al*[53]	129	196			CN	M > F
Buckwalter *et al*[55]	72	87	17.8	16.5	CN	M > F
McPherson *et al*[77]	23	36	23.3	22.2	LF (F, A, S)	NS
					CF (a)	NS
					CN	M > F
Ripich *et al*[78]	29	31			LF (F, A, S)	NS
					CN	M > F
Bayles *et al*[79]	30	33	15.2	15.9	LF (A, S)	NS
					CF (a, f)	NS
					CN	NS
Perneczky *et al*[81]	50	43	23.9	23.0	CF (a)	NS
					CN	M > F
Henderson *et al*[136]	26	27	13.8	11.8	LF (F, A, S)	NS
					CF (a)	NS
					CN	NS

F: Female; M: Male; NS: Not significant; MMSE: Mini mental state examination; CF: Category fluency; CN: Confrontation naming; LF: Lexical fluency; CF: Categories; a: Animals; b: Birds; bg: Buildings; bp: Body parts; c: Clothing; f: Fruit; fl: Flowers; fu: Furniture; i: Insects; k: Kitchen utensils; mi: Musical instruments; n: First names; s: Supermarket items; t: Tools; tr: Trees; v: Vehicles; ve: Vegetables.

verbal memory and episodic memory[22], while others have found no sex differences in verbal memory[61] or for delayed word learning[47,50]. Some evidence also documents that elderly men have better visual memory[38], working memory and episodic memory[24]. In summary, sex-differences in the memory ability of the healthy elderly where described, appear to be significantly dependent upon the specific task.

We have reviewed the evidence for sex and age-related differences in cognitive tasks within the normal population and principally within the healthy elderly population. It is important to have a clear understanding of such patterns, and the extent to which they are well-replicated, if we are to make sense of any sex differences in cognitive abilities that may emerge after the onset of AD.

VERBAL ABILITIES IN AD

Semantic memory impairments emerge early in AD neuropathology[62]. Category fluency deficits may be apparent as much as five years prior to diagnosis[63] and mild AD patients are more impaired than those with Mild Cognitive Impairment (MCI) and elderly controls on category fluency[64,65]. Two meta-analyses[66,67] have highlighted large significant category fluency deficits in people with AD compared to healthy elderly controls,

with Laws *et al*[67] calculating an extremely large effect size in 92 studies (d = 2.10).

The pattern with regard to lexical fluency deficits, however, is more varied with some finding no impairment[68,69], but others a worsening of performance[70]. Nonetheless, meta-analytic studies[66,67] confirm a reliable AD deficit in lexical fluency, with[67] estimating a large effect size (d = 1.46).

Compared to the healthy elderly, AD patients also have far greater difficulties on confrontation naming tasks[64,69,71-75]. A meta-analysis of 56 naming studies assessing 2607 AD patients and 2285 healthy controls by Laws *et al*[67] obtained a large effect size deficit in AD patients (d = 1.54).

SEX DIFFERENCES IN VERBAL ABILITIES IN AD

While AD is characterised by decline in the verbal and semantic domains, does any evidence suggest that the relative performance of men and women differs from the pattern in the healthy elderly? A search of the literature reveals a modest number of relevant studies (Table 1).

Several studies in the 1990s reported that, compared to men with equivalent AD severity, women manifest a more profound impairment of semantic memory[46,76-78].

Table 2 Studies assessing sex differences in visuospatial abilities for Alzheimer's disease patients

Ref.	Sample size		MMSE		Tasks	Finding
	M	**F**	**M**	**F**		
Beinhoff *et al*[45]	26	23	25.6	24.7	FC	M > F
Henderson *et al*[46]	22	24	Not given		FC	NS
Henderson *et al*[46]	270	377	17.5	17.3	FC	NS
Buckwalter *et al*[55]	72	87	17.8	16.5	BD	NS
					FC	NS
Perneczky *et al*[81]	50	43	23.9	23.0	FC	NS
Cushman *et al*[90]	22	12	24.03		JLO	NS
Heun *et al*[101]	171	267	15.5	16.3	FC	M > F
Millet *et al*[102]	20	20			Corsi,	NS
					Corsi (b),	M > F
					VPT	
Henderson *et al*[136]	26	27	13.8	11.8	FC	NS

M: Male; F: Female; NS: Not significant; MMSE: Mini mental state examination; BD: Block design; Corsi: Corsi block tapping; Corsi (b): Corsi block tapping (backwards); FC: Figure copying; JLO: Judgment of line orientation; VPT: Vecchi's pathway task.

In particular, female AD patients name fewer items correctly in confrontation naming tasks[46,55,77,78]. Others have reported higher naming scores by men, but no significant sex differences[45,79]. Crucially, the significant sex differences in AD patients remain after controlling for the effects of age, education, and duration of illness[46,55,76,78]. In their meta-analysis, Laws *et al*[67] confirmed a male naming advantage by showing larger effect sizes in studies containing a greater proportion of female AD patients.

Several studies have reported no sex differences in lexical fluency performance in AD patients[42,77-80] but a more ambiguous profile has emerged with category fluency tasks and may reflect the specific choices of categories. Perhaps surprisingly, men with AD significantly out-perform women on the most commonly used category of animals[46] as well as for insects, trees, tools, musical instruments, vehicles[49] and birds[41]. Nevertheless, others have found no significant sex differences for: Animals[45,46,49,77,79,81], fruits[49,79], furniture[41,49] supermarket items or first names[42]. Only one study[42] has asserted a female semantic fluency advantage, though this was for the total of animals, fruits and vegetables combined.

VISUOSPATIAL ABILITIES IN AD

While verbal and memory problems in AD are widely acknowledged, the prevalence of visuospatial deficits has been relatively underplayed, and may be important given the link between visuospatial abilities and functional competence in healthy older individuals[82] as well as those with dementia[83]. Some evidence also intimates that visuospatial tests may be useful in staging the disease process, differentiating mild from moderate dementia in AD[84].

People with AD fare worse than the healthy elderly on visuospatial tasks, including, for example, tests of mental rotation[85-88]. Lineweaver *et al*[88] posited that since mental rotation involves the parietal cortex, and AD results in extensive damage to this region, AD patients would be impaired at this task. This was indeed the case when compared with healthy elderly controls[88].

Salmon *et al*[89] claimed that the visuospatial deficits associated with AD are usually evident in visuoconstructional tasks (*e.g.*, block design) and visuoperceptual tasks [*e.g.*, judgement of line orientation (JLO) task]. In line with this, several studies show that AD patients are worse than elderly controls on the JLO task[88,90-92], although Finton *et al's*[93] AD patients presented with no problems on this task. Impairments have been reported both for block design[91,94] and for figure copying[95,96]. Even participants with mild AD score lower than elderly controls on a figure-copying task[97,98] and on drawing a complex figure from memory[98].

Visuospatial deficits are also apparent for some years prior to AD diagnosis. Backman *et al*[99] found that healthy elderly individuals, who were later diagnosed with AD, performed worse on visuospatial tasks than those who remained free of dementia at follow-up. In a related vein, Laukka *et al*[100] identified an increase in the rate of visuospatial decline in elderly participants up to 10 years prior to AD diagnosis.

SEX DIFFERENCES IN VISUOSPATIAL ABILITIES IN AD

Although AD reduces performance on a range of visuospatial tasks, does the performance on these tasks differ between men and women? If AD affected men and women equivalently, then we might expect to see a continuing male advantage on most visuospatial tasks, as an extension of the typical healthy elderly profile.

Of 16 studies that have considered sex differences in AD patient cognition, nine included at least one visuospatial ability task (Table 2). Only one used a spatial perception task[90], which was primarily concerned with navigation (they also included the JLO task) and no significant sex differences emerged. All other papers examined spatial visualisation tasks where, as already discussed, findings in the general population are variable and seem to be contingent on the specific task used. Buckwalter *et al*[55] found no differences between male and female AD patients on block design. Beinhoff *et al*[45], however, reported that AD males outperformed AD females at a drawing task measuring visuospatial episodic memory. These findings were unlikely to reflect a generalised visuospatial skill advantage as no sex differences emerged for visuospatial memory span. Most other researchers, however, have failed to discern any difference between AD men and AD women at copying a geometric figure[46,55,80,81,101]. Heun *et al*[101] did report a male superiority on visuoconstructive tests that involved copying geometrical figures such as cubes, but this did not reach significance.

In summary, the visuospatial abilities of men and women with AD do not parallel the male-female divergence seen in the healthy population. To date however, no paper has examined mental rotation performance in AD patients, which as discussed earlier, is the most sensitive visuospatial task to sex differences in the general population. Given the complexity of rotation tasks, researchers may feel they are less useful with AD patients. As noted, Lineweaver *et al*[88] did use a simplified rotation task, but they did not report male and female performance separately.

By contrast, some studies suggest that sex-based cognitive differences may disappear or even reverse in AD. For example, Perneczky *et al*[81] reported no significant sex differences in patients with mild AD on either verbal or visuospatial tests. One possible interpretation is that a proportionally greater deterioration of verbal and visuospatial ability occurs, respectively, for women and men. However, some contend that the male visuospatial advantage remains in AD sufferers, possibly on tasks that tasks requiring active manipulation of visuospatial information[102]. And Beinhoff *et al*[45] reported that AD males were superior at learning and retaining visuospatial information, though no sex differences in visuospatial memory span emerged. Turning to verbal abilities, Chapman *et al*[103] found greater accuracy in AD men on the Logical Memory test, which assesses verbal episodic memory, and this was a reversal of the profile in their healthy elderly controls. Surprisingly perhaps, male AD patients are also superior on naming tasks[46,55,77], and verbal fluency[55]. It is evident then that findings relating to sex differences in AD patient cognition are somewhat inconsistent, and the results of individual studies may even be misleading on this issue. Moreover, a failure to find significant sex differences in some studies may reflect insufficient statistical power; and thus, meta-analysis is a useful approach in this area.

Surprisingly few studies present neurocognitive data separately on males and females with AD. Irvine *et al*[104] identified just 15 published studies presenting data from a total of 828 men and 1238 women with AD. Unsurprisingly, AD studies contained more female than male patients (60% *vs* 40%) and although most researchers test both male and female patients, they do not routinely report between-sex comparisons, and so any differences have gone unnoticed. In earlier work, we tried to circumvent this methodological drawback by assessing the "proportion" of male participants per study as a moderator of effect sizes. This approach showed that picture-naming effect sizes increase in AD patients as the proportion of female patients increase[67]. Similarly, our meta-analysis[67] of 92 studies examining semantic and 96 examining phonemic fluency in over 4500 and 3000 AD patients respectively, found that the proportion of females significantly predicted both effect sizes. In other words, as most studies of AD patients have more females, studies will tend to significantly inflate effects, and differences in the proportions of male and female participants will increase variability in findings across studies.

Irvine et al[104] *meta-analysis*

The meta-analysis by Irvine *et al*[104] uncovered small, but significant male advantages across each of five cognitive domains examined (Cohen's *d*, 95%CIs, Table 3): Episodic memory ($d = -0.17$, 95%CI: -0.33 to -0.01) semantic memory ($d = -0.25$, 95%CI: -0.42 to -0.07) verbal ($d = -0.27$, 95%CI: -0.37 to -0.16), non-semantic ($d = -0.14$, 95%CI: -0.26 to -0.02) and visuospatial ($d = -0.24$, 95%CI: -0.43 to -0.05). In terms of consistency, 49 of 52 (94%) effect sizes calculated by Irvine *et al*[104] were in the direction of worse female performance across varied cognitive domains. Furthermore, moderator regression analyses revealed that these deficits were not predicted by differences in age, education or overall dementia severity (as measured by MMSE). Hence, the worse cognitive performance of women with AD is not attributable to obvious demographic confounds.

What are the possible reasons for AD affecting the cognitive abilities in women more than in men?

One reason for the more pronounced decline in women might relate to men having greater cognitive reserve. Cognitive reserve has been defined as the amount of brain damage an individual can tolerate before reaching a clinical threshold for impairment[105]. Individuals with greater reserve are hypothesized to sustain more AD-related neuronal damage before onset of symptoms and clinical diagnosis. Consistent with this hypothesis, several recent neuroimaging studies have reported differences in brain function for male and female AD patients who are at the same disease stage. In accord with the greater age related cognitive decline in men, corresponding brain imaging evidence points to greater age-related brain deterioration in males than females[36,106]. Magnetic resonance imaging has detected greater age-related brain atrophy (as indicated by increased cerebrospinal fluid volume) in males than females[108]. In terms of specific regions and structures, greater age-related frontal and temporal lobe volume reductions have been described in males[109], while others[110] have reported a more specific reduction in hippocampal volume across early adulthood in males but not in females. A more recent and novel study using diffusion tensor imaging[111] assessed patterns of white matter connectivity - the connectome - in a large sample of males ($n = 428$) and females ($n = 521$) aged from 8 to 22, finding that females displayed stronger interhemispheric connections, while intrahemispheric connections seemed stronger in males. Although the study found no age-by-sex interaction, suggesting no sex differences in the developmental trajectory of connectivity, the duration covered was relatively limited.

In their post mortem analyses of 141 brains from the religious orders study, Barnes *et al*[21] found the association between AD pathology and clinical AD was significantly more likely to be expressed in women than

Table 3 Cohen's *d* effect sizes (95%CI) in different cognitive domains

Ref.	Sample size			Semantic	Non-semantic	Verbal	Visual-spatial	Memory
	M	F	Total	*d*	*d*	*d*	*d*	*d*
Marra *et al*[41]	85	168	253	-0.23		-0.23		
Beinhoff *et al*[45]	26	23	49	-0.07	-0.44	-0.22	-0.60	-0.37
Hendersen *et al*[46]	22	24	46	-0.37	-0.37	-0.37	-0.18	
Hendersen *et al*[46]	270	377	647	-0.30	-0.12	-0.30	-0.11	-0.12
Moreno-Martinez *et al*[50]	28	33	61	-0.42		-0.42		
Buckwalter *et al*[55]	72	87	159	-0.46	-0.24	-0.46	-0.24	
McPherson *et al*[71]	23	36	59	-0.24	-0.54	-0.35		-0.71
Ripich *et al*[78]	29	31	60	-0.74		-0.74		
Bayles *et al*[79]	30	33	63	-0.10		-0.10		
Perneczky *et al*[81]	50	43	93	-0.24	-0.12	-0.20	0.02	-0.17
Heun *et al*[101]	17	76	93		-0.18		-0.62	-0.04
Millet *et al*[102]	20	20	40		-0.40	0.08	-0.63	-0.40
Laiacona *et al*[107]	11	15	26	-0.29		-0.29		
Hendersen *et al*[136]	26	27	53	-0.26	-0.22	-0.09	-0.44	-0.15
Hebert *et al*[137]	119	245	364	-0.23	0.04	-0.09		
Total	828	1238	2066	-0.25 (-0.42 to -0.07)	-0.14 (-0.26 to -0.02)	-0.27 (-0.37 to -0.16)	-0.24 (-0.43 to -0.05)	-0.17 (-0.33 to 0.01)

Negative effect sizes favour men and positive effect sizes favour women; numbers in parenthesis are 95%CIs. M: Male; F: Female.

in men. Indeed, each unit of AD pathology (based on neuritic plaques, diffuse plaques, and neurofibrillary tangles in areas sampled from four cortical regions) increased the odds of clinical AD by more than 20 times in women compared with a 3-fold increase in men. Furthermore, with each additional unit of AD pathology, the cognitive function scores for episodic memory, semantic memory, working memory, perceptual speed, and visuospatial ability were significantly more reduced in women than in men.

Although male and female AD patients show some commonalities in functional imaging studies, differences in regional cerebral blood flow (rCBF) emerge, with women showing a more severe decrease of rCBF in the medial temporal region and frontal lobe[112]. Neuroimaging studies reporting sex differences in brain function for males and females at the same stage of the disease are consistent with a reserve hypothesis. Perneczky *et al*[81,113] found that despite being at the same disease stage; and showing no significant cognitive differences, men with AD had more pronounced and extensive pathology affecting the frontal, temporal and insular cortex, as well as the hippocampus in the right hemisphere. Moreover, women were more likely to clinically express reductions of regional cerebral metabolic rate as dementia. The authors suggest that this could be because the brain reserve capacity serves as a stronger counterweight to neurodegeneration in men than in women.

Early neuropathological progression appears to be independent of sex, but female MCI patients show an increased vulnerability to cognitive impairment earlier in the illness course; and women with AD show greater cognitive impairment than men, despite an apparent equivalence in brain atrophy[114]. Perneczky *et al*[113] reported more pronounced and extensive AD pathology for men than women in the frontal, temporal and insular

cortices as well as the right hippocampus, despite being at the same disease stage and showing no significant differences in general cognitive abilities.

The apolipoprotein E (APOE) ε4 allele is an established genetic risk factor for AD[115]. Although estimates vary somewhat across studies and ethnicity, the *APOE ε4* allele is present in > 50% of AD patients and approximately only 15% of healthy elderly controls[116]. Crucially, *APOE ε4* affects the probability of developing AD more in women than men[117-120]. This common polymorphism increases the risk of clinical conversion more in women than in men both in the conversion from healthy aging to MCI/AD and in the conversion from MCI to AD[120]. Lin *et al*[121] have recently examined longitudinal rates of change over eight years in a large sample of 398 MCI subjects (141 females and 257 males), finding faster rates of cognitive and functional decline in women than men and this effect was greater in female *APOE ε4* carriers. In the healthy population, the impact of APOE ε4 on cognitive performance is more pronounced in women[122,123], and has been specifically linked to hippocampal atrophy in female MCI sufferers[124]. A large post mortem study (*n* = 729) established that AD-related abnormalities such as neurofibrillary tangles and senile plaques are affected by a complex interaction between the aging process, sex, and genetic (*APOE ε4*) risk factors[125]. These findings are consistent with a relatively greater semantic and verbal impairment in female AD sufferers that differs from, and is greater than, any pre-existing sex differences in cognition[101].

Estrogen has been implicated in the pathobiology of AD[126]. Indeed, findings suggest that verbal sex differences in AD may arise *via* an estrogen deficiency in women. Further evidence shows that Estrogen therapy prevents the decrease in verbal memory when administered immediately following the surgical

removal of both ovaries in premenopausal women[127]. Women with AD who receive estrogen hormonal therapy perform as well on naming and verbal short-term memory tasks as men and significantly better than AD women not receiving such therapy. Further evidence suggests that duration of estrogen use is related to the rate of global cognitive decline and visuospatial ability in non-demented elderly women, although not to semantic or episodic memory[37]. Loss of estrogen alone cannot fully explain the poorer cognitive performance of women with AD; otherwise we would expect the same deficits seen in women with AD (for verbal fluency and verbal episodic memory) to be evident in the healthy elderly and this is not the case.

Following the menopause, cognitive abilities in healthy elderly women may be adversely affected by estrogen loss, albeit primarily on verbal tasks[127-130]. Indeed, women show significant changes in cognitive function during pregnancy and the postpartum period, principally in verbal free-recall and working memory[131,132], word fluency and word list learning[133]. Recent evidence suggests that during the third trimester and the early postpartum period, verbal recall deteriorates in pregnant women[134]. Furthermore, a longitudinal study of pregnant women showed they performed worse than non-pregnant controls on two tests of verbal memory, a visuospatial task, and on a task of processing speed[135]. These findings support the view that changes in sex hormone production within the physiological range that occur during reproductive events modify performance on a variety of cognitive functions - but principally on verbal tasks.

CONCLUSION

Although not unanimous, the evidence presented in this review converges on the multiple cognitive abilities being more adversely affected by AD in women than in men. This conclusion is strengthened by our own recent meta-analyses consistently affirming that men with AD outperform women with AD across a range of cognitive domains.

The literature on verbal abilities in the elderly reveals either an advantage for women or no sex difference - crucially, not one paper documents a male advantage in this domain. Findings are somewhat inconsistent in studies of cognitive decline under normal aging, suggesting something specific about AD neuropathology that disadvantages females. Some limited evidence suggests that females deteriorate faster than males in the earlier stages of the disease. Possible explanations are for a hormonal influence, possibly due to estrogen loss in women or a greater cognitive reserve in males, which provides protection against the disease process. Future studies which examine sex differences on a longitudinal basis, may provide greater clarity on these issues.

The unequivocal finding from the Irvine *et al*[104] meta-analysis of AD patients is that men modestly

but significantly outperform women in all of the five cognitive domains assessed. Moreover, most papers report better male performance within every domain (only three had a female superiority in any single domain and the effect sizes were close to zero). Neither any differences in age nor dementia severity (as measured by MMSE) could account for the male advantage. Overall, the findings indicate that in women with AD, multiple cognitive functions are affected both more severely and more widely than men.

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REVIEW

Systems psychopharmacology: A network approach to developing novel therapies

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Abstract

The multifactorial origin of most chronic disorders of the brain, including schizophrenia, has been well accepted. Consequently, pharmacotherapy would require multi-targeted strategies. This contrasts to the majority of drug therapies used until now, addressing more or less specifically only one target molecule. Nevertheless, quite some searches for multiple molecular targets specific for mental disorders have been undertaken. For example, genome-wide association studies have been conducted to discover new target genes of disease. Unfortunately, these attempts have not fulfilled the great hopes they have started with. Polypharmacology and network pharmacology approaches of drug treatment endeavor to abandon the one-drug one-target thinking. To this end, most approaches set out to investigate network topologies searching for modules, endowed with "important" nodes, such as "hubs" or "bottlenecks", encompassing features of disease networks, and being useful as tentative targets of drug therapies. This kind of research appears to be very promising. However, blocking or inhibiting "important" targets may easily result in destruction of network integrity. Therefore, it is suggested here to study functions of nodes with lower centrality for more subtle impact on network behavior. Targeting multiple nodes with low impact on network integrity by drugs with multiple activities ("dirty drugs") or by several drugs, simultaneously, avoids to disrupt network integrity and may reset deviant dynamics of disease. Natural products typically display multi target functions and therefore could help to identify useful biological targets. Hence, future efforts should consider to combine drug-target networks with target-disease networks using mathematical (graph theoretical) tools, which could help to develop new therapeutic strategies in long-term psychiatric disorders.

Core tip: Pharmacotherapies of mental disorders have to take into account their multifactorial origins. Therefore, combinatorial drug therapies have to be devised

targeting multiple molecules in disease networks. Mathematical approaches can aid in learning more about molecular interactions in disease networks and, by in silico simulations, discover dynamic changes in network properties. Targeting important nodes by drugs should be avoided to preserve network integrities. Efforts to find efficient combinations of drugs could be supported by more research into compounds contained in herbal extracts.

INTRODUCTION

It is widely accepted that many diseases, in particular disorders of the Central Nervous System are multifactorial of origin. Consequently, reasonable pharmacotherapies should aim at addressing those multiple factors causing and sustaining the diseases, simultaneously. However, only recently strategies of "polypharmacology" (one drug adressing several targets) have appeared in research and in the literature. Apparently, the drug industry has relied for a long time on a small number of targets that had already been validated, subsequently generating an abundance of "follow-on" drugs. Statistical analyses of large component size, degree distribution and clustering coefficient quantitatively confirm this bias. This bias has been reinforced by the conviction, that the "major" targets known for most mental disorders were sufficient for the development of efficient pharmacotherapies. However, with the advent of modern sequencing technologies facilitating the sequencing of whole genomes within reasonable times and declining costs, intensive efforts to search for more targets, preferably on the gene level, have gained new steam.

GENOME-WIDE ASSOCIATION STUDIES

The idea behind genome-wide association studies (GWAS) was to identify all genetic changes eventually causing a disease. Until very recently, however, browsings of whole genomes for sites of associations with a mental disorder have not met with the expectations, because they did not stand up to rigorous statistical analysis or were not sufficiently specific for only one mental illness[1]. Moreover, it has to be mentioned, that these tremendous efforts have been burdened by the fact, that an estimated 99% of all single nucleotide polymorphisms (SNPs) are benign and without adverse effects. Nevertheless, it has to be acknowledged that these are unbiased approaches with the potential to discover new pharmacological targets.

Many so-called "candidates" have surfaced in schizophrenia (SCZ) research (such as COMT, NRG, dysbindin), but for instance in a larger European ancestry sample, where 14 candidate genes had been detected[2], associations were equivocal. In another more recent study on psychosis, the authors came to the conclusion, that "No individual SNP showed compelling evidence for association with psychosis"[3]. Furthermore, in a recent study on anorexia nervosa (AN) encompassing 5551 AN cases and 21080 controls, there were "No findings that reached genome-wide significance", concluding that the sample, the largest yet reported for this disorder, was underpowered for their detection[4]. Apparently, these statistical hurdles were overcome by the efforts of the Psychiatric Genetics Consortium, resulting in the identification of 22 loci significant for association to SCZ, and considered to be statistically sufficiently robust[5]. It was estimated that more than 8000 SNPs independently contribute to SCZ, which confirms that SCZ is a highly polygenic disorder[6]. A subsequent analysis by the same consortium encompassing very large samples (approximately 37000 cases and 113000 controls) revealed 128 independent associations spanning 108 defined loci (including at least 600 genes) that are genome-wide significant for SCZ[7].

In an attempt to assign a physiological meaning to some genes belonging to those loci, genes linked to voltage-gated calcium channel subunits (*CACNA1C*, *CACNB2*, and *CACNA1I*), involved in glutamatergic neurotransmission and synaptic plasticity (*GRM3*, *GRIN2A*, *SRR*, *GRIA1*) and the dopamine D2 receptor gene (*DRD2*), "the target of all effective anti-psychotic drugs" have been discussed. Apart from doubts raised recently that the symptoms of psychosis or SCZ are caused by the over activity of dopamine[8], hypothesis-free investigations should rather serve to broaden our view of the disorder by searching for additional genes of interest not belonging to the main stream of thinking. Dopamine and glutamate neurotransmission appear to be involved in too many essential brain functions as to be suitable for drug therapies specific for SCZ. Another very popular category of genes identified in the GWAS loci were genes with important functions in the immune system, nourishing the belief brought up in quite some publications before, that SCZ is an autoimmune disorder[9]. However, the data published in this respect are weak and controversial, so that repetition of this hypothesis does not render it more likely. Moreover, a recently published investigation on SCZ and multiple sclerosis (MS) samples[10], focusing exactly on risk alleles of the HLA group of genes, reports on associations of these genes with both SCZ and MS, but with an opposite directionality of effect of associated HLA alleles (that is, MS risk alleles were associated with decreased SCZ risk). Our studies may also be in this line of results, revealing that many more genes of the immune system were downregulated in post-mortem brains of SCZ patients, than upregulated. However, more importantly, as pointed out in our paper, many of

those gene products may have functions in the Central Nervous System distinct from their immunological functions[11]. This notion had already been raised before by other groups who discovered the involvement of *MHC* genes in signal transduction of glutamate receptors and synaptic plasticity[12,13], which attributed a feature to MHC distinct from its immune function in the aetiology of SCZ[14].

Since all these recent studies required enormous logistic coordination and substantial financial support, they deserve to be scrutinized for their cost-benefit ratio in more detail.

As already alluded to above, associations have been found with genomic regions ("loci"), not with single genes. Genomic loci typically encompass more than one gene, so that it remains elusive, which gene is affected. Moreover, a SNP may or may not influence expression of genes within - or outside - the locus. And SNPs may or may not interfere with non-protein-coding genes (or unknown genes), or with annotated protein-coding genes[15].

Very unlikely the identified SNP is the causative SNP, but only tags the stretch of DNA where the causal variant is located. Furthermore, all the SCZ -associated SNPs show up in non-coding regions of DNA (*e.g.*, intergenic, or intronic) or are synonymous exonic polymorphisms[16]. And above all, it has been underscored in a recent publication, that within approximately 7300 GWAS associations to common diseases or traits, only 20 could be clearly attributed to a causal variant[17].

Typically (and expectedly) the odds ratios associated with each SCZ risk SNP are around 1.10, indicating a very small effect on disease risk.

Some of the SNPs are not specific for the disorder, but also show associations *e.g.* with bipolar disorder or, to a lesser extent, with major depression, with attention-deficit hyperactivity disorder, and with autism (genetic pleiotropy)[18,19]. Hence, the clinical overlap between these disorders may arise to some extent from a shared genetic predisposition.

It is well known that SCZ is a broad spectrum psychiatric disorder which poses substantial problems of reliabilities of inclusion/exclusion criteria of patients according to diagnostic manuals ICD-10 or DSM IV, manuals that have been repeatedly critisized and are in the course of being thoroughly revisited[20-22]. In this light, the extensive increase of *n*-numbers in GWAS is bound to increase the probability of irreproducibility of results. Therefore, it remains at least controversial, to what extent further increases in sample sizes will improve our understanding of the disorder[23,24].

It is still surprising that even from apparently robust "candidate gene" approaches of the pre-GWAS era[25,26], almost all GWAS studies, including Ripke *et al*[5] were unable to replicate any of those genes. For these reasons, it is still a matter of debate to what extent genome-wide statistical significance can be reliably used to decide which gene is a risk gene or not[27].

Only a minor part of the heritability of SCZ can be explained by the existing results[28]. As concerns the major part, one assumption implies, that rather than simply arising from cumulative effects of multiple independent genes, gene-gene interactions (epistasis) may have higher impacts on the genetic risk to develop the disorder[29].

There are preliminary clinical[29,30], and experimental data[31,32] supporting a role for epistasis in SCZ. Unfortunately, systematic investigations on epistatic events in SCZ are not available, and GWAS studies do not help that matter[33].

Conversely, the majority of genetic heritability cannot be explained by GWAS studies, neither individually nor collectively[34,35]. And for most of the associated variants no functional links have been provided[36].

Therefore, GWAS results are of limited value for closer insights into molecular mechanisms of the identified loci/genes, and far away from applications in translational medicine or pharmacotherapeutic interventions. A very important, additional point to be made here is that all those studies depend on statistical significant results, which require prohibitively high numbers of samples due to multiple testing and other statistical problems, which in turn increase the "noise" level churning up the spiral more and more. On the other hand, gene modifications that do not result in any statistical significances because of their subtle effects, may synergize metabolically on the gene product level with each other or with additional genes not modified to exert significant contributions to the development of the disorder, meaning that not only genes reaching statistical significance are important. Very likely, there is much more passing undetected in the "noise" (see below, summary). Along these lines, it has to be assumed, that variation anywhere in the genome affects every character. In keeping with this, the notion was put forward, that there are "no special genes for psychosis". Instead, the "normal gene" model proposes that any gene or allele that influences the development of the human brain can tentatively act as vulnerability gene or allele, as well[37].

The disappointing failure to identify even one etiological candidate gene during many years of genetic studies on psychosis may be explained by the possibility that genetic vulnerability to psychosis is due to random mutations. Therefore, given the hypothesis that gene alleles that are endowed with general functions in brain development could also act as vulnerability gene alleles, it appears plausible that there is no need to postulate the existence of specific "psychosis genes or polymorphisms".

FROM GENETICS TO EPIGENETICS

Moreover, epigenetic modifications of various susceptibility genes with minor effects may well reinforce the development of psychosis[38]. Possibly, the combined effects of those genes along with their interaction with environmental factors results in a number of distinct
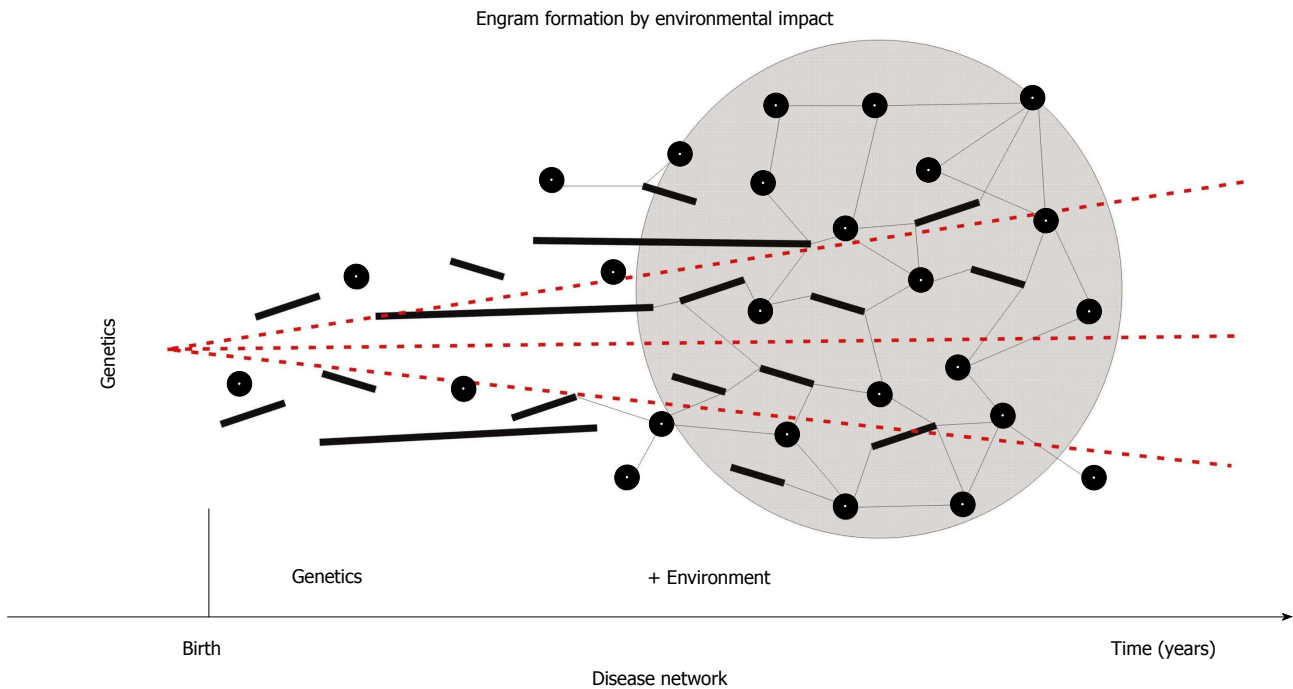
Engram formation by environmental impact

Figure 1 Theoretical formation of a disease network in human brain. Short-term (*e.g.*, traumatic = circles), medium-term (short bars), and long-term (*e.g.*, family = long bars) adverse impact emboss molecular engrams that eventually synergize to form a disease module or network (as shown by connections between nodes). Because the development of the human brain occurs predominantly postnatally, these environmental (epigenetic) influences appear to have more importance than the underlying genetic vulnerability (dashed lines).

phenotypes. In other words, a wide array of genes is working through various intermediate developmental and physiological pathways on different molecular levels. Changes of gene expression impact on protein expression, which interferes with cell metabolism (*e.g.*, in neurons)[39]. However, often directionality is reversed feeding back from "higher" levels down to ongoing activities at lower levels - thus the interactions are bidirectional[37]. This would account, at least in part, for the substantial heterogeneity repeatedly observed in the psychotic phenotype. As a matter of fact, there is strong evidence for environmental interference with regulation of neurodevelopment by means of epigenetic modifications. What is more, it has to be recalled that almost 80% of human brain growth falls in the postnatal period of life - this includes axonal growth, arborisation of dendrites, synapse formation, and myelination of axons. Therefore, this life span provides ample room for adaptation to environmental conditions and to organize brain development on the epigenetic level[40]. Apparently, the complex construction of brain, mind, and consciousness to enable the organism to respond adequately in a world of social interactions can better be optimized after the individual is born. Needless to mention, that the development of human intelligence and many more typical human characteristics (writing, language, abstraction, anticipation, *etc.*) are subject to environmental impact. Therefore, it comes of no surprise, that also the majority of psychiatric disorders arise primarily through problems of social functioning, social navigation, or social understanding. It appears

safe to say that psychosis is not just a gene-driven by-product of brain evolution. Eventually, each society with its inherent adverse conditions of life gives rise to its specific psychosomatic or brain disorders. In this way, psychosis is an example of "socially sensitive" diseases and reflects structural deficits inherent of human societies. But also "simple" traumatic events, such as lack of oxygen supply during delivery may leave behind a traumatic "imprint" or "engram" that may be accelerated and reinforced by multiple, additional adverse events in childhood and adolescence (short or long-lasting, such as the influence of the family) and eventually result in the formation of a molecular "disease module" or a neuronal "disease circuit" (Figure 1).

This reasoning does not deny the presence of a genetic vulnerability being modified or reshaped postnatally by environmental influences and contributing its burden to the progression of the disease. However, even if a defect (polymorphism, copy number variant, *etc.*) is detected in a gene of known function, the phenotypic impact of that defect can only be seen in the context of the functions of the gene product's interaction partners, *i.e.*, by its network context[41]. There is no question that the most immediate reactions on environmental stimuli are located on the cellular level. These reactions may change or modify cellular components or subcellular, molecular interconnectivities. They may not only impact on predisposed genetic abnormalities but influence transcription on the epigenetic level (DNA methylations and posttranslational modifications of histone proteins)[39,40] and post-transcriptional events,

such as editing of mRNAs or mRNA degradation by small interfering RNAs or micro (mi)RNAs[42]. The latter way of posttranscriptional regulation of gene expression has become very popular to experimentally silence single genes as an alternative to produce gene knock-out animals. In the context of this review, however, it appears to be even more interesting, because those short RNAs (21-25 nucleotides in length) typically are not specific for one mRNA, hence display multi-target functions[43]. Expression levels of several hundreds of mRNAs can be modified by one miRNA, which results in "fine-tuning" of target gene expression. There are several reports delineating the occurrence of altered miRNA expression profiles in psychiatric disorders[44,45]. For example, miRNAs 1202 and 135 turned out to be involved in major depression disorder (MDD), supporting their role in influencing higher brain functions[46,47]. Owing to this relatively new field of research, the molecular mechanisms leading to altered miRNA expression in those disorders are largely unknown. Until recently, experimental and/or computational methodologies capable of detecting accurately and with high resolution miRNA gene transcription start sites (TSSs) were not available, although efforts in this direction have been made several years ago[48,49]. The latter, however, lacked reliable accuracy in experimental techniques, or presented *in silico* algorithms providing low resolution/high false positive rate predictions and heuristics. The first algorithm that surpassed the barrier of 54% sensitivity and 64.5% precision in miRNA TSS identification of the earlier studies by achieving 93.6% sensitivity and 100% precision is MicroTSS[50]. MicroTSS can accurately identify miRNA TSSs in single nucleotide resolution[51]. The interconnection between miRGen v3.0 and other DIANA resources enables users to identify *in silico* as well as experimentally verified miRNA targets on lncRNAs with LncBase[52]. This is very promising progress in getting more insight into regulatory mechanisms of miRNA gene expression and their influence on target mRNAs. A great challenge will be to identify methylation patterns and posttranslational modifications of histones of these genes in health and disease. As a result, effects on protein expression and disturbances of molecular networks in brain disorders may be better understood. Along these lines, it would be important to know, to what extent the products of genes targeted by miRNAs belong to disease networks (see below). Pharmacological interventions on miRNA gene expression would then be reasonable strategies to tackle the problem of the multifactorial origin of chronic psychiatric disorders. Consequently, a key hypothesis is that various pathobiological processes interacting within complex networks are continuously embossing a disease phenotype[39]. Generally speaking, it can be concluded that reductionistic biology (concentrated on single molecules) will not provide insights into the workings of those interconnected networks nor result in improvements of therapeutical solutions.

MOLECULAR NETWORKS IN HEALTH AND DISEASE

There are quite some efforts to abandon reductionistic biology and pave the way for a broader understanding of the maintenance of health and the initiation and progress of disease. Models of oscillating molecular networks could play key functions in identifying weak, but crucial variations in molecular interactions characterizing disease processes. For example, genome-scale metabolic (GSM) networks[53] have been used to investigate metabolic interactions at the cellular level. Additionally, as an attempt to improve the work with GSMs by computational modelling, "Flux Balance Analysis" (FBA) has been elaborated. This constraint-based modelling approach, or constraint-based reconstruction and analysis method, characterizes and predicts aspects of an organism's metabolism[54]. As a matter of fact, efforts to integrate mRNA expression data into metabolic networks could be significantly improved using FBA as an analytical platform[55]. For example, when growth of mutant *E. coli* was simulated with FBA, 86% of the mutant phenotypes (*i.e.*, growth or no growth) were accurately predicted[56]. Current GSMs stand out by their large sizes and by a rich source of annotations, but can be applied only to model protein relationships and reactions. More detailed modelling would need more sophisticated resources, which are hard to recover within a drug industry setting. FBA uses rates of uptake of extracellular metabolites and their production as input. The most important challenge appears to reside in the unclear relationship between gene expression and reaction flux[57]. As an advantage, this strategy can be used without biochemical data of enzyme kinetics or concentrations of intracellular metabolites. However, modelling a system using GSM networks is restricted to conditions of a pseudo-steady state, assuming, for instance, that cell proliferation is constant. There is quite some disagreement that the pseudo-steady state assumption is valid. Exact reaction kinetics, which would better reflect *in vivo* activity[58], cannot be reconciled with this approach. Nevertheless, these mathematical tools to study network behaviour paved the way to describe intracellular molecular interactions. They could be further improved, if they were replenished by modules of protein-protein interactions (PPIs) and by the influence of modules of signal transduction pathways. Overall, studies in this area would greatly benefit from the development of a good metabolite resource[59].

Some time ago, the total number of protein interactions within the so-called human "interactome" has been estimated to fall in the range of 130000-650000 interactions[60,61]. The wide range of variance is due to the fact that only subsets of these interactions have been experimentally identified. Networks have not only been used to gain insight into disease mechanisms[62,63], but also to study comorbidities[64], and to analyze the
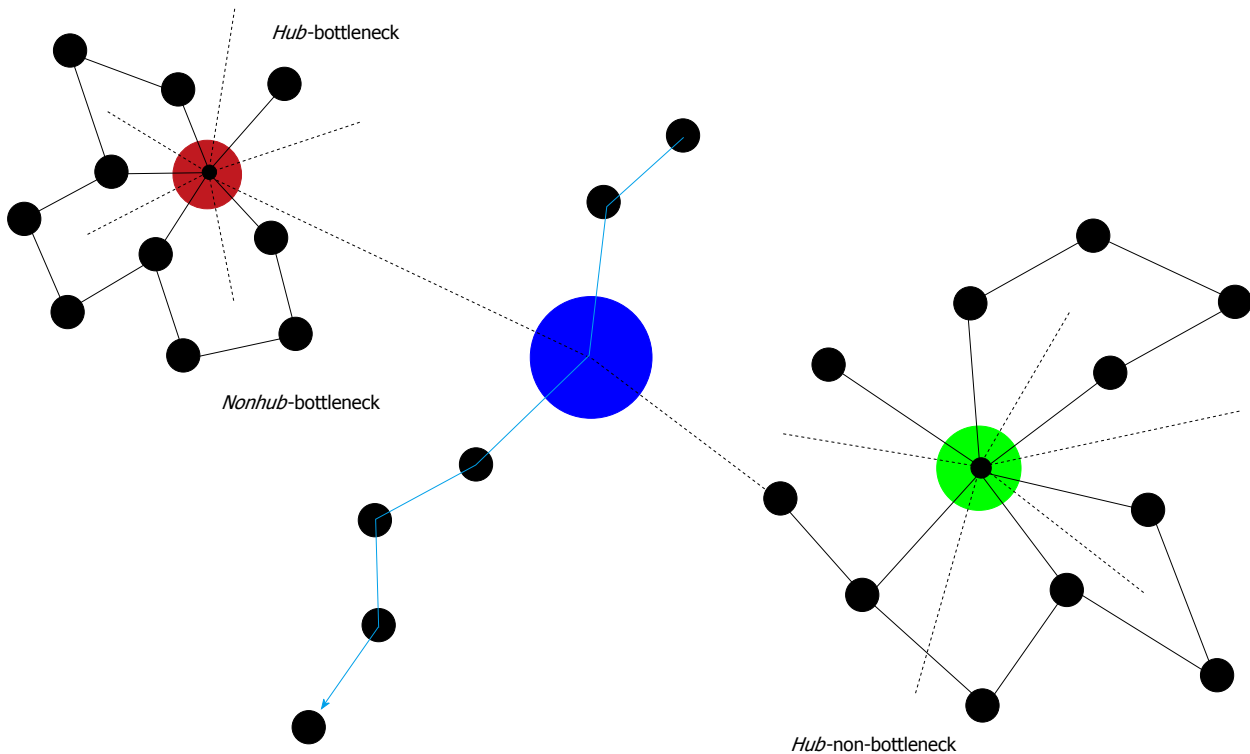
Figure 2 Some characteristics of protein-protein interaction networks. Hubs are important nodes (proteins) due to their numerous connections (dashed lines = connections in 3rd dimension). Directly inserted into a biochemical pathway, they can also represent a bottleneck (*Hub*-bottleneck). Otherwise, they are *Hub*-non-bottlenecks. *Nonhub*-bottlenecks are nodes inserted in biochemical pathways, but lacking numerous connections. Evidently, disturbances of any of these three important types of nodes result in serious consequences. Therefore, although associations of chronic mental illness with these types of nodes may exist, more unwanted side effects than benefits would ensue from targeting these types of nodes by pharmacotherapy.

actions of drugs and effects on their targets[65,66].

Some topological properties and mathematical tools to analyse those networks (Figure 2): Degree distribution in a network shows the number of connections of each single node, hence, identifies nodes with many connections as opposed to nodes with only few connections. In random networks, this distribution would look like a Gaussian curve of distribution, where few nodes have low numbers, and few have high numbers of connections whereas the majority of nodes have similar (mean) numbers of connections. Biological networks are different in that typically most of their nodes have very low connections (degrees), and with increasing connections there is a rapid dropoff of number of nodes with only very few nodes displaying high numbers of connections, obeying the mathematics of a power law distribution. For instance, the degrees of Scale-Free networks, as observed often in social networks, like Facebook, follow a power law, where a small number of nodes (people) are highly connected (hubs, see below). Hence, the low end of a power law distribution may indicate increasing importance within a network. However, the question arises here, if the importance of a node in a network is only dependent on its number of connections, which leads us to measures of centrality. A node with a high degree may occupy a central position within a network, or a peripheral position. Conversely, a node with a low degree (very few connections), may also be located in a central position. Hence, the

question arises if it is possible to compute the centrality of a given node or how close any node is to any other node in a network. Closeness centrality is the most easiest way to determine it. It measures the average of the shortest pathlength of one node to every other node in the network. The result of this may be that nodes with low degrees may display higher closeness centrality, or display more importance, than nodes with higher degrees. Consequently, network structure and function may be affected more easily by attacks on these types of nodes compared to others with less closeness centrality. It may also give some hints as to the efficiency of transmission of information from those nodes to any other nodes of the network. The most widely used centrality measure is betweenness centrality[67], characterizing a node's influence. It is the percentage of shortest paths from every pair of nodes in the network. It gives us an idea what amount of information has to pass through each individual node. It has been proposed that edges located "between" highly connected subgraph clusters (so-called "community structures") are edges with high betweenness; consequently, a network could be disrupted by disabling these edges[68].

Eigenvector centrality is more sophisticated in adding weights to nodes (*e.g.*, ranking web pages) enabling identification of heavily used nodes *vs* nodes being used infrequently. Another important topological feature of networks is their clustering coefficient, which is an indication of neighbourhood connectivities.

In metabolic and other biological networks these connectivities are not random but some are favoured over others. Clustering algorithms are used to group sets of proteins in PPIs showing greater similarities among proteins of the same cluster than in different clusters which identifies functional protein modules or densely connected subgraphs. Several methods have been developed to search for such complexes. The Markov Cluster algorithm simulates a flow on the graph by calculating successive powers of the associated adjacency matrix[69]. Restricted Neighborhood Search Clustering is a cost-based local search algorithm calculated according to the numbers of intra-cluster and inter-cluster edges, that explores the solution space to minimize a cost function[70]. Molecular Complex detection is based on node weighting to isolate densely connected regions by local neighborhood density and outward traversal from a locally dense seed protein[71]. Spirin et al[72] developed a means to detect highly connected subgraphs (cliques) in combination with Monte Carlo optimization. The authors described two types of clusters: Protein complexes and dynamic functional modules. Furthermore, a highly connected subgraphs algorithm was used for discovery of protein complexes by Przulj et al[73], and spectral clustering for generating modules, and possible functional relationships among the members of the cluster for predicting new protein-protein connections has been proposed by Sen et al[74]. Along these lines, networks displaying small world characteristics should be mentioned. Compared to random networks, small world networks show intense local connectivities combined with more or less frequent large connectivities, displaying both low average shortest path lengths of random graphs and high clustering coefficients. The wiring of neuronal networks as well as of PPI networks shows features typical of small world graphs[75].

Hubs: A typical feature of hubs is their multi-connectedness or degree. Hub proteins appear to be encoded by essential genes[76]. These genes are older than genes encoding non-hub proteins and they are more stable over time[77]. Reportedly, products of essential genes are located in hub-like positions or, if expressed in multiple tissues, in functional centers of metabolic networks[78]. Due to this importance of hubs, the hypothesis arose that hub proteins in human molecular networks ought to be encoded by disease genes. However, it turned out later, that in human cells essential genes, but not disease genes, are encoding hubs.

Bottlenecks: Interestingly, protein clusters in interaction networks constructed by the method of edge betweenness show a strong tendency to display related functions[79]. These high-betweenness proteins were called bottlenecks[80] in contrast to hubs as proteins with high degree. Betweenness reflects the important role nodes would play in information transmission in the network. Proteins encoded by essential genes very often are positioned as bottlenecks (both nonhub-bottlenecks and hub-bottlenecks), whereas, surprisingly, proteins in positions of hub-nonbottlenecks are expressed by non-essential genes. The majority of proteins expressed by these genes are structural proteins, whereas proteins located in hub-bottlenecks rather belong to signal transduction pathways. A large part of proteins of nonhub-bottlenecks do not belong to complex members but to regulatory proteins or to proteins of the signal transduction machinery. Their coexpression with their neighbors in the networks is less well correlated than with proteins of nonhub-nonbottlenecks,, which is in agreement with the finding that betweenness is a good predictor of average correlation with neighbors. Apparently, inhibition or blockage of both hubs and bottleneck nodes may severely affect network integrity and function (Figure 2).

One critical feature to be considered in PPIs are post-translational modifications (PTM). More than 200 different types of PTMs have been identified, such as phosphorylations, glycosylations, methylations, acetylations, amidations, as detailed in http://www.uniprot.org/docs/ptmlist curated by UniProt[81] and other databases, like dbPTM, PTMCuration, PTMcode. For additional systematic searches of individual protein interactions, more databases, such as STRING[82], MINT and IntAct[83,84], for protein interactions within pathways, Wikipathways[85], Reactome[86] and Ingenuity (Ingenuity Pathway Analysis: http://www.ingenuity.com) are available. The volume of data held by the IntAct database alone, which includes just under 10% of the estimated human interactome[60], and currently encompassing a range of 50000 binary human interactions, may grow to 750000 binary interaction evidences in the next 5 years. More recently the issue was investigated if proteins exhibiting a particular type of PTM from a collected series of protein sets (displaying 12 types of PTMs) showed characteristic PPI network properties, such as scope of impact (interaction degree), diversity of responses (clustering coefficient), or position in a signalling pathway (closeness centrality)[87]. Interestingly, the 12 PTM-types could be grouped into 2 major groups with (1) sumoylation, nitrosylation, methylation, acetylation, phosphorylation, ubiquitination, and (2) disulfide bond, carboxylation, hydroxylation, proteolytic cleavage, glycosylation, and amidation. Not surprisingly, it turned out that there is a considerable overlap of PTMs, occurring in the same protein, especially with methylations, acetylations, and phosphorylations, indicating their joint associations with histones and their epigenetic functions. Results show that all PTM-types show a tendency of higher degrees, lower clustering coefficients, and higher closeness centralities than protein sets devoid of the respective PTMs. Furthermore, high degree proteins carrying the PTMs acetylation, phosphorylation, ubiquitination showed larger overlaps with human disease proteins than proteins with low degree.

Additionally, it is a challenge to involve the dynamic aspect into network studies by integrating complex data sets across time, space and different organizational

levels, providing a systems-level understanding. Therefore, in contrast or in extension to the above mentioned pseudo-steady state approaches, a recent review[88] welcomes various strategies to include the dynamics of biological networks and assumes that these approaches will be the de-facto network modelling in the future. As a matter of fact, the MINT-IntAct consortium is just beginning to implement this in their database, generating dynamic interaction data, in which dynamic changes in protein complex composition in response to stimuli are to be presented as animations driven by radio-buttons. All those mathematical tools and more sophisticated algorithms developed in the future will pave the way to analyze cellular (neuronal) and molecular (gene or protein) interaction networks in great detail, and identify modules or single nodes pivotal for their normal functioning. Moreover, simulations will provide insights into temporary profiles of those networks and detect sites of malfunction that may accumulate over time and set the stage of modules of disease, which reiterates to the model depicted in Figure 1.

DISEASE NETWORKS

Increased attention has been payed by the bioinformatics society to establish disease networks. These can be grouped into the following types of molecular networks: PPIs networks, whose nodes are proteins linked to each other *via* physical (binding) interactions; metabolic networks, whose nodes are metabolites that are linked if they participate in the same biochemical reactions; regulatory networks, or protein signalling networks whose directed links represent regulatory relationships, such as links between a transcription factor and a gene, or by other signalling molecules on downstream events or on PTM, such as those between a kinase and its substrates; and RNA networks, encompassing RNA-DNA interactions, such as small non-coding miRNAs and siRNAs in regulating gene expression.

PPI networks in particular have become very popular in this context[41]. PPIs entail binding characteristics between proteins and can also include PTMs and protein-protein dimerizations (see below).

Closer insights into the influences of molecular interconnectedness on disease progression could reveal gene products linked to disease and disease pathways, which, in turn, could offer more suitable targets for drug development. Additionally, these new targets could serve as better biomarkers that more accurately monitor the functional integrity of the network perturbed by the diseases. In this way, they could directly impact on clinical practice to achieve better classifications of disease and enabling earlier diagnosis and prognosis, which eventually aims at personalized therapies and treatment[20]. Therefore, analyses of disease networks are believed to permit a better understanding of the pathophysiology of chronic psychiatric diseases with the potential to design combinatorial pharmacotherapies[59].

Moreover, if infections have to be included in the consideration of disease progression, efforts have been undertaken to develop host-pathogen PPI networks[89]. These networks can lead to a better understanding of host-pathogen interactions and to identification of pivotal points for pharmacotherapeutic treatments. In all these approaches, the available databases have to be standardized to seamlessly enable sharing of data, such as being developed in BioPAX[90]. Along those lines, attempts were made to discover functional subnetworks tentatively related to the progression of colorectal cancer by combining analysis of mRNA expression with PPI data[91]. Here, a new computational algorithm was used to search for subnetworks embedded in a PPI network, that entailed genes differentially expressed in the disease. In another study, trying to elaborate predictions on metastasis in breast cancer, it has been shown that markers differentially expressed in subnetworks were more precise than single gene markers[92]. Hence, analysis of PPI networks is very useful to identify candidate biomarkers, to get more insights into disease mechanisms, and to obtain a better understanding of their biology. PPI network analysis also revealed significantly elevated protein interactions specific for the disorder. Two hundred and ninety of such interactions were identified, which corresponded to a 10-fold increase compared to random expectation ($P < 10^{-6}$)[93]. In other studies, similar results have been reported, *i.e.*, there are significantly increased, direct interactions of gene products associated with disorders of similar phenotypes[94,95]. From those observations, it can be concluded that once some disease components have been identified in the network, more disease-related components should be located in their neighbourhood. In other words, it seems likely that there are interactomes linked to diseases that are embedded in PPI-networks in well-circumscribed neighbourhoods. These interactomes frequently are named disease modules.

Along these lines, three distinct network modules should be considered (Figure 3): (1) topological modules; (2) functional modules; and (3) disease modules. (1) Topological modules stand out due to locally densely connected neighbourhoods of the interactome, *i.e.*, intramodular nodes preferentially interact within the module rather than with nodes outside of the module. In this respect, topological modules represent a pure network property; (2) Functional modules are distinguished for their significant segregation of nodes of related function (shown as circles in Figure 3, and connected by short dashed lines), and thus require to define some nodal characteristics. Their belonging to the same network neighbourhood is grounded on the assumption that intensities of nodal interactions are determined by their joint cellular functions; and (3) A disease module is a group of nodes showing changes (of expression, due to mutations, or epigenetic modifications) connected to a specific disease phenotype (drawn as squares in Figure 3, and connected by long, dashed lines).

The tacit assumption in network medicine is that the topological, functional, and disease modules partially
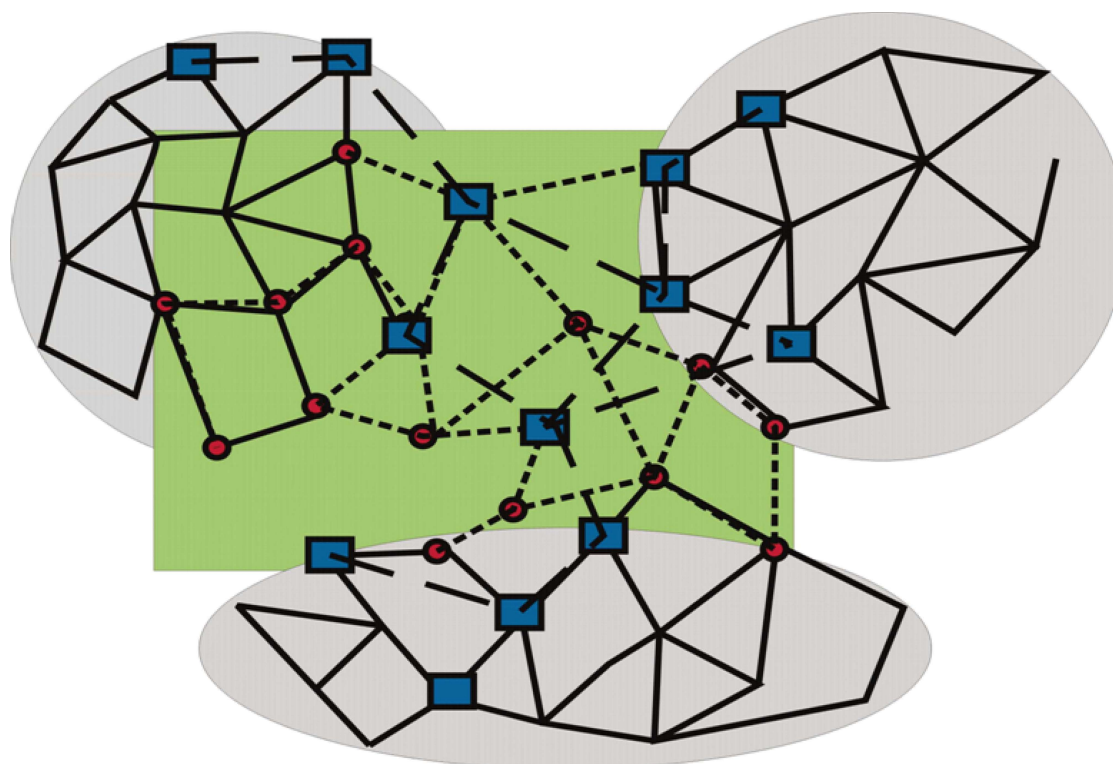
Figure 3 Topological, functional, and disease modules. Locally densely connected topological modules (grey circles) contrast with functional modules (circles connected by short, dashed lines), showing more (upper left), less or no overlap (center) with topological modules. The latter are preferentially associated with signal transduction pathways. Disease modules (nodes shown as dark squares, and connected by long, dashed lines) overlap with topological and functional modules, but may be less intensively connected and occupy more peripheral sites of networks.

overlap: Cellular components that form a topological module have closely related functions, thus being part of a functional module; and a disease is a result of disturbances in some functional module, which means that a functional module is also part of a disease module. However, several characteristics of disease modules are important to bear in mind. As pointed out, a disease module likely overlaps with the topological and/or functional modules, but, because a disease module is defined in relation to a particular disease, each disease has its own unique module. Optionally, a gene, protein, or metabolite can be implicated in several disease modules. There is general agreement in network medicine of mutual, partial overlaps of the topological, functional, and disease modules, which means that on the cellular level, topological modules typically are also closely related in their functions, hence being part of a functional module; and a disease results from disturbances in some functional module, which means that a functional module is also part of a disease module. However, it has to be pointed out, that albeit overlapping features of disease modules with the other modules, the definition of disease modules is specified by each particular disease, endowing these modules with some unique characteristics. Finally, when looking at single genes, proteins or metabolites in a particular disease module, it should be mentioned that each of these components can be a part of other disease modules.

A few years ago, PPI networks (disease networks) were constructed using data from genes differentially expressed in some psychiatric disorders[96]. The study revealed several disease markers (nodes or vortices) characteristic for SCZ (SBNO2), for bipolar disorder (SEC24C), and for MDD (SRRT). Furthermore, similar networks were constructed for Parkinson's disease (PD), using proteins differentially expressed only in substantia nigra and frontal cerebral cortex[97]. Construction of those networks was based on the following assumptions[96]: (1) There is a positive correlation between expression levels of most proteins and mRNAs in brain; (2) Proteins with similar expression patterns more likely interact with each other; and (3) The abundance of proteins correlates with their participation in biological processes.

In this way, thirty seven unreported disease marker genes were identified. Eight of them belonged to the core functional modules and four were strongly associated with some neurotransmitters, including dopamine. The results of this study may pave the way for addressing new targets in search for more efficient pharmacotherapy of PD. A more general study on the animal model of PD induced by the chemical MPTP used proteomics meta-data from the literature where neuronal alterations due to the metabolite of MPTP MPP+ had been reported[98]. The topological analysis of the protein networks generated on physical or functional interactions revealed a close interaction between nodes
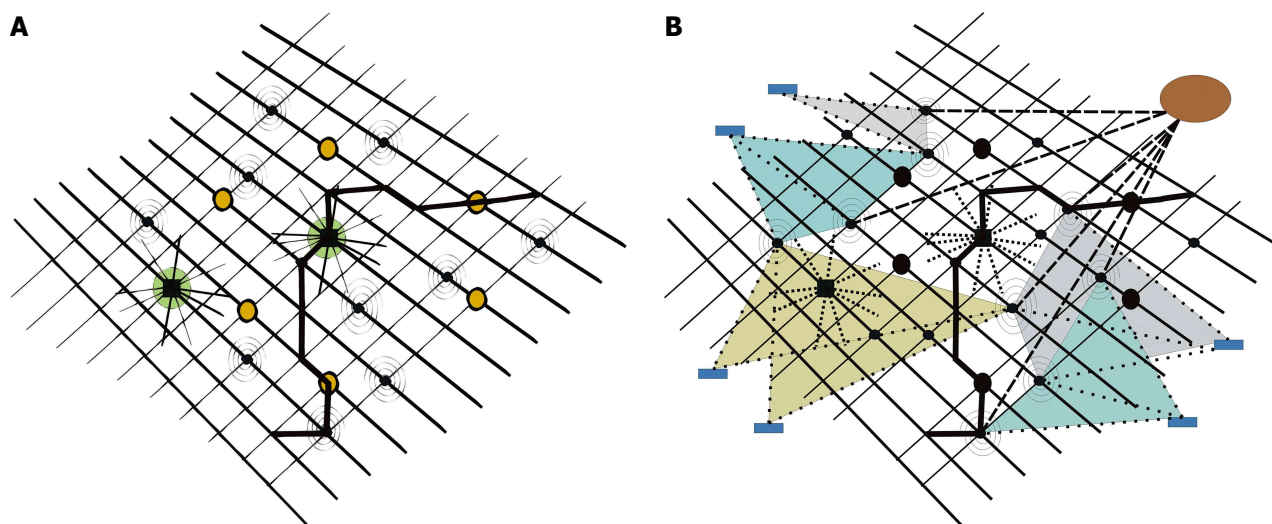
Figure 4 Molecular disease network. A: With two "hubs" (big, dark circles, showing high connectivity profiles) and six "bottlenecks" (dark circles), inserted in pathways (one pathway highlighted in bold), plus additional molecular nodes, tentatively disturbing network oscillations (spiral nodes, belonging to disease network); B: Subtle disturbances of network harmony by molecular nodes of minor importance (spiral nodes) may substantially interfere on a long-term scale in their summation with network oscillations and result in disease. Drugs with multi-target properties (circle upper right, strong, dashed lines), or multiple drugs with specificities for only one or a few targets (rectangles in periphery, weak, dashed lines) may address nodes of disease networks and reset disease networks to networks characteristic of healthy states.

as identified by an average shortest path length smaller than in random networks. Moreover, specific alterations in the mitochondrial proteome underlined in what way this model can recapitulate some pathogenic events of PD.

As mentioned above, the construction of those networks was based on the assumption that there is a positive correlation between expression levels of most proteins and mRNAs in brain. This may not be true in any case. Moreover, there are more steps between transcription of a gene and its product, such as post-transcriptional and-translational regulations, all of which complicate the correspondence between expression of a gene and its protein product.

DRUG-TARGET NETWORKS AND NETWORK PHARMACOLOGY

Traditionally, the focus in drug development is to interfere with the activity of one target molecule tentatively crucially involved in the onset or the progression of a disease. Typically, they evoke additional biological responses in patients, some of them leading to adverse or even toxic effects. Others may be benign. These benign effects may result from additional drug effects on additional targets beyond specificity ("dirty drug"). Research to identify those additional targets may open up new therapeutic options for the drug, which is well known in pharmacology as "drug repositioning"[99]. These basic principles constitute the basis of drug-target networks (DTN). Drug discovery can benefit from concepts of DTN in two ways: As polypharmacology and computational drug repositioning. Polypharmacology takes into account the above mentioned features of

many drugs to be "promiscuous" in their specificities addressing more than one target molecule[100]. Specific changes of gene expression profiles in a cell are results of direct or indirect responses to a drug or a disease. Disease-induced disturbances of expression profiles could be conceived as perturbations of the dynamic, equilibrium state of a PPI. Drugs ideally may (re-) organize these molecular networks (drug-induced profile) and reverse disease-induced profiles into a state towards the dynamic, equilibrium state (Figure 4). Often, drug discovery is accompanied by investigating biochemical pathways. Because, however, in complex diseases multiple pathways may not be functioning in dynamic equilibrium, it may be advisable to use DTN to search for targets not necessarily being connected at the pathway level, but interacting more specifically on the disease level. Drugs expressing multiple target specificities with little adverse effects or displaying better tolerance have frequently been discovered in natural sources. In contrast to many currently approved drugs, natural products can be viewed as multi-component complex systems with therapeutic potential for a variety of diseases. They display many biological activities and good drug-like properties, show vast chemical diversity and can interact with multiple cellular target proteins[101]. Moreover, biologically active natural products are able to influence disease-related pathways, could provide selective ligands for disease-related targets[102], and could eventually shift the biological network from disease status to the healthy status.

One very well-known drug in this respect is acetyl-salicylic acid. Salicylates have been known for centuries as anti-inflammatory substances and were already used as extracts from the willow tree (*Salix Alba*) in the form of tea in times when these active substances

had not been identified. While the anti-inflammatory properties of salicylates are due to their inhibition of the NF-κB pathway[103], the serendipitous modification of salicylic acids by an acetyl group endows aspirin with a variety of additional characteristics. The substance inactivates cyclooxygenases through acetylation of serine residues[104]. Some 33 cellular proteins have been identified as acetylation targets of aspirin, one being the tumor suppressor protein, p53 at K382, inducing expression of its target genes[105]. Furthermore, enzymes of the glycolytic pathway (such as glyceraldehyde-3-phosphate dehydrogenase, enolase, aldolase, pyruvate kinase M2, and lactate dehydrogenase A and B chains), cytoskeletal proteins, histones, ribosomal and mitochondrial proteins are targets of aspirin modification. Aspirin also acetylates enzymes involved in ribonucleotide biosynthesis, such as glucose-6-phosphate dehydrogenase and transketolase[106]. Additionally, induction of apoptosis by activation of p38 kinases[107], and catabolism of polyamines[108] have been related to anti-cancer mechanisms of aspirin[109,110]. Regular intake of aspirin has been shown to reduce the risk of cancer of the esophagus by 73%, of the colon by 63%, of the stomach by 62%, of the breast and prostata by 39%, and of the lung by 36%[111]. The inhibition of G6PDH with increasing concentrations of aspirin is believed to be a crucial event in its anti-cancer effect, because this enzyme regulates the synthesis of nucleotides and nucleic acids. Along these lines, the activation of the ERK pathway mediated by the high levels of Ras mutations observed in many cancers[112], has been shown to be inhibited by aspirin, as well. Antiproliferative effects have also been reported from actions of salicylic acid, that is able to reduce mitochondrial calcium uptake[113]. Finally, aspirin has been shown to activate CREB, facilitating its binding to a cAMP-response element in the promoter of the neurotrophic factor CNTF and increasing its gene expression[114]. Aspirin (salicylates) is only one example of multi target effects exerted by many natural substances. Very likely, many other substances occurring in plants may be superior to synthetic drugs with high specificity for one target. For that reason, it may be more beneficial to learn from nature in what ways pharmacotherapies could become more efficient. Polypharmacology, indicating multi-target strategies in pharmacotherapies, encompasses those attempts to identify the multi-target nature of natural substances, but also to develop synthetic drugs with multiple target properties[115]. Especially in chronic brain disorders, these drugs may offer higher efficiency and less unwanted adverse effects[116]. In these terms, successful drug discovery in the pharmaceutical industry is still in its infancy, slowly trying to abandon the point of view that successful drugs should interact only with single, individual targets and approaching concepts of systems biology models of human metabolic networks[117]. Investigations on DTN on one hand, and disease networks on the other hand can aid to find overlapping targets and achieve better understanding

of the mechanisms of action of multi-target (natural) compounds. The goal, hence, is to understand drug targets in the context of cellular and disease networks using systems pharmacological approaches[118].

Generally, hundreds of different biologically active substances are found in herbal extracts[119] and in contrast to most of synthetic drugs designed to bind single targets, most of the ingredients of herbal formulae display only weak to moderate effects, but address multiple cellular targets during treatment of complex diseases[120]. Normally, the underlying mechanisms are not clear. As a newly emerging field, network pharmacology[121] could help to understand the mechanisms of multiple action drugs across multiple scales from the molecular and cellular level to the tissue and organism level by analyzing the features of biological networks[122]. Network pharmacology is supposed to integrate polypharmacology and network biology and considered as upcoming paradigm in drug development[123].

It is exciting to study network pharmacology in product-target networks using natural products. Recent results on network properties suggested a marked enrichment of polypharmacology with respect to nodes (compounds) with large degree and high betweenness centrality. These nodes turned out to be highly influential in the whole network. Despite a slow change of direction in drug research and development, it has to be acknowledged that until recently approximately every second drug approved by the FDA was a natural product or a derivative thereof[124]. That means, that also in the industrial context increased efforts have been made to explore in more detail the mechanisms of action of herbal formulae employing network pharmacological approaches, such as DTN[125], PPI networks[126], metabolic networks[127], or disease networks (see above)[128]. In order to speed up virtual screening of natural products on a large scale, the Universal Natural Products Database has been constructed. Until now, it entails 197201 natural products (http://pkuxxj.pku.edu.cn/UNPD). Due to the complexity of studies using network pharmacology approaches, most of them are based on static networks[129]. However, network pharmacology also provides a systems-level approach to understanding the development and pathogenesis of disease, taking into account the dynamics of biological networks[130].

The mechanisms of action of natural products have been studied by well established, modern technologies, such as gene expression microarrays[131], technologies in proteomics[132], and metabolomics[133]. The concept of "network targets" emerging from these investigations extends the widely used concept of a drug tailored for a single biological component to the concept of a drug or group of drugs exerting multiple effects on a biological network[134]. The problem presently remaining is to develop mathematical algorithms able to configure herbal formulae holistically as a gestalt, whose emergent and tentatively synergistic properties no longer rely on analyzing each substance separately. Exemplarily, components of the Liu-Wei-Di-Huang pill

have been investigated completely *in silico* to predict their effects and mechanisms[135].

After analyzing the composition of chemical groups in LWDH, their chemical characteristics and distribution in chemical space were studied. Then their pharmacological properties were explored. Based on these results, predictions were made as to what biological molecules could be targeted by LWDH components. It was suggested that a biological molecule would be a "good" candidate target if there were several components specific for that biological molecule in the natural product. The results revealed that PPI networks constructed from the predicted candidate targets of LWDH displayed high intranet connectivity. Therefore, a compound-target network was constructed from the PPI network, where compounds were connected to their targets[136]. Then a disease network could be constructed by adding edges between candidate target proteins and a disease if the target protein's gene was in a gene list related to the disease. The results show that some target proteins belonging to hormone signalling, such as ESR1, NCOA1 and AR, are not only highly connected to the components of LWDH (high degree), attributing hub-like properties to them, but also highly connected to disease. However, a disease might be influenced by many biological processes that are targeted by different groups of ingredients, and hormone signalling is only one example. Like in many studies before, this study reveals, as well, that often "key players", here hubs and bottlenecks in molecular networks, become a focus of major interest.

CONCLUSION

There are numerous mathematical tools available to analyze networks both on the static and dynamic levels, and to distinguish structural components required for maintenance of their connectivities or responsible for their malfunctioning. Apparently, one major challenge for future studies is to improve algorithms taking into account changes of PTM over time and incorporate many more molecular nodes into these observations than just some "important" ones. The notion to be delineated here, is that efforts to identify "key" players in molecular networks may be misleading. Although they are often involved in maintenance and progression of a chronic mental disorder, they are also crucial for maintenance of many other metabolic functions independent of the disease. Therefore, interference by drugs with these crosspoints may easily destroy network integrity and, hence, be accompanied with numerous unwanted adverse effects. We rather want to conclude from the features of molecular networks outlined above, that it is more desirable to search for targets with more subtle effects on network functions, but specifically perturbed in a mental illness, and hence belonging to a disease network, as well (Figure 3). Along these lines, Lamb[137] have shown that mere node connectivity (degree) might not be the only influential

parameter to characterize biological networks. And Goñi *et al*[138] reported that in case of neurodegenerative diseases, less extensively connected proteins are much more appropriate therapeutic targets than highly connected ones, as the critical role of highly connected nodes (hubs) in the network modules prevents them from substantial fluctuation. Exceptions probably are genes of miRNAs displaying "hub" features as well, that nevertheless may be good pharmacological targets. Moreover, it was shown that the above mentioned betweenness centrality can also be used as an important parameter to search for lowly connected nodes[80]. Chronic degenerative disorders of the brain extend over considerable periods of time, which strongly argues for disturbances of multiple nodes with weak influences, each. Or conversely, if abnormally functioning hubs or bottlenecks were the only players, the disorders would not be long-lasting and chronic. Consequently, in order to correct "damages" in disease networks, future pharmacological strategies to treat mental disorders may be aimed at targeting "peripheral" molecules with only subtle effects using polypharmacological approaches. The great challenge, hence, is to identify those "peripheral" targets and develop wide spectrum drugs aimed at those targets. In summary, it has to be kept in mind, that the human brain both in health and disease is a biological system distinguished by its extremely high complexity especially on the molecular level. Mathematical approaches to investigate changes on this level still require many improvements, and even greater challenges are confronted when it comes to address dynamic changes of the system. Because, however, this is at the core of biological systems, there is no way around. Reductionistic attempts to understand molecular mechanisms of mental illness are not able to address these issues adequately.

ACKNOWLEDGMENTS

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Are the changes in the peripheral brain-derived neurotrophic factor levels due to platelet activation?

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Abstract

Brain-derived neurotrophic factor (BDNF) plays an important role in central nervous system development, neurogenesis and neuronal plasticity. BDNF is also expressed in several non-neuronal tissues, and it could play an important role in other processes, such as cancer, angiogenesis, *etc*. Platelets are the major source of peripheral BDNF. However, platelets also contain high amounts of serotonin; they express specific surface receptors during activation, and a multitude of pro-inflammatory and immunomodulatory bioactive compounds are secreted from the granules. Until recently, there was insufficient knowledge regarding the relationship between BDNF and platelets. Recent studies showed that BDNF is present in two distinct pools in platelets, in α-granules and in the cytoplasm, and only the BDNF in the granules is secreted following stimulation, representing 30% of the total BDNF in platelets. BDNF has an important role in the pathophysiology of depression. Low levels of serum BDNF have been described in patients with major depressive disorder, and BDNF levels increased with chronic antidepressant treatment. Interestingly, there is an association between depression and platelet function. This review analyzed studies that evaluated the relationship between BDNF and platelet activation and the effect of treatments on both parameters. Only a few studies consider this possible confounding factor, and it could be very important in diseases such as depression, which show changes in both parameters.

Key words: Platelets; Brain derived neurotrophic factor; Depression; Antidepressants; Biomarkers

Core tip: Brain-derived neurotrophic factor (BDNF) is expressed in neuronal and non- neuronal tissues and is stored peripherally in platelets. Platelet BDNF is present in α-granules and cytoplasm and only BDNF of granules is released by agonist stimulation. Little is known about mechanisms related to BDNF release in human platelets. Depressive disorders are associated with BDNF and platelet dysfunction. Low levels of serum BDNF have been described in major depression and they increased

with antidepressant treatment. Only a few studies have evaluated the relationship between platelet activation and peripheral BDNF values. This review suggests that platelet reactivity may partly explain the alterations in BDNF.

INTRODUCTION

Major depressive disorder (MDD) is a common and invalidating mental illness, with a global point prevalence of 4.7%[1]. Depressive disorder is one of the leading causes of disability, and it has been suggested to become the disease with the second highest burden[2]. The lack of diagnostic and treatment markers in depression is one of the most important problems in clinical practice. Researchers are deeply involved in the identification of validated markers, particularly biomarkers that could be useful in clinical practice. Evidence suggests that brain-derived neurotrophic factor (BDNF) is relevant in the pathophysiology of depression and that it may be more useful as a biomarker for diagnostic and prognostic purposes than the other potential biomarkers[3]. BDNF is expressed in the nervous system and in non-neuronal tissues. The relationship between both is inconsistent, and will be analyzed in this work. Another factor that it has not been studied extensively is the storage and release of BDNF from platelets. Furthermore, depressed patients exhibit enhanced platelet reactivity and increased expression of activation markers, which may influence the characterization of BDNF in depression. The following review will focus on the relationship between platelet activation and BDNF, particularly in depression. This review will examine current literature regarding this topic until August 2015.

BDNF

BDNF, a member of the nerve growth factor family, is expressed in the central and peripheral nervous system and is widely distributed across subregions of the hippocampus and the frontal cortex, brain regions that are of crucial importance in the regulation of emotion, learning and memory[4-8]. BDNF-containing secretory vesicles are present in both the axon terminals and dendrites of neurons[9]. BDNF is also synthesized and released by astrocytes[10]. BDNF plays an important role in central nervous system development, neurogenesis, neuronal survival, migration, cell differentiation, growth of axons and dendrites and synapse formation[11-14], as well as in the synaptic process of hippocampal long-term potentiation (LTP) that play an important role in

memory[4,15]. It also provides protection against learning and memory impairments under conditions of chronic stress[16].

The BDNF gene encodes a precursor protein (prepro-BDNF) that is cleaved into the smaller 35-kDa precursor, proBDNF, in the endoplasmic reticulum. ProBDNF need to be folded correctly, sorted and transported to the appropriate subcellular compartment. ProBDNF moves *via* the Golgi apparatus into the trans-Golgi network, where two kinds of secretory vesicles are generated: Those of the constitutive secretory pathway and those of the regulated pathway whose secretion is activity-dependent. ProBDNF can then be further cleaved into mature BDNF (mBDNF)[9]. In neurons, both proBDNF and mBDNF are preferentially sorted and packaged into vesicles in the activity-dependent secretory pathway. Activity-dependent secretion is believed to be an important feature because it may reflect the nature of the nervous system to respond to and form synaptic modulations based on experiences, and may be a cellular manifestation of memory and learning[17,18]. BDNF can also act *via* autocrine and paracrine mechanisms, depending on the site of the cell surface receptors through which it signals[19].

ProBDNF is either proteolytically cleaved by intracellular enzymes such as furin or pro-convertases and secreted as the 14 kDa mBDNF, or secreted as proBDNF and then cleaved by extracellular proteases[9]. The extent of the intracellular and extracellular processing of proBDNF is not exactly clear, but proBDNF is less efficiently processed by intracellular proteases compared to other neurotrophins, and the secretion of proBDNF seems to prevail over mBDNF[20-22]. It becomes important to identify the specific extracellular proteases that cleave proneurotrophins and understand their regulation. Several matrix metalloproteinases (MMP), including MMP3 and MMP7, have been shown to cleave pro nerve growth factor and proBDNF[23]. However, the most significant protease that cleaves proneurotrophins is the serine protease plasmin[23,24], which is generally expressed as an inactive plasminogen that must be activated by proteolytic cleavage by tissue plasminogen activator (tPA). In the brain, plasminogen is exclusively expressed in neurons and is present in the extracellular space, particularly at the synaptic cleft. tPA is secreted from axon terminals into the extracellular space, and this secretion depends on high-frequency neuronal activity[25]. Therefore, it is conceivable that tPA is the key trigger for the tPA-plasmin-proneurotrophin cascade. The regulation of MMP and plasmin expression or activation could regulate neurotrophin signaling in a spatially and temporally controlled manner. Other work has suggested that proBDNF (35 kDa) and tPA are secreted in an activity-dependent manner, and the extracellular conversion of proBDNF to mBDNF by the tPA/plasmin protease system is critical for late-phase LTP[24-26].

ProBDNF is not an inactive precursor and has been shown to have effects in the central nervous system

that are independent of mature BDNF, as it acts at a separate receptor. Once released, proBDNF preferentially binds to the pan neurotrophin receptor p75 (p75NTR), and mBDNF preferentially binds to both pre- and post-synaptic tropomyosin-related kinase receptors (TrkB), activating different intracellular secondary messenger cascades and affecting distinct cellular responses[27]. The binding of BDNF with TrkB results in intracellular phosphorylation and the activation of intracellular signaling cascades that trigger the so-called pro-survival pathways, inactivate pro-apoptotic signaling and promote neurogenesis[8,28]. ProBDNF binds to p75NTR, which leads to apoptosis and initiates long-term depression of synaptic transmission[29], causing a reduction in the complexity and density of dendritic spines in hippocampal neurons. Proteolytic cleavage of proBDNF represents an important mechanism by which the opposing cellular actions of proBDNF and mBDNF may be regulated[25].

PERIPHERAL BDNF

Platelets are the major source of peripheral BDNF[30,31], and they are important for storing the BDNF that is secreted from other tissues[32,33]. The BDNF and TrkB mRNAs are expressed in several non-neuronal tissues, including muscle, thymus, heart, liver, vascular smooth muscle cells, lung and spleen[34-38]. BDNF is also produced in monocytes, lymphocytes[39,40] and eosinophils. The latter cells produce BDNF *via* the autocrine system and utilize it to evoke and extend the allergic reaction[41,42]. BDNF has been shown to play a pivotal role in the growth, survival and chemoresistance of tumor cells in various types of cancers, including Hodgkin lymphoma, myeloma, hepatocellular carcinoma and neuroblastoma[43-47]. BDNF also mediates the survival and activation of endothelial cells through its interaction with TrkB[48-50], suggesting its potential role in angiogenesis. Many non-neuronal cells, such as smooth muscle cells, fibroblasts and astrocytes, may not express the molecular components of the regulated secretory pathway and therefore only secrete neurotrophins constitutively.

PLATELETS

Platelets are small unnucleated blood cells with a size of approximately 3 μm that originate from mega-karyocytes (MK) in the bone marrow, from which they are released into the blood system. They circulate for an average of seven to 10 d. Platelets contain many structures that are critical to stop bleeding. They contain proteins on their surface that allow them to adhere to breaks in the blood vessel wall and each other. They possess several important organelles: Three types of platelet secretory granules (α-granules - the most abundant, dense granules, and lysosomes), an open canalicular system and proteins similar to muscle proteins that allow them to change shape when they

become sticky[51,52]. The platelets' organelle content is primarily taken up from the plasma; however, platelets are also able to synthesize molecules, such as platelet-derived growth factor (PDGF), platelet factor 4 (PF4), β-thromboglobulin (β-TG) and thrombospondin[53]. Figure 1A shows a resting platelet.

α-granules are essential for normal platelet activity. In platelets, the α-granules fuse with the plasma membrane upon activation, releasing their cargo and increasing the platelet surface area. N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) and SNARE accessory proteins control α-granule secretion, and hundreds of bioactive proteins are released from α-granules. The presence of distinct subpopulations of α-granule suggests that platelets may regulate the differential release of distinct classes of α-granules. This breadth of proteins implies a versatile functionality, and the α-granules participate in thrombosis and hemostasis, inflammation, atherosclerosis, antimicrobial host defense, wound healing, angiogenesis, and malignancy. Many aspects of the formation, structure, content, protein sorting, transport and release of α-granules are poorly understood[54]. Figure 1B and C show stimulated platelets.

Platelets are an essential component of the hemostatic process. Platelets must have additional roles in several physiological and pathophysiological regulatory processes[55]. They express many immunomodulatory molecules and cytokines, and they have the ability to modulate the immune system through interactions with various cells[56]. The binding of biochemical agonists (thrombin, collagen, ADP, epinephrine, arachidonic acid) or mechanical disruption of blood vessels initiates localized hemostatic responses that involve interactions of the vascular endothelium, platelets, red blood cells, coagulation and fibrinolysis. Platelet thrombus formation involves three main steps: Platelet adhesion to the damaged endothelium, activation, and aggregation. Platelet activation involves the generation of intracellular chemical signals that are initiated by platelets through specific surface receptors (platelets express certain surface markers, such as the active form of the glycoprotein receptor GPⅡb/Ⅲa, P-selectin and CD40 ligand). These signals cause dramatic morphological changes, such as the extension of pseudopodia, platelet-platelet aggregation, and granule secretion, resulting in the generation of a platelet thrombus.

BDNF IN PLATELETS

Ninety percent or more of blood BDNF is stored in platelets[32]. A close relationship has been found between BDNF and platelets under physiological conditions, where platelets are an important source of BDNF storage. In addition, there is an approximately 100- to 200-fold difference between the plasma and serum levels of BDNF because platelets release BDNF during the clotting process[32,57]. It has been elegantly demonstrated that the amount of BDNF in serum is nearly identical to the
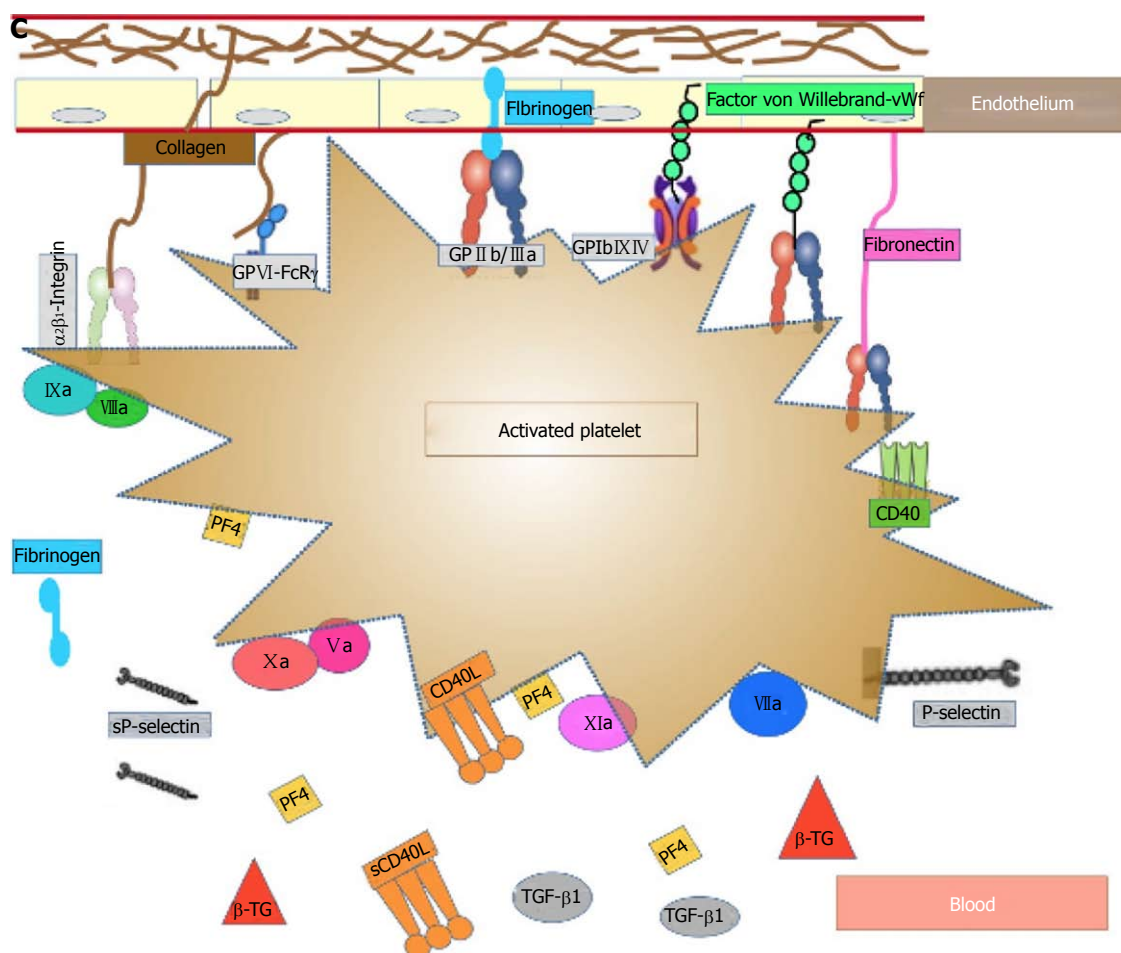
A Resting platelet

B
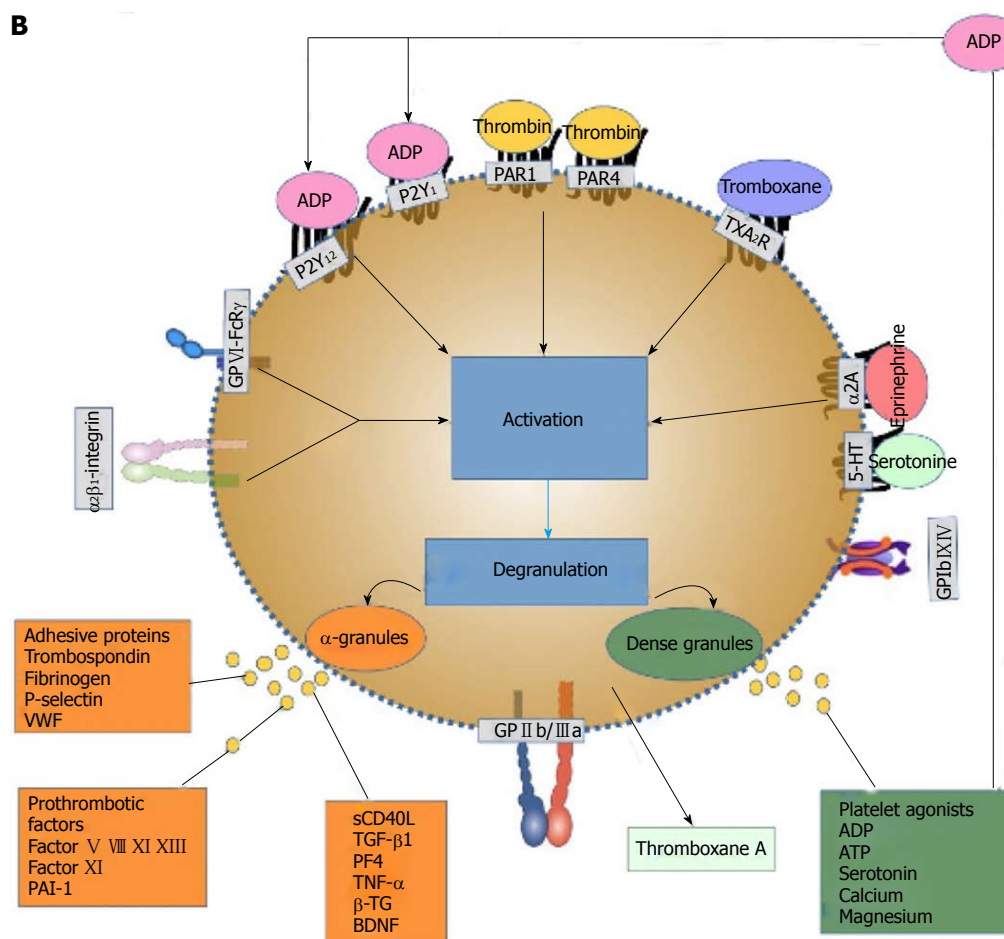
Figure 1 Representation of platelets before and after activation. A: Resting platelet: The discoid form of resting platelets is supported by a circumferential coil of microtubules lying just below the surface membrane whereas α-granules and dense bodies are irregularly dispersed in the cytoplasm. Platelet membrane contains receptors for ADP, thrombin, vWF, fibrin, fibronectin, epinephrine, PAF, thromboxane A2, Prostacyclin, trombospondin, serotonin and gycosil transferase; B: Platelet activation: Platelets may be activated *via* different agonists, through several receptor-mediator pathways (thrombin, TXA2, collagen, epinephrine, *etc*.). Collagen exposed within the denuded area of vascular endothelium stimulates platelet activation and adhesion to the vessel wall. These agonists bind and activate their respective receptors, which in turn stimulate the activation of associated G-proteins, ultimately activating GP IIb/IIIa receptors and promoting the interaction of adjacent platelets within the clot. When the platelet is stimulated a transient influx of calcium occurs, followed by extrusion of platelet storage granule contents. The dense bodies contain serotonin (5-HT), ADP, ATP and calcium, whereas the α-granules contain the highest amount of proteins, including vWF, fibronectin, fibrinogen, P-selectin, PF-4, β-TG, RANTES, CD40, CD40L, PDGF, amyloid precursor protein, MMP, adhesion molecules (ICAM, VCAM and PECAM), various coagulation and growth factors (such as BDNF) and inflammatory markers. All these phenomena are calcium (Ca^{2+})-dependent, amplify platelet activation and induce irreversible platelet-platelet aggregation and thrombus formation; C: Platelet activation-aggregation: Platelet aggregation following platelet activation is marked by a shape change that increases their surface area available for adhesion and gives platelets the ability to bind fibrinogen and vWF *via* the active form of the surface glycoprotein GP IIb/IIIa receptors, leading to platelet aggregation and thrombus formation. Platelets express certain surface markers such as P-selectin and CD40 ligand and these expressed activation markers are cleaved, promoting the circulation of soluble CD40L and soluble P-selectin. Platelets also expose phosphatidylserine providing attachment sites for coagulation factors. Vascular injury also exposes subendothelial tissue factor, which forms a complex with factor VIIa and sets off a chain of events that culminates in the formation of the prothrombinase complex. Prothrombin is converted to thrombin, which subsequently converts fibrinogen to fibrin, generating a fibrin-rich clot. The coagulation cascade contributes to the stabilization of the thrombus. BDNF: Brain-derived neurotrophic factor; ADP: Adenosine-diphosphate; GP: Glycoprotein; ATP: Adenosine-triphosphate; PF-4: Platelet factor-4; β-TG: β-thromboglobulin; PDGF: Platelet-derived growth factor; vWF: Von Willebrand factor; MMP: Matrix metalloproteinases.

amount of BDNF found in washed platelet lysates[32]. Thus, the difference between the serum and plasma BDNF levels seems to reflect the amount of BDNF stored in circulating platelets, and the BDNF in platelets might serve as a reservoir for circulating BDNF. The BDNF in platelets may play a role during tissue trauma or nerve injury, releasing their contents into the circulation at the site of the injury[58].

In the first studies, BDNF was not expressed in or produced from the megakaryocyte precursor cell of the mature platelets, in which protein synthesis is generally absent, but was sequestered from the circulation[32,34]. Recently, one study found that BDNF is present in the cytoplasm of platelets and in α-granules, suggesting that BDNF is either produced in platelets or passed down from MK[59]. A second study found that a megakaryocyte progenitor line (MEG-01) produces BDNF upon thrombopoietin stimulation, also expressed in the bone marrow from the liver and kidney[60], and the levels of BDNF in MEG-01 cells increased in a time-dependent mannerly[61]. It was the first report of the production of BDNF in a megakaryocyte cell line and led to the

hypothesis that BDNF potentiates the cell proliferation of the megakaryocyte lineage *in vivo*. It is likely that there is a receptor for BDNF on the MEG-01 cell surface, but the TrkB receptor was not detected in MEG-01 cells or human platelets[32,59,62]. Therefore, there should be an unidentified novel receptor in MKs or platelets.

Some agonists, such as thrombin, collagen, Ca^{2+} and shear stress, could induce a rapid release of BDNF from platelets. Even with agonist stimulation, only approximately half of the BDNF in platelets is secreted, which suggests that platelets maintain a stable pool of BDNF as a buffer system[30]. However, another study found that platelet-mediated BDNF release in depressed patients was independent of platelet reactivity[31]. There was little knowledge about the relationship between BDNF and platelets and only a few studies have assessed issues such as platelet activation mechanism that allows the release of BDNF and BDNF localization within platelets.

The rate of BDNF release paralleled the secretion of 5-HT from the dense granules and PF4 from the α-granules, although a greater proportion (90%) of the total 5-HT and PF4 were released compared to BDNF. Because only 40%-60% of the total content of platelet BDNF was released by maximal platelet activation, some authors postulated that platelets either have a non-releasable pool of BDNF, or that the released BDNF is sequestered by binding to a transporter or receptor on the platelet surface[32]. Such binding could promote the internalization of BDNF by the platelets, as has been reported for 5-HT[63,64] and for astrocyte recycling of BDNF[65,66]. The binding of BDNF to washed platelets was confirmed by microscopy and FACS analysis, as well as confocal microscopy, suggesting that platelets bind exogenous BDNF[32]. However, a recent study found two different locations where it is stored BDNF: In the α-granules and in the cytoplasm. Using immunoelectron microscopy, BDNF was clearly detected in the same fractions as P-selectin, an α-granule marker, and protein kinase C, a cytoplasmic marker[59].

BDNF is predominantly released from the platelets through protease-activated receptor 1 (PAR1) activation during thrombin stimulation, along with vascular endothelial growth factor but not endostatin stimulation. Platelets stimulated with concentrations of the PAR1-activator peptide showed a dose-response curve of BDNF release, exhibiting a two-phase pattern. The first phase is a drastic release phase at low level activation which is completely inhibited by Prostaglandin (PGE1) pretreatment suggesting that this phase depends on calcium mobilization. The second phase is a mild release phase at high level activation which is not affected to PGE1 pretreatment, suggesting another signal that is not affected by PGE1[59]. BDNF response curve was similar to that of PF4[59]. There was no significant difference in BDNF release between the non-stimulated and PAR4-AP-stimulated cells. Interestingly, PAR1 activation promotes the release of proangiogenic factors and these results support the action of BDNF as a proangiogenic factor[47-50,67,68].

Another important finding of this study is that the BDNF in the α-granules is released upon platelet activation, whereas the cytoplasmic BDNF is not[59]. The maximum BDNF release is approximately 30%-40% with stimulation and the remaining 70% of BDNF is equivalent to that found in the cytoplasm, which is not released, similar to study of Fujimura[32].

MEASUREMENT OF BDNF IN PERIPHERAL SAMPLES

BDNF levels have been linked with various diseases, such as depression[69,70], schizophrenia[71,72], Alzheimer's disease[73] and anorexia[74]. Some available non-invasive options for evaluating BDNF include measuring the BDNF concentrations in the whole blood, serum, plasma and platelets. The results of BDNF in one sample cannot be directly generalized to other peripheral BDNF parameters because there are only modest statistically significant associations among these peripheral measurements. Studies found that the correlations between the plasma and serum BDNF concentration are between $r = 0.2$ to $r = 0.70$[75-77]. The distinct biological significance of the serum and plasma BDNF levels has already been noted[78,79].

Human serum contains BDNF at far higher concentrations than human plasma. Because of the storage of BDNF in platelets, the concentrations of BDNF in serum and plasma differ by a factor of 200[58]. There are some confounding factors regarding the serum BDNF levels. Serum BDNF showed strong associations with race, platelet count, and depression after adjusting for other factors. Females, blacks, smokers, and those with high platelet counts had higher serum BDNF levels[80]. The platelet count is considered the factor with the strongest association with the serum BDNF concentrations[81,82] and this strong association is consistent with theory that the BDNF in serum is derived directly from the platelets[80]. The serum BDNF concentrations largely reflect the activation-dependent release of BDNF from platelets[32,57]. Interindividual differences in the serum BDNF concentrations are mediated by changes in the activity of the blood platelets caused by medications or pathological conditions[83].

The BDNF protein circulates in plasma for less than an hour[84,85]. The measures of BDNF in plasma samples are quite unstable, and previous studies reported a large degree of dispersion, which could reflect the different cellular sources of plasma BDNF (endothelia, central nervous system, *etc.*) and the influence of different factors on the expression and secretion of BDNF from different sources[57]. Lower levels in males was observed and age, body weight and cholesterol-LDL showed a negative correlation with the plasma BDNF levels[57]. This study showed that the BDNF plasma levels significantly decreased with increased age or weight, whereas the platelet counts did not. However, one study

found that the platelet count is the most important predictor of plasma BDNF concentrations in children and adolescents, and the plasma BDNF concentrations in children may need to be interpreted with age-specific and platelet count-specific standards[86]. Another study found the plasma BDNF levels were positively associated with the platelet count and negatively associated with the fibrinogen level in patients with angina pectoris. Similar to BDNF, fibrinogen is a major storage protein of the platelet α-granules and is delivered to the α-granules by endocytosis. These associations may be associated with the level of BDNF release from platelets in inflammatory states, and the low plasma BDNF concentration in patients with angina pectoris may be primarily due to platelet release[87].

Another consideration is that the plasma BDNF levels have a very low retest stability when measured twice within one year, and there are substantial changes, even throughout one day. Recently, one study raised methodological concerns regarding the assessment of the BDNF levels in plasma and recommends measuring the BDNF levels in serum[88]. However, the plasma BDNF levels could represent the currently biologically active BDNF as a state-dependent marker, and it appeared to be influenced by inflammation mediators[89].

Platelets circulate for up to 11 d in the peripheral blood[84,85]. The platelet BDNF levels could represent a long-term marker of the varying plasma BDNF levels over a period of several days. The BDNF stored in platelets is likely obtained from both the circulating plasma pool, from cells in the brain or other organs and from megakaryocites. Variation in BDNF production in specific organs[57] may produce changes in platelet BDNF content and it measure could be a circulating indicator of altered production. Differences in platelet function and release of or sequestering BDNF from the blood may result in the differences between the serum and plasma BDNF concentrations[70]. The platelet and serum levels have been shown to strongly correlate, and it has been proposed that the BDNF released from platelets directly correlates to the serum BDNF levels; however, in recent studies, only 30% of the BDNF in platelets is secreted into the blood[59]. The platelet BDNF levels did not correlate with age, weight, cholesterol or long-term storage but changed with menstrual cycle[57,90].

Another limitation of measuring BDNF in the periphery is that the ELISA kits used in most studies quantified the total BDNF concentrations in serum and did not make the distinction between the pro- and the mBDNF variant, but the two BDNF variants are functionally different. Moreover, this raises the question of which parameter best serves as a mirror for the neurotrophic action in the brain. Some authors have suggested that the leukocyte BDNF mRNA content could more closely reflect central BDNF dynamics because of its short half-life[91] and therefore may be less subject to the peripheral confounding factors. In addition, it has been argued that a combination of peripheral BDNF indices may have advantages over a single index. Assessing both the platelet and serum BDNF concentrations could be particularly relevant[3].

CORRELATIONS BETWEEN THE CEREBRAL AND PERIPHERAL BDNF LEVELS

Because the central BDNF levels are difficult to obtain for methodological and ethical reasons, there is a great interest in peripheral BDNF measures in relation to psychiatric illness. There are indications that the BDNF measured in peripheral tissues reflects BDNF activity in the brain. These indications include preclinical findings that BDNF crosses the blood-brain barrier[92] and positive correlations between the peripheral and central BDNF concentrations[57,93,94]. Referring to the blood-brain barrier, some rodent studies have shown that peripheral BDNF administration promotes the regeneration of spinal cord injury[95], has an effect on depressive-like behaviour[96] and could increase BDNF levels in the brain[97]. Other studies did not find these results but found that BDNF protein may have poor or null blood-brain barrier penetrability[98-100]. Systemically administered BDNF in rodents showed that BDNF signaling pathways were activated only in disrupted regions[101]. However, studies also point towards a role for vascular endothelial impairment in MDD[102]. A meta-analysis found an increased risk of MDD in those with major vascular diseases including diabetes, cardiovascular disease and stroke[103].

Evidences regarding the positive correlation between the peripheral and central BDNF concentrations[57,93,94] are as follows. Human post-mortem studies had indicated similar alterations in BDNF concentrations in the brain and periphery of persons who were depressed at the time of death[104]. In one human study, the BDNF levels were higher in blood that was derived from the internal jugular veins compared to the arterial blood[105], suggesting that the source of BDNF in the peripheral tissues can be found in the brain. These studies indicated that neurotrophic function can be estimated from the periphery in a rather non-invasive manner by taking advantage of this "window to the brain". However, the specificity, extent and relationship between the peripheral BDNF levels to disease activity are not fully known. Due to the large variation in the amplitude between the serum and plasma BDNF levels, it is unlikely that it will translate as a useful biomarker of disease activity in clinical practice[106]. Future studies should determine the ratios of the BDNF concentrations in the cerebral spinal fluid (CSF), serum and plasma in acutely ill and remitted patients and controls.

Other studies report null findings with regard to an association between the peripheral BDNF concentrations and the more central parameters for BDNF activity, namely an absence of correlations between the plasma and CSF concentrations of BDNF[107]. In an older

adult study, blood-based measures of BDNF are not representative of the CSF BDNF levels[80]. A study comparing the CSF and serum BDNF measurements in a sample of patients with Alzheimer's disease found that they were not correlated[73]. Moreover, the concentrations of BDNF are much higher in the serum (> 1000 ×) or plasma (approximately 10 ×) than in cerebrospinal fluid, which may reflect peripheral synthesis[106-108]. One explanation is that tissues other than the brain, including the immune system, liver, smooth muscle and vascular endothelial cells serve as sources of BDNF[34,35]. Neurons and glia cells of the central nervous system might originate a substantial portion of the circulating BDNF, but our current knowledge does not allow us to distinguish whether the peripheral sources produce or release less BDNF or if decreased synthesis or release in the brain are responsible for the lower plasma and serum levels[92,106]. Another explanation is that these changes in the BDNF levels may represent a counter-regulatory response to other etiological factors of the illness, such as metabolic and redox factors[109]. Another reason to criticize this relationship between the brain and peripheral BDNF levels could be that the expression of BDNF is specific to a particular local and time[110], and animal studies have shown that the levels of BDNF are increased in some brain regions and decreased in others in some diseases[111].

RELATIONSHIP BETWEEN BDNF AND PLATELET ACTIVATION

The relationship between platelet activation and the peripheral BDNF level is poorly documented in humans. No evidence shows the interaction between BDNF and platelets under pathological conditions, such as tumor growth and metastasis[32]. Therefore, we will review the studies examining the relationship between BDNF and different markers of platelet activation.

Transforming grown factor β1

Transforming grown factor β1 (TGF-β1) is abundant in platelets and is stored in the α-granules[112]. It plays an important role in regulating the immune response, cell proliferation and tissue fibrosis, and it recruits inflammatory cells to the wound area. Lommatzch *et al*[57] found a stronger correlation between the serum BDNF levels and the serum TGF-β1 levels than with the serum 5-HT levels and the first two could be anatomically and functionally related in the platelet both located in α-granule. The postoperative abdominal surgery changes in the serum BDNF levels virtually paralleled the changes in platelet number and the platelet mediator TGF-β1. The platelet numbers and TGF-β1 concentrations decreased in the immediate acute response and increased later. A positive relationship was observed between the serum BDNF and TGF-β1 values at all times after surgery[89].

Fibrinogen

Similarly to BDNF, fibrinogen is a major storage protein of platelet α-granules and is delivered to the α-granules by endocytosis[113]. Recent studies reported an association between elevated plasma fibrinogen levels and psychological distress and depression in individuals from the general population after adjusting for confounders[114], but the effect size of plasma fibrinogen could be small[115]. Another study found higher fibrinogen levels in non-responders than in responders in major depression patients, suggesting that baseline plasma fibrinogen levels can serve as a biomarker to gauge the success of antidepressant treatment response[116]. Hattori *et al*[115] found that a subpopulation of patients with MDD had high CSF fibrinogen levels compared with controls and those patients with a high fibrinogen level had white matter tract abnormalities. The increased CSF fibrinogen in patients could represent a trace of blood-brain barrier disruption induced by neuroinflammation, which is in accordance with the mild inflammation hypothesis in the aetiology of MDD[117,118]. The plasma BDNF levels were negatively associated with the fibrinogen levels in patients with angina pectoris[87]. The exact reason for the association between the decreased plasma BDNF levels and these factors is unclear, but it may be related to the level of BDNF release from platelets in inflammatory states.

P-selectin

P-selectin (CD 62-p) is primarily located in the α-granule membrane of resting platelets and is only found on the platelet surface after platelet activation. P-selectin is perhaps the most cited of the biologically active molecule that appears on the platelet surface after activation and secretion. P-selectin on platelets or endothelial cells has a key role in inflammation. The soluble P-selectin (sP-selectin) levels in blood represent a measure of platelet and/or endothelial cell activation. Elevated sP-selectin levels were associated with enhanced generation of tissue factor-expressing microparticles, leading to shorter plasma clotting time and a pro-coagulant phenotype, which facilitated fibrin generation and also altered the blood-brain barrier permeability and exacerbated stroke[112]. One study in cardiac patients showed that the median serum BDNF levels were higher in the myocardial infarction (MI) group than in the stable angina pectoris (SAP) group. In the MI patients, there was a significant correlation between the BDNF and sP-selectin levels. In contrast, no such correlation was observed in the SAP patients. The study suggested that the BDNF serum levels in MI patients could be related to platelet activation and the inflammatory response[119].

Soluble CD-40-ligand

CD40L appears to be localized to the granule membranes. Upon platelet activation, it is translocated to the platelet surface, where it is cleaved and acts by associating with the αIIbβ3 integrin. This soluble form

[soluble CD-40-ligand (sCD40L)] is predominantly derived from activated platelets and thus represents a circulating marker of platelet activation. Platelets are the main source of sCD40L and are responsible for > 95% of the circulating sCD40L levels. A study by Lorgis et al[119] in patients with coronary artery disease did not find a significant correlation between the serum BDNF levels and sCD40L in either the MI or SAP patients. The controversial results between the sCD40L and sP-selectin levels could be explained by methodological issues regarding the measurement of both markers[120,121].

PF4
PF4 is a platelet-specific protein that is stored in the α-granules and secreted into the plasma upon platelet activation[64]. PF4 is considered an index of platelet reactivity. Patients with depression showed increased PF4 plasma levels with respect to the controls, but there were no differences in the serum PF4 levels. The total PF4 levels obtained by completely clotting the platelets are the same in both groups. Alterations in the serum and plasma BDNF levels in depression are not related to the changes in either the whole blood BDNF levels or in the platelet release of the activation marker, PF4[31]. The plasma PF4 levels were elevated, indicating increased platelet reactivity without a change in the total PF4 levels in the serum. These results suggested that there are independent regulatory mechanisms for platelet BDNF and PF4 release. However, one question is whether it is possible for two proteins that are supposed to be stored in the same granules to be independently released or if there are different subcellular localizations for these proteins. Another study found that the rate of BDNF release paralleled the secretion of 5-HT from the dense granules and of PF4 from the α-granules, although a greater proportion (90%) of the total 5-HT and PF4 were released compared to BDNF[32].

β-TG
The plasma β-TG levels were significantly decreased in patients with Alzheimer's disease compared to the healthy controls. In Alzheimer's disease patients, the serum BDNF concentrations were significantly correlated to the β-TG and plasma BDNF values. In contrast, the plasma BDNF and β-TG values were not significantly correlated. The levels of BDNF and β-TG in the blood of patients with Alzheimer's disease are decreased compared to the controls. These results confirm an association between the serum BDNF concentration and the degree of platelet activation, as measured by the plasma β-TG levels[122].

EFFECTS OF ANTIDEPRESSANTS OR ANTI-PLATELET DRUGS ON BDNF RELEASE FROM PLATELETS
The release of BDNF from platelets is a polemical issue. It seems likely that methodological differences may

markedly affect the results, including the methodology for isolating the platelets (pH, use of diverse inhibitors, etc.); anticoagulation (using EDTA/citrate tubes, heparin a, etc.); buffers with or without calcium; acute/chronic treatment; receptor profile or mechanism of action of the drug; drug doses; animal or human studies; and direct study of platelet BDNF levels or calculating the difference between the serum and plasma BDNF levels. For example, an elevation in the calcium concentrations plays an important role in platelet activation and secretion[123]. High concentrations of calcium and thrombin lead to an immediate release of diverse growth factors, including PDGF and TGF-β1[124,125]. Under calcium-free conditions, only a low amount of 5-HT and BDNF (approximately 10%-16% of the total content) was released after 10 and 60 min; however, almost all of the nerve growth factor was released[126,127].

Animal studies and single doses of an antidepressant
One study investigated the direct influence of antidepressants on BDNF release from platelets and their effects on the serum levels. Platelet BDNF release was studied using samples of washed platelets prepared from rat blood that they had incubated with sertraline, paroxetine, fluvoxamine and milnacipran, and the BDNF levels were determined at different time points. The changes in the serum BDNF concentrations were studied after single intravenous injection of antidepressants in rats at 1, 2 and 5 h following the injection. The BDNF from platelets was released by these antidepressants, and there were no differences between the effects of serotonin-norepinephrine reuptake inhibitors and selective serotonin re-uptake inhibitors (SSRIs). Antidepressants promoted BDNF release from platelets within 1 h, and the changes in BDNF release depended on the amount of the antidepressant and they were specific for each antidepressant. Sertraline was the most effective antidepressant in promoting platelet BDNF release[128], but other study with human controls did not found it[129]. The BDNF released represented approximately 20% of the total BDNF in platelets, similar to other agonists[32,59]. The serum BDNF concentration increased 1 h after sertraline injection; this change exhibited a significant difference at 5 h and was dose-dependent from 0.03 μmol/L to 0.3 μmol/L of sertraline in rat platelets. BDNF release from platelets is affected by antidepressants, which means that the administration of antidepressants might affect the changes in the BDNF in the peripheral tissues, such as platelets, and the serum BDNF concentrations. The authors suggested that the decreased serum BDNF concentrations in depressed patients may reflect reduced platelet BDNF levels[128].

Another study showed that treatment of rat platelets with sertraline, citalopram, paroxetine and indomethacin did not influence the release of BDNF after 10 and 60 min independently calcium conditions. BDNF release was significantly reduced by ibuprofen, an anti-inflammatory drug, after 10 and 60 min[127] but only when calcium was present, similarly with the work of

Fujimura *et al*[32].

Glutamatergic modulator riluzole have been proposed as a strategy for the treatment of mood disorders. Riluzole could stimulate BDNF release, acting directly on these cells. When platelets of healthy controls were incubated with riluzole at low concentrations for 4 h, but not for 24 h, riluzole stimulated the release of BDNF. This acute effect, but not later, suggests that its effect derived from evoking neurotrophin release. Moreover, mean platelet volume and platelet distribution width did not change during study, so platelets used in this study were not activated at the time of exposure to riluzole[129].

Chronic antidepressant use in humans

One study evaluated the changes in the platelet BDNF levels in patients with major depression when they were treated with s-citalopram. The platelet BDNF levels of the untreated patients appeared significantly lower than those of the healthy subjects, and antidepressant treatment with an SSRI normalized the platelet BDNF levels. The platelet BDNF levels were normalized earlier (at eight weeks of treatment) than the plasma BDNF levels[70]. Another study evaluated the platelet BDNF levels in patients with MDD and childhood trauma. They were treated with antidepressant medications for three months, including escitalopram, mirtazapine, and duloxetine, without intensive psychotherapy. The platelet and serum BDNF levels showed a significant increase from baseline at the 3-mo follow-up in the patient group. Conversely, the plasma BDNF levels were not significantly different between the two time points in the patient group. There were no significant differences in BDNF levels between the different antidepressant treatment groups[76] in either of the peripheral samples analysed.

Anti-platelet regimens in humans

A study by Stoll *et al*[130] investigated the impact of common anti-platelet drugs on the BDNF concentrations in serum and plasma and on the release of BDNF from platelets in a group of healthy volunteers. They showed that a single oral dose of clopidogrel but not aspirin significantly reduced the release of BDNF from platelets in healthy volunteers. The platelet α-granule marker TGF-β1 was also significantly reduced in the serum after clopidogrel treatment but not after aspirin administration. In addition, the decrease in the serum TGF-β1 concentrations correlated with the decrease in the serum BDNF concentrations at 24 h after clopidogrel administration. Aspirin and clopidogrel had no significant effects on the plasma BDNF levels[130]. Another study found a reduction in the release of BDNF from platelets when treated with aspirin (a non-specific cyclooxygenase-inhibitor)[131]. These drugs act through different mechanisms; aspirin acts by inhibiting cyclooxygenases, and clopidogrel irreversibly binds to the membrane adenosine diphosphate receptor and

impacts platelet α-granule degranulation[130]. The study by Stoll *et al*[130] is consistent with the decrease of the platelet α-granule marker TGF-β1 after clopidogrel treatment and the correlation between the effects of clopidogrel on the BDNF and TGF-β1 concentrations but not those after aspirin administration.

SIGNIFICANCE OF PLATELET BDNF RELEASE IN DEPRESSION

Many attempts have been made to generate reliable blood-derived candidate biomarkers based on the current models of disease pathogenesis. 5-HT and BDNF are known to modulate behavioral responses to stress and to mediate the therapeutic efficacy of antidepressant agents, and they interact at different levels[132]. BDNF has been implicated in the pathophysiology of depression[133], and it has been studied as biomarker of this disease. A large number of clinical studies have reported that the BDNF levels in serum[83,134-136] are significantly decreased in depressed patients and that this decrease is normalized by antidepressant treatments[135,137-140], which was confirmed by meta-analysis[141,142]. In some of these studies, the BDNF levels correlated with higher scores on scales for assessing depression[141], although there are studies that have found no such correlation[108,143]. A recent published meta-analysis[3] concluded that there are low concentrations of BDNF in the serum of patients with untreated depression, but the size of the effect becomes substantially smaller than in previous studies. However, no consistent associations were found between the serum concentrations of BDNF and the severity of the depressive symptoms. An aspect to consider is that the serum BDNF levels are dependent on the release of BDNF from platelets[32,57], which has not been evaluated in most studies. Another aspect to consider is that other diseases, such as schizophrenia, bipolar disorder, and anorexia, among others, have shown decreases in the serum BDNF levels; thus, this finding is not specific enough for a diagnostic marker.

With respect to the plasma levels, some studies showed lower plasma BDNF levels in depressive patients[78,144,145] or did not observe changes[146,147]. Our group reported that the plasma BDNF levels were significantly higher in the depressed patients compared to the healthy controls and that they were similar in both groups when the symptoms remitted[70]. One possible explanation could be that BDNF is released from platelets and increases the plasma BDNF levels. However, the BDNF in plasma has a short half-life, and platelets contain greater concentrations of BDNF than the plasma. The plasma BDNF levels also appeared to be influenced by inflammation mediators[89].

There are few studies that have evaluated the platelet BDNF levels in depression. Two studies that directly evaluated the platelet levels showed reductions in the platelet BDNF levels in depression, which may be associated with lower serum BDNF levels in patients

with major depression, but these two studies did not evaluate the effects of treatment[148,149]. Another study showed that the platelet BDNF levels were significantly decreased with respect to the controls, but treatment with SSRIs normalized the levels to the levels of the controls[70]. These data are consistent with a preactivation state of platelets in major depression. Treatment with an SSRI would improve this preactivated state and explain the increase in the BDNF levels inside the platelets[70]. The remaining studies evaluated the platelet BDNF levels indirectly by the difference between the serum and plasma levels[76].

A disruption in serotonergic signaling in the brain is believed to be involved in the pathophysiology of depression. It is well known that 99% of the 5-HT found in the human body is stored in platelets and that 5-HT can induce downstream platelet aggregation and coronary vasoconstriction[150]. In contrast with arachidonic acid, 5-HT is a weak platelet agonist that requires co-stimulation with other agonists to induce full platelet activation[151]. Increasing evidence indicates that there is an association between depression and platelet function[152-154]. A relationship between depressive symptoms and increased platelet activity has been established in physically healthy depressed patients[155] and, in post-MI depressed patients[156]. Elevated platelet reactivity has been found in depressed patients, as indicated by the increased plasma levels of either PF4 or β-TG or the increased expression of procoagulant platelet surface receptors[157-159]. Our group demonstrated the existence of a prothrombotic endophenotype in the platelets of depressed patients before treatment that was characterized by a statistically significant increase in the average volume of platelets and high expression of glycoprotein GPIb and antigen markers. Compared to the controls, the patients' platelets showed a significantly enhanced aggregation response to arachidonic acid. The clot firmness and procoagulant activity of platelet-associated tissue factor were also significantly elevated, which can contribute to the increased prothrombotic profile of platelets and precipitation events in ischemic vascular lesion sites[160]. Studies with circulating blood revealed increased fibrin formation and thrombin generation in depression compared to the blood of healthy donors, when exposed to a thrombogenic surface in flow conditions[161]. Other observed alterations in platelet parameters in patients with major depression included a reduction of 5-HT transporter [3H]-imipramine binding sites in platelets[162] and increased in 5-HT₂ receptor binding sites on the platelet surface compared to the controls[163]. Platelet monoamine oxidase activity has been shown to be elevated in depressed patients[164]. Heightened membrane expression of glycoprotein Ⅱb/Ⅲa and the P-selectin receptors has also been reported in depressed patients without heart disease[156]. These alterations have been proposed as a possible mechanism that contributes to the elevated cardiac risk associated with the diagnosis of major depression[165]. Several

mechanisms could explain the platelet abnormalities observed in major depression[159]. Although many studies have shown exaggerated platelet activation in patients with depression, several have shown no such relationship[152].

During platelet activation, 5-HT stimulation also accelerates the exocytosis of the platelet α-granules, which secrete procoagulant molecules into the plasma. One of these molecules is plasminogen activator inhibitor-1 (PAI-1), which is released at the site of thrombus formation. The levels of PAI-1 in arterial clots are 2-3 times higher than those observed in venous clots, and the relative content of PAI-1 determines the resistance to thrombolysis. PAI-1 inhibits the bioavailability of tPA and plasmin, which are the proteases that cleave proBDNF to mBDNF; therefore, the elevated synthesis of PAI-1 reduces the production of mBDNF. Multiple lines of evidence have shown that split proBDNF is central to the pathophysiology of major depression and the mechanisms of action of antidepressants[166]. The inadequate split of proBDNF may increase the risk of mood disorders[167]. In fact, patients with major depression show increased levels of proBDNF and decreased levels of mBDNF[168]. Moreover, PAI-1 inhibits the production of plasmin, preventing the dissolution of blood clots in atherosclerotic plaques, and one would expect that patients with depressive disorders have a higher risk of cardiovascular events.

In platelets, SSRIs results in a decrease in the 5-HT storage in dense granules, and thus could affect platelet aggregation[169]. 5-HT can definitely potentiate platelet mediated thrombogenesis[151]. Continued treatment with the SSRI may modulate not only the circulating levels of 5-HT but also the presence and activity of the serotonergic mechanisms and inhibit the release of 5-HT during platelet aggregation[170,171]. Clinical data have also shown that antidepressant treatment influenced platelet activation by lowering the plasma PF4 and β-TG levels[158,159], but other studies did not find this association[172]. Citalopram inhibited human platelet aggregation *in vitro* in response to different agonists, with the strongest effect observed in response to ADP and ADP + 5-HT[151]. *Ex vivo* escitalopram treatment of blood resulted in a significant inhibition of ADP- or collagen-induced platelet aggregation[165,173,174]. In aggregation experiments with collagen or arachidonic acid, a significant decrease in the aggregation intensity was noted in the platelets of SSRI-treated patients[175]. The addition of ADP to platelets from patients treated with SSRIs induced an inhibition of ATP release and the secondary wave of platelet aggregation[176]. Furthermore, a significant decrease in the platelet activation markers CD62-P and annexin-V-binding was reported after preincubating platelets with citalopram, but the release of factor V/Va from the α-granules was not noticeably affected[151]. Treatment with SSRIs for 24 wk normalized the majority of the altered parameters in patients with depression, but it accentuated the

expression of GPⅡb/Ⅲa and the viscoelastic properties of the clots formed under low shear rate conditions[161]. SSRI treatment rapidly and effectively counteracted the enhanced fibrin or aggregate formation observed under flow conditions, confirming the previous *in vitro* results in the clinical setting[151]. However, the viscoelastic parameters showed a progressive acceleration of clotting time and enhanced clot strength during the treatment with escitalopram, but these results were obtained in almost static conditions[161]. Patients treated with SSRIs seem to have fibrinogen and PAI-1 levels in plasma that are similar to those of the healthy controls and lower than depressed patients who do not receive serotonergic antidepressants[177]. Overall, these results could explain why the patients treated with SSRIs show a reduction in cardiovascular disease risk when compared with patients not receiving antidepressants[178,179].

CONCLUSION

To the best of our knowledge, only a few studies have assessed both BDNF and platelet activation in depression[31]. BDNF has been implicated in the pathophysiology of depression[133], and it has been studied as a biomarker of this disease. A large number of clinical studies have reported that the BDNF levels in serum[83,134-136] are significantly decreased in depressed patients and that this decrease is normalized by antidepressant treatments[135,137-140], which was confirmed by meta-analysis[141,142]. Serum levels are influenced by platelets and plasma levels results are inconsistent. Ninety percent or more of blood BDNF is stored in platelets, but these studies did not consider the platelet alterations observed in depression. Increasing evidence indicates that there is an association between depression and platelet function[152-154], with an elevated platelet reactivity, a prothrombotic endophenotype and increased of plasma substance levels excreted from α-granules in depressed patients. Some authors propose that the lower peripheral BDNF concentrations in depression and their upregulation over the course of antidepressant treatment may be an epiphenomenon resulting from an altered BDNF metabolism or expression by these peripheral organs[3]. One possible explanation could be that alterations in peripheral BDNF levels in depression depend more on platelet reactivity that excretes more BDNF from α-granules than on alterations of central BDNF. A serious question remains to be answered of whether the relationships found between BDNF and depression may be mediated primarily by the relationship between depression and platelet activation. Further studies are required to evaluate the complexity of the relationship between BDNF and platelet reactivity and its possible influence on the peripheral levels in certain diseases, such as depression. Another group of studies is required to evaluate the implications of anti-platelet and antidepressant drugs in the relationship between BDNF and platelet activity.

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REVIEW

Biomarkers in schizophrenia: A focus on blood based diagnostics and theranostics

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Abstract

Identifying biomarkers that can be used as diagnostics or predictors of treatment response (theranostics) in people with schizophrenia (Sz) will be an important step towards being able to provide personalized treatment. Findings from the studies in brain tissue have not yet been translated into biomarkers that are practical in clinical use because brain biopsies are not acceptable and neuroimaging techniques are expensive and the results are inconclusive. Thus, in recent years, there has been search for blood-based biomarkers for Sz as a valid alternative. Although there are some encouraging preliminary data to support the notion of peripheral biomarkers for Sz, it must be acknowledged that Sz is a complex and heterogeneous disorder which needs to be further dissected into subtype using biological based and clinical markers. The scope of this review is to critically examine published blood-based biomarker of Sz, focusing on possible uses for diagnosis, treatment response, or their relationship with schizophrenia-associated phenotype. We sorted the studies into six categories which include: (1) brain-derived neurotrophic factor; (2) inflammation and immune function; (3) neurochemistry; (4) oxidative stress response and metabolism; (5) epigenetics and microRNA; and (6) transcriptome and proteome studies. This review also summarized the molecules which have been conclusively reported as potential blood-based biomarkers for Sz in different blood cell types. Finally, we further discusses the pitfall of current blood-based studies and suggest that a prediction model-based, Sz specific, blood

oriented study design as well as standardize blood collection conditions would be useful for Sz biomarker development.

Key words: Schizophrenia; Peripheral blood; Biomarker; Diagnostics; Schizophrenia-associated phenotype; Treatment response; Clinical courses

Core tip: In recent years, there has been search for blood-based biomarkers for schizophrenia (Sz) as a valid alternative. However, it must be acknowledged that Sz is a complex and heterogeneous disorder which needs to be further dissected into subtype using biomarkers. The scope of this review is to critically examine published blood-based biomarker of Sz, focusing on possible uses for diagnosis, treatment response, and their relationship with schizophrenia-associated phenotype. We suggest that a prediction model-based, Sz specific, blood oriented study design as well as standard blood collection procedures would be useful for development of Sz biomarkers.

INTRODUCTION

Identifying biomarkers that can be used as diagnostics or predictors of treatment response (theranostics) in people with schizophrenia (Sz) will be an important step towards being able to provide personalized treatment and would support efforts to develop new drug treatments[1-3]. Sz is a psychiatric disorder and there have been great efforts to the study of potential neuronal and glial abnormalities that may provide the basis of the aetiology of the disorder. However, findings from such studies have not yet been translated into biomarkers that are practical in clinical use[4] because brain biopsies are not acceptable and neuroimaging techniques are expensive[5] and the results are inconclusive. Thus, in recent years, there has been search for blood-based biomarkers for Sz as a valid alternative[5,6].

Blood-based biomarkers are regarded as a feasible option because the dysregulation of gene expression, epigenetic patterns, protein quantities, metabolic and inflammatory molecules in peripheral blood have been shown to have distinct patterns in people with Sz[4,5,7-11]. In addition, the strong heritability of Sz suggests that there may be genetic markers detectable in peripheral tissue[12]. Finally, there are data that suggest changes in gene expression[13,14], epigenetic patterns[15], proteo-

mic/metabolic markers[16-18] and functional cellular pathways[16,19-21] are present in both the peripheral and central nervous system (CNS) tissue. More recently our concepts about the interactions between the brain and periphery have been expanded with data suggesting that the CNS may influence gene expression and metabolism in the peripheral blood *via* cytokines, neurotransmitters, or hormones[22,23], while immune-related alterations in the CNS may in turn originate from peripheral blood[10,24] (Figure 1).

Whilst there are some encouraging preliminary data to support the notion of peripheral biomarkers for Sz, it must be acknowledged that Sz is a complex and heterogeneous disorder[25,26] which needs to be further dissected into subtypes using biological based and clinical markers[27]. Therefore it is probable that some types of peripheral blood biomarkers may only define sub-sets of people with Sz. In addition, whilst most studies have focused on diagnosing Sz, biomarkers that can indicate the clinical course or drug treatment response will also be of clinical use. Another concern is that whilst the use of convergent functional genomics methods[12,28] is a good idea, so far the selection of most candidate biomarkers has been based on findings related to the brain. This approach would be likely to miss any periphery oriented dysregulations which could be associated with Sz. The scope of this review is to critically examine published studies of blood-based biomarkers for Sz, focusing on possible uses for diagnosis, drug treatment response, or their relationship with phenotypes associated with Sz in different blood cell types.

GENERAL DESCRIPTION OF THE INCLUDED STUDIES

Manuscripts were identified by searching PubMed (http://www.ncbi.nlm.nih.gov/pubmed) using the keywords "schizophrenia" and "peripheral" and "biomarker", in addition any articles whose title included "schizophrenia" and "peripheral" were also included. Only articles written in English and published between year 1995 and 2015 were included, the details of which are given in supplementary table. Briefly, of the 79 identified studies, 35% were performed in serum/plasma; 24% in peripheral blood mononuclear cells (PBMC) including monocytes and lymphocytes; 16% in lymphocytes; 13% in whole blood; 7% in white blood cells (leukocyte) including monocytes, granulocytes, and lymphocytes; 4% in platelets; and 1% in red blood cells (Figure 2A). In terms of experimental targets of analysis, nearly 40% examined differences in protein levels including cytokines and metabolites; 28% examined mRNA levels; with the remaining studies focused on enzyme activity assays (11%), DNA methylation patterns (7%), cell numbers (5%), miRNA levels (5%), or others (3%) (Figure 2B).

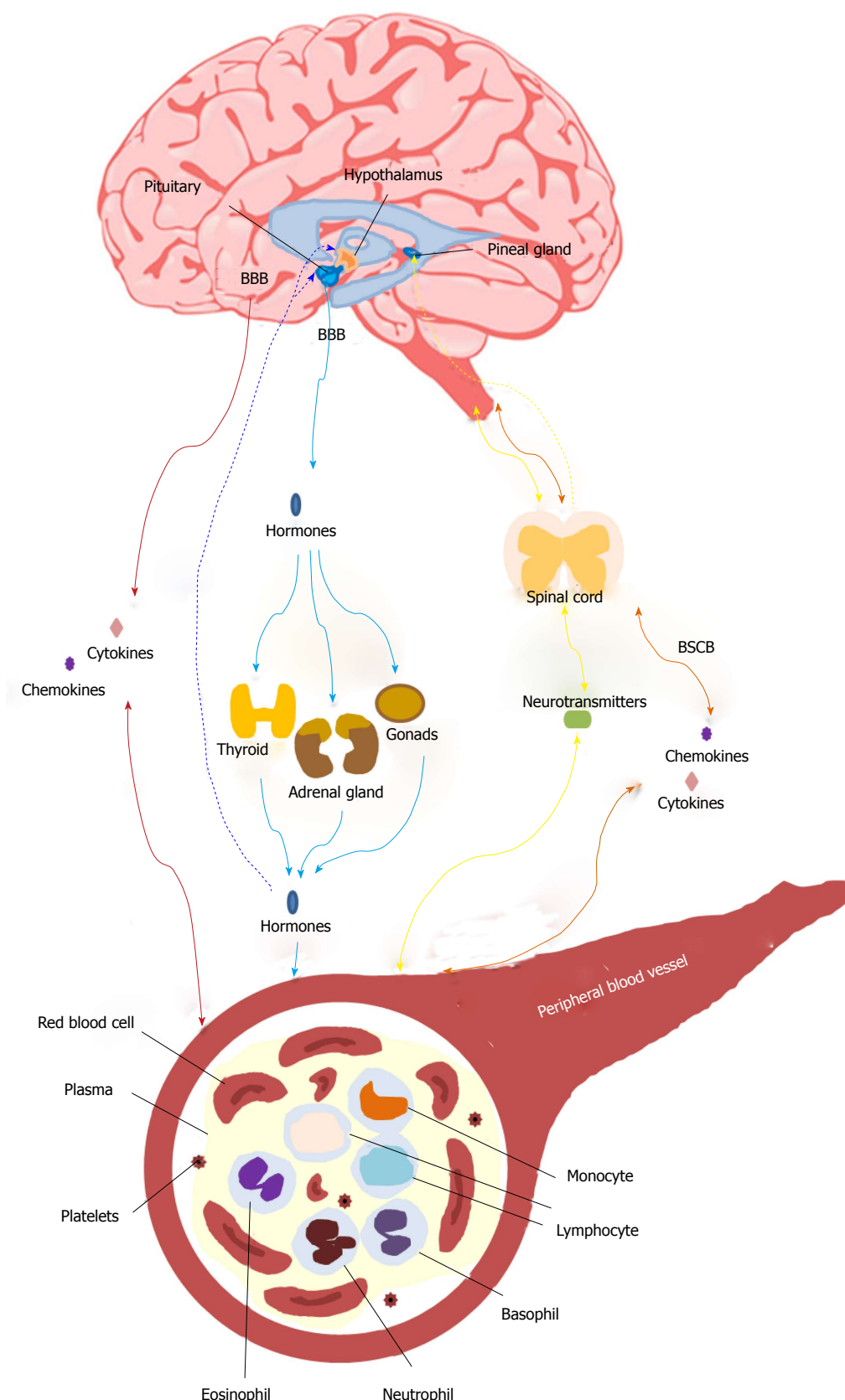Overall, the studies can be sorted into categories which include: (1) brain-derived neurotrophic factor

Figure 1 A schematic representation of central nervous system-peripheral blood tissue interactions. CNS stress may influence gene expression, DNA methylation, and cell metabolism in the peripheral blood *via* cytokines, neurotransmitters, or hormones with different transportation methods. Cytokines or chemokines can transport across the BBB (red lines) or BSCB (orange line) either from CNS to peripheral blood tissue or vice versa. The hormones are exerted by CNS and transported across the BBB *via* blood system to the target tissue (blue lines) and in turn regulate CNS through negative feedback (blue dashed lines). Another connection is the stimulated (yellow line) or negative feedback inhibition (yellow dashed line) *via* spinal cord *via* the parasympathetic or sympathetic nervous system. It is noted that there are several blood cell types with their own features in the peripheral blood vessel. The figure is an extension of Figure 1 in Marques-Deak *et al*[23]. BSCB: Blood-spinal cord barrier; CNS: Central nervous system; BBB: Blood-brain barrier.
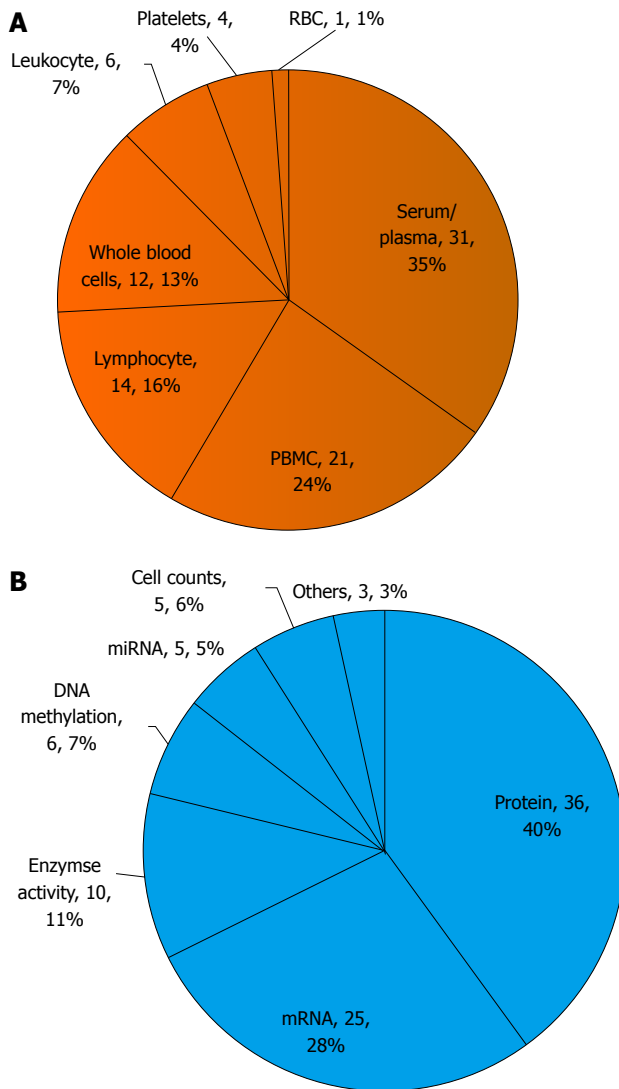
Figure 2 A summation of studies into peripheral biomarkers for schizophrenia. Studies are classified based on A: Blood cell types (*e.g.*, serum/plasma, PBMC, lymphocytes, whole blood cells, leukocyte, plateles or red blood cells); B: Research focus (*e.g.*, proteins, mRNA, enzyme activity assays, DNA methylation, or miRNA). The numbers indicate the number of studies in the categories, and its overall percentage. PBMC: Peripheral blood mononuclear cells; RBC: Red blood cell.

(BDNF); (2) molecules involved in inflammation and immune function; (3) neurochemistry; (4) oxidative stress response and metabolism; (5) epigenetic regulation and miRNA; and (6) transcriptome and proteome studies. In the following sections, we organized the narration based on biological functions and pathways while emphasizing (1) the biomarkers for diagnosis (D); (2) drug treatment response; (3) their correlations with phenotypes (*i.e.*, clinical symptom, cognitive function, or brain imaging) present in people with Sz.

BDNF

It has been postulated that Sz is associated with altered neurotrophin levels in blood, particularly lowered levels of BDNF[29,30]. A recent meta-analysis[30] of a total of 41

studies and more than 7000 participants indicated that the serum/plasma BDNF levels were moderately reduced in Sz compared with controls. Using DNA from whole blood, it has also been suggested that the promotor region of the *BDNF* gene has higher methylation levels in people with Sz[31] and this alteration may also be present in the brain[32]. Although the meta analysis showed that serum/plasma BDNF levels did not correlate with either the positive or negative symptom in Sz people[30], the plasma BDNF levels were positively correlated (r^2 = 0.14) with certain cognitive functions (*e.g.*, semantic generation tasks)[33] and auditory processing after computerized cognitive training[34]. Notwithstanding the significant result in Sz studies, plasma BDNF is unlikely to prove to be a specific biomarker for the disorder because reduced plasma BDNF is also present in bipolar disorder[35,36] and major depressive disorder[36,37].

Combining the study of peripheral blood levels and neuroimaging, it has been shown that BDNF levels in the serum/plasma positively correlate with N-acetylaspartate (r^2 = 0.15)[38], a marker of neuronal integrity, and brain activity (r^2 = 0.2)[39], which is measured by functional magnetic resonance imaging, in the parietal cortex. This means that peripheral BDNF levels may be a prognostic marker, as they return to normal during remission from acute states of bipolar disorder and major depressive disorder[36]. Whether people with Sz show similar patterns throughout different phases of their illness warrants further investigation.

The question of whether antipsychotic treatment itself can affect BDNF levels has resulted in conflicting data. A meta-analysis reported that antipsychotic treatment increased BDNF levels in plasma[30] but this was not seen in studies which examined levels in serum. However, it should be noted that the heterogeneity across studies in meta-analyses was high and the increase in BDNF levels after antipsychotic treatment was small (Hedges' g = 0.24). Furthermore, most of the studies evaluating changes of BDNF levels with antipsychotic treatment produced negative result for people with Sz[40-43] and those with treatment resistant Sz[44]. More detailed longitudinal studies will probably be required to determine if increased BDNF levels in the serum/plasma is a trait biomarker for the disorder or has some prognostic value as a disease state biomarker that can predict improvements in symptoms with treatment.

INFLAMMATION AND IMMUNE FUNCTION

Another category of biological pathways that has been receiving a lot of attention in the search for markers for Sz are those involved in immunological/inflammatory processes[9,10,45]. A comprehensive review[46] of serum/plasma biomarkers that allow the differentiation of people with Sz from controls suggests that more than 70% of potential biomarkers for the disorder are involved in the inflammatory response[46]. The

immune related biomarkers from the review include S100 calcium-binding protein B (S100B), interleukin 6 (IL-6), IL-2, and tumor necrosis factors (TNFs). The cytokines most frequently reported as changed in the serum of people with Sz include up-regulation of pro-inflammatory cytokines (*i.e.*, IL-6, IL-2, TNF, IL-1β and IL-8) or some of their receptors [*i.e.*, IL-2 receptor-α (IL-2Rα), IL-1 receptor antagonist[46,47]]. In addition, levels of mRNA of *TNF-α* and *IL-1β* genes have been shown to be increased in PBMC from people with Sz and their siblings, compared to controls[48], whereas, reduced interferon-γ (IFN-γ) mRNA has been identified in PBMC of people with Sz[49]. However, it should be noted that people with bipolar disorder also showed increases in some of the pro-inflammatory cytokines such as IL-6, TNF, and IL-1 in serum. Similarly, when a study examined the cell cycle-related histone biochemical properties (*i.e.*, total histone synthesis rates and H2A and H3 histone variant patterns of the nuclear extracted histone fraction) of lymphocytes, there are alterations that are common to both Sz and bipolar disorders[50]. Finally, in terms of cell numbers, T lymphocytes and monocytes have been found to be increased in Sz[51,52]. Taken together, these results indicate that people with Sz showed increases in pro-inflammatroy cytokines as well as lymphocyte numbers. However, some of these changes in "inflammation" components are shared with people who have bipolar disorder and this may reflect commonalities between these two disorders.

It is worth noting that, contrary to what occurs in people with Sz, a study investigating systemic inflammatory condition in a large cohort of individuals undergoing their first episode of psychosis (n = 117) showed that the majority of soluble elements of the pro-inflammatory are not significantly altered in PBMC[53]. However, they did find significant increases in intracellular molecules involved in pro-inflammatory pathways in PBMC (*i.e.*, nuclear factor κB, inhibitory complex IκB, inducible isoforms of nitric oxide synthase, cyclooxygenase) alongside significant decreases in some anti-inflammatory proteins (*i.e.*, prostaglandin 15-dexoy-PGJ$_2$ and peroxisome proliferator activated receptor-γ). The results indicate that the peripheral markers may change at different disease stages, but together suggest that the peripheral immune system is over-activated in both individuals undergoing their first episode of psychosis and people with Sz. However, it is important to note that the immune system is dynamic and will be sensitive to changes of cellular environment or medication status, which are confounds often not controlled for in the studies in Sz[10,45,46,54].

Evidences have shown that antipsychotic drugs can modulate components of the inflammatory-related pathways[55-57]. For example, serum levels of pro-inflammatory cytokines (*i.e.*, IL-2)[58,59] and anti-inflammatory cytokines (*i.e.,* IL-1RA and IL-10)[60] have been reported to be lower after treatment with typical or atypical antipsychotic drugs[58,59]. In lipopolysaccharides (LPS)-induced or polyinosinic: Polycytidylic acid-stimulated PBMC cultures, antipsychotic treatment altered immune function by suppressing the levels of IFN-γ and MCP-1 and raising the levels of IL-4, IL-10, IL-18 and RANTES[61,62]. Immune cell counts can also change in people with Sz after antipsychotic drug treatment; haloperidol increased CD3+ T cells, CD4+ T cells, and IL-2-secreting cells, together with CD4/CD8 ratio after 12-wk of treatment[63]. In addition, clozapine treatment initially (12 wk) increased CD34+ T cells, neutrophils and leukocytes. Over longer term, slightly elevated plasma IL-6 levels were observed in chronically clozapine monopharmacy-receiving patients, compared to controls[64]. Therefore, whilst it is still too early to draw any firm conclusions, current data indicate that decreased levels of cytokines are associated with antipsychotic treatment, whereas, T cells numbers were generally elevated by antipsychotic treatment.

The immune responses to changes of cellular environment have been measured and reported to be altered in people with Sz[62,65,66]. For example, out of 107 immune molecules, less IL-18 and more S100A12 (ENRAGE) were secreted by blood cells in *ex vivo* cultures obtained from first-onset people with Sz compared to controls following stimulation condition with a T cell stimulator (*i.e.*, anti-CD28/CD49d)[66], an effect replicated in an independent cohort[66]. In addition, T cells from people with Sz had significantly lower proliferative responses following stimulation with anti-CD3, compared to controls[65], a response not affected by antipsychotic medication. When PBMC from people with Sz were stimulated with bacterial LPS they released more protein of MCP-1, MIP-1α, IL-8 and IL-18 but less RANTES (for "regulated upon activation normal T cell expressed and secreted", a pro-inflammatory chemokine) and IFN-γ[62]. Collectively, the data suggests that changes of immune response to changes of cellular environment in Sz people could be complex.

There is evidence to suggest that the levels of some inflammatory molecules in blood are correlated with symptom severity in people with Sz[10,33,48,60,63,67]. For example, plasma levels of IL-2 in people with Sz were reported to be positively correlated with the scores in cognitive tests of digit span test (r^2 = 0.17) and intelligence (r^2 = 0.19), and negatively correlated with the presence of negative symptoms (r^2 = 0.2[67]), levels of IL-1β and TNF-α mRNA in PBMC were positively correlated (r^2 = 0.17) with scores on the general psychopathology factor of the PANSS[48] and changes in serum IL-10 levels were significantly correlated with improvements in symptoms [*i.e.*, negative, general psychopathology and total score (r^2 = 0.1-0.2)] among people with first-onset Sz[60]. Levels of the chemokine, CCL-11, were not only higher in people with Sz but were negatively correlated with performance on the working memory test (r^2 = 0.16) and positively correlated with a cognitive flexibility task (r^2 = 0.26[33]). Moreover, increased S100B positive natural killer cells in blood from people with acute Sz correlated with the perception of stress (r^2 = 0.08[68]) whilst increases in

CD4 positive cells correlated with an improvement of clinical symptoms in people with Sz ($r^2 = 0.1$)[63].

In summary, people with Sz demonstrate a distinct profile of immune peptides, aberrant immunological responses, and changes of immune cell counts. For example, higher plasma levels of IL-2 appears to be a promising Sz specific biomarker[47] which may reflect the clinical symptom as well as cognitive function[67] particularly since the levels decrease following treatment with antipsychotic drugs[58,59]. Some of these changes in the immune system markers correlate with treatment responses and symptom profiles, suggesting they may be suitable theranostic markers. However, the majority of the studies focused on cross-sectional group differences, and since the immune process is highly dynamic and changes are often transient, more research into the longitudinal patterns of immune profiles will be helpful to determine whether markers of this system are good candidates for indicating treatment responses.

NEUROCHEMISTRY

Monoamine pathways

Monoamine neurotransmitters such as dopamine (DA), norepinephrine (NE) or serotonin [5-hydroxytryptamine (5-HT)] have been postulated to associate with pathogenesis of Sz, primarily as a result of the pharmacological profiles of the drugs used to treat the disorder[69]. A number of dopaminergic markers have been evaluated in blood from people with Sz, including DA receptors (*i.e.,* DRD$_2$, DRD$_3$, and DRD$_4$[70]), DA transporter (DAT), and other molecules associated with the dopaminergic system [*i.e.,* tyrosine hydroxylase (TH)]. Both mRNA expression and receptor binding of DRD$_2$ were increased in lymphocytes from people with Sz who were drug-naive[71,72], however the up-regulation of DRD$_2$ mRNA was not replicated[73,74]. The levels of lymphocyte DRD$_3$ mRNA was reported to be elevated in both people with chronic Sz[73,75] and people with Sz who were drug-naïve[76]. However, it was also reported to be down-regulated in people with Sz and people with bipolar disorders[77]. For DRD$_4$, the mRNA has been reported to be either down-regulated in CD4 positive T cells[73] or not different in people with Sz[74]. It is noteworthy that higher DRD$_3$ mRNA has been reported in people with heroin addiction, whilst lower DRD$_4$ mRNA has been reported in people with major depression as well as during alcohol and heroin withdrawal[70].

There is evidence to suggest that lymphocyte DRD$_2$ mRNA levels positively correlate ($r^2 = 0.2$) with positive symptoms of Sz[74]. This provided some indirect support for the report that DA uptake by platelets correlated with delusional state of people with Sz[78]. In addition, the changes of lymphocyte DRD$_3$ and DRD$_5$ mRNA were associated with symptom severity (effect size = 1.46)[76].

As well as these changes in markers for DA receptors, DAT mRNA is reported to be higher in lymphocytes from people with chronic Sz compared to controls[74], by

contrast, reduced DAT binding was reported in people with Sz[79], suggestive of a lower number of the DAT protein. TH mRNA has been reported as increased in PBMC of both people with Sz and their siblings compared to controls[48]. Other changes such as elevation of plasma homovanillic acid (HVA), a breakdown product of DA, were noted among people with Sz[80], people in the prodromal phase[81] and those with schizotypal personality disorder[82] but not in people with bipolar disorder[80], suggesting some disorder specificity. In addition, protein of DA- and cAMP-regulated neuronal phosphoprotein of 32 kDa (DARPP-32), a critical downstream target of DRD$_1$ and DRD$_5$-mediated signaling, was decreased in CD4 positive T lymphocytes and CD56 positive natural killer cells from people with Sz in comparison to control group[83], indicating lymphocytes may therefore function as an easily accessible model to study the DA intracellular signaling in the cells of people with Sz. Overall, some of the markers (*i.e.,* TH and HVA) may prove to be markers for a high risk population, such as people with a family history of the disorder as they were not only dysregulated in people with Sz[48,80] but also in their siblings[48] as well as in prodromal individuals of Sz[81] or people with schizotypal personality disorder[82].

One caveat of using monoamine related molecules as biomarkers is that most of the antipsychotics would block their receptors[69]. This effect is not limited to the CNS, therefore the drugs may dynamically change the peripheral profile of monoamine-related receptors and metabolites. Thus monoamine related molecules in the blood may prove to be state markers instead of the stable trait markers that are suitable for diagnosis. However, studies on the longitudinal changes in peripheral monoamine related molecules necessary to address this hypothesis are scarce. Thus, in terms of the effect of antipsychotics, a single study found that lymphocyte DRD$_2$ and platelet 5-HT$_{2A}$ receptor binding were both reduced after treatment with antipsychotics[84]. In addition, lymphocyte DRD$_3$ and DRD$_5$ mRNA was reported to show dynamic, non-linear changes in people with SZ who were drug-naïve in the beginning of follow-up[76]. Briefly, mRNA of the DA receptors peaked at week 2 after taking antipsychotics, after which it decreased but was above baseline at week 8[76,77]. Finally, it has been reported that both risperidone and clozapine elevate plasma NE levels, with risperidone producing a smaller effect[85]. Logically, considering their close ties with the pharmacological properties of antipsychotics, peripheral monoamine related molecules might be a good indicator for treatment response but current evidence to support this posit is lacking.

Glutamatergic pathways

Because glutamatergic dysregualtion may play a role in the pathogenesis of Sz[69], there is a body of research focusing on peripheral levels or functions of the amino acids, such as glutamate, D-serine, L-serine, glycine, and agmatine, that activate N-methyl-D-aspartate

(NMDA) glutamate receptors[86-89] and are involved in modulating the glutamatergic neuronal pathways.

Most studies focused on peripheral glutamate levels in people with Sz. In a meta-analysis[86] of 10 studies, higher levels (standardized mean difference = 0.64; 95%CI = 0.21-1.06) of glutamate have been reported in blood from people with Sz compared to controls, however, similar dysregulations have been reported in the small number of studies that focused on major depressive disorder[90,91], suggesting the alteration of glutamate levels is not specific to the diagnosis of Sz. Interestingly, the response of platelet intracellular calcium to glutamate was greater in people with Sz than in controls, suggesting NMDA receptor may be more sensitive in these people[87]. A single study has reported that people with Sz had higher levels of plasma agmatine, an endogenous substance that is synthesized from L-arginine and is proposed to be a new neurotransmitter, than controls[88], and that the levels correlated with their PANSS score, suggesting that plasma agmatine levels may be a potential diagnostic biomarker for Sz. Lower plasma D-serine, D-/L-serine ratio were found in people with Sz who were treatment-resistant[89], but chronically people with Sz showed higher plasma D-serine and glycine levels[92]. In addition, the plasma levels of D-serine were associated with improvements in positive symptom[92], but not with cognitive functions in people with Sz[93]. A study examining the effects of clozapine treatment on D-serine, L-serine, and glycine in people with Sz who were treatment-resistant, showed glycine levels and the glycine/L-serine ratio were significantly increased following clozapine treatment, suggesting that plasma L-serine and glycine could be potential therapeutic markers[89]. In summary, higher levels of blood glutamate has some potential to be a diagnostic marker for Sz, but its correlation with major depressive disorder warrants further investigation in order to determine the specificity of the changes.

Other neurochemicals

Of other neuronal chemicals associated with the pathogenesis of Sz, levels of plasma γ-aminobutyric acid have been reported to be lower in people with Sz as well as bipolar disorders compared to the control group[80]. By contrast, plasma reelin[94], which is involved in the migration of neurons; and adenosine deaminase activity[95], a homeostatic modulator that affects brain DA and glutamate activities, were higher in people with Sz. It is also of note that acute metabolic stress in the brain, induced by pharmacological doses of 2-deoxyglucose (2DG), a glucoprivic agent transported across the blood-brain barrier into brain tissue where it inhibits intracellular glucose metabolism and produces a clinical state similar to hypoglycaemia[96], appears to affect the peripheral vesopressinergic system *via* the pituitary-adrenal axis[96,97]. In addition, after 2DG treatment people with Sz had higher 2DG-induced

plasma adrenocorticotropic hormone levels[97], HVA, and 5-hydroxyindoleacetic acid levels[96]. These data suggest that central acute metabolic stress may alter the metabolism in peripheral dopaminergic and serotonergic systems *via* hormones (Figure 1). In PBMC, both people with first episode of psychosis[98] and chronic Sz[99] show decreased protein levels of cannabinoid receptor 2 (CB2), however, levels of CB2 were influenced by the medications and cannabis use history[98], suggesting endocannabinoid system presented at the initial phases of psychosis could be contributing to the pathophysiology of the disease and constitutes a possible treatment response biomarker of psychotic disorders[98]. In the cholinergic system, a decrease of a7 acetylcholine receptor (AChR) mRNA levels has been reported in lymphocytes from people with Sz[100] that was not affect by antipsychotic treatment, suggesting AChR as a peripheral trait makers of Sz. Finally, a single study reported that the density of the peripheral-type benzodiazepine receptors in platelets is a predictor for aggressive behaviors in people with Sz[101] showing an association with higher scores for overt aggression, hostility and anxiety. This particular marker may prove useful for defining a subgroup within the syndrome of Sz. However, considering limited evidence so far, it is too early to predict the robustness of these markers. All of the markers require validation in larger cohorts and need to be assessed for specificity before their usefulness can be assessed.

OXIDATIVE STRESS RESPONSE AND METABOLISM

The pattern of oxidative stress markers in the plasma was examined in several studies. Psychotic patients in general are in a significantly increased long-term condition of oxidative stress[102,103]. Oxidative stress markers, such as pentosidine[104], glycer-AGE[105] and thiobarbituric acid reactive substances[103,106] were found to be higher among people with Sz and carbonyl stress was found to be lower in people with the disorder[104,105,107]. However, those oxidative markers have overlapping levels between people with Sz and healthy individuals, suggesting the discriminability of those markers is low[102]. One group suggested that only a subgroup of study participants with the glyoxalase I (GLO1) deficits showed distinct patterns of oxidative stress abnormality in the plasma[104], suggesting GLO1 deficits and oxidative stress may contribute to development of a certain subtype of Sz. However, evidence also suggests these oxidative markers show dynamic non-linear changes during hospitalization and levels could be associated with the dosage of antipsychotics irrespective of the treatment responses[107]. If this finding is replicated, the utility of these markers may be restricted to determining medication compliance.

In light of the tendency of people with psychiatric disorders, particularly Sz, to develop metabolic syn-

drome, several studies have examined molecules involved in glucose homeostasis[95,108-113]. In a study where PBMC were stimulated *ex vivo* with staphylococcal enterotoxin B, eight out of total of 18 proteins examined were differentially expressed in cells from people with Sz who were first onset and drug-naive Sz compared to controls. These proteins belonged to the glycolytic pathway[110], indicating that impaired glycolytic response could be a potential early stage of diagnostic biomarker for Sz. Mitochondrial complex Ⅰ, the first enzyme in the mitochondrial respiratory chain for the oxidation of glucose, was found to have increased activity in the platelets from people with Sz[108] and to have higher mRNA levels in blood from people with Sz[111,113]. It has also been reported that the activity of platelet mitochondrial complex Ⅰ is associated with positive symptoms and clinical disease course in people with Sz[108]. Significantly, the activity of platelet mitochondrial complex Ⅰ is not altered in people with bipolar disorder[106] and mRNA levels for platelet mitochondrial complex Ⅰ is not altered in white blood cells from people with autism spectrum disorder[113]. These latter findings are an indicator that the activity of platelet mitochondrial complex Ⅰ is associated with positive symptoms and clinical disease course may be specific to Sz and a potentially useful biomarker for those features of the disorder.

The utility of these markers is thrown into doubt by a study in T-lymphocytes which reported that antipsychotic drugs affect the expression of a large number of genes and that some of those genes were related to oxidative stress and metabolic disease[114]. In addition, it has been suggested that molecules involved in glucose homeostatsis may also be predictors for people who develop extrapyramidal symptoms after receiving antipsychotics[115]. Thus, it may be that rather than being markers for diagnosis, some of the molecules involved in glucose homeostatsis might be potential markers for either susceptibility to side-effects or for medication compliance.

EPIGENETICS AND MICRORNA

Epigenetic factors can potentially alter susceptibility to Sz[116]. Thus, it is not suprising that studies have evaluated the global methylation pattern in people with Sz[117-120]. Overall, leukocyte DNA from people with Sz had lower methylated deoxycytidine (mC) content compared to controls[120]. In addition, a genome-wide study showed there were 234 CpG sites (0.04% of CpG sites) that were differentially methylated in both people with Sz who were drug-naïve compared to controls and in people with Sz compared to their discordant monozygotic twin sibling[118], with most of the sites located in promoter regions of genes. Further studies have reported that in PBMC from people during their first onset of Sz there were 603 CpG sites (2% of CpG sites) that were differentially methylated[119], the genes that were particularly affected were related

to the nuclear lumen, transcription factor binding, and nucleotide binding. Using whole blood, a methylome-wide association study showed that 139 CpG sites (0.003% of CpG sites involved in analyses) were differently methylated in people with Sz, highly implicating an exon and 3'UTR of FAM63B and an intron of RELN[117]. Overall, differentially methylated CpG sites can be detected in peripheral blood of people with Sz and many of the methylation was reported in the promoter regions of genes, therefore they may affect transcription of these genes. However, it remains to be determined if the changes in methylation are correlated to changes in levels of gene expression, the mechanism by which methylation predominantly regulates gene activity.

The central regulatory role of microRNA (miRNA) in gene expression[121] makes them promising biomarkers for Sz. Whilst there have been a number of studies focusing on miRNA in brain samples[122], relatively few studies have examined them in peripheral blood. Of these studies, reported changes in miRNA include up-regulation of hsa-miR-34a[123-125] and down-regulation of hsa-miR-432[123,126] in PBMC from people with Sz, which have been replicated in independent studies[125]. However, the data for other miRNAs are conflicting. For example, hsa-miR-21 and hsa-miR-30e have been reported to up-regulated[125,127] and down-regulated[126] in PBMC from people with Sz. Likewise, levels of hsa-miR-30e and hsa-miR-181b were altered in opposite directions[124-126] in studies using plasma from people with Sz. It has been reported that antipsychotic treatment is associated with changes of miRNA levels in human plasma[124,128] and T-lymphocyte cell lines[114]. The expression levels of hsa-miR-365, hsa-miR-520c-3p, and hsa-miR-181b in plasma were significantly down-regulated after antipsychotic treatment[124,128], of these, miRNA-181b expression levels were positively correlated with the improvement of negative symptoms[124]. Finally, an *in vitro* antipsychotic treatment response study in T-lymphocyte cell lines demonstrated that miR-200c-3p and miR-28-5p were down-regulated in haloperidol-treated cell lines compared to controls[114]. Given the variance in data regarding levels of peripheral miRNAs, the consistent findings of hsa-miR-34a and hsa-miR-432 as well as their expressions levels were not changed following treatment, these have implied their potential as diagnostic markers.

In sum, peripheral epigenetic markers might be a promising direction for finding biomarkers for Sz. The methylation patterns and miRNA expression patterns have both shown differential patterns between people with Sz and controls. However, considering the discrepant results mentioned above, the consistency between studies warrant further investigation.

THE HUMAN TRANSCRIPTOME AND HUMAN PROTEOME STUDIES

Modern technologies allow the investigation of levels of

thousands of mRNAs and proteins at the same time. The studies at the levels of mRNA are known as studies of the transcriptome whereas studies at the levels of proteins are known as studies of the proteome.

Most studies of the transcriptome have involved measuring levels of mRNA is gene expression microarrays. In psychiatry, such studies have involved the use of whole blood[129-131], lymphocytes[132] or PBMC samples[133-135] to identify potential biomarkers for Sz. Other studies examined proteomics information in RBC[109], T cells[136], and serum[61,109,137,138] in attempts to answer the same question. This approach has typically led to a large set of mRNAs or proteins being proposed as possible biomarkers in Sz but most findings await replication.

Briefly, in a whole blood study from people during their first onset of Sz, the expression of glucose transporter (SLC2A3) and actin assembly factor (DAAM2) were increased, whereas zinc metallopeptidase, neurolysin 1 and myosin C were significantly decreased, compared to controls[129], indicating peripheral mRNA of these genes could be a potential biomarkers in early stage of disease course. A genome-wide expression analysis in PBMC samples from people with Sz was characterized by alterations of genes with immune system function[133,135] with some differentially expressed genes, such as argonaure 2, myocyte enhancer factor 2D, Enah/Vasp-like, peptidase inhibitor 3, S100 calcium binding protein A12 (S100A12), defensin α4 (DEFα4[133]) as well as AKT1[135] being confirmed as changed using qPCR, the result supporting the hypothesis that the disorder has a significant immunological component in its etiology. In addition, a microarray study identified six genes [including B cell translocation gene 1, glycogen synthase kinase 3α (GSK3α), HLA-DRB1, heterogeneous nuclear ribonucleoprotein A3, selenium-binding protein 1 (SELENBP1), and splicing factor] which were differentially expressed in both brain and peripheral blood of people with Sz[13]. Of these, SELENBP1 was identified as the strongest candidate biomarker among all genes differentially expressed in Sz, because it was the only gene showing significant differential expression in a similar direction in both brain and blood[13]. However, the result of up-regulation of SELENBP1[139] and up-regulation of GSK3A[112] in peripheral blood was not replicated in the other studies. Another study reported several genes related to nucleosome and histone structure were dysregulated in PBMC of both people with Sz and their siblings, suggesting a potential epigenetic mechanism underlying the risk state for the disorder[134]. Other differentially expressed genes in PBMC were involved in pathways such as cell adhesion and neuronal guidance, neurotrophins, oxidative stress and glucose metabolism, and apoptosis and cell-cycle regulation[135], all of which have some face value for the pathophysiology associated with Sz.

For the studies reporting discriminability of the markers, a study using whole blood from people with Sz who were drug naïve identified 14 probes which annotated 11 genes (listed in supplementary material)

that predicted diagnosis with 91.2% accuracy in the training set and 87.9% accuracy in the validation set[131], however, this study lacked qPCR analysis to validate the microarray data. A microarray using white blood cells of discordant sib-pairs for either Sz or bipolar disorder identified a set of 35 transcripts which can discriminate people with Sz from people with bipolar disorders and their unaffected sib-pairs with 95% accuracy[130]. However, only few genes [*i.e.*, Serotonin receptor type 4 (5HT4), transcription factor-like 4, and neuregulin 1] were confirmed using qPCR and there has been no subsequent validation study for the prediction model. Collectively, the inconsistent findings of this type of study may result from the heterogeneity of the preparations used, the people with Sz being at different clinical courses of the disorder (*i.e.*, first onset or chronic stage) and the fact that Sz is a spectrum rather than a discrete illness.

Of the studies discussed, only one followed up the differentially expressed RNA levels in whole blood of first onset Sz people for 12-24 mo[129]. DAAM2, one of five differentially expressed genes, returned to control levels in the whole blood when people were in remission after their first psychotic episode, suggesting this mRNA could be altered by antipsychotics and may be a potential marker for treatment response. Taken together, there is only down-regulation of intercellular adhesion molecule in people with Sz that were replicated in at least two microarray studies[138,140]. Other studies have identified markers that may be of use in identifying people who are at risk of developing the disorder, such as the up-regulation of DEFA in T cells and plasma from people with Sz, and both their affected or unaffected monozygotic twin[136]. Of interest is the down-regulation of apolipoprotein A1 identified in both red blood cells of people with Sz and serum of people with Sz who were drug-naive[109]. Not only does this suggest it might be a trait marker but a similar pattern was shown in postmortem brain tissue[109], suggesting that the peripheral changes reflect central pathophysiology.

Proteomics studies have resulted in a signature comprised of 34 serum protein analytes that have 60%-75% discriminant accuracy in separating people with Sz from those with major depression, bipolar disorder, Asperger syndrome and controls[140], suggesting these proteins in blood serum as a potential biomarker for Sz. In addition, 27 serum proteins were identified as discriminating between people with Sz and healthy controls[138] and highlighted some proteins among them with important roles in the immune system. It has been reported that dysregulation of 4 serum analytes [up-refulation of 2-piperidinec carboxylic acid, along with the down-regulation of 6-deoxy-mannofuranose, galactoseoxime, and a serum peptide (m/z 3177)] have the best discriminating value between people with Sz, and controls[141], indicating that metabolomics and proteomic approaches can be used in the biomarker research.
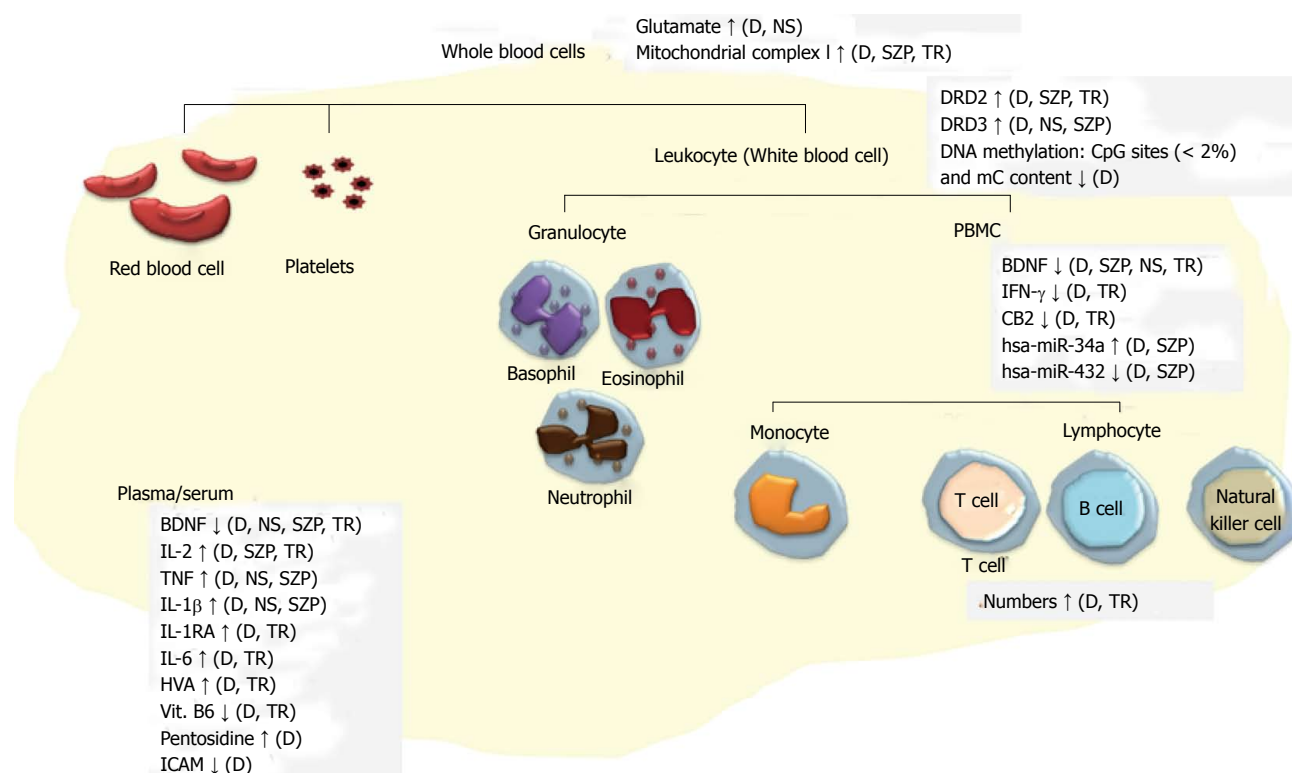
Overall, the outcome of current omic studies has

Figure 3 Representation of potential peripheral biomarkers for schizophrenia. Only markers consistently reported in two or more individual peripheral studies are listed in the figure. The markers were classified as either diagnostic (D), antipsychotic TR or both. SZP indicates those markers have been reported to correlate with phenotypes (*i.e.*, clinical symptom or cognitive function) in people with schizophrenia. Some markers were NS for schizophrenia. PBMC: Peripheral blood mononuclear cell; BDNF: Brain-derived neurotrophic factor; IL: Interleukin; TNF: Tumor necrosis factor; HVA: Homovanillic acid; Vit. B6: Vitamin B6 (pyridoxamine); ICAM: Intercellular adhesion molecule; DRD$_2$: Dopamine receptors D$_2$; IFN: Interferon; CB2: Cannabinoid receptor; TR: Treatment response; NS: Not specific.

been the identification of a number of potential biomarkers in peripheral blood with a cluster of reports suggesting that markers associated with immune function/inflammation might be of interest, however the utility of these need to be confirmed.

CONCLUSION

As can be seen from the précis of studies to date, the search for biomarkers of Sz in peripheral samples is flourishing. Markers that have consistently been altered in two or more individual studies are summarized in Figure 3. It is possible that some of the differential patterns reported to exist in people with Sz can be used for diagnostic purposes. However, only a few studies reported the sensitivity and specificity of the discriminability of their markers[108,123,125,130,138,140,141], leaving doubt as to the potential of the other molecules to act as markers. To try and assess the utility of these disparate markers, performing a meta-analysis based on the categories we used here might be helpful to identify potential markers that have large and consistent effect size across studies. On the other hand, as some studies have shown, peripheral biomarkers may be more useful as state indicators. The changes of some biomarkers appear to be associated with clinical outcome, treatment responses, and the changes of clinical symptoms, suggesting they might be useful for specific clinical

events, an opportunity that has been ignored in the search for diagnostic markers.

An important factor that hasn't been considered in many studies is whether or not the candidate peripheral biomarkers showed similar patterns in other psychiatric diseases. It is important to recognize that several psychiatric diseases can share some of the same components with Sz[12,142,143]. Based on the limited studies so far, many of identified markers were not specific to Sz but were shared with other psychiatric diseases such as bipolar disorders[47,50,70,80]. The next step of peripheral biomarker development would be examining the candidate markers in other psychiatric diseases. Having Sz specific biomarkers can increase the efficiency of disease diagnosis as well as improving treatment strategies.

One question raised by this review is the blood-brain relationship. Most of the studies mentioned above (approximately 70%) were prompted by findings related to the brain. For instance, neurotransmitters apparently behave differently in the peripheral system than in the brain, yet many studies have focused on the neurotransmitters implicated from neuroscientific studies of Sz. This often leads to inconsistency among studies, and patterns found in the brain oftentimes fail to be found in the peripheral blood. Nevertheless, even if the neurotransmitters were to behave differently in the periphery, some evidence suggests they can

be biomarkers for treatment effect. We suggest the biomarkers in the peripheral blood should be initially simply viewed as whether or not they have clinical utility rather than whether they may be tied to the aetiology of Sz. If a similar dysregulation can be seen in the brain or the aberration of the peripheral molecules were related to clinical symptoms it would add more information for interpreting the potential peripheral biomarker. In this respect, given it is known that many biomarkers have been shown to be affected by factors such as fasted/fed state, diurnal and seasonal rhythm and disturbances in sleep[57] efforts should develop standardized blood collection procedures in order to decrease study to study variation. Alternatively, the exact specimen collection protocol, time of collection, anticoagulants used and methods of blood processing should be accurately described in any blood study seeking to report findings on potential biomarkers in Sz.

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ORIGINAL ARTICLE

Retrospective Study

How does a real-world child psychiatric clinic diagnose and treat attention deficit hyperactivity disorder?

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Abstract

AIM: To investigate child and adolescent psychiatrists' (CAPs) attention deficit hyperactivity disorder (ADHD) and oppositional defiant disorder (ODD) diagnoses and treatments in real-world clinical practice.

METHODS: The medical records of 69 ADHD children (mean age = 9.5 years), newly referred to the ADHD clinic, were reviewed for their scores of parent- and teacher-reported Vanderbilt ADHD Diagnostic Rating Scales (VADRSs), CAPs' diagnoses of ADHD and ODD, and CAPs' treatment recommendations. Among 63 ADHD subjects who completed both parent and teacher VADRSs, we examined the agreement of the parent and teacher VADRSs. We also examined the concurrent validity of CAPs' ODD diagnoses against the results from the VADRSs. In addition, we compared CAPs' treatment recommendations against established ADHD and ODD guidelines.

RESULTS: Among 63 ADHD subjects, the majority of the subjects (92%) met full ADHD diagnostic criteria at least in one setting (parent or teacher) on the VADRSs. Nearly half of the patients met full ADHD diagnostic criteria in two settings (parent and teacher). Relatively low agreement between the parent and teacher VADRSs were found (95%CI: -0.33 to 0.14). For 29 children who scored positive for ODD on the rating scales, CAPs confirmed the ODD diagnosis in only 12 of these case-positives, which is considered as a fair agreement between CAPs and VADRSs (95%CI: 0.10-0.53). For 27 children with no ODD diagnosis made by either CAP or VADRS, more than half of them were recommended for medication only. In contrast, where CAPs made the diagnosis of ODD, or where the parent or teacher VADRS was positive for ODD, almost all of the patients received recommendations for medication and behavior therapy.

CONCLUSION: CAPs' ADHD diagnoses have strong concurrent validity against valid rating scales, but ADHD's most common comorbid condition - ODD - may be under-recognized.

Key words: Attention deficit hyperactivity disorder; Oppositional defiant disorder; Vanderbilt attention deficit hyperactivity disorder Diagnostic Rating Scale; Quality assessment; Clinical practice

Core tip: Given the concerns about possible attention deficit hyperactivity disorder (ADHD) over-diagnosis and over-treatment, within a newly diagnosed sample of consecutive ADHD patients, we examined the concurrent validity of child and adolescent psychiatrists' (CAPs) ADHD and oppositional defiant disorder (ODD) diagnoses against the results from the Vanderbilt ADHD Diagnostic Rating Scales. We also evaluated CAPs' ADHD and ODD treatment recommendations and discussed clinical implementations of the established treatment guidelines into CAPs' practice. In our samples, CAPs diagnosed ADHD strongly agreeing with the rating scales, but given our results showing the relatively low prevalence rates of ODD diagnosis within ADHD, ODD may be under-recognized.

INTRODUCTION

In view of national concerns about the rising rate of diagnoses of attention deficit hyperactivity disorder (ADHD), increased understanding of the diagnostic procedures and accuracy of clinicians' diagnoses of ADHD and its associated conditions is needed[1,2]. Although diagnostic criteria for ADHD and other psychiatric disorders are clearly specified in the DSM-5; Diagnostic and Statistical Manual of Mental Disorders 5th ed[3], it is less clear whether clinicians use these criteria in clinical practices. Relatedly, ADHD frequently occurs with comorbid conditions, particularly oppositional defiant disorder (ODD), its most common comorbidity with 54% to 84% of prevalence in ADHD patients reported in the American Academy of Child and Adolescent Psychiatry (AACAP) practice parameter[4,5]. However, relatively lower prevalence of ODD in ADHD children has been reported in different samples most likely due to heterogeneity of outcome measures[6]. Given the greater risks for substance use, academic disability, and social dysfunction in ADHD children comorbid with ODD[7,8], identification and accurate diagnosis of ODD in the early stages is essential, to improve the prognosis[5,9,10].

However, implementing these guidelines in real-world clinical practice can be a time-consuming task[4,5,10,11]. To make the process more practical and feasible, many rating scales comprised of the DSM diagnostic criteria have been published as valid assessment tools for both ADHD and ODD[5,10]. Among many ADHD rating scales, the Vanderbilt ADHD Diagnostic Rating Scale (VADRS), published in 2002 by the AAP and National Institute for Children's Healthcare Quality, was designed to capture standardized ADHD symptom information from parents and teachers reporting on children's behaviors[12]. This rating scale was also designed to assist providers in screening ODD and other common comorbidities[13,14].

Furthermore, the parent VADRS has shown high concurrent validity with ADHD diagnoses (γ = 0.79) made *via* structured diagnostic interviews (C-DISC-IV) in elementary school children[12,15]. Recently, Wolraich *et al*[12] evaluated the validities of parent and teacher VADRS in a larger sample of children and concluded that the agreement between the teacher VADRS's symptoms and DISC-IV was statistically significant (P < 0.05; inattention γ = 0.33, hyperactive γ = 0.29), but of less magnitude of agreement compared to the parent VADRS with the DISC-IV[16,17]. The parent and teacher VADRSs have also been evaluated for their utility in assessing comorbid conditions such as ODD/CD and learning disorders[13,14]. Similarly, Becker *et al*[13] noted that a total score > 10 of the 8 ODD items on the parent VADRS demonstrated high sensitivity and specificity (0.88, 0.85 respectively) against C-DISC-IV diagnoses, with 91% of true positive cases identified.

The AAP and AACAP guidelines for ADHD[4,5] recommend that ADHD diagnoses be made *via* an intensive process that requires a careful diagnostic interview and review of other records (*e.g.*, school evaluations, other medical reports, rating scales, *etc*.). Ultimately, the physician's "best clinical judgment" must integrate all this information to render the final diagnoses. Despite

of the importance of the physician's "best clinical judgment" final step, little research has examined clinicians' best judgments in diagnosing ADHD and ODD[7,8,18], comparing clinicians' diagnostic judgments with the results from parent and teacher rating scales with a validated measure such as the VADRS.

Assuming that the correct diagnosis has been rendered, additional questions arise: Do physicians' recommendations follow treatment guidelines in clinical practice of ADHD? While ADHD guidelines generally recommend medication as a first-line treatment, combining medication with behavior therapy is frequently recommended and is the best option for most cases. Behavior therapy is a first-line intervention for ODD, which is also advisable as an initial approach for some ADHD cases, such as younger children with ADHD. Behavior therapy promotes positive parenting and provides an opportunity for social training for disruptive children, which may not be achieved by medication treatment only[2,19-24].

Given previous research showing the high agreement of specific VADRS thresholds against DISC-derived diagnoses[13,16,17], we hypothesized that physicians' clinical diagnoses of ADHD and ODD might be evaluated by comparing the results of the parent and/or teacher VADRSs with clinicians' final diagnosis of these conditions. We also reasoned that we could evaluate physicians' treatment recommendations by comparing them with the treatment guidelines for ADHD and ODD.

MATERIALS AND METHODS

Sampling frame
From June 1 to November 30, 2011, all records of pediatric patients newly referred to the ADHD Clinic at the Mayo Clinic in Rochester, Minnesota for initial evaluation were identified (*n* = 120, age range 3-18, and mean 9.4 years). Of these, 69 patients were selected through the inclusion and exclusion criteria described below.

Inclusion criteria
Patients, living in the immediate geographic area, who received their routine care either within the Mayo Clinic or its larger regional system, Mayo Clinic Health System, were included. Across the system, electronic medical records were available for examination by the study team. Thus, to be eligible, patients must have had an initial evaluation by CAPs within the ADHD clinic, and received their follow-up care either within the ADHD clinic or in the surrounding Mayo primary care settings.

Exclusion criteria
All patients who were followed up by non-Mayo Clinic providers were excluded (40 subjects). All patients not receiving an ADHD diagnosis after clinical evaluation were excluded as this study was a part of the naturalistic study to assess clinical implementations of VADRSs for diagnoses and treatment outcomes in ADHD children in

the ADHD clinic. No comorbid conditions were used as exclusion criteria.

Records abstraction
Eight months after their initial diagnostic visit, patients' medical records were reviewed in order to gather diagnostic and treatment-related data, *i.e.*, ADHD and comorbid diagnoses, medications, therapies, and VADRS scores.

Sample characteristics
The mean age of the 69 children identified with ADHD was 9.5 years (age range, 4-18). As expected, males predominated, with a male/female ratio of 2.8:1. Eighty-two percent were Caucasians, 6% were African-American, 3% were Asian, and 6% were another ethnicity. Expected proportions of the various ADHD subtypes were found (combined subtype 41%, inattentive subtype 32%), except that nearly one-quarter of children were diagnosed with ADHD - not otherwise specified.

VADRS
As a part of its standard of care, the Parent and Teacher VADRS were used to assist in the assessment of ADHD symptoms and comorbid conditions at initial visits to the ADHD clinic. Among 69 patients, all of them had a completed parent VADRS at the initial visit, except for two incomplete ODD ratings. Six patients did not have teacher VADRS available for scoring at the initial visit. Among 63 subjects who had both parent and teacher VADRS completed, 59 subjects had completed parent and teacher ODD rating scales available for scoring. Accepted procedures for scoring the VADRS were used[25] as follows: On the VADRS, all ADHD and ODD symptoms were rated on a 0 to 3 scale (0 = none, 1 = minimal, 2 = often, 3 = very often). For any symptom to be considered "positive" for diagnostic purposes, it must be scored at a 2 or 3 (often, very often). When scoring the parent and teacher VADRS for diagnostic purposes, each of the two ADHD symptom subtypes (inattentive and hyperactive-impulsive) is considered to be screen-positive if > 6 of 9 of their respective symptoms are scored at 2 or 3. Additionally, the ADHD and ODD scores in each rating scale were added up to calculate the total ADHD and ODD scores (range of total possible ADHD symptom scores: 0-54; range of possible ODD symptom scores: 0-24 Parent VADRS, 0-30 Teacher VADRS). We applied the cut point of a total score > 10 of the eight ODD items on the parent VADRS considering its high sensitivity and specificity for a screening of ODD. However, for teacher VADRS, ODD is considered to be screen-positive when > 3 of 10 of their ODD symptoms are scored at 2 or 3 on the teacher VADRS.

Additional items on the VADRS also ascertain the presence or absence of impairment in functioning, another pre-requisite before making any ADHD or ODD diagnoses. Both the parent and teacher VADRSs have

Table 1 Comparison of vanderbilt attention deficit hyperactivity disorders Diagnostic Rating Scales parent *vs* teacher results *n* (%)

		Parent vanderbilt rating scale		Total
		Negative	Positive	
Teacher Vanderbilt	Negative	5 (8)	17 (27)	22 (35)
Rating scale	Positive	13 (21)	28 (44)	41 (65)
Total		18 (29)	45 (71)	63 (100)

eight items to assess children's performance levels, which are rated on a 1 to 5 scale (1 = excellent, 2 = above average, 3 = average, 4 = somewhat of a problem, 5 = problematic). The performance domains in the parent rating scale range from academic performance of reading, writing, and mathematics to social skills by assessing relationship qualities with parents, siblings, and peers as well as behavioral skills to attend organized activities. The performance domains in the teacher rating scale similarly include academic performance of reading, mathematics, and written expression, as well as social skills in terms of relationships with peers, but also behavioral skills such as following directions, not disrupting class, assignment completion and organizational skills. To consider the performance score as a functional impairment either at home or school, at least one item among 8 items must be scored at a 4 or 5 (somewhat of a problem, problematic).

In summary, we considered it as a positive diagnostic result when either parent or teacher's VADRS was positive (> 6 of 9 on either or both of the inattentive and hyperactive-impulsive subscales), along with the presence of impairment. For an ODD screening, either a total ODD score > 10 on parent VADRS or > 3 of 10 ODD items scored at 2 or 3 on teacher VADRS.

Clinical process to diagnose ADHD and ODD

As a part of the initial assessments, comprehensive assessments were conducted by board-certified child and adolescent psychiatrists (CAPs), capturing information from the child's guardians pertaining to the child's growth and development, medical and psychosocial history, school history and performance, test scores, and mental status examinations, as well as semi-structured diagnostic interviews to the parents for ADHD and related conditions, using DSM-IV criteria. The CAPs were free to examine the results of both the parents and teachers VADRS, which were collected not only at the initial evaluation but throughout the 8-mo period to track the child's symptoms and treatment response. No steps were taken to require CAPs to include the VADRS results as a part of their final diagnostic considerations, however.

Analytic approach

For all subjects, we determined the number and pro-

portion of them who met criteria for ADHD and ODD in either a parent or teacher rating scale as well as in both of the scales, based on the recognized scoring criteria for parent and teacher VADRS. We also calculated the number of symptom criteria (items scored at 2 or 3) for ADHD and ODD scales as well as the numerical totals for ADHD and ODD symptoms scales, reporting separate counts for both parent and teacher informants. Then, we computed cross-tabulations of psychiatric diagnoses of ADHD and ODD, referencing CAPs' diagnoses against ADHD and ODD diagnoses derived from the parent and teacher VADRS scores. Cohen's Kappa was calculated to evaluate the degree of concordance between the VADRS and CAP diagnoses. To understand diagnostic discrepancies between CAP and VADRS-derived diagnoses, we further examined ADHD and ODD total scores as well as the numbers of impairment domains from parent and teacher VADRS, comparing groups of patients with/without CAP diagnosis of ODD, using ANOVAs and *t*-tests to evaluate these subgroup differences in VADRS scores. Finally, to evaluate whether CAPs' treatment practices are consistent with recognized guidelines for management of ADHD and ODD, we compared their recommendations for medication and/or behavior therapy as a function of the presence or absence of ODD, examining these recommendations against CAP-rendered ODD diagnoses *vs* VADRS-rendered ODD diagnoses.

RESULTS

ADHD diagnosis and the VADRS

As hypothesized, an initial comparison of parent and teacher VADRS scores indicates that a majority of CAP-diagnosed subjects (92%) met full ADHD diagnostic criteria on either the parent or teacher VADRS. Nearly half of the children (44%) met ADHD criteria on both the parent and teacher VADRSs (Table 1). Five children did not meet full DSM criteria regardless of the informant. A single chi-square for the overall table was $\chi^2 = 0.6$, df = 1, $P = 0.5$. The observed percentage agreement between parent and teacher VADRSs was 52.4%, slightly less than expected percentage agreement by chance alone (56.4%). Calculated agreement by Cohen's Kappa was -0.09, indicating "disagreement" between parents and teachers (95%CI: -0.33 to 0.14).

Of note, DSM requires that a child meets full diagnostic criteria, with sufficient symptoms and impairment in at least one setting, and that the child also presents several ADHD symptoms in at least one other setting. Thus, for 28 of 63 subjects where both parent and teacher rating scales were positive, the child psychiatrists' diagnoses were strongly supported, with evidence of concurrent validity with strong evidence of functional impairments in both parent and teacher impairment domains (Table 2)[5]. Of note, there were 30 cases in Table 1 with only one of the two informants'

Vanderbilt rating scale	ADHD	*n*	Parent VADRS total ADHD score	Teacher VADRS total ADHD score	No. of parent's impairment domains	No. of teacher's impairment domains
Parent	Negative	5	16.6	10.2	3.4	1.8
Teacher	Negative				(SD ± 0.9)	(SD ± 1.5)
Parent	Negative	13	26.4	32.6	1.1	4.4
Teacher	Positive				(SD ± 0.5)	(SD ± 1.9)
Parent	Positive	17	33.7	16.6	2.9	1.7
Teacher	Negative				(SD ± 0.4)	(SD ± 1.6)
Parent	Positive	28	37.3	34.0	3.7	5.7
Teacher	Positive				(SD ± 1.8)	(SD ± 1.6)
Statistics			11.3	21.4	7.5	24.7
F ratio, df = 3			(*P* < 0.0001[1])	(*P* < 0.0001[1])	(*P* < 0.0003[1])	(*P* < 0.0001[1])
Total mean score (%)			32.4	27.1	2.9	4.0

[1]Statistically significant at a significance level of 0.05. ADHD: Attention deficit hyperactivity disorder; VADRS: Vanderbilt ADHD diagnostic rating scale.

		Psychiatrist ODD diagnosis		Total
		Negative	Positive	
Vanderbilt ODD screening	Negative	27 (46)	3 (5)	30 (51)
	Positive	17 (29)	12 (20)	29 (49)
Total		44 (75)	15 (25)	59

ODD: Oppositional defiant disorder.

ratings met full diagnostic symptom criteria (17 parent positive/teacher negative; 13 parent negative/teacher positive).

To better understand whether these cases showed sufficient evidence of a presence of several ADHD symptoms in at least two settings, we computed the parent and teacher total ADHD scores (adding all 18 ADHD items) for all groups as a function of positive/negative VADRS scores. The results in Table 2 indicate that even when one informant shows "negative results", that informant's total scores are still highly elevated, generally just below diagnostic threshold, providing supportive evidence of concurrent validity of CAPs' ADHD diagnoses for these 30 cases.

ODD diagnosis and the VADRS

Among 69 ADHD patients, 59 subjects had completed ADHD and ODD rating scales. Twenty-nine children scored positive for ODD on the VADRS, but CAPs confirmed the ODD diagnosis in only 12 (less than half) of these case-positives, as well as in three additional ODD-negative children. As seen in Table 3, the actual number of observed agreements between CAP and VADRS was 39 (66%), a slight improvement in the number of agreements expected by chance. The calculated agreement by Cohen's Kappa was 0.32,

which is considered "fair" (95%CI: 0.10-0.53). A single χ^2 of the over all table was $\chi^2 = 7.7$ ($P = 0.0057$).

To better understand the discrepancies seen in the VADRSs' and CAPs' ODD diagnoses (Table 3), we calculated the total ODD scores from the parents and teachers' VADRS, comparing their mean total scores across the 4 ODD diagnostic agreement/disagreement categories. Four parents' total ODD scores and two teachers' total ODD sores were missing for incomplete scoring. As seen in Table 4, when comparing parent and teacher total ODD scores, parents rated children with more ODD symptoms than teachers across all of the agreement/disagreement categories. As expected, when both the CAP and the VADRS scales were in agreement for a positive ODD diagnosis (12 cases, fourth row), total parents and teachers' VADRS scores were elevated. However, surprisingly, another 17 cases had similarly elevated parent ODD scores and evident functional impairments on the VADRS, but they were not diagnosed by CAPs with ODD, a particularly surprising finding in view of the fact that CAPs did diagnose 5 additional subjects with ODD, even though their average parents and teachers' ODD scores and number of impairment domains were generally lower than the aforementioned 17 subjects.

Treatment for ADHD Children with ODD symptoms

As seen in Table 5, medication therapy was recommended to almost all (54/59) patients regardless of their ADHD/ODD diagnostic groupings. For 27 patients who did not have ODD, neither by VADRS nor CAP diagnosis, a slight majority (15) received recommendations for medication treatment alone, with most of the remainder receiving recommendations for combined treatment (medication and behavior therapy). In contrast, for the three other diagnostic groups with either a positive VADRS for ODD, a CAP diagnosis of ODD or both, the major treatment recommendation made by CAPs was for combined treatment. A single chi-square for the overall table was Pearson $\chi^2 = 22.8$, df = 6, $P = 0.0009$.

Table 4 Total parent and teacher oppositional defiant disorder scale scores, grouped by positive/negative oppositional defiant disorder diagnostic criteria, child and adolescent psychiatrist *vs* vanderbilt attention deficit hyperactivity disorder diagnostic rating scale

Diagnostic source	ODD	n	Parent VADRS total ODD score	Teacher VADRS total ODD score	No. of parent's impairment domains	No. of teacher's impairment domains
CAP	Negative	27	6.1	1.1	2.3	3.7
VADRS	Negative					
CAP	Negative	17	14.2	4.0	3.7	3.9
VADRS	Positive					
CAP	Positive	3	4.0	2.0	3.3	2.3
VADRS	Negative					
CAP	Positive	12	15.2	10.3	3.2	5.4
VADRS	Positive					
Statistics *F* Ratio, df = 3			24.9 ($P < 0.0001^1$)	12.0 ($P < 0.0001^1$)	2.4 ($P = 0.07$)	1.9 ($P = 0.14$)
Total mean score (%)			10.2 (10.2/24 = 43%)	3.8 (3.8/30 = 13%)	2.9	4.0

[1]Statistically significant at a significance level of 0.05. ODD: Oppositional defiant disorder; CAP: Child and adolescent psychiatrist; VADRS: Vanderbilt ADHD diagnostic rating scales.

Table 5 Psychiatric treatment recommendations for attention deficit hyperactivity disorder patients with *vs* without oppositional defiant disorder comorbidity (*n* = 59)

Diagnostic source	ODD	n	Medication therapy only	Behavior therapy only	Combined treatment
CAP	Negative	27	15 (25%)	1 (2%)	11 (19%)
VADRS	Negative				
CAP	Negative	17	3 (5%)	0 (0%)	14 (24%)
VADRS	Positive				
CAP	Positive	3	0 (0%)	1 (2%)	2 (3%)
VADRS	Negative				
CAP	Positive	12	0 (0%)	3 (5%)	9 (15%)
VADRS	Positive				
Total (%)			18 (30%)	5 (9%)	36 (61%)

ODD: Oppositional defiant disorder; CAP: Child and adolescent psychiatrist; VADRS: Vanderbilt ADHD Diagnostic Rating Scales.

To examine this relationship, we did a posthoc analysis using a 2 × 2 contingency table to examine the frequency of behavioral therapy recommendations (either alone or with combined treatment) as a function of the presence/absence of an ODD diagnosis; thus, 29 of 32 (91%) subjects with a possible ODD diagnosis received a treatment recommendation that included behavior therapy *vs* only 12 of 27 (44%) subjects without a possible ODD diagnosis (Pearson's $\chi^2 = 14.7$, $P = 0.0001$).

DISCUSSION

Our study examined the CAPs diagnostic practices and treatment recommendations for ADHD and its most common comorbidity, ODD, comparing their clinical diagnoses with the results of parent- and teacher-completed VADRS. We also examined CAPs' treatment recommendations against current ADHD treatment guidelines. To our best knowledge, this is the first study to examine CAPs' decision-making processes in a real-world, clinical practice setting where highest quality, specialized care for ADHD might be expected.

ADHD diagnostic findings
As anticipated, in this sample of children presenting

to the ADHD clinic and diagnosed with ADHD by child psychiatrists, 91% of subjects had completed parent and teacher VADRSs available for scoring at the initial visit. Among them, the vast majority of subjects (92%, 58 of 63) met full ADHD diagnostic criteria in at least one setting (parent or teacher) on the rating scales (Table 1). Nearly half of the patients even met full ADHD diagnostic criteria in more than one setting (*e.g.*, home and school). Of note, in the case of ADHD, five conditions must be met before a diagnosis should be made: (1) The child must manifest sufficient symptoms of inattention and/or hyperactivity-impulsivity; (2) several ADHD symptoms must be present in at least two settings (*e.g.*, home, school, with peers, *etc.*); (3) the symptoms must be of sufficient duration (> 6 mo); (4) symptoms must begin during childhood (DSM-IV - before age 7, DSM-5 - before age 12); and (5) other likely or possible explanations for the symptoms must be ruled out during the evaluation (*e.g.*, vision or hearing problems, child abuse, family chaos, *etc.*). Although the DSM diagnostic criteria require that a child meets the symptom criteria threshold (*e.g.*, at least six of nine symptoms), they do not specify that the minimum six be met in more than one setting. Instead, the criteria require the presence of several ADHD symptoms in more than one setting. This two-setting requirement

serves to eliminate "setting-specific" ADHD, which may be associated more with environmental factors rather than intrinsic factors.

To examine whether the 30 children evincing full ADHD symptoms in only one setting had some ADHD symptoms in one other setting, we examined the parents and teachers' total ADHD scores, comparing those children with potentially "setting specific" ADHD to those meeting full criteria in both home and school settings, and finding comparably high symptom levels in all groups (Table 2). These findings provide supportive evidence for the concurrent validity of CAPs' ADHD diagnoses. Furthermore, the fact that similar numbers of patients were identified as meeting full criteria by either parents (*n* = 17, 27%) or teachers (*n* = 13, 21%), may reflect equivalent referral pressures from both sources, and does not support uninformed claims as seen in the media that ADHD is due to single causes, such as bad parents or unskilled teachers. Furthermore, among these 30 subjects, parents or teachers reported ADHD symptom scores were associated with the scores of performance domains reported by the same informant. In ADHD subjects with both screenings positive on the parents and teachers' VADRSs, their functions were severely impaired across the settings. Indeed, previous studies showed that ADHD symptoms were associated with executive function (EF) impairments, which are closely related to performance levels in daily activities in both home and school settings[26]. Yet, we should not ignore the variable behaviors reported by different sources in different settings, which are most likely due to interactions of intrinsic factors and environmental factors such as parent-child relationship qualities or expectation levels of parents or teachers. The considerations of these environmental factors could be more important when providers organize individualized treatment plans. Karpenko *et al*[27] concluded that without having significant treatment response to ADHD symptoms, some of the functional domains still improved reliably. This study suggests that treatment goals should focus not only on ADHD symptoms but also on better functioning in different settings[27].

Only 8% of the patients (*n* = 5) had both negative parent and teacher ADHD VADRS scores. Among these five cases, an in-depth review indicated that most of these patients were classified as ADHD, inattentive subtype, and tended to be comorbid with learning disorders or other chronic diseases. The parents of these five subjects reported relatively high impairments in their children's functions at home.

ODD diagnostic findings
Our findings related to CAPs' ODD diagnoses raise interesting questions: In this ADHD sample, CAPs diagnosed ODD in one-fourth of cases, substantially less than the expected 54% to 84% prevalence rates of ODD found within ADHD patients reported in other clinical sample studies[4]. In contrast to CAPs' diagnoses,

nearly half of our subjects had a positive ODD screening result in either the parent or teacher VADRS, consistent with the possible conclusion that CAPs under-diagnosed ODD in the patients referred to the clinic.

Further evaluation of the 17 cases with a positive VADRS ODD rating scale but not diagnosed with ODD by CAPs revealed that these cases had much higher ODD rating scale scores on the parent VADRS than on the teacher VADRS (Table 4). Although the teacher VADRS ODD scores were lower in these cases, they were nonetheless quite comparable to the average teacher scores reported in three cases where CAPs did make the diagnosis of ODD despite negative ODD screening results. Additionally, the impairment domains reported by parents and teachers were similarly high in all groups, which is an interesting finding considering the association of ADHD score results and performance levels.

In reviewing these findings, we considered the possibility that if only the parents experience disruptive symptoms in their children's behaviors, CAPs might consider the disruptive symptoms to reflect problems in family functioning and parenting skills *vs* a disorder more intrinsic to the child. Another possible consideration that might lead to ODD "under-diagnosis" could be that CAPs do not follow ODD diagnostic criteria per se and are reluctant to make the diagnosis if teachers do not also highly rate ODD symptoms. The fact that most of the CAP-diagnosed, ODD-positive cases had both high parent and high teacher ODD symptom scores (Table 4) is consistent with these interpretations.

To further understand these findings, we considered the possibility that CAPs might tend to dismiss the possibility of an ODD diagnosis if children's ODD symptoms were noted only (or principally) by parents rather than by teachers. To examine this possibility, we conducted a posthoc analysis to assess the likelihood of CAPs making an ODD diagnosis with *vs* without a positive teacher VADRS ODD score. Accordingly, these analyses indicated that CAPs made the ODD diagnosis in 57% (4 of 7) cases where the teacher VADRS ODD score was positive, in 36% (8 of 22) cases with only a positive parent VADRS ODD score, and in 10% (3 of 30) cases with neither parent nor teacher positive VADRS ODD scores (Pearson χ^2 = 8.9, df = 2, *P* = 0.01).

These results suggest that negative ODD screening results from the parent and teacher VADRS has high specificity (27/30 = 90%) in ruling out ODD among patients with ADHD, consistent with earlier reports[13]. However, in this study, CAPs do not appear to rely on parent VADRS ODD screening results to rule ODD in, but do appear to place significant weight on teachers' ODD screening results - a possible decisional component that is NOT found in the DSM criteria. Moreover, the generally low prevalence rate of ODD, as diagnosed by CAPs in this study, raises questions for future research about how, when, and why CAPs make the diagnosis of ODD.

Treatment recommendation findings

In regard to treatment recommendations, for 27 children with no ODD diagnosis made by either CAP or VADRS, more than half of them were recommended for medication only, which can be seen as a conservative approach for an academic psychiatry center. In a previous study examining community-based pediatric care for ADHD, only 17% of children received behavior therapy[28]. In contrast, where CAPs made the diagnosis of ODD, or where the parent or teacher VADRS was positive for ODD, almost all of the patients received recommendations for combined therapy (medication plus behavior therapy), except for a few cases. In four cases, CAPs recommended behavior therapy only without medication for children with ODD symptoms - a group of patients consisting of younger children (mean age of 6.4 years, range 4 to 8 years).

In summary, our findings suggest that CAPs appear to follow diagnostic criteria for ADHD but not for ODD, given our results showing a relatively low prevalence rate of ODD diagnosis for ADHD, and discrepancies between CAPs' ODD diagnoses, with positive ODD screening results on the VADRS. Despite CAPs' apparent failure to make ODD diagnoses, for most of these children with ODD symptoms, whether detected by the CAPs or VADRS, CAPs nonetheless recommended behavior therapy. However, their failing to make ODD diagnoses when appropriate could lead others to underemphasize or even overlook the importance of the role of behavior therapy, *e.g*., neglecting the education of parents in understanding disruptive behaviors and learning necessary parenting skills to manage such behaviors.

Our findings suggest that the rating scales are important in real-world clinical practices as efficient and reliable means of obtaining valid information from both parents and teachers to assist CAPs in making better diagnoses of ADHD and its most common comorbidity, ODD.

We note several limitations intrinsic to this study. First, this was a retrospective study in which non-ADHD patients were excluded. Although we acquired highly completed parent and teacher VADRSs, collected at the initial evaluation in the ADHD clinic, and conducted rigorous medical-record evaluations to assess the accuracy of diagnoses in the ADHD patients, for all that, our results can only explain the accuracy of the diagnoses in the ADHD samples but not the reasons for which ADHD was not diagnosed in the non-ADHD samples. Second, our sample size was relatively small, and subjects were drawn from a single clinic, which may limit the generalizability of our findings. However, the fact that this study was conducted in an academic center psychiatric clinic, where practice standards presumably should be fairly uniform and high, suggests that future studies are needed across a broader range of clinics and clinical settings. Thus, additional research should further describe and evaluate CAP's diagnoses and treatments for ADHD within larger samples and a wider range of community-based settings. Moreover, future studies should address these same issues within the outpatient practices of primary care providers, where even greater discrepancies might be noted.

CAPs' ADHD diagnoses have strong concurrent validity against valid rating scales, but ADHD's most common comorbid condition - ODD - may be under-recognized.

COMMENTS

Background

The American Academy of Child and Adolescent Psychiatry and AAP guidelines for attention deficit hyperactivity disorders (ADHD) practices have emphasized the importance of accurately diagnosing ADHD and its comorbidities to provide appropriate treatment options for ADHD children. However, its most common comorbidity, oppositional defiant disorder (ODD), has been reported with a wide range of prevalence rates due to a heterogeneity of the research outcome measures of ODD symptoms. Because ADHD children comorbid with ODD are at greater risk for social and academic dysfunctions, diagnosing ODD within ADHD early on is critical to prevent the externalizing behaviors from progressing. Furthermore, implementations of the established diagnostic and treatment guidelines for ADHD practices have been examined in primary care settings but not in a setting of real-world child and adolescent psychiatric practice.

Research frontiers

To make the time-consuming diagnosing process more efficient and accurate, the parent- and teacher-reported Vanderbilt ADHD diagnostic rating scales, (VADRSs) known to have high concurrent validity with ADHD diagnoses (γ = 0.79), were introduced to ADHD clinical practices. It has been suggested that the current comorbidity-scoring system in the VADRSs is excellent at ruling out ODD/CD but not at ruling in ODD/CD. However, Becker *et al*[13] examined the utility of the VADRSs to assess ODD/CD in relation to ADHD and found that a total score > 10 of the 8 ODD items on the parent VADRS, which was not in the original scoring instruction, showed high sensitivity and specificity against C-DISC-IV diagnoses for ODD.

Innovations and breakthroughs

Given the previous studies' results of high agreement of specific VADRSs thresholds against DISC-derived diagnoses, The authors compared the results of the parent and/or teacher VADRSs with clinicians' final diagnoses of these conditions. The authors also reasoned the physicians' treatment recommendations by comparing them with established treatment guidelines for ADHD and ODD practices. Theirs findings suggest that child and adolescent psychiatrists (CAPs) follow the guidelines for ADHD diagnoses and treatments but not entirely for ODD.

Applications

The VADRSs are an efficient tool to assist ADHD practices in diagnosing ADHD and ODD. CAPs and primary care physicians can gain more benefits through understanding the strengths and weaknesses of the VADRSs. Clinicians'

attentions to the ODD scorings will help to identify more ODD cases within ADHD. Further research to examine the best practice of ODD in ADHD children is required.

Terminology

ADHD: ADHD is a condition which has difficulties with attention, increased activity, and impulsivity; Cohen's Kappa: Cohen's Kappa is a statistic measure of the agreement between two groups who rate categorical data as agreed or disagreed, considering the agreement by chance alone; Concurrent validity: Concurrent validity is a measure of the extent to which a particular test correlates with a previously established measure; Confidence interval (CI): CI is a type of interval estimate of a population parameter; ODD: ODD is a condition which includes an ongoing pattern of defiant behavior toward authority figures that disturb the children's daily functioning.

Peer-review

Very nice written paper with high clinical impact.

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Retrospective Study

Poor CD4 count is a predictor of untreated depression in human immunodeficiency virus-positive African-Americans

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Abstract

AIM: To determine if efforts to improve antiretroviral

therapy (ART) adherence minimizes the negative impact of depression on human immunodeficiency virus (HIV) outcomes.

METHODS: A cross-sectional study of a clinic-based cohort of 158 HIV seropositive (HIV+) African Americans screened for major depressive disorder (MDD) in 2012. CD4 T lymphocyte (CD4+) counts were obtained from these individuals. Self-report on adherence to ART was determined from questionnaire administered during clinic visits. The primary outcome measure was conditional odds of having a poorer CD4+ count (< 350 cells/mm^3). Association between CD4+ count and antidepressant-treated or untreated MDD subjects was examined controlling for self-reported adherence and other potential confounders.

RESULTS: Out of 147 individuals with available CD4+ T lymphocyte data, 31% hadCD4+ count < 350 cells/mm^3 and 28% reported poor ART adherence. As expected the group with > 350 cells/mm^3 CD4+ T lymphocyte endorsed significantly greater ART adherence compared to the group with < 350 cells/mm^3 CD4+ T lymphocyte count ($P < 0.004$). Prevalence of MDD was 39.5% and 66% of individuals with MDD took antidepressants. Poor CD4+ T lymphocyte count was associated with poor ART adherence and MDD. Adjusting for ART adherence, age, sex and education, which were potential confounders, the association between MDD and poor CD4+ T lymphocyte remained significant only in the untreated MDD group.

CONCLUSION: Therefore, CD4+ count could be a clinical marker of untreated depression in HIV+. Also, mental health care may be relevant to primary care of HIV+ patients.

Key words: Human immunodeficiency virus positive; Depression; CD4 T lymphocyte count; Antiretroviral Therapy; African Americans

Core tip: A retrospective data review was done on human immunodeficiency virus+ patients of a primary care clinic. We examined data on depression diagnosis of patients over a two-year period. Antiretroviral therapy (ART) adherence and major depressive disorder were associated with CD4+ lymphocyte counts. Non-treatment of depression was associated with poor CD4+ lymphocyte count independent of ART adherence.

INTRODUCTION

Human immunodeficiency virus (HIV) epidemic is a major public health concern, with significant clinical, social and economic impacts, disproportionately affecting minority populations who have less access to care. Approximately 1.2 million people are living with HIV in the United States[1] with 50000 new infections yearly and 1 out of 5 infected people unaware of their status[2]. Since its discovery, HIV/AIDS (acquired immunodeficiency syndrome) has been studied and researched widely and there have been significant improvements in our knowledge of HIV disease and its treatment. The advent of highly active antiretroviral therapy (HAART) has made it possible to suppress the infection, thus prolonging life and putting HIV in the class of chronic long-term infections for many individuals.

Evidence strongly suggests significant association between HIV infection and depression[3]. Prevalence rates of depression are significantly higher in HIV-infected (HIV+) individuals than in the general population[4-7]. The prevalence of depressive symptoms or mood disorders in HIV+ individuals may be about 30% in the United States[4,8]. These prevalence rates vary across specific population groups in HIV+ individuals, with studies reporting higher prevalence rates in HIV+ women than in HIV+ men[3,9,10] and increased depression risk among HIV+ individuals with substance abuse disorders[4,11,12] and among those who have same-sex sexual partners[13,14]. While the prevalence rate of depression decreases with increasing age for the general adult population, this may not be the case for HIV+ adults as evidenced in some studies[15]. Although earlier studies failed to depict this relationship, recent longitudinal studies, especially since the advent of HAART, have observed significant associations between depression and markers of poorer HIV disease outcomes including lower CD4 counts and higher viral loads[16]. The reported associations between depression and HIV disease severity might be mediated by risky behaviors, such as substance abuse, unsafe sexual practices or ART non-adherence, which may further increase transmission risk or exacerbate infection in HIV+ individuals - especially with new drug-resistant viral strains. Indeed, majority of current studies on the subject suggest that co-morbid depression may negatively impact adherence to ART, while non-adherence to HAART, depression and lack of antidepressant use have been associated with accelerated progression of HIV[16-18] and increased mortality[19-21]. On the other hand, given the growing body of scientific evidence implicating important role of immunologic mechanisms in the pathophysiology of major depressive disorder (MDD)[22-24], especially in people with chronic illnesses, it is plausible that the association between MDD and CD4+ outcome could be driven by immunologic mechanisms that are independent from ART adherence and other relevant health behaviors.

This study was conducted with a clinical cohort

of African-American HIV+ individuals with or without MDD comorbidity, who reside in a community that has received intense focus and concerted efforts to improve HIV screening, treatment and ART adherence. In an effort to determine if integrating specialized depression management is needed in addition to intense ART adherence promotion, we collected clinical and immunologic data to examine the indirect and direct associations between MDD and CD4+ counts, adjusting for ART adherence. We hypothesized that non-adherence to ART and non-treatment of co-morbid depression would be independently associated with poor CD4+ levels on admission.

MATERIALS AND METHODS

Study site and sample
The study sample consisted of adult HIV+ African Americans who received care at the Family and Medical Counseling Service (FMCS), Inc. during years 2011 and 2012, and who were formally screened for depression during their intake registration. The FMCS, Inc. is a primary care center that provides health services for the largely African American communities living in the South-East zone district of Washington, DC. A blood specimen was collected for each patient at entry, for measurement of viral load, immunologic profiles and other routine tests. All participants completed a detailed biopsychosocial form, comprised of sociodemographic, behavioral, and medical information. All collected data were stored in an encrypted and password-protected electronic medical record (EMR). To search for additional information missing in the EMR, such as names of ART prescribed prior to intake admission and names of abused substances, we reviewed patient paper documents and charts with handwritten notes. This retrospective study was approved by the Howard University Institutional Review Board.

Assessment of depression
As part of their intake registration into the clinic between 2011 and 2012, patients were interviewed with the Substance Abuse and Mental Illness Symptoms Screener (SAMISS), a well-validated 16-item clinician-administered questionnaire that screens for patterns of substance abuse and key psychiatric syndromes, including manic and major depressive episodes, generalized anxiety disorder, panic disorder, post-traumatic stress disorder and adjustment disorder[25]. Additionally, the SAMISS inquires about use of antidepressants in the past year. It is usually administered in 10 min, and has exhibited good psychometric properties[26]. All intake clinical personnel at FMCS were trained to administer the SAMISS in a reliable manner. Patients interviewed with the SAMISS were asked if they had experienced a period lasting two weeks or more in the past year when they felt depressed, and then subsequently asked if they received a diagnosis or treatment(s) for depression. An affirmative response to a two-week period of depression

was used for classification of MDD in this study. All patients who screened positive for MDD in the past year either received antidepressants or a physician's diagnosis of MDD, thereby providing some support to the validity of self-report of MDD in this questionnaire.

Other assessments
CD4+ T lymphocyte counts were measured from blood specimen collected from each patient on the day of clinic intake and during follow-up visits. These results were abstracted from the EMR, were all clinical and laboratory data were stored. We used the most recent CD4+ count for the purpose of this study.

ART adherence and other potential correlates of CD4+ outcome, including age, gender, education, monthly income, housing, and insurance status were derived from the biopsychosocial section of the EMR. As part of their intake interview and during subsequent primary care visits, all patients regardless of their HIV status were asked if they knew about their HIV status, and if they were receiving HIV treatments. Patients who acknowledged being HIV positive were subsequently asked what percentage of time they took their HIV treatments as prescribed by their physicians. For the purpose of this study, we used 80% adherence as threshold for classification of adequacy of ART adherence: $\geqslant$ 80% adherence was classified as good or adequate adherence and < 80% was classified as poor or inadequate adherence. Adherence status for subjects who recently became seropositive for HIV was classified as unknown. Substance abuse and pattern of alcohol use were determined from responses to questions in the SAMISS. Problem drinking was defined as heavy drinking (*i.e.*, > 4 drinks per drinking episode) in addition to having experienced difficulty cutting down on drinking or social problems as a consequence of drinking. Additionally, two questions in the SAMISS specifically assessed abuse of prescription and nonprescription drugs in the past year. Presence or absence of substance abuse history was determined from questions in SAMISS inquiring about problem drinking, illicit drug use, and abuse of prescription drugs.

Statistical analysis
The STATA statistical package, version 12 was used to carry out this analysis. The CD4 count data was incomplete with 11 missing values. Since our outcome of interest is CD4 count differences across groups, we excluded subjects with missing information on CD4 count from the regression analyses. We could efficiently do so because the individuals with missing CD4 count values had similar distribution as non-missing across other variables in the data set.

Age, monthly income, and CD4 T lymphocyte count were grouped into categories because of their highly skewed distributions. Age was thus categorized into < 35 years, 35-55 years and > 55 years. Monthly income was grouped into categories according to quartiles. CD4 count was categorized into < 350 cell/mm^3

Table 1 Demographic and behavioral characteristics of study participants with and without a CD4 count of < 350 cells/mm³			
Characteristics	**CD4 count > 350** (*n* = 102) %	**CD4 count < 350** (*n* = 45) %	***P***
Gender			
Male	62.75	51.11	0.19
Female	37.25	48.89	
Age groups (yr)			
< 35	12.75	17.78	0.16
35-55	65.69	73.33	
> 55	21.57	8.89	
Monthly income ($)			
0-200	35.11	27.27	0.72
204-670	15.96	22.73	
672-739	25.53	25	
743-2650	23.4	25	
Educational status[1]			
College	22.22	42.31	0.049
No college	77.78	57.69	
HIV treatment adherence			
No	20.59	44.44	0.004
Yes	50	44.44	
Unknown	29.41	11.11	
Substance abuse			
No	38.24	44.44	0.48
Yes	61.76	55.56	
Problem drinking			
No	63.73	61.36	0.79
Yes	36.27	38.64	
Depression			
No	66.67	46.67	0.02
Yes (treated)	24.51	28.89	
Yes (untreated)	8.82	24.44	

[1]Data on education was missing in 49 patients. HIV: Human immunodeficiency virus.

("poor CD4+ count") and > 350 cells/mm³ based on earlier findings in studies, that observed that deferring treatment until CD4 count was < 350 cells/mm³ was associated with worse prognosis and mortality[27-29].

χ^2 and Fisher's exact test were used to compare social, demographic and behavioral factors by CD4+ status. For all bivariate analyses, a *P* value of < 0.05 was regarded as threshold for significance. With the exception of education status and insurance status, missing response rates were less than 8%. Educational status was missing in 33% of individuals and insurance status was missing in 55% of individuals; hence, these latter variables were not included in our multivariate analysis.

We used multiple logistic regression analysis to determine the independent association of MDD with HIV immunological status, adjusting for MDD treatment, ART adherence, age and gender. Several epidemiologic studies have implicated age and gender as key determinants of MDD and HIV outcomes; hence our decision to include these two variables in our multivariate model[30,31]. Ethnicity, another key determinant was not included because our study base consisted of 99% African Americans. The primary outcome measure of interest was the adjusted OR of poor CD4+ outcome comparing MDD treatment groups to nonMDD controls.

RESULTS

Sample characteristics

From October 2011, when depression screening with SAMISS was first implemented in the FMCS, to July 2012, a total of 158 HIV+ patients were enrolled into treatment, and all 158 (100%) of them completed the SAMISS. The 11 individuals who were missing information on CD4+ counts were excluded, resulting in the final sample size of 147. Table 1 depicts the distribution of demographic and behavioral variables by CD4+ count categories among the 147 individuals included in the analysis. As shown, a total of 45 (31%) patients had baseline CD4+ count < 350 cell/mm³. Majority of the patients were males, between 35 and 55 years of age, and with low monthly income; no significant differences for age, sex or monthly income were observed by CD4+ categories. Group differences were, however, observed for education attainment and ART adherence. Twenty-two percent of those with CD4+ count > 350 cells/mm³ received some college education, compared to 42% among those with CD4+ count < 350 cells/mm³ (*P* < 0.05). However, data on education was missing in 49 patients. As depicted in Table 1, among the patients with CD4 count > 350 cells/mm³, 21% reported poor ART adherence and 50% reported good ART adherence. The ART adherence status of the remaining 29% of patients was classified as "not applicable", since this group consists of patients with recent HIV+ diagnosis, who have not been prescribed ART. Among the category of patients with poorer CD4+ counts (*i.e.*, < 350 cells/mm³), the proportion with poor adherence is reversed, accounting for 44%. Approximately 24% of patients in the poor CD4 group had untreated depression in the past year, compared to 9% of untreated depression among those with CD4+ count > 350 (Table 1). In addition, 53% of patients in the poor CD4 group had past year MDD diagnosis, compared to 33% past year MDD in those with CD4 count > 350 cells/mm³.

Table 2 shows the comparison of ART adherence and other potential demographic and behavioral correlates of poor CD4 outcome between patients with treated MDD, untreated MDD and without MDD. Patients with untreated MDD had the lowest ART adherence rate. No significant differences were observed between these groups in ART adherence. ART adherence was highest among those with treated MDD. Problem drinking rates 55% for the untreated MDD, 46% for treated MDD and 28% for patients without MDD (*P* < 0.02). No significant differences were observed in age, gender, income, education and substance abuse between the MDD categories.

Relationship between depression and CD4+ counts

Table 3 depicts the result of our multiple logistic regression analyses for determination of the independent

Table 2 Demographic and behavioral characteristics of study participants with or without depression

Characteristics	No MDD ($n = 89$) (%)	MDD treated ($n = 38$) (%)	MDD untreated ($n = 20$) (%)	P
Gender				
Male	63.27	50	60	0.35
Female	36.73	50	40	
Age groups				
> 55	24.49	5	15	0.12
35-55	62.24	77.5	70	
< 35	13.27	17.5	15	
Monthly income				
0-200	32.61	30.77	33.33	0.32
204-670	17.39	25.64	5.56	
672-739	21.74	30.77	27.78	
743-2650	28.26	12.82	33.33	
Educational status				
No college	70.59	78.57	72.73	0.73
College	29.41	21.43	27.27	
Accommodation				
Yes	60.42	72.50	70	0.35
No	39.58	27.50	30	
HIV treatment adherence				
No	26.53	25	45	0.51
Yes	48.98	52.50	40	
Unknown	24.49	22.50	15	
Substance abuse				
No	41.84	30	45	0.37
Yes	58.16	70	55	
Problem drinking				
No	72.45	53.85	45	0.02
Yes	27.55	46.15	55	

HIV: Human immunodeficiency virus; MDD: Major depressive disorder.

Table 3 Logistic regression analysis of potential risk factors for lower CD4 count of < 350 cells/mm^3

Characteristic	OR (P-value, 95%CI)	AOR (P-value, 95%CI)[1]
Depression		
No	REF	REF
Yes (treated)	1.68 (0.22, 0.73-3.87)	1.35 (0.52, 0.54-3.38)
Yes (Untreated)	3.96 (0.008, 1.44-10.88)	3.12 (0.04, 1.07-9.11)
Age		
> 55	REF	REF
35-55	2.71 (0.09, 0.86-8.54)	3.22 (0.03, 1.14-9.11)
< 35	3.38 (0.09, 0.85-13.55)	4.25 (0.03, 1.15-15.69)
Gender		
Male	REF	REF
Female	1.61 (0.19, 0.79-3.28)	1.48 (0.33, 0.68-3.21)
HIV treatment adherence		
No	REF	REF
Yes	0.41 (0.03, 0.18-0.92)	0.38 (0.03, 0.17-0.89)
Unknown	0.18 (0.003, 0.06-0.54)	0.17 (< 0.01, 0.05-0.57)

[1]Model includes: MDD, age sex and adherence. REF: Reference category; HIV: Human immunodeficiency virus; MDD: Major depressive disorder; AOR: Adjusted odds ratio.

DISCUSSION

In this study of HIV+ individuals of African-American ethnic background, we found a 39% prevalence of positive screen for depression in the past year, higher than the prevalence rates in the general population and in keeping with previous studies. Untreated depression was associated with lower CD4 T lymphocyte counts, suggesting a significant relationship between untreated depression and immune status in HIV+ individuals. Poorer immunological status was also significantly associated with younger age and non-adherence to ART. These findings are very important as the ultimate result of lower CD4 T lymphocyte counts in HIV disease is progression to AIDS and mortality[32,33].

CD4 T lymphocytes counts are reported as the number of cells in a cubic millimeter of blood. A normal CD4 count is from 500 to 1500 cells per cubic millimeter of blood. CD4 T lymphocytes are primarily targeted by HIV viral cells and play an important role in host immune defenses against opportunistic infections[34]. With progressive HIV disease, CD4 count levels drop to low levels resulting in AIDS defining illness and increasing mortality. As such, CD4 count is one of the most important prognostic markers of HIV-1 disease progression and an important measure in the decision for commencement of ART in HIV+ patients. Our finding of reduced CD4 T lymphocyte count in association with untreated depression has potential clinical significance, as it suggests that CD4 count could potentially be used as clinical marker of untreated co-morbid depression in HIV+ individuals, in addition to its well-established value as marker of HIV disease severity[35].

Efforts to understand the role of the immune system in major depression have been going on for 3 decades. The majority of studies have focused on the

association between MDD and poor CD4+ outcome, adjusting for ART adherence, age and gender. The result of simple logistic regression for each variable in the multiple regression was included to aid comparison of indirect versus direct (*i.e.*, independent) associations. In the un-adjusted analysis (second column of Table 3), the odds of having poor CD4 count in the treated MDD group were 1.68 times that of the non-MDD group, and this difference was not significant ($P < 0.3$). On the other hand, there was an almost 400% increase in odds of poor CD4 outcome in the untreated MDD group compared to the reference (nonMDD) group ($P < 0.008$). Adjusting for age, gender and ART adherence, the odds ratio (OR) of poor CD4 comparing untreated MDD to non-MDD patients was 3.12 ($P < 0.04$). The unadjusted analysis revealed a 60% decreased odds of poor CD4 count outcome comparing the group with good ART adherence to the group with poor ART adherence ($P < 0.03$). Also in the multivariate analysis, there was a significant inverse association between ART adherence and poor CD4 counts (OR = 0.38, $P < 0.03$). Our multiple logistic model revealed significant associations between the younger age groups (*i.e.*, < 35 years and 35 to 55 years) and poor CD4 outcome, treating the older age group (*i.e.*, > 55 years) as reference group.

role of activated innate immune system in depression, with fewer studies on the role of T lymphocyte cells and other adaptive immune responses[36]. It is of note that some initial studies on T lymphocyte responses in hospitalized depressed patients, revealed a reduced proliferation of T cells in response to T cell mitogens - phytohemagglutinin, concanavalin A[37-39], but these findings were not always reproducible. To elucidate this discrepancy, an extensive metaanalysis of more than 180 studies comprising greater than 40 immune measures, revealed mild reductions in Natural Killer cell count, decreased T cell count and T cell proportions and reduced lymphocyte proliferation responses in depressed patients compared to their controls[40].

The evidence based data depicting T lymphocyte dysfunction in depressed individuals, furthered studies on the role of depression in HIV progression. An extensive literature review on studies carried out in both pre-HAART and post-HAART eras showed depression exacerbates HIV progression to AIDS, with at least three studies further depicting an independent association of depression with decreased CD4 T lymphocyte count in individuals with HIV[32]. An example is a longitudinal study of a multiethnic 177 HIV positive men and women, with CD4 counts of between 150 and 500, followed for 2 years. After adjusting for age, gender, antiretroviral treatment and adherence, cumulative depression and hopelessness were associated with decreases CD4 count and increase in viral load[41].

Our results also show a significant association between ART treatment adherence and CD4+ T lymphocyte count, which is in keeping with previous studies[32,42]. Individuals who were adherent were approximately 60% less likely to have a poor CD4 count in the univariate analysis and after controlling for depression, age and gender. Other studies examining different populations have found depression to be associated with poor ART adherence[41-43]. One of such studies of HIV infected injecting drug users, revealed an association between depression and ART non-adherence as a strong indicator of clinical progression[44]. Another study on indigent low income ethnic minorities with HIV, consisting of mainly African-American population, revealed a significant association between depression and poorer ART adherence[45]. Thus, some studies also discussed the possibility of ART adherence being a potential mediator of sorts in the relationship between MDD and poor CD4+ T lymphocyte outcomes[32,40,41].

In lieu of the mediating effect of ART adherence on the relationship between depression and CD4 count, our study examined if the strength of the relationship between depression and CD4 count diminished by adjusting for ART adherence. Our results confirmed an independent relationship between depression and CD4 count. There was however a 21% decrease in the odds of poorer CD4 count in the untreated depression group after adjusting for ART adherence. Hence, our study shows that ART adherence may be a confounder in the association between untreated depression and poorer

CD4 count, especially in light of the fact that nearly 50% of individuals with untreated depression also were non adherent to ART. However, larger prospective studies will be beneficial in further examining the nature of these relationships.

This study has some limitations that should be acknowledged. The cross-sectional design and small sample size limit our ability to make inferences about causal direction of the observed associations and their significance, respectively. The study sample consisted exclusively of African-American individuals from a specific urban community. While this limits the generalizability of the findings, it also provides natural control for potential sociodemographic (*e.g.*, poverty, health disparities) and cultural, racial or community-bound factors that could have confounded the results. It also provides clues that may help inform future clinical and research efforts to adapt evidence based-psychological and social interventions, including mental health screening and treatment monitoring protocols in a manner that will accommodate the sociocultural dimensions of HIV, depression, and salient health behaviors that may be unique to predominantly low-income, African-American urban communities.

In summary, we found that depression is highly prevalent in this population of HIV+ individuals and untreated depression and poor ART adherence are independently associated with poorer CD4 T-lymphocyte outcomes. Furthermore, it contributes to growing appreciation of other possible pathways linking depression to HIV outcome, other than the mediating role of ART adherence. Our findings need to be validated in a larger cohort with follow up over time. There is also a need for further research into the factors that may predispose this community to poorer outcomes. The lower mean income and educational attainment in this clinical cohort also suggests the potential relevance of integrating specialized mental health and case management services into community primary care centers in for better management of HIV+ patients.

ACKNOWLEDGMENTS

COMMENTS

Background

Human immunodeficiency virus (HIV) epidemic is a major public health concern. Approximately 1.2 million people are living with HIV in the United States with 50000 new infections yearly. Evidence strongly suggests significant association between HIV infection and depression. Prevalence rates of depression are significantly higher in HIV-infected individuals in comparison to the general population. The reported associations between depression and HIV disease severity might be mediated by risky behaviors, such as substance abuse,

unsafe practices or antiretroviral therapy (ART) non-adherence. Consequently, these factors may further increase transmission risk or exacerbate infection in HIV+ individuals - especially with new drug-resistant viral strains. Recent studies have shown significant association between depression and markers of poorer HIV disease outcomes including lower CD4 counts and higher viral loads. In contrast, the association between major depressive disorder (MDD) and CD4+ outcome could be driven by immunologic mechanisms that are independent from ART adherence. To examine the indirect and direct associations between MDD and CD4+ count, adjusting for ART adherence, the authors collected clinical and immunologic data from the cohort of African-American HIV+ individuals with or without MDD comorbidity. The aim of this study is to see the association between non-adherence to ART and non-treatment of co-morbid depression with poor CD4+ levels on admission.

Research frontiers

Majority of the current studies suggest that co-morbid depression may negatively impact adherence to ART, while non-adherence to highly active ART, depression and lack of antidepressant use have been associated with accelerated progression of HIV and increase mortality. On the other hand, given the growing body of scientific evidence implicating important role of immunologic mechanisms in the pathophysiology of MDD, especially in people with chronic illnesses, it is plausible that the association between MDD and CD4+ outcome could be driven by immunologic mechanisms that are independent from ART adherence and other relevant health behavior.

Innovation and breakthroughs

Efforts to understand the role of the immune system in major depression have been going on for 3 decades. The majority of studies have focused on the role of activated innate immune system in depression, with fewer studies on the role of T lymphocyte cells and other adaptive immune responses. In this study, the authors found 39% prevalence for depression, which is higher than the general population. Untreated depression was associated with lower CD4 T lymphocyte counts, suggesting a significant relationship between untreated depression and immune status in HIV+ individuals. Poorer immunological status was also significantly associated with younger age and non-adherence to ART.

Applications

ART adherence may be a confounder in the association between untreated depression and poorer CD4 count. This study provides the evidence that non-adherence to ART and non-treatment of co-morbid depression would be independently associated with poor CD4+ levels on admission. Practical application: To determine if recent efforts to improve ART adherence among HIV+ African Americans attending a primary care clinic sufficiently minimizes the negative impact of depression on HIV outcomes.

Terminology

Epidemics: Widespread outbreak of infectious disease in a population or community; Cohort: Group of people with common characteristics.

Peer-review

The manuscript explores interesting relationship between CD4 count and depression. It found that there is association between poor CD4 count and untreated depression.

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Prospective Study

Isotretinoin was not associated with depression or anxiety: A twelve-week study

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Abstract

AIM: To investigate the frequency and severity of depression and/or anxiety in isotretinoin (ITT)-treated subjects and in a non-ITT control group.

METHODS: Sixty consecutively-admitted non-psychiatric outpatients with acne were assigned to either ITT at a fixed dose of 30 mg/d ($n = 36$) or "other treatment" group (OT; $n = 24$). The Zung depression or anxiety scales (with cut-off points), two locally developed scales for depression (GeDepr) and anxiety (Ansilet) (without cut-off points) and clinical global impression scales of acne severity were administered at baseline and at weeks 6 and 12 of treatment. Data was analyzed with the chi-squared test and covariance analysis.

RESULTS: Gender distribution, age, marital status and education level did not differ between both treatment groups. The frequency of depression, as defined by the Zung scale cut-off points was similar in the ITT and in the non-ITT groups: Weeks 6 and 12: 8.3% in both groups, $P = 0.9$. The frequency of anxiety was similar in the groups as well: Week 6: ITT = 8.3%; OT = 0.0%, $P > 0.05$; week 12: ITT = 11.1%, OT = 4.2%, $P > 0.05$. The scores in both scales' sets did not differ between the treatment groups at any evaluation time point (P

> 0.05). Five ITT-treated subjects (13.8%) and two from the OT-treated group (8.3%) developed clinically significant anxiety and/or depression during treatment ($P > 0.05$).

CONCLUSION: Our study confirms the safety of ITT regarding psychological side effects in regular dermatological patients. Susceptible subjects may exist but their identification requires additional strategies.

Key words: Isotretinoin; Short-term; Psychopathology; Depression; Anxiety; Other treatments

Core tip: Isotretinoin (ITT) is frequently used for acne therapy, particularly in young people, but concerns exist regarding the risk of depression and suicide attempts. We conducted a 12-wk prospective study administering a fixed ITT dose in non-psychiatric acne patients an in a non-ITT control group. We used categorical and continuous scales for the assessment of depression and anxiety. The frequency and severity of psychopathology was similar in both treatment groups, stressing the safety of ITT in typical dermatological patients. However, 13.8% ITT subjects and 8.3% of the non-ITT developed clinically significant anxiety and/or depression. Hence, susceptible subjects exist, who deserve further investigation and assistance.

INTRODUCTION

Isotretinoin (ITT) is a vitamin A-related compound widely used in the treatment of severe cystic acne vulgaris[1]. It is less frequently used to treat squamous-cell carcinoma, brain or pancreatic cancer and severe ichtyosis[2].

The safety of ITT on mental functioning has been a concern since its introduction into clinical practice. For example, as early as in 1983, clinically significant depression was reported in a case series[3].

The association between ITT treatment and psychopathology, particularly with depressive symptoms, comes from accumulated evidence of case reports, temporal association, challenge-re-challenge tests, dose response/class effect studies, and biological plausibility[4]. As a matter of fact, ITT was among the top five drugs with the most frequent spontaneous reports of depression in a recent survey conducted in the United Kingdom[5]. Collectively, these results have been criticized for not discriminating between the worsening of pre-existing psychopathology and the development of *de novo* psychopathology[4].

The frequency of depression during ITT administration observed in group studies ranges from 1% to 11%[4]. The low frequency of depression obtained in some studies has been associated with protocols based on self-reports of psychological symptoms. Research has shown that patients tend to underestimate the magnitude of depression in comparison to their family controls[6]. Besides, numerous negative studies lacked standardized diagnostic protocols and/or control groups[4].

Current research trends suggest that the risk of ITT-related depression maybe particularly relevant in a minority of patients who are also susceptible to other ITT neural side effects, such as headache[4,7]. Besides, subjects with bipolar, anxiety or obsessive-compulsive disorders may be at risk of clinical worsening during ITT administration[4,8]. By contrast, numerous practitioners and research studies report that ITT may rather improve the psychological status in patients with severe acne[4]. These results are not in contradiction with the finding of a specific minority of subjects with increased susceptibility to ITT-related psychological side effects.

In this longitudinal 12-wk study, we evaluated depression and anxiety symptoms in acne patients receiving either ITT or antibiotics by using not only the well-known Zung scales for depression/anxiety but also a set of instruments developed and previously validated at Los Andes University, Mérida, Venezuela.

MATERIALS AND METHODS

This is a naturalistic, longitudinal, open-labeled study conducted in a non-probabilistic sample from February 2013 to June 2013 at the Dermatology Department of Los Andes University Hospital in Mérida, Venezuela. It was approved by the Ethics Committee and by the Institutional Review Board of the Department of Psychiatry, Los Andes University Medical School, Mérida, Venezuela. No formal trial registration system exists in this country.

Subjects and treatments

Consecutively admitted patients consulting for acne were invited to participate in the study and filled an informed consent of voluntary participation. Treatments were assigned by a dermatologist (YC) according to disease severity and patient's income. The participating subjects were assigned either to the ITT-group (30-40 mg/d) or to the OT-group that consisted in the administration of a combination of oral or topic antibiotics and the antibacterial agent benzoil peroxide. Exclusion criteria were previous administration or intolerance to ITT, < 18 years of age, and/or explicit refusal to participate in the study.

Evaluation

Acne severity was assessed by the dermatologist at

Table 1 Demographic features

Isotretinoine (*n* = 36)		Other treatments (*n* = 24)	
Gender [*n* (%)][1]			
Females	Males	Females	Males
16 (44.4)	20 (55.6)	11 (45.8)	13 (54.2)
Age (mean ± SD)[2]			
23.0 ± 3.2	20.3 ± 2.9	22.9 ± 3.8	23.4 ± 4.4
Marital status [*n* (%)][3]			
Single	Others	Single	Others
34 (94.4)	2 (5.6)	15 (62.5)	9 (37.5)
Education [*n* (%)][4]			
College	Others	College	Others
6 (16.6)	35 (83.4)	4 (16.7)	(83.3)

[1]χ^2 (1) = 0.01, P = 0.9; [2]F (3) = 2.9, P = 0.04; post-hoc Tukey HSD test = 0.07; [3]χ^2 (1) = 7.8, P = 0.005; [4]χ^2 (1) = 0.00, P = 1.

baseline only by using a 5-point, likert-type, clinical global impression scale, from 0 as "minimally severe" to 5 as "very severe". At weeks 6 and 12, the patients and the treating physician assessed the changes in acne severity compared to baseline by using a 7-point, likert-type scale, from 0 as "markedly worst" to 7 as "very much improved".

Depression and anxiety were evaluated at baseline and at weeks 6 and 12 with categorical and continuous self-administered scales. The former consisted in the validated Spanish versions of the Zung depression and anxiety scales that provided a categorical classification[9-11]. Every scale consisted of 20 items with positive or negative valence that explored the frequency of depression or anxiety signs and symptoms, as follows: From 1 = rarely to 4 = always. Each subject was then classified as non-depressed or non-anxious when scoring < 50 points, or slightly, moderately or severely depressed and/or anxious when scoring 50-59, 60-69 and ≥ 70 points, respectively.

The continuous scales corresponded to the previously mentioned Zung scales plus the following two scales for continuous quantification of depression or anxiety levels. The "Ge-Depr" is a two-factor scale consisting of 16 depression-related items. It was validated in 249 Venezuelan subjects and reported a Cronbach alpha coefficient of 0.88 and Pearson correlation coefficients of 0.65 *vs* an aggression scale, of 0.68 *vs* an anxiety scale, and of 0.65 *vs* a general scale of psychological maladjustment[12].

The "Ansilet" is a one-factor scale consisting of 15 anxiety-related items. It was validated in 264 Venezuelan university students and reported a Cronbach alpha coefficient of 0.82 and a Pearson correlation coefficient of 0.59 *vs* the self-esteem Rosemberg scale[13].

Both local scales were scored with a 6-point likert-type scale from 0 = complete disagreement to 6 = complete agreement and no neutral score. These scales did not include a cut-off point but are aimed to assess changes over time in a continuous scale.

A separate analysis was conducted with the Zung depression scale items that explore suicide-related

ideation (items 17 and 19).

Statistical analysis
Categorical data (frequency of depression or anxiety according to the Zung scales from baseline to week 12 with intra-group and inter-group comparisons) were analyzed with the χ^2 test.

Continuous data (depression and anxiety scores) were analyzed with the univariate general linear model with treatment and time as between- and within-group variables, respectively, and age as covariate.

Bivariate correlation analysis was conducted with the Pearson correlation coefficient. Results were considered significant when $P < 0.05$.

RESULTS
Sixty consecutively admitted patients were assigned either to the ITT-group (*n* = 36) or to the OT-group (*n* = 24). No subject was excluded in any group. Gender distribution and education level were similar in both groups, but the ITT-treated males tended to be younger, and the proportion of single (unmarried) subjects was significantly higher in the ITT group (Table 1). Most patients (35 out of 36) received 30 mg/d of ITT; hence, the treatment protocol consisted of a fixed-dose schedule.

Acne severity was higher in the ITT - than in the control group. Severe and very severe acne[9] was only observed in the ITT group: 9 subjects (25%) *vs* 0 subjects (0%): Mann Whitney test: Z = 2.6, P = 0.008.

Depression and anxiety frequency and scores
The frequency of depression or anxiety categorically defined according to the Zung scales and the scores obtained in the continuous scale analysis were similar in both treatment groups at baseline and did not display significant changes over time (Tables 2 and 3). The scores in the depression Zung scale suicide related items were similar in both treatment groups at baseline and at weeks 6 and 12: Item 17: F (1) = 0.001, P = 0.9; item 19: F (1) = 0.5, P = 0.5 (data not shown).

The Zung depression and anxiety scale scores showed a highly significant positive correlation with the GeDepr and Ansilet scale scores, respectively: The r coefficient value was over 0.60, P = 0.000 in all the analyses (data not shown).

Correlation analysis
Clinical assessment of acne severity and depression/anxiety scores: For the sake of concision, we only present the data related to the GeDepr and Ansilet scale scores.

No significant correlations were observed between the clinical severity assessment of acne at baseline and the depression and anxiety scores at any time-point in the ITT group. However, in the OT group, significant correlations were observed in the depression scores,

Table 2 Frequency of depression and anxiety according to the Zung scales at baseline and during treatment

	ITT			OT		
	Basal	Week 6	Week 12	Basal	Week 6	Week 12
Categorical level of depression [*n* (%)][1]						
Mild	1 (2.8)	3 (8.3)	3 (8.3)	2 (8.3)	2 (8.3)	2 (8.3)
Moderate	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Severe	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	1 (2.8)	3 (8.3)	3 (8.3)	2 (8.3)	2 (8.3)	2 (8.3)
Categorical level of anxiety [*n* (%)][2]						
	Basal	Week 6	Week 12	Basal	Week 6	Week 12
Mild	1 (2.8)	2 (5.5)	1 (2.8)	1 (4.2)	0 (0.0)	0 (0.0)
Moderate	0 (0.0)	1 (2.8)	2 (5.5)	0 (0.0)	0 (0.0)	0 (0.0)
Severe	0 (0.0)	0 (0.0)	1 (2.8)	0 (0.0)	0 (0.0)	1 (4.2)
Total	1 (2.8)	3 (8.3)	4 (11.1)	1 (4.2)	0 (0.0)	1 (4.2)

Values represent number and percentage in parentheses of subjects with depression or anxiety. [1]Within-group comparisons: ITT group: χ^2 (2) = 1.2, P = 0.5; OT group: χ^2 (2) = 0.0, P = 1; between-group comparison: χ^2 (5) = 1.3, P = 0.9; [2]Within-group comparisons: ITT group: χ^2 (2) = 4.6, P = 0.6; OT group: χ^2 (2) = 4.0, P = 0.4; between-group comparison: χ^2 (5) = 4.8, P = 0.4. OT: Other treatments; ITT: Isotretinoine.

and marginally significant correlations were detected in the anxiety value sets (Table 4).

Patient and physician perceived changes in acne severity *vs* depression/anxiety scores: Several negative correlations reached statistical significance in the ITT group but not in the OT group (Table 5). The negative correlations reveal the improvement in psychological symptoms (decreased depression or anxiety scores) along with the decrease in acne severity (magnitude of the improvement perceived by the patient and the physician at a given time point).

Narrative description of the subjects who developed anxiety and/or depression during treatment

Five ITT-treated subjects (13.8% of the ITT group) and two receiving other treatments (8.3% of the OT group patients) developed clinically significant anxiety and/or depression during treatment [χ^2 (1) = 0.06, P = 0.8] (Table 6). In the five subjects of the ITT group, three were females (60%) and two (40%) reported previous symptoms of depression and/or anxiety.

One subject in each group increased their scores in the suicide-related items in the Zung depression scale (items 17 and 19, data not shown). However, because of the very small number of subjects (5 only), an association between gender and previous psychiatric disorders with ITT-induced psychopathology cannot be inferred.

DISCUSSION

This study showed that oral ITT in a fixed-dose schedule of 30 mg/d, compared to a control group of subjects treated with antibiotics or antibacterial agents, was not associated with a significant increase in the frequency of depression or anxiety or in the scores of

the corresponding scales up to 12 wk of treatment. This result points to a safe profile of short-term ITT administration in a typical group of non-psychiatric outpatients with acne.

The correlation analysis showed complex results in both treatment groups: On the one hand, only the OT group showed significant correlations (positive ones, mainly in the depression scores) between the physician basal evaluation of acne severity and the psychopathological scales over time (Table 4). This may reveal a poor response to OT because one might have expected a trend toward negative correlations, that is the greater the physician appreciation of the acne severity at baseline, the lower the depression or anxiety scores after successful treatment.

On the other hand, only the ITT-treated patients showed the expected negative correlations between the changes in acne severity observed by the physician and those perceived by the patients (higher scores over time) and the scores in depression and anxiety at each evaluation time point (lower punctuations along time (Table 5). As the correlation data in Table 4 show, this result may imply a better response of the ITT-treated group. However, this is only speculative, because the present investigation was not designed to properly assess the comparative treatment response.

Five ITT-treated subjects developed clinically significant psychopathology: Two patients with pure anxiety, one with pure depression and two with both diagnoses. Personal antecedents of depression or anxiety or significant symptoms at baseline were observed only in two of these five ITT-treated patients. Hence, this small sub-sample of patients does not allow us to explore the predisposing features that favored the development of psychopathology during ITT administration.

The frequency of depression in the ITT group, alone or in combination with anxiety, was 8.3% (3 out of 36 subjects). This value is within the range reported in the literature which is between 1% and 11%[14].

Most research on the psychological deleterious effects of ITT has focused on depression development or aggravation and suicide ideation and/or suicide attempts during treatment[4,5,15-27]. Most recent reviews point to a safe profile of ITT regarding depression and/or suicide[28-31] with the exception of a study conducted by an Australian research group[32]. According to a comprehensive review[4], important methodological differences among the studies would explain the divergent results.

The present research agrees with the conclusion that depression occurs in a minority of ITT-treated patients[4]. Recent studies suggest that these depression-prone patients may be susceptible to other neural side effects of ITT, such as headache[6]. Interestingly, preliminary data suggest that subjects with bipolar disorders may be particularly susceptible of symptom worsening during ITT treatment[33,34]. Since migraine is common in bipolar disorder, particularly in the predominantly depressive type[35], and since psychoses, panic attacks

Table 3 Depression and anxiety levels at baseline and during treatment

	ITT			OT		
	Depression scores					
	Basal	Week 6	Week 12	Basal	Week 6	Week 12
GeDep scale[1]	38.3 ± 2.5	38.1 ± 2.3	36.8 ± 2.3	36.9 ± 3.1	34.9 ± 2.9	34.9 ± 2.9
Zung scale[2]	35.2 ± 1.3	33.8 ± 1.4	34.2 ± 1.4	34.9 ± 1.6	34.6 ± 1.7	35.0 ± 1.7
	Anxiety scores					
	Basal	Week 6	Week 12	Basal	Week 6	Week 12
GegDep scale[3]	43.3 ± 2.4	41.9 ± 2.3	40.8 ± 2.3	40.9 ± 2.9	38.1 ± 2.9	36.8 ± 2.9
Zung scale[4]	37.2 ± 1.1	36.7 ± 1.4	36.6 ± 1.6	34.5 ± 1.4	32.4 ± 1.7	32.8 ± 1.9

Values represent mean ± SD scores in the depression and anxiety scales. [1]Within-subject effect: $F_{(2)} = 0.5$, $P = 0.6$; between-subject effect: $F_{(1)} = 0.3$, $P = 0.6$; [2]Within-subject effect: $F_{(2)} = 0.6$, $P = 0.6$; between-subject effect: $F_{(1)} = 0.6$, $P = 0.5$; [3]Within-subject effect: $F_{(2)} = 0.5$, $P = 0.6$; between-subject effect: $F_{(1)} = 0.9$, $P = 0.4$; [4]Within-subject effect: $F_{(2)} = 0.8$, $P = 0.5$; between-subject effect: $F_{(1)} = 3.1$, $P = 0.09$. OT: Other treatments; ITT: Isotretinoine.

Table 4 Correlation matrix between the physician assessment of the acne severity at baseline and the depression and anxiety scores before and during treatment

Treatment group		Depression score (baseline)	Depression score (week 6)	Depression score (week 12)	Anxiety score (baseline)	Anxiety score (week 6)	Anxiety score (week 12)
ITT	PAB *vs*	0.1 (0.4)	0.08 (0.6)	0.02 (0.9)	0.1 (0.5)	0.1 (0.4)	0.05 (0.8)
OT	PAB *vs*	0.48 (0.01)[1]	0.5 (0.01)[1]	0.5 (0.01)[1]	0.25 (0.2)	0.39 (0.054)	0.37 (0.07)

PAB: Physician assessment of acne severity at baseline with a scale ranging from minimal = 0 to very severe = 5. Values are the Pearson correlation coefficient and its associated probability in parentheses. [1]Significant association. OT: Other treatments; ITT: Isotretinoine.

Table 5 Correlation matrix of the patient and physician assessments of the acne severity change and the depression and anxiety scores during treatment

Treatment group		Depression score (week 6)	Depression score (week 12)	Anxiety score (week 6)	Anxiety score (week 12)
ITT	Patient evaluation *vs*	-0.31 (0.067)	-0.43 (0.009)[1]	-0.43 (0.01)[1]	-0.38 (0.02)[1]
	Physician evaluation *vs*	-0.28 (0.1)	-0.33 (0.049)[1]	-0.35 (0.03)[1]	-0.32 (0.057)
OT	Patient evaluation *vs*	-0.1 (0.6)	-0.-08 (0.7)	-0.3 (0.9)	0.25 (0.2)
	Physician evaluation *vs*	-0.15 (0.5)	-0.18 (0.4)	-0.03 (0.9)	-0.08 (0.7)

The assessment in acne severity change was conducted with a Likert-type scale ranging from severely worst = 1 to very much improved = 7. Values are the Pearson correlation coefficient and its associated probability in parentheses. [1]Significant association. OT: Other treatments; ITT: Isotretinoine.

Table 6 Demographic and clinical features of the subjects who developed clinically significant depression or anxiety

Treatment	Age (yr)	Gender	Personal history of depression	Personal history of anxiety	Diagnosis before treatment (categorical Zung scales)	Diagnosis during treatment (categorical Zung scales)
ITT	18	M	Yes	Yes	-	Anxiety
ITT	28	M	No	Yes	Anxiety and depression	Anxiety and depression
ITT	25	F	No	No	-	Anxiety and depression
ITT	23	F	Yes	No	-	Anxiety
ITT	23	F	No	No	Depression	Depression
OT	18	M	Yes	Yes	Anxiety and depression	Anxiety and depression
OT	24	M	Yes	No	Depression	Depression

ITT: Isotretinoin; OT: Other treatments; F: Female; M: Male.

and obsessive doubting worsening have also being reported during ITT treatment[8,36-38], the predictive value of headache as a relatively specific marker for ITT-induced depression should be assessed in future studies.

This research field would also benefit from animal studies that would explore the pathways involved in the induction of depression-related behaviors in rodents, such as changes in brain monoamine transmission and corticotrophin-releasing hormone[39,40]. Finally, it is worthwhile mentioning that genetic studies are currently under way to identify genetic polymorphisms in the retinoic receptors that may predispose to or protect from ITT-unintended effects[41].

The present study has the following strengths: A longitudinal evaluation, a control group, the administration of scales for psychopathological assessment that provided categorical and continuous evaluation and the use of locally-developed scales that provided an adequate wording for this specific clinical population. However, it has several limitations: A relatively small sample size and a relatively short period of psychological assessment; the patients were older than those who typically request acne therapy (*i.e.*, adolescents); evaluations were not blind; treatment assignment was not random, and hence, the acne severity was higher in the ITT group than in the control group, and there was no formal scale to assess such a severity.

Further research should take into account that, while random treatment assignment appears problematic from an ethical point of view, semi-quantitative scales for acne severity have been developed[42]. Since severe acne is in many cases a chronic and severe condition and since ITT is often prescribed in doses higher than 30 mg/d and for prolonged periods, long-term studies with high ITT doses are also needed. In those studies, other physical ITT side effects must be assessed. The influence of ethnicity and previous psychiatric disorders on ITT safety should also be examined in further research.

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COMMENTS

Background
Concerns exist about the risk of depression, suicide risk and/or anxiety during isotretinoin (ITT) administration in acne-treated patients.

Research frontiers
Limited information exists about the psychopathology background and the pretreatment mental status of subjects who developed clinically significant depression/anxiety during ITT administration.

Innovations and breakthroughs
Few studies in ITT-treated subjects include an adequate control group, locally-developed psychopathological scales and a pretreatment evaluation. The authors aimed to overcome these limitations in the present study.

Applications
Approximately one out of ten subjects treated for acne either with ITT or antibiotics in the short term may develop clinically-significant depression or anxiety. Subjects at risk for psychopathology must be identified before starting ITT administration.

Terminology
ITT is a vitamin. A-related compound widely used in the treatment of severe cystic acne vulgaris.

Peer-review
Well written manuscript.

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Internet addiction and problematic Internet use: A systematic review of clinical research

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Abstract

AIM: To provide a comprehensive overview of clinical studies on the clinical picture of Internet-use related addictions from a holistic perspective. A literature search was conducted using the database Web of Science.

METHODS: Over the last 15 years, the number of Internet users has increased by 1000%, and at the same time, research on addictive Internet use has proliferated. Internet addiction has not yet been understood very well, and research on its etiology and natural history is still in its infancy. In 2013, the American Psychiatric Association included Internet Gaming Disorder in the appendix of the updated version of the Diagnostic and Statistical Manual for Mental Disorders (DSM-5) as condition that requires further research prior to official inclusion in the main manual, with important repercussions for research and treatment. To date, reviews have focused on clinical and treatment studies of Internet addiction and Internet Gaming Disorder. This arguably limits the analysis to a specific diagnosis of a potential disorder that has not yet been officially recognised in the Western world, rather than a comprehensive and inclusive investigation of Internet-use related addictions (including problematic Internet use) more generally.

RESULTS: The systematic literature review identified a total of 46 relevant studies. The included studies used clinical samples, and focused on characteristics of treatment seekers and online addiction treatment. Four main types of clinical research studies were identified, namely research involving (1) treatment seeker characteristics; (2) psychopharmacotherapy; (3) psychological therapy; and (4) combined treatment.

CONCLUSION: A consensus regarding diagnostic criteria and measures is needed to improve reliability across studies and to develop effective and efficient treatment approaches for treatment seekers.

Key words: Internet addiction; Problematic Internet use; Gaming addiction; Internet Gaming Disorder; Clinical studies; Treatment seekers; Treatment; Therapy

Core tip: Internet addiction has appeared as new mental health concern. To date, reviews have focused on clinical and treatment studies of Internet addiction and Internet Gaming Disorder, limiting the analysis to a specific diagnosis of a potential disorder that has not yet been officially recognised, rather than a comprehensive investigation of Internet-use related addictions (including problematic Internet use) more generally. This systematic literature review outlines and discusses the current empirical literature base for clinical studies of Internet addiction and problematic Internet use. A total of 46 relevant studies on treatment seeker characteristics, psychopharmacotherapy, psychological therapy, and combined treatment were identified.

INTRODUCTION

Over the last 15 years, the number of Internet users has increased by 1000%[1], and at the same time, research on addictive Internet use has proliferated. Internet addiction has not yet been understood very well, and research on its etiology and natural history is still in its infancy[2]. Currently, it is estimated that between 0.8% of young individuals in Italy[3] and 8.8% of Chinese adolescents[4] are affected. The reported higher prevalence rates in China suggest Internet addiction is a serious problem in China, and the country has acknowledged Internet addiction as official disorder in 2008[5].

A comprehensive systematic review of epidemiological research of Internet addiction for the last decade[6] indicated Internet addiction is associated with various risk factors, including sociodemographic variables (including male gender, younger age, and higher family income), Internet use variables (including time spent online, using social and gaming applications), psychosocial factors (including impulsivity, neuroticism, and loneliness), and comorbid symptoms (including depression, anxiety, and psychopathology in general), suggesting these factors contribute to an increased vulnerability for developing Internet-use related problems. Despite the gradually increasing number of studies concerning Internet addiction, classification is a contentious issue as a total of 21 different assessment instruments have been developed to date, and these are currently used to identify Internet addiction in both clinical and normative populations[6]. Conceptualisations vary substantially and include criteria derived from pathological gambling, substance-related addictions and the number of problems experienced. In addition to this, the cut-off points utilised for classification differ significantly, which impedes research and cultural cross-comparisons and limits research reliability.

Increasing research efforts on Internet addiction have led the American Psychiatric Association (APA) to include Internet Gaming Disorder in the appendix of the updated version of the Diagnostic and Statistical Manual for Mental Disorders (DSM-5) in 2013 as condition that requires further research before it can be accepted for inclusion in the main manual[7]. This has resulted in researchers commencing efforts to reach an international consensus for assessing Internet Gaming Disorder using the new DSM-5 approach based on an international expert panel[8]. However, various limitations to this recently proposed "consensus" have been identified, including the lack of a representative international community of experts in the field, the voting method used to arrive at the consensus, the criteria and nosology identified, lack of critical measurement of the disorder and lack of field testing[9]. For the purpose of a comprehensive and inclusive understanding of the potential disorder, in this systematic literature review, Internet addiction will be referred to as encompassing Internet-use related addictions and problematic Internet use, including Internet Gaming Disorder. It is argued that until this concept is understood more fully (including nosology, etiology and diagnostic criteria), limiting our understanding of Internet-use related addictions to Internet gaming-related problems does neither pay sufficient respect to the affected individuals' personal experience nor to the variety of online behaviours that can be engaged in excessively online. For example, other potential online addictions and Internet-use related disorders have been recently reviewed[10], suggesting that limiting a diagnosis to online gaming exclusively misses out many cases of individuals who experience negative consequences and significant impairment due to their Internet use-related behaviours.

For some individuals, their online behaviours are problematic and they require professional help as they cannot cope with their experiences by themselves, suggesting treatment is necessary. Based on in-depth interviews with 20 Internet addiction treatment experts from Europe and North America, Kuss and Griffiths[11] found that in inpatient and outpatient clinical settings, Internet addiction and Internet-use related problems are associated with significant impairment and distress for individuals, which have been emphasised as the criteria demarcating mental disorders[12]. This suggests that in the clinical context, Internet addiction can be viewed as mental disorder requiring professional treatment if the individual presents with significant levels of impairment. Psychotherapists treating the condition indicate the symptoms experienced by the individuals presenting for treatment appear similar to traditional substance-related addictions, including salience,

mood modification, tolerance, withdrawal, conflict and relapse[11]. This view is reflected by patients who seek treatment for their excessive gaming[13].

In 2002, the South Korean government-funded National Information Society Agency has opened the first Internet addiction prevention counselling centre worldwide, and has since developed large-scale projects (including prevention, training, counselling, treatment, and policy formulation) to tackle the pervasive problem of technology overuse[14]. Across the United States and Europe, Internet addiction treatment is not funded by the government, often leaving individuals seeking help either for other primary disorders or through private organisations, although new clinical centres that specialise in treating Internet-use related problems are being developed[15]. Based on the available evidence, recent research furthermore suggests that the best approach to treating Internet addiction is an individual approach, and a combination of psychopharmacotherapy with psychotherapy appears most efficacious[16].

To date, reviews have focused on clinical and treatment studies of Internet addiction[16-19] and Internet Gaming Disorder[2]. This arguably limits the analysis to a specific diagnosis of a potential disorder that has not yet been officially recognised in the Western world, rather than a comprehensive and inclusive investigation of Internet-use related addictions (including problematic Internet use) more generally. Previous reviews relied on overly restrictive inclusion criteria, and this has led to ambiguities in the conceptualisation of the problem, and consequently resulted in limitations regarding both validity and reliability. In order to overcome these problems, the aim of this literature review is to provide a comprehensive overview of clinical studies on the more inclusive clinical picture of Internet-use related addictions from a holistic perspective.

MATERIALS AND METHODS

Between July and August 2015, a literature search was conducted using the database Web of Science. This database is more comprehensive than other commonly used databases, such as PsycINFO or PubMed because it includes various multidisciplinary databases. The following search terms (and their derivatives) were entered: "Internet addict*", "Internet gaming addiction", "gaming addiction", "Internet Gaming Disorder", "compuls* Internet use", "compuls* gam*", "pathological Internet use", "excessive internet use", or "problematic Internet use", and "clinic*", "diagnos*", "treat*", "therap*", or "patient*". Studies were selected based on the following inclusion criteria. Studies had to (1) contain quantitative empirical data; (2) have been published after 2000; (3) include clinical samples and/or clinical interventions for Internet and/or gaming addiction; (4) provide a full-text article (rather than a conference abstract); and (5) be published in English, German, Polish, Spanish, Portuguese, or French as the present authors speak these languages. The initial search yielded 152 results. Following a thorough inspection of

the articles' titles and abstracts, the articles that did not meet the inclusion criteria were excluded. The search strategy is presented in Figure 1.

Additional articles were identified through searching the citations in the literature selected, resulting in the inclusion of another eight studies[20-27].

RESULTS

A total of 46 studies met the inclusion criteria. These studies are presented in Table 1. The included studies used clinical samples, and focused on characteristics of treatment seekers and online addiction treatment. Four main types of clinical research studies were identified, namely research involving (1) treatment seeker characteristics; (2) psychopharmacotherapy; (3) psychological therapy; and (4) combined treatment. The results section will outline each of these.

Treatment seeker characteristics
A total of 25 studies[19,26,27,32,43,50,62,72,78,79,93,106,109,111,112,118,124,130,133,143,146,163,164,188,204] investigated the characteristics of treatment seekers. Here, treatment seekers are defined as individuals seeking professional support for online addiction-related problems. The following paragraphs will outline the treatment seekers' sociodemographic characteristics, Internet/gaming addiction measures used to ascertain diagnostic status in the respective studies, differential diagnoses and comorbidities.

Sociodemographic characteristics
In the included studies, sample sizes ranged from a case study of a male in Australia presenting with the problem of generalised pathological Internet use[112] to a total of 1826 clients sampled from 15 inpatient alcohol addiction rehabilitation centres in Germany, of which 71 also presented with Internet addiction and were then compared to a control group of 58 patients treated for alcohol addiction only[188]. Ages ranged from 16 years[112] to a mean age of 30.5 years[72]. The majority of studies used male participants, with one study using female participants only[50]. Most studies included individuals seeking treatment for Internet addiction and/or problematic Internet use in specialised inpatient and outpatient treatment centres. A number of studies included particular samples, such as individuals sampled *via* phone consultations (*i.e.,* including 86% relatives of the affected individuals)[43], patients sampled in alcohol rehabilitation centres[130], patients diagnosed with obsessive compulsive disorder (OCD)[46], and female patients treated for eating disorders[50].

Treatment seekers were sampled from various continents. Within Europe, samples included treatment seekers in Germany[43,78,124,130,133,164,188,197], The Netherlands[50], Italy[26,27,32], and Greece[79]. In North America, a Canadian sample was included[72]. In South America, samples included individuals from Perú[62], Puerto Rico[118], and Brazil[139]. In Western Asia, Turkish individuals were sampled in two studies[143,146], whereas in East Asia, participants were from China[163,204], South
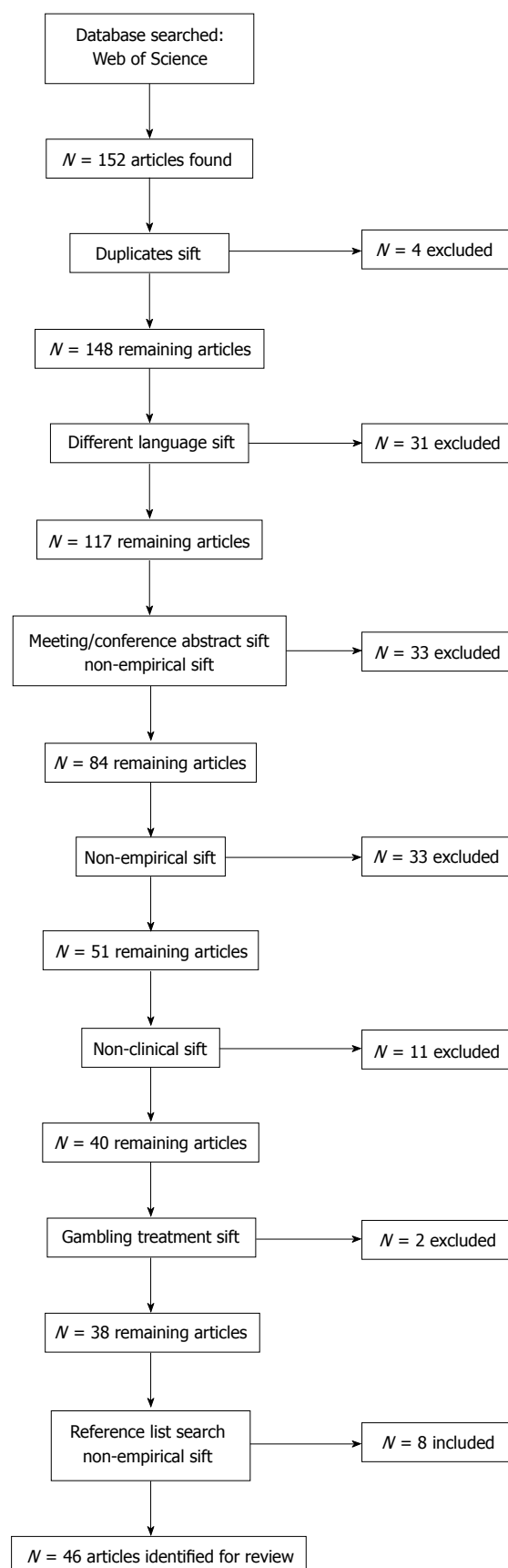
Figure 1 Flow chart displaying the search process.

Korea[93,106,109], and Taiwan[113]. One case study included an Australian adolescent[112].

Internet/gaming addiction

Internet and/or gaming addiction were measured with a number of different psychometric tools in the included studies, sometimes combined with structured clinical interviews. Clinical interviews were explicitly mentioned in the reports of eight studies[32,50,62,93,106,109,164,204], and these consisted mostly of the Structured Clinical Interview for DSM-IV[64], a semi-structured interview for DSM-IV Axis I diagnoses for mental disorders.

In terms of psychometric measures, in the majority of studies, Young's popular Internet Addiction Test[48], the IAT, was used[26,32,72,93,106,109,118,143,146]. The IAT is a 20-item self-report scale that measures the extent of Internet addiction based on criteria for substance dependence and pathological gambling[51], and includes loss of control, neglecting everyday life, relationships and alternative recreational activities, behavioural and cognitive salience, negative consequences, escapism/mood modification, and deception. Significant problems due to Internet use are identified if individuals score between 70-100 on the test, and frequent problems when they score between 40-69[48]. However, previous research has suggested that across studies, different cut-off scores for the IAT have been used to classify individuals[6], impairing comparisons across studies.

Another popular measure appeared to be the Assessment of Internet and Computer Game Addiction Scale (AICA-S)[44,194], which was used in seven studies[43,78,124,130,133,188,197]. The AICA-S is a 16-item scale and includes questions about the frequency of specific Internet usage, associated negative consequences and the extent to which use is pathological from a diagnostic point of view. Fourteen out of the total sixteen main questions are used to calculate a clinical score, and to distinguish normal from potentially addictive use[211].

Other measures included the Compulsive Internet Use Scale (CIUS)[55], a 14-item unidimensional self-report questionnaire including loss of control, preoccupation (cognitive and behavioural), withdrawal symptoms, coping/mood modification, and conflict (inter- and intrapersonal). The CIUS classification is based on the DSM-IV TR diagnoses for substance dependence and pathological gambling[12], and was used in one study[50]. Moreover, in one study[79], the Online Cognitions Scale was used[80], which is a 36-item questionnaire that measures cognitions related to problematic Internet use, and includes subscales on loneliness/depression, diminished impulse control, social comfort, and distraction. In another study[113], Chen's Internet Addiction Scale[117] was administered, which is a 26-item self-report measure of core Internet addiction symptoms, including tolerance, compulsive use, withdrawal, and related problems (*i.e.,* negative impact on social activities, interpersonal relationships, physical condition, and time management). Another study[164] used the Internet Addiction Scale[212], as well as a combination of Young's[213] and Beard's[66] Internet addiction criteria, including preoccupation, tolerance, loss of control, withdrawal, overall impairment, deception,

Table 1 Clinical studies reviewed

Study	Aims	Sample and design	Treatment approach	Instruments	Results
Atmaca[28]	To describe a case of problematic Internet use successfully treated with an SSRI-antipsychotic combination	Case report *n* = 1 male 23-yr old single 4th year medical student	SSRI-antipsychotic combination: Citalopram 20 mg/d increased to 40 mg/d within 1 wk, continued for 6 wk; then quetiapine (50 mg/d) added and increased to 200 mg/d within 4 d	SCID-IV to assess Axis I psychiatric comorbidity[29] YBOCS[30,31]	Y-BOCS score decreased from 21 to 7 after treatment Nonessential Internet use decreased from 27 to 7 h/wk; essential Internet use decreased from 4.5 to 3 h/wk Improvement maintained at 4 mo follow-up with the same medication
Bernardi *et al*[32]	To describe a clinical study of individuals with Internet addiction, comorbidities and dissociative symptoms	*n* = 50 adult outpatients self-referred for internet overuse in Italy (age M = 23.3, SD = 1.8 yr) 9 women and 6 men scored ≥ 70 on Internet Addiction Scale; 19 with "possible Internet addiction" (scoring 40-69 on IAT)	N/A	Youngs Internet Addiction Scale IAS[33] Clinical interview DES[34] CGI[35] Sheehan Disability Scale[36] Structured Clinical Interviews for DSM-IV (SCID I and II)[37,38] Hamilton Rating Scale for Depression[39] Hamilton Rating Scale for Anxiety[40] Liebowitz Social Anxiety Scale[41] YBOCS[30] CAARS:S[42]	Clinical diagnoses included 14% ADHD, 7% hypomania, 15% generalized anxiety disorder, 15% social anxiety disorder; 7% dysthymia, 7% obsessive compulsive personality disorder, 14% borderline personality disorder, and 7% avoidant personality disorder, 2% binge eating disorder IAD associated with higher perception of family disability and higher Yale-Brown Obsessive Compulsive Severity score Scores for the Dissociative Experience Scale were higher than expected and related to higher obsessive compulsive scores, hours per week on the Internet, and perception of family disability
Beutel *et al*[43]	To present the assessment and clinical characterisation of individuals seeking help for computer and Internet addiction *via* a telephone hotline	N = 346 phone consultations (85.8% relatives, 14.2% persons affected) *n* = 131 patients (M = 21.9, SD = 6.6, range 13-47 yr, 96.2% male) Specialised clinic for behavioural addictions in Germany	Telephone consultations First diagnostic interview with expert clinicians	Skala zum Computerspielverhalten [CSV-S (Scale for the Assessment of Pathological Computer Gaming)][44] Symptom-Checklist SCL-90-R[45]	Consultation mainly sought by relatives (86% mothers) 48% reported achievement failure and social isolation, lack of control (38%), family conflicts (33%) 96% of patients (*n* = 131) met criteria for pathological computer gaming
Bipeta *et al*[46]	To compare control subjects with or without Internet addiction with patients with pure obsessive compulsive disorder with or without Internet addiction	*n* = 34 control subjects with or without Internet addiction (age M = 26.9, SD = 6.6 yr) *n* = 38 patients with obsessive compulsive disorder with or without Internet addiction (age M = 27.0, SD = 6.1 yr)	OCD patients treated for 1 year with standard pharmacological treatment for OCD (TAU), received clonazepam, tapered off in three weeks, and an SSRI or clomipramine IA OCD group: 5 received 150-200 mg fluvoxamine/d, 4 received 150-200 mg sertraline/d, 1 received 60 mg fluoxetine/d, 1 received 200 mg clomipramine/d Non-IA OCD group: 8 received 150-300 mg fluvoxamine/d, 5 received 100-200 mg sertraline/d, 11 received 40-80 mg fluoxetine/d, 3 received 150-200 mg clomipramine/d	Youngs Diagnostic Questionnaire[47] IAT[48] Diagnostic and Statistical Manual of Mental Disorders, DSM-IV (psychiatric interview)[12] BIS-11[49] YBOCS[30]	11 OCD patients (28.95%) diagnosed with IA compared to 3 control subjects OCD group, no difference in OCD scores btw IA/OCD and non-IA/OCD groups IA scores higher in IA/OCD group Treatment improved test scores At 12 mo, 2/11 patients with OCD fulfilled IA criteria

Claes *et al*[50]	To investigate the association among CB, CIU, and reactive/regulative temperament in patients with ED	*n* = 60 female patients with eating disorders in the Netherlands (38.3% with Anorexia nervosa, 6.7% with Anorexia binging-purging type, 26.7% with bulimia nervosa, and 28.3% with Eating Disorder not otherwise specified; age range 15-57 yr, mean age = 27.8, SD = 9.8 yr)	N/A	DSM-IV, standardised clinical interview[51] EDI-2[52,53] CBS[54] Dutch Compulsive Internet Use Scale[55] BIS/BAS scales[56,57] DAPP[58,59] Adult Temperament Questionnaire-Short Form[60,61]	Positive association btw CB and CIU, emotional lability, excitement seeking, lack of effortful control (lack of inhibitory and lack of activation control) 11.7% of CB patients with IA No significant differences between ED subtypes regarding CIU
Cruzado Díaz *et al*[62]	To describe clinical and epidemiological characteristics of inpatients in a clinical centre in Perú between 2001-2006	*n* = 30 patients with "IA" 90% devoted themselves to online games) in Perú All single males from 13 to 28 yr old (M = 18.3, SD = 3.8), 63.3% with secondary education completed and 66.7% dropped out Descriptive, retrospective and transversal study	N/A	Reviewed 30 clinical registers through FEIA[63], a semi-structured instrument for psychiatric evaluation applied to clinical histories Patients completed a brief survey through an interview regarding information about their Internet use and online behaviours	Patient characteristics: Young age (18.3 ± 3.8 yr old) Extensive daily Internet use (50% remained online for more than 6 h/d) Primary Internet use: Online gaming (43.3% excessive gaming and 6.7% excessive gambling) Comorbidities (DSM-IV): High frequency of psychopathic behaviours (antisocial personality traits: 40%), 56.7% affective disorders (30% major depression and 26.7% dysthymia), 26.7% other addictions (13.3% gambling, 10% alcohol, 10% marihuana, 6.7% nicotine and 3.3% cocaine), 16.7% antisocial disorders (13.3% ADHD, social phobia 10% and 3.3% dysmorphic corporal disorder)
DellOsso *et al*[20]	To assess the safety and efficacy of escitalopram in IC-IUD using a double-blind placebo-controlled trial	*n* = 19 adult subjects (12 men, mean age = 38.5, SD = 12.0 yr) with IC-IUD (as primary disorder) 19 wk prospective trial with 2 consecutive phases: 10 wk treatment phase (*n* = 17, 11 men, mean age = 37.5, SD = 12.0 yr) and 9 wk randomised double-blind placebo controlled trial (*n* = 14, 10 men, mean age = 40.0, SD = 11.5 yr)	Escitalopram started at 10 mg/d, increased and maintained at 20 mg/d for 10 wk Subsequently, participants randomly assigned to placebo or escitalopram for 9 wk	Structured Clinical Interview for DSM-IV Axis I[64] Time spent in non-essential Internet use (hours/wk) CGI-I[35] BIS[49] IC-IUD version of YBOCS[30]	Following double-blind phase, there were no significant differences in weekly non-essential Internet use and overall clinical response between treatment and placebo group Side effects: Fatigue and sexual side effects in treatment, but not placebo group

Du *et al*[65]	To evaluate the therapeutic effectiveness of group CBT for Internet addiction in adolescents	*n* = 56 adolescents with IA (age range 12-17 yr) *n* = 32 active treatment group (28 male, mean age = 15.4, SD = 1.7 yr)	Group cognitive behavioural therapy: Active treatment group: 8 1.5-2 h sessions of multimodal school-based group CBT with 6-10 students/group run by two child and adolescent psychiatrists (topics: Control, communication, Internet awareness, cessation techniques); group CB parent training; psychoeducation delivered to teachers	Beards Diagnostic Questionnaire for Internet addiction[66] Internet Overuse Self-Rating Scale[67,68] Time Management Disposition Scale[69] Strength and Difficulties Questionnaire (Chinese edition)[70]	Internet use decreased in both groups Only treatment group had improved time management skills and better emotional, cognitive and behavioural symptoms
		n = 24 clinical control group (17 male, mean age = 16.6, SD = 1.2 yr)	Clinical control group: No intervention	SCARED[71]	
Dufour *et al*[72]	To describe the sociodemographic characteristics of Internet addicts in a CDR, and to document their problems associated with other dependencies (alcohol, drugs, game practices), self-esteem, depression and anxiety	*n* = 57 Internet addiction treatment seekers (88.4% males, 11.6% females; age range = 18-62 yr (M = 30.5, SD = 11.8 yr). Canada	N/A	IAT[48,73] Becks Anxiety inventory[74] Becks Depression inventory[75] DÉBA-Alcohol/Drugs/Gaming[76] Self-esteem[77]	88% of Internet addicts were male, with a mean age of 30, living with their parents with low income M = 65 h of Internet use per week: 57.8% MMORPGs, 35,1% video streaming, and 29.8% chat rooms Rosenberg test: 66.6% weak and very weak self-esteem; Depression in only 3.5% and anxiety in 7.5% 45.6% received pharmacological treatment for mental disorders (psychotropic) and 33.3% had a chronic physical problem
Duven *et al*[78]	To investigate whether an enhanced motivational attention or tolerance effects are reported by patients with IGD	*n* = 27 male clinical sample from specialised behavioural addiction centre in Germany (*n* = 14 with IGD, *n* = 13 casual computer gamers) Semi-natural EEG designed with participants playing a computer game during the recording of event-related potentials to assess reward processing	N/A	AICA-S[44] SCL-90-R[45]	Attenuated P300 for patients with IGD in response to rewards relative to a control group Prolonged N100 latency and increased N100 amplitude, suggesting tolerance during computer game play, and gaming reward attention uses more cognitive capacity in patients
Floros *et al*[79]	To assess the comorbidity of IAD with other mental disorders in a clinical sample	*n* = 50 clinical sample of college students presenting for treatment of IAD in Greece (39 males, mean age = 21.0, SD = 3.2 yr; 11 females, mean age = 22.6, SD = 4.5 yr) Cross-sectional study	N/A	OCS[80] DSQ[81] ZKPQ[82,83] SCL-90[84,85]	25/50 presented with comorbidity of another Axis I disorder (10% with major depression, 5% with dysthymia and psychotic disorders, respectively), and 38% (19/50) with a concurrent Axis II personality disorder (22% with narcissistic, and 10% with borderline disorder) The majority of Axis I disorders (51.85%) were reported before IAD onset, 33.3% after onset
Ge *et al*[86]	To investigate the association between P300 event-related potential and IAD	*n* = 41 IAD subjects (21 males, age M = 32.5, SD = 3.2 yr) *n* = 48 volunteers (25 males, age M = 31.3, SD = 10.5 yr) Experimental task	CBT	Standard auditory oddball task using American Nicolet BRAVO Instrument	IA individuals had longer P300 latencies, but similar P300 amplitudes compared to controls Following treatment, P300 latencies decreased significantly, suggesting cognitive function deficits associated with IAD can be ameliorated with CBT

Han and Renshaw[21]	To test whether bupropion treatment reduces the severity of EOP and MDD	n = 50 male subjects with EOP and MDD (aged 13-45 yr)	Random allocation to either bupropion and EDU group or placebo and EDU group	Structured Clinical Interview for DSM-IV[64]	During active treatment period, Internet addiction, gaming, and depression decreased relative to placebo group
		n = 25 treatment group (mean age = 21.2, SD = 8.0 yr, range = 13-42)	12-wk treatment (8 wk active treatment phase and 4-wk post treatment follow-up period)	Youngs Internet Addiction Scale[87,88]	During follow-up, bupropion-associated reductions in gaming persisted, while depressive symptoms recurred
		n = 25 placebo group (mean age = 19.1, SD = 6.2 yr, range = 13-39)			
		Randomised controlled double-blind clinical trial	150 mg/d Bupropion SR given and increased to 300 mg/d during first week	Becks Depression Inventory[89]	
Han *et al*[24]	To test the effects of bupropion sustained release treatment on brain activity for Internet video game addicts	n = 11 IAG (IAG; mean age = 21.5, SD = 5.6 yr; mean craving score = 5.5, SD = 1.0; mean playing time = 6.5, SD = 2.5 h/d; mean YIAS score = 71.2, SD = 9.4)	Placebo group started with one pill and then raised to two pills	Structured Clinical Interview for DSM-IV[64]	Bupropion sustained release treatment works for IAG in a similar way as it works for patients with substance dependence
		n = 8 HC (HC; mean age = 11.8, SD = 2.1 yr; mean craving score = 3.9, SD = 1.1; mean Internet use = 1.9, SD = 0.6 h/d; mean YIAS score = 27.1, SD = 5.3) in South Korea	Buproprion sustained release treatment: 6 wk	Beck Depression Inventory[89]	During exposure to game cues, IAG had more brain activation in left occipital lobe cuneus, left dorsolateral prefrontal cortex, left parahippocampal gyrus relative to HC
				Youngs Internet Addiction Scale[87]	
		Experimental design	Participants underwent 6 wk of bupropion sustained release treatment (150 mg/d for first week, 300 mg/d afterwards)	Craving for Internet video game play: 7-point visual analogue scale Brain activity measured at baseline and after treatment using 1.5 Tesla Espree fMRI scanner	After treatment, craving, play time, cue-induced brain activity decreased in IAG
Han *et al*[22]	To assess the effect of methylphenidate on Internet video game play in children with ADHD	n = 62 children (52 males, mean age = 9.3, SD = 2.2 yr, range = 8.12), drug-naïve, diagnosed with ADHD, and Internet video game players in South Korea	Treatment with Concerta (OROS methylphenidate HCl, South Korea) Initial dosage: 18 mg/d, and maintenance dosage individually adjusted based on changes in clinical symptoms and weight	YIAS-K[87,88]	Following treatment, Internet addiction and Internet use decreased
				Korean DuPaul's ADHD Rating Scale[90,91] Visual Continuous Performance Test using the Computerised Neurocognitive Function Test[92]	Changes in IA between baseline and treatment completion correlated with changes in ADHD, and omission errors from the Visual Continuous Performance Test
Hwang *et al*[93]	To directly compare patients with IA to patients with AD regarding impulsiveness, anger expression, and mood	n = 30 patients with IA (mean age = 22.7, SD = 6.7 yr)	N/A	Korean version of Youngs IAT[48,94]	IA and AD groups showed lower agreeableness and higher neuroticism, impulsivity, and anger expression compared to the HC group (all related to aggression)
		n = 30 patients with AD (mean age = 30.0, SD = 5.9 yr)		SCID[64]	Addiction groups had lower extraversion, openness to experience, and conscientiousness, were more depressive and anxious than HCs
		n = 30 HCs (HCs, mean age = 25.3, SD = 2.8 yr)		Alcohol Use Disorder Identification Test-Korean version[95]	Severity of IA and AD positively correlated with these symptoms
		Outpatient clinic in South Korea		Korean version of the NEO-PI-R[96,97]	
				Korean version of the BIS-11[98,99]	
				Korean version of the STAXI-K[100,101]	

Kim[23]	To examine the effect of a reality therapy (R/T) group counselling programme for Internet addiction and self-esteem	*n* = 25 university students in South Korea (20 males, mean age = 24.2 yr) Randomised controlled trial/quasi- experimental design	Treatment group (*n* = 13, 10 males): Participated in R/T group counselling programme, 2 60-90 min sessions/wk for 5 consecutive weeks (with the purpose of taking control and changing thinking and behaviours) Control group (*n* = 12, 10 males): No treatment	K-IAS[102] CSEI[103]	Treatment programme reduced addiction level and increased self-esteem
Kim *et al*[25]	To evaluate the efficacy of CBT combined with bupropion for treating POGP in adolescents with MDD	*n* = 65 adolescents with MDD and POGP in South Korea (aged 13-18 yr)	*n* = 32 CBT group (medication and CBT): 8 wk intervention; 159 mg bupropion/d for 1 wk, then 300 mg/d for 7 wk; participated in 8 session weekly group CBT; weekly 10 min interviews	BDI[89] BAI[74] YIAS[87,88] Modified-School Problematic Behaviour Scale[104]	Internet addiction decreased and life satisfaction increased in CBT and medication group relative to medication only group, but no changes in depression Anxiety increased in medicated group
		Prospective trial	*n* = 33 clinical control group (medication only, as above)	Modified Students Life Satisfaction scale[105]	
Kim *et al*[106]	To investigate the value of Youngs IAT for subjects diagnosed with Internet addiction	*n* = 52 individuals presenting with Internet addiction at university hospital in South Korea (47 males; mean age = 21.7, SD = 7.1 yr, range: 11-38)	N/A	Clinical interview Youngs IAT[107,108] Classification of IA severity *via* DSM-IV-TR[12]	Samples mean IAT score below cut-off (70) IAT detected only 42% of sample as having Internet addiction No significant differences in IAT scores between mild, moderate and severe Internet addition found No association between IAT scores and Internet addiction duration of illness found IAT has limited clinical utility for evaluating IA severity
Kim *et al*[109]	To compare patients with IGD with patients with AUD and HC regarding resting-state ReHo	*n* = 45 males seeking treatment in South Korea *n* = 16 IGD patients (mean age = 21.6, SD = 5.9 yr) *n* = 14 AUD patients (mean age = 28.6, SD = 5.9 yr) *n* = 15 HCs (mean age = 25.4, SD = 5.9 yr)	N/A	Youngs IAT[87] SCID[64] AUDIT-K[110] BDI[89] BAI[74] BIS-11[111] FMRI resting data acquired via Philips Achieva 3-T MRI scanner using standard whole-head coil, obtaining 180 T2 weighted EPI volumes in each of 35 axial planes parallel to anterior and posterior commissures	Significantly increased ReHo in PCC of the IGD and AUD groups Decreased ReHo in right STG of IGD, compared with AUD and HC groups Decreased ReHo in anterior cingulate cortex of AUD patients Internet addiction severity positively correlated with ReHo in medial frontal cortex, precuneus/PCC, and left ITC in IGD Impulsivity negatively correlated with ReHo in left ITC in IGD Increased ReHo in PCC: Neurobiological feature of IGD and AUD Reduced ReHo in STG: Neurobiological marker for IGD specifically relative to AUD and HCs
King *et al*[112]	To present a case study of an individual with GPIU	*n* = 1, 16-yr old male in Australia	N/A	N/A	PIU identified due to: (1) use of several different Internet functions; (2) social isolation; (3) procrastination and time-wasting tendencies
		Case study			Problems unlikely to have occurred without the Internet

Ko *et al*[113]	To evaluate the diagnostic validity of IGD criteria, and to determine the cut-off point for IGD in DSM-5	*n* = 225 adults in Taiwan (*n* = 75 individuals with IGD (63 males, mean age = 23.4, SD = 2.6 yr), no IGD (63 males, mean age = 22.9, SD = 2.5 yr), and IGD in remission (63 males, mean age = 23.8, SD = 2.9 yr), respectively)	N/A	Diagnostic interview based on DSM-5 IGD criteria[7] DC-IA-C[114] Chinese version of the MINI[115] QGU-B[116] CIAS[117]	Diagnostic accuracy of DSM-5 IGD items between 77.3% and 94.7% (except for deceiving and escape), and differentiated IGD from remitted individuals Meeting ≥ 5 IGD criteria: Best cut-off point to differentiate IGD from non-IGD and remitted individuals
Liberatore *et al*[118]	To describe the prevalence of IA in a clinical sample of Latino adolescents receiving ambulatory psychiatric treatment	*n* = 71 adolescent patients in Puerto Rico (39 males, aged 13-17 yr), 39.4% diagnosed with disruptive disorder, 31.0% with mood disorder, 19.7% with mood and disruptive disorder	N/A	Spanish version of the Internet Addiction Test (IAT)[87]	Sample did not involve any cases of severe IA 71.8% of the sample had no IA problem 11.6% discussed Internet use with therapists IA correlated with mood disorders
Liu *et al*[119]	To test the effectiveness and underlying MFGT	*n* = 92 (46 adolescents with 12-18 yr old, and 46 parents, aged 35-46 yr old) 2 groups: 1 experimental (EG; MFGT adolescents and parents) and 1 control (CG; waiting-list similar adolescents and parents) EG: Adolescents: 17 males and 4 females (age: M = 15, SD = 1.73); Parents: 5 males and 16 females (age: M = 40.9, SD = 2.85) CG: Adolescents: 21 males and 4 females (age: M = 15.7, SD = 1.2); Parents: Idem to EG (no sign. Diff). China Quasi-experimental design	MFGT is a new approach to treat Internet addiction (IA) behaviours that has not been tested before MFGT = group therapy for families, both adults and adolescents that have the same problem (IA) Advantage: Peer group (support and learn from peer confrontation) Transference reactions occur within and between families	Structured questionnaires at pre-test (T1), post-test (T2) and follow-up (T3): Adolescents scales: Adolescent Pathological Internet Use Scale APIUS[120] Parents scales: Closeness to Parents[121] Parent-Adolescent Communication Scale[122] College Students Psychological Needs and Fulfillment Scale[123]	Significantly decreased IA in EG at T2 and maintained in T3 (adolescents IA rate dropped from 100% at baseline to 4.8% after intervention, then remained at 11.1%) Significantly better reports in the EG from adolescents and parents compared with those in the CG Underlying mechanism of less IA was partially explained by adolescent satisfaction of their psychological needs and improved parent-adolescent communication and closeness
Müller *et al*[124]	To characterize German treatment seekers and to determine the diagnostic accuracy of a self-report scale for IA	*n* = 290 mostly male (93.8%) treatment seekers between 18 and 64 yr (M = 26.4, SD = 8.22) Germany	Treatment of behavioural addictions Non-experimental design	SCL-90R[125] PHQ[126] GAD-7[127] CDS-2[128] AICA-S[129]	71% met clinical IA diagnosis Displayed higher levels of psychopathology, especially depressive and dissociative symptoms Half met criteria for one further psychiatric disorder, especially depression Level of functioning decreased in all domains AICA-S showed good psychometric properties and satisfying diagnostic accuracy (sensitivity: 80.5%; specificity: 82.4%)
Müller *et al*[130]	To compare personality profiles of a sample of patients in different rehabilitation centres	IA group: 70 male patients with an addiction disorder that additionally met the criteria for IA; M =29.3 yr (SD = 10.66; range 16-64) AD group: 48 male patients suffering from AD; M = 31.7 yr; SD = 9.18; range 17-65) Germany	N/A Non-experimental design	Computer game Addiction (AICA-S)[129] NEO-FFI[131] BDI-II[132]	Patients with comorbid IA can be discriminated from other patients by higher neuroticism and lower extraversion and lower conscientiousness After controlling for depressive symptoms, lower conscientiousness turned out to be a disorder-specific risk factor

Müller *et al*[133]	To evaluate the relationships between personality traits and IGD	*n* = 404 males aged 16 yr and above	N/A	AICA-S[44]	IGD associated with higher neuroticism, decreased conscientiousness and low extraversion
		4 groups:	Experimental design: Characteristics of people selected for assigning them to two groups, non-random allocation		The comparisons to pathological gamblers indicate that low conscientiousness and low extraversion in particular are characteristics of IGD
		IGD group: 115 patients with IGD		AICA-C[134]	Etiopathological model proposed for addictive online gaming
		Clinical CG: 74 controls seeking treatment for IGD, but not diagnosable		Berlin Inventory for Gambling[135]	
		Gambling group: 115 gambling patients		NEO Five-Factor Inventory[131]	
		Healthy CG: 93 individuals with regular or intense use of online games			
		Germany			
Park *et al*[136]	To examine the effectiveness of treating an Internet-addicted young adult suffering from interpersonal problems based on the MRI interactional model and Murray Bowen's family systems theory	1 family case study consisting of husband (age 50), wife (age 50), 2 sons (ages 22, 23), older son with Internet addiction and interpersonal problems		Comparative analysis method	Characteristics of the parents family of origin and dysfunctional communication pattern associated with interpersonal problems revealed by participants
		South Korea		Miles and Huberman's matrix and network[137]	Both the MRI model and Bowen's family systems theory produced effective treatments
Poddar *et al*[138]	To describe a pilot intervention using MET and CBT principles to treat IGD in an adolescent	*n* = 1	Initial therapy session: Rapport building with patient, detailed interview, primary case formulation	IQ ESDST,	IGD due to child neglect and boredom, consolidated by subsequent negative reinforcements
		14-yr-old boy	Subsequent sessions: Psychoeducation, cost/ benefit analysis of behaviour (motivation level improved)	BVMGT, and TAT	
		India	Progressive muscle relaxation because gaming urge accompanied by physiological/emotional arousal	IAT	Individual interventions encouraged as there are varied antecedents and consequences for IGD development
		Case study			MET-CBT principles for IGD resulted in improvement
			Subsequently: Game addiction assessment, contract for behaviour modification (reduce gaming time, increase other activities)		Therapy terminated when gains had consolidated
			Tokens introduced as positive reinforcement		Good exam scores achieved
			Less time spent gaming on weekdays, but excess on weekends		Weekend gaming times reduced
			Patient recorded Thoughts, Emotions and Behaviors (TE and B) contributing to gaming (result: Gaming due to boredom)		IAT score reduced to 48 (from 83)
			Non-gaming behaviour reinforced *via* scooty rides		

Santos *et al*[139]	To describe a treatment of a patient with PD, OCD (both anxiety disorders) and IA involving pharmacotherapy and CBT and test its efficacy	Case report *n* = 1 24-yr-old Caucasian woman A patient with PD, OCD and IA Brazil	Pharmacotherapy and CBT CBT 1x/week for 10 wk Pharmacotherapy [clonazepam (0.5 mg) and sertraline (50 mg) once daily] Both (pharmaco and CBT) started together CBT focus: Teach patient how to deal with anxiety and internet use (*i.e.*, breathing retraining with diaphragmatic breathing exercise, education about PD and OCD symptoms and internet use, time management, identifying PIU triggers, changing habits, cognitive restructuring, exposure and response prevention, social support promotion, building alternative activities, functional internet use promotion)	Hamilton Anxiety Scale (HAMA-A)[40] Hamilton Depression Scale (HAM-D)[39] Chambless BSQ[140] Bandelow PA[141] IAT CGI[142]	Treatment effective for anxiety and IA
Senormanci *et al*[143]	To investigate the attachment styles and family functioning of patients with IA	*n* = 60 2 groups: EG: 30 male patients with IA [age: M = 21.6 (18-20) yr] CG: 30 healthy males without IA Non-experimental	N/A	IAT[48] BDI[89] Experiences in Close Relationships Questionnaire-r [144] Family Assessment Device[145]	Patients with IA had higher BDI and higher attachment anxiety sub-scores on the ECR-r compared with those in the CG IA patients evaluated their family functioning as more negative and reported problems in every aspect addressed by the FAD Scores on the FAD behaviour control, affective responsiveness, and problem-solving subscales (and on the FAD communication, roles, and general functioning subscales) significantly higher in patients compared with CG
Senormanci *et al*[146]	To determine the predictor effect of depression, loneliness, anger and interpersonal relationship styles for IA in patients diagnosed with IA	*n* = 40 male IA patients with at least 18-yr-old Turkey	N/A	IAT[48] BDI[89] STAXI[100] UCLA Loneliness Scale[147] IRSQ, subscale "Contributing and inhibiting styles"[148]	Duration of Internet use (hours/day) and STAXI anger in subscale predicted IA. Although the duration is not adequate for IA diagnosis, it predicts IA It is helpful for clinicians to regulate the hours of Internet use for patients with excessive or uncontrolled internet use Psychiatric treatments for expressing anger and therapies focussing on emotion validation may be useful

Shek *et al*[149]	To described an indigenous multi-level counselling programme designed for young people with IA problems based on the responses of clients	*n* = 59	Indigenous multilevel counselling program designed to provide services for young people with Internet addictive behaviour in Hong Kong:	3 versions of IA Young's assessment tools[150]: 10-item, 8-item and 7-item measures[151-153]	The outcome evaluation, pretest and posttest data showed IA problems decreased after joining programme
		58 male and 1 female Most in early adolescence (aged 11-15 yr; *n* = 29) and late adolescence (aged 16-18 yr; *n* = 27), while 3 were over 18 China	(1) Emphasis on controlled and healthy use of the Internet; (2) Understanding the change process in adolescents with Internet addiction behaviour; (3) Utilization of motivational interviewing model; (4) Adoption of a family perspective; (5) Multi-level counselling model; (6) Utilization of case work and group work	Goldberg's framework[154] Chinese Internet Addiction Scale (CIA-Goldberg)	Slight positive changes in parenting attributes
				Items for assessing beliefs and behaviours for using Internet: 7 items from Computer Use Survey[155] 6 items from OCS[80] 6 items from Internet Addiction-Related Perceptions and Attitudes Seale[156] 2 items from IAD-Related Experience Scale[157] 33-item C-FAI developed[158] Chinese Purpose in Life Questionnaire[159] Chinese Beck Depression Inventory[160] Chinese Hopelessness Seale[161] Chinese Rosenberg Self-Esteem Seale[162]	Participants subjectively perceived the programme was helpful
Tao *et al*[163]	To develop diagnostic criteria for IAD and to evaluate the validity of proposed diagnostic criteria for discriminating non-dependent from dependent Internet use in the general population	3 stages: Criteria development and item testing; criterion-related validity testing; global clinical impression and criteria evaluation;	N/A	N/A: Authors developed the proposed Internet addiction diagnostic criteria, which have been one of the main sources for the APAs IGD criteria	Proposed Internet addiction diagnostic criteria: Symptom criterion (7 clinical IAD symptoms), clinically significant impairment criterion (functional and psychosocial impairments), course criterion (duration of addiction lasting at least 3 mo, with at least 6 h of non-essential Internet use per day) and exclusion criterion (dependency attributed to psychotic disorders)
		Stage 1: *n* = 110 patients with IA in SG, M = 17.9 SD = 2.9 yr (range: 12-30 yr), 91.8% (*n* = 101) males; 408 patients in IA in TG, M = 17.6, SD = 2.7 yr (range: 12-27 yr), 92.6% (*n* = 378) male; Stage 2: *n* = 405; Stage 3: *n* = 150 (M = 17.7, SD = 2.8, (92.7% males) China			Diagnostic score of 2 + 1, where first 2 symptoms (preoccupation and withdrawal symptoms) and min. 1/5 other symptoms (tolerance, lack of control, continued excessive use despite knowledge of negative effects/affects, loss of interests excluding Internet, and Internet use to escape or relieve a dysphoric mood) was established Inter-rater reliability: 98%

Te Wildt *et al*[164]	To examine the question whether the dependent use of the Internet can be understood as an impulse control disorder, an addiction or as a symptom of other psychiatric conditions	EG: *n* = 25 patients (76% male, M = 29.36 yr, SD = 10.76)	2 groups matched: The EG and CG	Preliminary telephone interview to test inclusion criteria with Young's and Beard's IA criteria[48,66]	Compared to controls, patient group presented significantly higher levels of depression (BDI), impulsivity (BIS) and dissociation (DES)
		CG: Matched for age (M = 29.48; SD = 9.56), sex (76% males) and school education, and similar level of intelligence	Non-experimental design	Statistical Clinical Interview for DSM-IV[64] German Internet Addiction Scale ISS[165] German version of the Barratt Impulsiveness Scale BIS[49] Derogatis Symptom Checklist (SCL-90-R)[166,167] BDI[89,168] DES[169,170] SOC[171,172] IIP-D[173,174]	PIU shares common psychopathological features and comorbidities with substance-related disorders Should be viewed as diagnostic entity in itself within a spectrum of behavioural and substance dependencies
Tonioni *et al*[26]	To test whether patients with IA present different psychological symptoms, temperamental traits, coping strategies and relational patterns relative to patients with PG	Two clinical groups: 31 IA patients (30 males) and 11 PG patients (10 males) and a control group (38 healthy subjects; 36 males) matched with the clinical groups for gender and age were enrolled	N/A	IAT[48] Hamilton Anxiety Rating Scale[40] Hamilton Depression Scale[39] Global Assessment of Functioning[112] Snaith-Hamilton Pleasure Scale[175] Temperament and Character Inventory-Revised[176] Coping Orientation to Problems Experienced[177] Inventory of Parent and Peer Attachment[178]	IA and PG had higher scores than control group on depression, anxiety and global functioning IA patients had higher mental and behavioural disengagement associated with an important interpersonal impairment relative to PG patients IA and PG groups used impulsive coping, and had socio-emotional impairment
Tonioni *et al*[27]	To investigate psychopathological symptoms, behaviours and hours spent online in patients with IAD	*n* = 86: 21 clinical patients in hospital-based psychiatric IAD service (mean age=24, SD = 11 yr); 65 control subjects	N/A	Internet addiction interview[47] IAT[179] Symptom Checklist-90-Revised[125]	IAD patients had significantly higher scores on IAT relative to controls Only item 7 (how often do you check your e-mail before something else that you need to do?) showed a significant inverse trend SCL-90-R anxiety and depression scores and IAT item 19 (How often do you choose to spend more time online over going out with others?) positively correlated with weekly online hours in IAD patients

van Rooij *et al*[180]	To evaluate the pilot treatment for IA created for the Dutch care organization (to explore the possibility of using an existing CBT and MI based treatment programme (lifestyle training) from therapists experiences with 12 Internet addicts	*n* = 12 Internet addicts and *n* = 5 therapists treating them

The Netherlands | Treatment: A manual-based CBT

Standard Lifestyle Training programme, a manual-based treatment programme[181,182]

Therapy combines CBT and MI[183,184]

Focuses on eliciting and strengthening motivation to change, choosing a treatment goal, gaining self-control, relapse prevention, and coping skills training[185,186]
10 outpatient sessions of 45 min each, with 7 of these taking place within a period of 10 wk, the remaining 3 within a period of 3 mo
Each session: Introduction, evaluation of current status, discussing homework, explaining theme of the day, practicing a skill, receiving homework, and finally closing the session | Data sources:
(1) Session Reports;
(2) Case Review Meeting Minutes; (3) Questionnaires:
Compulsive Internet Use Scale (CIUS)[55]

Brief Situational Confidence questionnaire[187] | Therapists report programme (originally used for substance dependence and pathological gambling) fits problem of Internet addiction well
Interventions focused on controlling and reducing Internet use, and involved expanding (real life) social contacts, regaining proper daily structure, constructive use of free time, and reframing beliefs
Therapist report: Treatment achieved progress for all 12 treated patients
Patient report: Satisfaction with treatment and behavioural improvements |
| Wölfling *et al*[188] | To investigate whether IA is an issue in patients in addiction treatment | *n* = 1826 clients in impatient centres

Male patients meeting criteria for comorbid IA (EG; *n* = 71) compared with a matched control group of male patients treated for alcohol addiction without addictive Internet use (CG; *n* = 58)
Germany | N/A | Internet and Computer Game Addiction (AICA-S)[189,190]
Symptom Checklist 90R (SCL-90-R)[191]
PHQ[126]
GAD-7[127] | Comorbid IA associated with higher levels of psychosocial symptoms, especially depression, obsessive-compulsive symptoms, and interpersonal sensitivity
IA patients meet criteria for additional mental disorders more frequently and display higher rates of psychiatric symptoms, especially depression, and might be in need of additional therapeutic treatment |

| Wölfling *et al*[192] | To test the effects of a standardized CBT programme for IA | *n* = 42 patients with IA, all male from 16-yr-old (M = 26.1, SD = 6.60, range: 18-47) | Short-Term outpatient Treatment for Internet and Computer Game Addiction STICA (127) based on CBT techniques known from treatment programmes of other forms of addictive behaviour, consisting of 15 group sessions and additional 8 individual therapy sessions

Individual sessions dealt with individual contents; group sessions followed clear thematic structure: First third of programme: Main themes about development of individual therapy aims, identification of Internet application associated with symptoms of IA, conducting holistic diagnostic investigation of psychopathological symptoms, deficits, resources, and comorbid disorders Motivational techniques applied to enhance patients intention to cut down dysfunctional behaviour Second third: Psychoeducation elements; deepened Internet use behaviour analysis (focusing on triggers and patient reactions on cognitive, emotional, psychophysiological, and behavioural levels in that situation (SORKC scheme)[193] for development of a personalized model of IA for each patient based on interaction between online application, predisposing and maintaining factors of the patient (*e.g.*, personality traits) and the patients social environment Last stage: Situations with heightened craving for getting online further specified and strategies to prevent relapse developed | Inclusion criteria:

AICA-S[193,194]

Standardized clinical interview of IA (AICA-C; Checklist for the Assessment of Internet and Computer Game Addiction)[132]
GSE[195]
NEO Five-Factor Inventory[131]
Symptom Checklist 90R[196] | 70.3% of patients completed therapy

After treatment, symptoms of IA decreased significantly Psychopathological symptoms and associated psychosocial problems decreased |

Wölfling *et al*[197]	To investigate the occurrence of BSD in patients with excessive Internet use and IA	*n* = 368 treatment seekers with excessive to addictive Internet use screened for bipolar spectrum disorders Germany	N/A	AICA-S[194]	Comorbid BSD more frequent in patients meeting criteria for IA (30.9%) than among excessive users (5.6%)
				BSD assessed using MDQ[198] SCL-90R[199,200]	This subgroup showed heightened psychopathological symptoms, including substance use disorders, affective disorders and personality disorders
					Further differences were found regarding frequency of Internet use regarding social networking sites and online-pornography in patients with BSD who engage more frequently
					Patients with IA have heightened probability for meeting BSD criteria
					Recommendation: Implement BSD screening in patients presenting with IA
Young[201]	To investigate the efficacy of using CBT with Internet addicts	*n* = 114 Internet addicts in treatment (42% women (mean age = 38; men mean age = 46)	Sessions conducted between client and principle investigator	IAT[48]	Preliminary analyses indicated most clients managed their presenting complaints by the eighth session
			Initial sessions gathered familial background, nature of presenting problem, its onset and severity	Self-devised Client Outcome Questionnaire administered after 3rd, 8th, and 12th online session, and at 6 mo follow-up: 12 items regarding clients behaviour patterns and treatment successes during counselling process; questions rated how effective counselling was at helping clients achieve targeted treatment goals associated with Internet addiction recovery; questions assessed motivation to quit Internet abuse, ability to control online use, engagement in offline activities, improved relationship functioning, and improved offline sexual functioning (if applicable)	Symptom management sustained at 6-mo follow-up
			CBT utilized to address presenting symptoms related to computer use, specifically abstinence from problematic online applications and strategies to control online use		
			Counselling also focused on behavioural issues or other underlying factors contributing to online abuse, such as marital discord, job burnout, problems with co-workers, and academic troubles, depending on respective client		

Young[202]	To test a specialized form of CBT, CBT-IA	*n* = 128 clients to measure treatment outcomes using CBT-IA (65% male; age range: 22-56 yr)	CBT-IA: 3-phase approach including behaviour modification to control compulsive Internet use, cognitive restructuring to identify, challenge, and modify cognitive distortions that lead to addictive use, and harm reduction techniques to address and treat co-morbid issues associated with the disorder	IAT[48]	Over 95% of clients managed symptoms at the end of the 12 wk period 78% sustained recovery six months following treatment
			Administered in 12 weekly sessions Sessions conducted between client and principle investigator Initial sessions gathered familial background, symptoms of the presenting problem, its onset, and severity CBT-IA addressed presenting symptoms related to computer use, specifically abstinence from problematic online applications and strategies to control use CBT-IA also focused on cognitive issues and harm reduction for underlying factors contributing to Internet abuse such as marital discord, job burnout, problems with co-workers, or academic troubles, depending on respective client Internet use routinely evaluated and treatment outcomes evaluated after 12 sessions and at 1, 3 and 6 mo follow-up			CBT-IA ameliorated IA symptoms after 12 weekly sessions and consistently over 1, 3 and 6 mo after therapy
Yung *et al*[203]	To improve IAD involving Google Glass through residential treatment for alcohol use disorder	*n* = 1 (31-yr-old man who exhibited problematic use of Google Glass) Case report	Navys SARP All electronic devices and mobile computing devices customarily removed from patient during substance rehabilitation treatment	N/A regarding SARP and measures, only about his reactions (*e.g.*, withdrawal, craving, *etc.*)	Following treatment, reduction in irritability, movements to temple to turn on device, and improvements in short-term memory and clarity of thought processes
			United States 35-d residential treatment		Patient continued to intermittently experience dreams as if looking through the device

Zhou *et al*[204]	To examine whether Internet addicted individuals share impulsivity and executive dysfunction with alcohol-dependent individuals	*n* = 66 22 IAD, 22 patients with AD, and 22 NC (NC consisting of citizens living in the city) China Experimental design	N/A	BIS-11 Go/no-go task	Impulsiveness scores, false alarm rate, total response errors, perseverative errors, failure to maintain set of IAD and AD group significantly higher than that of NC group
				Wisconsin Card Sorting Test (Beijing Ka Yip Wise Development Co., Ltd, computerized version VI) Digit span task	Hit rate, percentage of conceptual level responses, number of categories completed, forward scores, backwards scores of IAD and AD group significantly lower than that of NC group
				Modified Diagnostic Questionnaire for Internet Addiction (YDQ)[66] Structured clinical interview (Chinese version) SADQ[205] Hamilton Depression Scale[206] Barratts Impulsivity Scale (BIS-11)[49]	No differences in above variables between IAD group and AD group Internet addicted individuals share impulsivity and executive dysfunction with alcohol-dependent patients
Zhu *et al*[207]	To observe the effects of CT with EA in combination with PI on cognitive function and ERP, P300 and MMN in patients with IA	*n* = 120 patients in China with IA randomly divided into 3 groups: *n* = 39 EA group (*n* = 40, 27 male, mean age = 22.5, SD = 2.1 yr)	Overall treatment period = 40 d EA applied at acupoints Baihui (GV20), Sishencong (EX-HN1), Hegu (LI4), Neiguan (PC6), Taichong (LR3), Sanyinjiao (SP6) and retained for 30 min once every other day	Internet Addiction Test[208] Wechsler Memory Scale (WMS)[209]	Following treatment, IA decreased in all groups Decrease stronger in CT group relative to both other groups
		n = 36 PI group (*n* = 25 male, mean age = 21.0, SD = 2.0 yr) *n* = 37 CT group (*n* = 40, 27 males, mean age = 22.5, SD = 2.3 yr)	PI with cognitive-behaviour mode every 4 d EA and PI used in CT group	ERP observation[210] using MEB 9200-evoked detector Latency and amplitude of MMN and P300 recorded *via* EEG	P300 latency depressed and amplitude raised n EA group MMN amplitude increased in CT group Short-term memory capacity and short-term memory span improved EA and PI improves cognitive function in IA *via* acceleration of stimuli discrimination and information processing on brain level

AD: Alcohol dependence; ADHD: Attention-deficit/hyperactivity disorder; AICA-C: Checklist for the assessment of internet and computer game addiction; AICA-S: Scale for the assessment of internet and computer game addiction; AUD: Alcohol use disorder; AUDIT-K: Korean version of alcohol use disorder identification test; BAI: Beck anxiety inventory; BDI: Beck depression inventory; BDI-Ⅱ: Beck depression inventory Ⅱ; BIS: Barratt impulsiveness scale; BIS-11: Barratt's impulsivity scale-11; BSD: Bipolar spectrum disorders; BSQ: Body sensations questionnaire; CAARS:S: Conners' adult ADHD rating scales self; CB: Compulsive buying; CBS: Compulsive buying scale; CBT: Cognitive behavioural therapy; CBT-IA: Cognitive-behavioural therapy for internet addiction; CDR: Centre for dependence rehabilitation; CDS-2: Cambridge depersonalization scale; C-FAI: Chinese family assessment instrument; CGI: Clinical global impression scale; CGI-I: Clinical global impressions-improvement scale; CIAS: Chen internet addiction scale; CIU: Compulsive internet use; CSEI: Coopersmith's self-esteem inventory; CT: Comprehensive therapy; DAPP: Dimensional assessment of personality pathology-short form; DC-IA-C: Diagnostic criteria of internet addiction for college students; DES: Dissociative experience scale; DSQ: Defense style questionnaire; EA: Electroacupuncture; EEG: Electroencephalogram; ED: Eating disorders; EDI-2: Eating disorder inventory 2; EDU: Education for internet use; EOP: Excessive online game play; EPI: Echo-planar image; ERP: Event-related potentials; GAD-7: Seven-item generalized anxiety disorder; GPIU: Generalised pathological internet use; GSE: General self-efficacy scale; HC: Healthy controls; IA: Internet addiction; IAD: Internet addiction disorder; IAG: Internet video game addicts; IAT: Internet addiction test; IC-IUD: Impulsive-compulsive internet usage disorder; IGD: Internet gaming disorder; IIP-D: Inventory of interpersonal problems; IRSQ: Interpersonal relationship styles questionnaire; ITC: Inferior temporal cortex; K-IAS: K-internet addiction scale; MDD: Major depressive disorder; MDQ: Mood disorder questionnaire; MET: Motivational enhancement therapy; MFGT: Mechanism of multi-family group therapy; MI: Motivational interviewing; MINI: Mini international neuropsychiatric interview; MMN: Mismatch negativity; MRI: Mental research institute; NC: Normal controls; NEO-FFI: NEO five factors inventory; NEO-PI-R: NEO personality inventory-revised; OCD: Obsessive compulsive disorder; OCS: Online cognitions scale; PA: Panic and agoraphobia scale; PCC: Posterior cingulate cortex; PD: Panic disorder; PG: Problematic gambling disorder; PHQ: Patient health questionnaire; PI: Psycho-intervention; POGP: Problematic online game play; QGU-B: Questionnaire on gaming urge-belief; ReHo: Regional homogeneity; SADQ: Severity of alcohol dependence questionnaire; SARP: Substance addiction recovery program; SCARED: Screen for child anxiety related emotional disorders; SCID: Structured clinical interview for DSM-Ⅳ; SCID-Ⅳ: Structured clinical interview for DSM-Ⅳ-patient version; SCL: Symptom checklist; SCL-90R: Symptom checklist 90-revised; SG: Survey group; SOC: Sense of coherence scale; STAXI-K: State-trait anger expression inventory; STG: Superior temporal gyrus; TG: Training group; YBOCS: Yale-brown obsessive compulsive severity scale; YIAS: Young's internet addiction scale; YIAS-K: Young's internet addiction scale, korean version; ZKPQ: Zuckermann-kuhlman personality questionnaire.

and escapism[164]. The latter was also used in another study[204].

A different approach was taken by Tao *et al*[163], who intended to develop diagnostic criteria for Internet Addiction Disorder (IAD) and to evaluate the validity of these criteria. Accordingly, in order to be diagnosed with IAD, patients had to fulfil the following criteria: The presence of preoccupation and withdrawal (combined with at least one of the following: Tolerance, lack of control, continued excessive use despite knowledge of negative effects/affects, loss of interests excluding the Internet, and Internet use to escape or relieve a dysphoric mood). In addition to this, clinically significant impairment had to be identified (*i.e.,* functional and psychosocial impairment), and the problematic behaviour had to last a minimum of three months, with at least six hours of non-essential Internet use a day. This study has been used as a basis for the APA's research classification of Internet Gaming Disorder in the DSM-5.

As this section demonstrates, a wide variety of measurements have been applied in order to ascertain Internet or Internet-use related addiction, sometimes involving an expert assessment by an experienced professional. As has been stated in previous research[6], no gold standard exists to measure Internet addiction with high sensitivity and specificity, which is exacerbated by the use of different cut-off points on the same measures across studies. To mitigate this diagnostic conundrum, a diagnosis of Internet addiction would significantly benefit from including a structured clinical interview administered by a trained professional[214], and this would help eliminating false positives and false negatives in the context of diagnosis.

Differential diagnoses/comorbidities

A number of studies investigated differential diagnoses and/or comorbidity of Internet addiction and other psychopathology. In terms of assessing potential comorbidities, the Structured Clinical Interview for DSM-IV mental disorders[64] was used by five studies[32,50,93,106,164]. Psychopathological symptomatology was also assessed using the Symptom-Checklist, SCL-90-R[125,191] and the Chinese version of the Mini International Neuropsychiatric Interview[115]. Personality disorders were identified by using the Dimensional Assessment of Personality Pathology-Short Form[58,59]. Other addiction-related assessments included alcohol and drug addiction measured with the DÉBA[76], the Alcohol Use Disorder Identification Test-Korean version[95], and the Severity of Alcohol Dependence Questionnaire[205], as well as shopping addiction, assessed *via* the Compulsive Buying Scale[54]. The presence of eating disorders was assessed using the Eating Disorder Inventory 2[52,53]. Mood disorders were assessed using the Hamilton Rating Scale for Depression[39], Beck's Depression Inventory[132], and the Mood Disorder Questionnaire[198]. Levels of anxiety were measured with the Hamilton Rating Scale for Anxiety[40], Beck's Anxiety Inventory[74],

and the Generalized Anxiety Disorder scale (GAD-7)[127]. Symptoms of Attention Deficit Hyperactivity Disorder (ADHD) were investigated by means of Conners' Adult ADHD Rating Scales Self (CAARS:S)[42]. Finally, dissociation and depersonalisation were measured using the Dissociative Experiences Scale[34] and the Cambridge Depersonalization Scale[128].

The results of comorbidity and differential diagnosis analyses revealed the following. Of 50 adult outpatients self-referred for their Internet overuse, 14% presented with comorbid ADHD, 7% hypomania, 15% GAD, 15% social anxiety disorder, 7% dysthymia, 7% obsessive compulsive personality disorder, 14% borderline personality disorder, 7% avoidant personality disorder, and 2% binge eating disorder[32]. Higher frequencies of comorbid psychopathology were reported in a sample of 30 male patients with Internet gaming addiction[62], namely 40% antisocial personality traits, 56.7% affective disorders (30% major depression and 26.7% dysthymia), 26.7% other addictions (13.3% gambling, 10% alcohol, 10% marihuana, 6.7% nicotine and 3.3% cocaine addiction), and 16.7% antisocial disorders (13.3% ADHD, social phobia 10% and 3.3% dysmorphic corporal disorder). Generally smaller prevalence rates were reported in a sample of 57 Internet addiction treatment seekers in Canada[72]: 3.5% presented with comorbid depression and 7.5% with anxiety.

Half of a sample of 50 students with Internet addiction[79] presented with a comorbidity of another Axis I disorder (10% with major depression, 5% with dysthymia and psychotic disorders, respectively). This finding was corroborated by another study of 290 male treatment seekers, half of whom met criteria for another psychiatric disorder[124]. In addition to this, of the former sample, 38% presented with a concurrent Axis II personality disorder (22% with narcissistic, and 10% with borderline disorder, respectively)[79]. Significantly higher levels of depression and dissociation were furthermore found in a sample of 25 patients with Internet addiction as compared to a matched healthy control group[164]. Moreover, relative to a control group of male patients treated for alcohol addiction, 71 male patients with alcohol addiction and comorbid Internet addiction presented with higher levels of depression and obsessive-compulsive symptoms[188]. Furthermore, another study[197] including 368 Internet addiction treatment seekers showed that 30.9% met the diagnostic criteria for bipolar spectrum disorders, and this study also evidenced generally increased psychopathological symptomatology (including substance use disorders, affective and personality disorders). Finally, significant positive correlations were reported between compulsive buying and compulsive Internet use, as 11.7% of a sample of 60 female patients displaying patterns of compulsive buying also presented with addictive Internet use. This study reported no differences between individuals presenting with different types of eating disorders regarding compulsive Internet use[50].

Moreover, patients with Internet addiction and patients with pathological gambling received higher scores on depression, anxiety[26,27], and lower scores on global functioning relative to healthy controls, used impulsive coping strategies, and experienced more socio-emotional impairment. Additionally, patients with Internet addiction differed from patients with pathological gambling in that the former experienced higher mental and behavioural disengagement, which was found to be associated with interpersonal impairments[26].

Overall, the presence of comorbidities for Internet-use related addiction in the clinical context appears to be the norm rather than an exception. Individuals seeking treatment for their Internet overuse frequently present with mood and anxiety disorders, and other impulse-control and addictive disorders appear common. This indicates Internet addiction treatment may benefit from therapeutic approaches that combine evidence-based treatments for co-occurring disorders in order to increase treatment efficacy and acceptability for the patient.

Psychopharmacotherapy

In five studies, psychopharmacotherapy[20,22,24,28,46] for online addictions was used. Atmaca[28] reported the case of a 23-year-old male 4th year medical student who presented with the problems of problematic Internet use and anxiety. The patient was treated with a combination of selective serotonine reuptake inhibitors (SSRI) and antipsychotic medication. The antidepressant citalopram was administered at a dose of 20 mg/d and was increased to 40 mg/d within the period of a week, which was continued for six weeks. Subsequently, quetiapine (an atypical antipsychotic typically used for schizophrenia spectrum disorders) was added to the treatment, starting with a dose of 50 mg/d, which was increased to 200 mg/d within four days. The treatment resulted in decreased Internet addiction as measured with the Y-BOCS[30] modified for Internet use, decreased non-essential and essential Internet use, and improved control over Internet use. The improvements persisted until four-month follow up.

Bipeta et al[46] compared 34 control subjects with or without Internet addiction assessed *via* Young's Diagnostic Questionnaire[48] with patients with pure OCD with or without Internet addiction (mean age = 27 years, SD = 6.5 years). OCD patients were treated with standard pharmacological treatment for OCD (treatment as usual) for one year, received the benzodiazepine clonazepam (often used in the treatment of anxiety disorders), which was tapered off in three weeks, an SSRI or the tricyclic antidepressant clomipramine for 12 mo. The individuals with Internet addiction in the OCD group received the following doses of medication: Five patients received 150-200 mg fluvoxamine/d, four received 150-200 mg sertraline/d, one received 60 mg fluoxetine/d, and the final one received 200 mg clomipramine/d. In the OCD group that included

individuals who were not addicted to using the Internet, the following doses of medication were administered: Eight patients received 150-300 mg fluvoxamine/d, five received 100-200 mg sertraline/d, eleven received 40-80 mg fluoxetine/d, and three received 150-200 mg clomipramine/d. Overall, the OCD treatment improved scores for both OCD and Internet addiction, while only two of the eleven OCD patients still fulfilled Internet addiction criteria after twelve months of treatment[46].

Dell'Osso et al[20] assessed the safety and efficacy of the antidepressant SSRI escitalopram (typically used for mood disorders) in 19 adult patients (12 men, mean age = 38.5, SD = 12.0 years) who presented with the problem of impulsive-compulsive Internet usage disorder assessed *via* the YBOCS[30], modified for Internet use. The trial consisted of a total of 19 wk, composed of a ten week treatment phase in which escitalopram was administered starting with 10 mg/d, and increased and maintained at 20 mg/d for 10 wk, and subsequent nine weeks of a randomised double-blind placebo controlled trial with or without administration of escitalopram at previous dosages. The treatment phase resulted in a significant decrease in Internet use. However, there were no differences in treatment effect between the treatment and placebo group following the second stage of the study. The authors also note that the group treated with escitalopram experienced negative side effects, including fatigue and sexual side effects, whereas side effects did not occur in the placebo group[20].

Han et al[26] used a controlled trial to test the effects of the antidepressant bupropion sustained release treatment (with a dose of 150 mg/d for the first week and 300 mg/d for five subsequent weeks) on the brain activity of eleven Internet video game addicts (mean age = 21.5, SD = 5.6 years), assessed *via* Young's Internet Addiction Scale[216]. The results indicated that the administered psychopharmacological treatment provided successful results for the video game addiction group, as it decreased craving, playing time, and cue-induced brain activity. These authors[22] also used the central nervous system stimulant concerta (methylphenidate commonly used for ADHD) in 62 video game playing children with ADHD (52 males, mean age = 9.3, SD = 2.2 years) who had not previously been given medication. Internet addiction was assessed using the Korean version of Young's Internet Addiction Scale[87]. The initial concerta dosage was 18 mg/d, with the maintenance dosage being individually adjusted based on the respective children's clinical symptoms and weight. Following treatment, Internet addiction and Internet use significantly decreased, as did ADHD symptoms and omission errors in a Visual Continuous Performance Test[22].

Taken together, the studies including psychopharmacological treatment for Internet addiction and/or gaming addiction showed positive effects in decreasing Internet addiction symptomatology and Internet/gaming use times. In the few studies conducted, antidepressant medication has been used most, suggesting mood

disorders may be comorbid with Internet use addiction. The research also indicated that if other (primary or secondary) disorders are present (specifically, OCD and ADHD), medication typically used to treat these disorders is also effective in reducing Internet addiction-related problems.

Psychological therapy

Ten studies[23,65,86,119,136,149,174,201-203] described some form of psychological therapy for treating Internet addiction. The majority of psychological therapies used an individual approach, which was applied to outpatients, apart from three studies that used group therapy approaches[23,65,119,136,149].

The most common approach used to treat Internet addiction was Cognitive Behavioural Therapy (CBT)[86,202]. This approach was usually individualised (apart from one study which used a group approach[65]). A further study used a combination of individualised and group therapy, namely Short-Term Treatment for Internet and Computer Game Addiction[192]. The typical CBT programme was administered for the duration of a few months, ranging from eight sessions[65] to 28 sessions, which included both group and individual sessions[192], and sessions lasted between one[86] and two hours[65]. The topics covered with patients in these sessions were: (1) identification of the Internet application associated with symptoms of addiction; (2) control issues (*e.g.*, examining the self, feelings, impulsivity, and the relation between the individual and the Internet to self-manage and self-restrain Internet use); (3) principles of healthy communication, namely interpersonal communication, such as between parent-children[65], and sharing success stories[86]; (4) Internet awareness (with regards to relationships established and developed through the Internet, and dealing with online content); (5) cessation techniques applied to the Internet (*e.g.*, recognizing the addictive behaviour and discontinuing it); and (6) additional elements (*e.g.*, college career planning, covering underlying factors contributing to Internet abuse, such as marital discord, job burnout, problems with co-workers, or academic problems). In general, CBT followed a number of stages, including team building or a probatory stage to review sessions or stabilization and relapse prevention. All sessions were run by therapists[119,192] or psychiatrists[86] who were supporting adults, apart from one case that involved children and adolescents[65].

The treatment outcomes were measured through scores on a number of psychometric scales covering excessive Internet use, including the Internet Overuse Self-Rating Scale[67,68], the Adolescent Pathological Internet Use Scale[120], the Internet Addiction Diagnostic Questionnaire[48], and the assessment of emotional, cognitive and behavioural symptoms. The following emotional skills and problems were measured in some studies. Anxiety was assessed using the Screen for Child Anxiety Related Emotional Disorders[71] and self-esteem was measured with Coopersmith's Self-Esteem

Inventory[103]. Cognitive skills covered were diverse, and measures included the Online Cognition Scale (OCS)[80], and the Time Management Disposition Scale[69]. The behavioural characteristics related to Internet addiction primarily concerned the individual, but also included their peer and family relationships, and were measured using the Chinese version of the Strength and Difficulties Questionnaire[70], and the Parent-Child Communication Scale[122]. Only one study[86] did not make use of questionnaires because it was a neuropsychological and electrophysiological study conducted using an event-related potential approach, focused on cognitive function by detecting a P300 component. The results of this study indicated there was a deficit in cognitive functioning in Internet addicts, which is a finding that has also been observed in other addictive disorders[215].

Four of the included group therapy approaches (out of five studies) included Internet addicts and family groups treated simultaneously. These included (1) a CBT modality called "multimodal school-based group" (MSBG)[65]; (2) a "multi-family group therapy" (MFGT), which was used for treating Internet addiction for the first time[119]; (3) a traditional family therapy for a young adult addicted to using the Internet[136]; and (4) a "multi-level intervention model" that is usually applied to substance abuse, which included family counselling and peer support groups[149].

The psychotherapeutic MSBG approach was applied in a school setting and involved students, parents and teachers. The group of Internet addicts were students treated using classical CBT in a group ranging from six to ten participants. The students' parents were also administered cognitive behavioural training to recognize their children's Internet addiction (through children's feeling states, communication and solving-problem skills in the family, and through controlling the parents' own feelings and behaviours to manage their children's excessive Internet use). Teachers were provided psychoeducation, which was delivered by means of workshops in didactic teaching, analysis and discussion, with the purpose of recognising and treating Internet addiction in students, and of supporting their parents.

MFGT is a new psychotherapy approach for adolescent Internet addicts[119]. This intervention provides therapeutic groups for both adults (parents) and adolescents (Internet addicts), and the aim is to provide peer support, allowing transferential reactions, engagement with the treatment and promoting family cohesion. The main goal of this form of psychotherapy is to reduce Internet addiction whilst improving parent-adolescent communication and closeness, and to fulfil the family members' psychological needs, rather than these needs being fulfilled by Internet use. Altogether, six active sessions were used, with a subsequent three-month follow-up to target potential relapse and discuss new issues and solutions to maintain the effectiveness of the intervention. Each of the sessions lasted for two hours and included five parts: a warm-up exercise, feedback on homework from the last session, a main structured

activity, a brief summary and the family assignment. The topics treated per session were: Understanding a family with the problem of excessive Internet use (session 1), parent-adolescent communication skills training (session 2), parent-adolescent communication practices related to the problem (sessions 2 and 3), parent-adolescent relationship building skills training (session 4), associations between psychological needs and Internet use, how to satisfy the unfulfilled need in the family relationships (session 5), and setting up healthy expectations for the family system (session 6).

The classical family therapy approach used in one study[136] was based on Bowen's[216] family system theory, which focused on the distinction of the self-inside from the self within the family constellation, and was based on an extensive analysis of family-of-origin problems and communication patterns. The treatment was focused on current interactions and changes in behaviour in the family system[217] to modify the family's communication method by changing behaviours that maintain problematic Internet use, and coping with Internet overuse related problems. The therapy focused on an undesirable online behaviour and replacing it with a healthy behaviour, which would simultaneously induce a change in the family relationships. The intervention lasted three months and included 15 sessions. It treated emotional problems to enhance control over Internet use, and included functional and emotional expression to solve interpersonal relationship problems associated with Internet addiction.

The multi-level intervention model included an individual-based counselling approach with motivational interviewing (MI), complementary techniques, and traditional family-based counselling[149]. It consisted of six phases, lasting between 15 and 19 mo. The phases included (1) emphasising controlled and healthy Internet use; (2) promoting understanding of the change process through different stages from pre-contemplation to relapse, (3) using the MI model[218] for Internet addiction; (4) adopting a family perspective by using a systemic approach; (5) applying a multi-level counselling model including the patient, his/her family and his/her peers; and (6) using individual and group therapy to facilitate the intervention.

The only group approach that did not include a family intervention was the R/T group counselling programme, which specifically addressed Internet addiction[23]. It consisted of ten group sessions (two per week) within the period of one month, which varied in length between one and 1.5 h. Accredited specialists provided this intervention for university students. The content included an introduction to the therapy goal, teaching, activities, homework assignment and sharing. Each session furthermore included four sections: The purpose, materials (*e.g.,* blank paper, topic-oriented games, posters, videos), strategies (*e.g.,* discussion topics, homework assignments) and session evaluation for both the individual and their family, in order to assess whether the aim of the sessions had been achieved.

Overall, the psychological studies which included a control group to compare the effect of the interventions achieved varying results, impeding a general analysis of psychotherapy impact. Du *et al*[65] did not find significant differences between experimental and control groups in the post-test measure of Internet overuse, although the intervention group improved their time management (efficacy and time control) and other skills (emotional symptoms, conduct problems, hyperactivity, peer relationships and prosocial behaviours) significantly, and this was maintained until a six-month follow-up. Other comparative findings included a longer P300 component duration in Internet addicts treated by CBT compared to healthy controls[86]. However, the amplitudes were similar in both groups. Moreover, although Internet addiction symptoms were reduced after treatment in the experimental group[149], this was not the case for the group's scores on beliefs and behaviours related to Internet use and psychological well-being, and there was only a small improvement in parental monitoring and functioning following treatment.

Only two studies (out of four experimental studies) showed a clear effectiveness of psychological therapy, and both of these used a group approach. Kim[23] used a quasi-experimental design and an intervention with a group psychotherapy approach, and found a significant reduction in Internet addiction and significantly higher self-esteem in the experimental group compared to the control group. Liu *et al*[119] found that their MFGT approach was effective in three aspects. It resulted in a significant reduction of time spent online (reduced by half in comparison to the controls), a decrease in the Internet addiction measure, and, from the parents' perspective, more satisfaction regarding their child's online behaviours. Moreover, the most important factor to reduce Internet addiction in this study was found to be the parent-adolescent relationship.

Combined therapy

Six studies used combined therapy to treat Internet addiction, consisting of some form of psychological treatment in combination with one of the following: Other psychological therapies[138,180], pharmacotherapy[21,25,139] or electroacupuncture therapy[221].

CBT was the most frequently applied psychological therapy to treat Internet addiction. Subsequently, add-ons to the CBT approach included in the identified studies will be elaborated on. Motivational Enhancement Therapy (MET) was developed by Poddar *et al*[138] and was tested in the context of treating IGD. This MET-CBT approach consisted of a series of stages: (1) a contemplation stage (*i.e.,* initial sessions of rapport building, a detailed interview and case formulation); (2) a preparation stage (*i.e.,* sessions delivered in an empathetic atmosphere to emphasise psychoeducation, including managing physiological and emotional arousal through relaxation techniques, and a cost-benefit analysis of game addiction); and (3) a contract stage with the patient, a parent and the therapist (*i.e.,* behaviour

modification of gaming, reducing time spent online and promoting healthy activities). By applying these stages, a reduction of IGD and online gaming was achieved, and school performance was improved.

Another case study[139] combined CBT with psychopharmacotherapy [*i.e.,* administering clonazepam (a benzodiazepine typically used to treat anxiety disorders) and sertraline (an SSRI antidepressant)] to treat Internet addiction. The intervention lasted for three months, and consisted of the following. The CBT approach aimed to support self-recognition and modify and restructure feelings and dysfunctional cognitions related to Internet use, with the goal to prevent relapse. CBT was administered for ten weekly sessions to teach the patient to handle her anxiety and other symptoms related to her Internet use (in this case panic and obsessive symptomatology, which was comorbid to her Internet addiction). Clonazepam (0.5 mg) and sertraline (50 mg) were also administrated once daily. The applied treatment proved effective for reducing both anxiety and Internet addiction.

A new treatment approach to treat Internet addiction combined CBT and MI with an on-the-job Lifestyle Training programme[180]. Treatment was delivered by qualified therapists who were supervised by a senior therapist for both main psychological therapies. The treatment consisted of eliciting and strengthening the motivation to change, choosing a treatment goal, gaining self-control, preventing relapse, and coping skills training. Ten outpatient sessions of 45 min were used, and seven of these took place within a period of 2.5 mo. The remaining sessions were optional and were administered as a follow-up within 3 mo. Each of the sessions had a fixed format: (1) introduction; (2) evaluation of current status; (3) discussing homework; (4) explaining the theme of the day; (5) practicing a skill; (6) receiving homework; and (7) closing the session. This study was the only study that provided three perspectives for data collection: The patients', the therapists' and the researchers' perspectives. This intervention, which is commonly used for other addictive disorders, was found to work well for Internet addiction as it reduced Internet use, increased social contacts, provided a daily structure, and encouraged alternative uses of free time and positive beliefs.

Moreover, CBT was most frequently used in combination with a psychopharmacological treatment, such as administering bupropion. The reason to select this medication is because a proportion of patients with major depressive disorder (MDD) are also excessive online gamers, and this drug has been previously evaluated as potential treatment for MDD and other drug-addictions. Recently, its effectiveness has been tested and confirmed experimentally[21,25]. Han and Renshaw[21] tested this combined treatment in Chinese male adolescent and adult patients with mood disorders and online gaming addiction, and treated them with bupropion sustained release (from 150 mg/d until 300 mg/d during 8 wk) and a psychological intervention (*i.e.,*

education for Internet use). The treatment resulted in significantly decreased depression and Internet addiction levels, and time spent playing online games compared with the control group. At follow-up (*i.e.,* four weeks post treatment), the reduction in gaming hours and level of Internet addiction was maintained, while the depression recurred.

Similarly, Kim *et al*[25] tested the effectiveness of CBT in an active treatment group *vs* a control group who did not receive CBT in Korean male adolescent patients with MMD and online gaming addiction. Both groups were treated using the same levels of bupropion. Following treatment, Internet addiction was significantly reduced in the CBT group and other measures showed improvement (*e.g.,* anxiety and life satisfaction), while depression severity did not change. These findings were maintained at follow-up. Therefore, the combination of psychotherapy with bupropion is effective in MDD patients with online gaming addiction in the long term only for online gaming addiction, and the time spent using online games. Both studies with bupropion were managed by psychiatrists, and one[25] used a multidisciplinary treatment team including a psychiatrist, nurse, psychologist, and social worker.

One study used clonazepam (0.5 mg/d) and sertraline (50 mg/d) combined with CBT to treat Internet addiction[139]. This study reported the case of a young Brazilian woman with Internet addiction and comorbid psychiatric disorders (*i.e.,* panic and OCDs). During the treatment period of ten weeks, both drugs were administered daily whilst CBT was provided once a week, and focused on teaching the patient how to handle anxiety and Internet use through breathing training with diaphragmatic exercises, education about both disorders' symptoms and about Internet use (*e.g.,* time management, triggers of problematic Internet use, changing habits, cognitive restructuring, exposure and response prevention, promotion of social support, alternative activities, and promotion of functional Internet use). This combined treatment was effective for all conditions treated.

Zhu *et al*[207] combined a psychological intervention (*i.e.,* CBT with sessions every four days for a total treatment period of 40 d) with electroacupuncture in 120 patients presenting with Internet addiction in China. They used three groups: 40 participants in the electroacupuncture group, 36 participants in the psychological intervention group, and 37 individuals participated in the comprehensive therapy group combining both treatment ingredients. Electroacupuncture was applied at acupoints Baihui (GV20), Sishencong (EX-HN1), Hegu (LI4), Neiguan (PC6), Taichong (LR3), and Sanyinjiao (SP6), and retained for 30 min once every other day. Overall, treatment was effective in all groups as Internet addiction symptomatology was successfully decreased, whereas this effect was significantly stronger in the combined therapy group relative to the other groups. The authors furthermore note that the combined treatment improved cognitive function in Internet

addiction by means of accelerating stimuli discrimination and information processing on the level of the brain.

Combined therapies have shown effective results for treating Internet addiction, including both post-treatment and follow-up measures. The use of electroacupuncture in combination with a psychological intervention improved treatment success for Internet addiction more than providing cognitive-behavioural treatment only, suggesting the novel therapy electroacupuncture may be beneficial in the treatment of Internet addiction. It is suggested to replicate this study to verify the positive results.

Conversely, given the results found by the included studies, psychopharmacotherapy does not always appear to be as efficacious for psychological problems, such as major depression, as it is for Internet and gaming addiction. This is an interesting finding, because it seems that Internet addiction is usually accompanied by other psychological disorders. Therefore, combining therapies may be a good option for some clients, and should be managed by interdisciplinary teams with structured mid-term interventions.

DISCUSSION

This systematic literature review has sought to provide an overview of the currently available clinical research on Internet addiction and problematic Internet use using a holistic perspective. Clinical studies concerning Internet addiction, problematic Internet use and excessive online gaming have been included to offer a comprehensive insight into the relevant research to date. A total of 46 empirical clinical studies were identified, which focused on treatment seeker characteristics and different types of therapy provisions. Treatments included psychopharmacotherapy, psychological therapy, and combined treatment. Each of these will be discussed subsequently.

In terms of treatment seeker characteristics, the included studies indicated that the published research ranged from case studies to including patients treated for problematic Internet use in both inpatient and out-patient settings across 13 countries and four continents. It is worth noting that a number of studies indicated that comorbidities appear to be the norm, rather than an exception for individuals who present with the problem of Internet addiction or problematic Internet use. Comorbid mood and anxiety disorders appear to be particularly common. A link between mood disorders and Internet addiction has been suggested in previous research, including both adolescent[88,210-227] and adult samples[228-233]. A possible explanation for this strong and frequent link may be the fact that as Internet use increases, online activities take up gradually more time in the lives of Internet users. This reduces the time available to participate in alternative enjoyable pastime activities and to engage with real-life family and friendship circles, which may lead to increased loneliness and stress[234]. Alternatively, Internet use and gaming may serve as a method to escape real-life problems, effectively resulting in avoidance coping, which may exacerbate stress and negative feelings, and lead to negative consequences, including addiction and depression[235].

Moreover, a number of earlier studies have shown that anxiety disorders and anxiety-related symptoms, including social phobia, phobic anxiety, and OCD co-occur with Internet addiction in adolescents[88,236-238] and adults[230,238]. Previous research including Internet addiction treatment experts from six countries indicated that a large percentage of individuals presenting with Internet addiction at both in-patient and out-patient treatment facilities suffer from comorbid anxiety disorders, most commonly social anxiety and social phobia[11]. This may be explained through the mechanism of compensation, suggesting individuals who have difficulties engaging and bonding with their peers in real life may instead use the Internet for social interaction, as the online space removes the embodied (and potentially anxiety-provoking) elements from the interaction. These elements include the individual's outward appearance and the exclusion of (often feared) face-to-face contact in favour of virtual (and often text-based) interaction. This may facilitate social interaction by increased likelihood of self-disclosure[239], online disinhibition[240], and hyperpersonal communication, characterised by the increased speed of developing social bonds and intimacy online[241].

The research presented indicated that comorbidities complicate treatment. This literature review has shown that comorbidities are very common in the context of Internet addiction, emphasising the necessity to investigate the extent to which Internet addiction can be considered a primary or a secondary disorder (*i.e.*, secondary to some other psychopathology). Researchers have suggested that given the presence of comorbidity, it is questionable whether Internet addiction deserves an individual diagnosis, as this may lead to other (primary) disorders being underdiagnosed. This may lead to problems regarding efficient treatment choices on behalf of the mental healthcare professionals given that efficacious treatments exist for the more prevalent disorders, such as anxiety and mood disorders[242], whereas the evidence base for Internet addiction treatment is still rather limited in comparison. However, research has also indicated that some symptoms of Internet addiction appear as stand-alone symptoms and can be differentiated from other psychopathology, providing empirical evidence for the discriminant validity and specificity of the Internet addiction construct[243]. If comorbidity is present in individuals presenting with Internet addiction or problematic Internet use, clinicians need to target both problems in treatment as research has indicated that individuals with comorbid psychopathology (specifically co-occurring Axis I mental disorders) present with more clinical problems[79].

In terms of psychopharmacotherapy, the five studies included in this systematic literature review showed that SSRIs (*i.e.,* citalopram, clomipramine, fluvoxamine, sertraline, fluoxetine, escitalopram), norepinephrine-dopamine reuptake inhibitors (NDRI; *i.e.,* buproprion), benzodiazepines (*i.e.,* clonazepam), antipsychotic medication (*i.e.,* quetiapine), and methylphenidate (*i.e.,* concerta) were used to treat Internet addiction and Internet-use related problems. Overall, in the included studies, the use of psychopharmacological treatment to alleviate Internet and gaming addiction symptomatology and time spent online appeared successful, suggesting that Internet addiction is an indication for the use of the administered medications[20,22,24,28,46].

The diverse range of administered medication corresponds with the diverse range of presenting problems of the samples included. For instance, concerta is a drug which is efficacious in treating ADHD and therefore commonly used in ADHD treatment[244] as it has been shown to improve inhibition, motivation and memory by increasing dopamine and norephinephrine concentrations in the brain[245]. Moreover, given the relatively high prevalence of both mood and anxiety disorders with comorbid Internet addiction as described above, it is not surprising that antidepressant medications and benzodiazepines are frequently used in the pharmacological treatment of Internet addiction. SSRIs are the method of choice for mood and anxiety disorders and related symptoms[246], and benzodiazepines have anti-anxiety and relaxing properties[247]. Despite their off-label status in countries including the United Kingdom and Australia, NDRIs are often prescribed for depression-related symptoms and disorders[248]. In sum, the studied psychopharmacological treatments for Internet addiction proved efficacious in decreasing both Internet addiction symptoms as well as symptoms of other psychopathologies for which the specific medications have been licensed. Even so, clinicians need to assess the costs and benefits of the medication they are prescribing for treating Internet addiction as some side effects may impact treatment acceptability and treatment adherence in patients.

Regarding psychological therapy for Internet addiction and problematic Internet use, ten studies were identified, most of which used a group therapy framework to support clients. Group therapy has a number of advantages over individual therapy. According to the American Psychological Association[249], the benefits of group therapy include establishing a support network of individuals who experience similar problems and are faced with similar difficulties. Other group members' stories may put the patients' own problems into perspective. Moreover, group therapy may create a safe environment in which the sensitive topic of Internet-use related addiction can be discussed openly. Group therapy has the benefit of offering the possibility to learn from others and consequently improve coping skills as individuals differ in their ways they face the world and deal with their lives. These benefits explain why group

therapy frameworks are popular psychological therapies for Internet addiction and Internet use-related problems.

The addition of the family network into therapy sessions as evidenced in studies on multimodal school based groups[65], MFGT[119], family therapy[136], and a multi-level intervention model[149] appears particularly fruitful for young patients, as families are important social groups supporting the young patients' development. Families teach values, offer emotional attachment, model appropriate behaviours, and discourage high-risk behaviours[250]. The efficacy of group-based and systemic therapy for adolescents with problems of substance use and addiction has been long established[251], and suggests that therapeutic frameworks derived from family-based therapies for these disorders may be similarly efficacious in the treatment of Internet addiction and problematic Internet use. The included studies have verified this contention, and therefore clinicians are advised to incorporate families in the psychological treatment of young patients (including adolescents and young adults).

The most commonly applied therapy form was CBT or some variation thereof (*e.g.,* CBT-IA)[202], which has frequently been used in an individual format. The primary goal of CBT is to change maladaptive cognitions and behaviours associated with Internet use, and this therapy form is in line with Davis'[252] cognitive-behavioural model of pathological Internet use. The model suggests cognitive factors are particularly important in the development and maintenance of Internet addiction. In the included studies, cognitive measures indicated that CBT is efficacious in reducing cognitive impairment associated with Internet addiction[86]. However, Winkler *et al*[17] examined the efficacy of different treatments for Internet addiction in a meta-analysis which included 13 studies, and their results showed that CBT did not perform significantly better than other psychological treatments, although CBT appears to be the most popular approach for treating Internet addiction.

Finally, a number of studies have simultaneously included different forms of therapy, namely psychological treatment supplemented with other types of psychological therapy[138,180], pharmacotherapy[21,25,139] or electroacupuncture therapy[219]. Taken together, all of the combined therapies were efficacious in treating Internet use-related problems, whereas the benefits for comorbid psychopathology (*e.g.,* depression) were limited. This suggests that in cases where comorbidity is present and psychopharmacological treatment is administered, the clinician and researcher need to carefully monitor the patient's progress, adjust the dosage of the medication and/or change the medication administered to achieve the best possible results for the patient. Moreover, as the new treatment modality of electroacupuncture outperformed psychological interventions, it is suggested that researchers replicate these positive results to ensure they hold across other samples.

A number of limitations need to be highlighted in

the included studies. Only a few studies (*e.g.,*[20,21,23,24, 26,27,46,65,78,93,109,119,133,143,188,204]) included a control group, making it difficult to ascertain whether the positive effects of treatment on Internet addiction symptom and related problem reduction were due to the administered treatment, or to non-specific factors of treatment [*i.e.,* the placebo effect (the improvement of symptoms with no treatment)], which can be due to natural history and statistical regression to the mean, among other factors[253]. Moreover, a lack of intention-to-treat analysis in the reported studies might have caused bias in the results due to treatment non-compliance, changes from the initial treatment protocol, or leaving out data from individuals who dropped out of the study before or during the course of treatment[254].

For future research, the need to utilise validated and reliable measures of Internet addiction and/or problematic Internet use needs to be stressed. Currently, the diagnostic and research landscape appears particularly broad, and diagnostic criteria used to identify the potential disorder are not globally agreed upon. Researchers are recommended to collaborate to establish a consensus regarding diagnostic criteria and measures in order to improve the reliability across studies and to develop effective and efficient treatment approaches for treatment seekers. This will furthermore contribute to providing an incentive for public policy and healthcare providers to offer funding for those who need professional help. Ultimately, research and clinical initiatives need to focus on providing the best possible care for individuals who experience significant impairment and distress as a consequence of their Internet use.

COMMENTS

Background
Over the last 15 years, the number of Internet users has increased by 1000%, and at the same time, research on addictive Internet use has proliferated. Internet addiction has not yet been understood very well, and research on its etiology and natural history is still in its infancy. In 2013, the American Psychiatric Association included Internet Gaming Disorder in the appendix of the updated version of the Diagnostic and Statistical Manual for Mental Disorders as condition that requires further research prior to official inclusion in the main manual, with important repercussions for research and treatment.

Research frontiers
To date, reviews have focused on clinical and treatment studies of Internet addiction and Internet Gaming Disorder. This arguably limits the analysis to a specific diagnosis of a potential disorder that has not yet been officially recognised in the Western world, rather than a comprehensive and inclusive investigation of Internet-use related addictions (including problematic Internet use) more generally.

Innovations and breakthroughs
The aim of this literature review is to provide a comprehensive overview of clinical studies on the clinical picture of Internet-use related addictions from a holistic perspective.

Applications
Researchers are recommended to collaborate to establish a consensus regarding diagnostic criteria and measures in order to improve the reliability

across studies and to develop effective and efficient treatment approaches for treatment seekers. This will furthermore contribute to providing an incentive for public policy and healthcare providers to offer funding for those who need professional help. Ultimately, research and clinical initiatives need to focus on providing the best possible care for individuals who experience significant impairment and distress as a consequence of their Internet use.

Terminology
Internet addiction is a condition that requires further research prior to official inclusion in the diagnostic manuals, with important repercussions for research and treatment. To date, reviews have focused on clinical and treatment studies of Internet addiction and Internet Gaming Disorder. This arguably limits the analysis to a specific diagnosis of a potential disorder that has not yet been officially recognised in the Western world, rather than a comprehensive and inclusive investigation of Internet-use related addictions (including problematic Internet use) more generally.

Peer-review
In this systematic review, the authors have presented a thorough and critical analysis of clinical research on Internet addiction related studies.

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SYSTEMATIC REVIEWS

White matter alterations in anorexia nervosa: A systematic review of diffusion tensor imaging studies

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Abstract

AIM: To identify findings concerning white matter (WM) fibre microstructural alterations in anorexia nervosa (AN).

METHODS: A systematic electronic search was undertaken in several databases up to April 2015. The search strategy aimed to locate all studies published in English or Spanish that included participants with AN and which investigated WM using diffusion tensor imaging (DTI). Trials were assessed for quality assessment according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses checklist and a published quality index guideline.

RESULTS: A total of 6 studies met the inclusion criteria, four of people in the acute state of the illness, one included both recovered and unwell participants, and one included people who had recovered. Participants were female with ages ranging from 14 to 29 years. All studies but one measured a range of psychopathological features. Fractional anisotropy and mean diffusivity were the main DTI correlates reported. Alterations were reported in a range of WM structures of the limbic

system, most often of the fornix and cingulum as well as the fronto-occipital fibre tracts, *i.e.*, regions associated with anxiety, body image and cognitive function. Subtle abnormalities also appeared to persist after recovery.

CONCLUSION: This diversity likely reflects the symptom complexity of AN. However, there were few studies, they applied different methodologies, and all were cross-sectional.

Key words: Eating disorder; Diffusion tensor imaging; Weight/shape overvaluation; Food anxiety

Core tip: The present systematic review identifies the latest research on white matter (WM) brain alterations in anorexia nervosa (AN). The WM architecture has been poorly understood due to its structure forming deep parts of the brain. It transmits information between cortical and subcortical structures. New advances in imaging methods with diffusion tensor imaging, allow its characterization and integrity analysis. Alterations in areas of fornix, cingulum, corpus callosum, cerebellum, superior longitudinal fasciculus and thalamus have been found in AN. They could be related to symptoms like anxiety, body image perception, reward processing and cognitive abilities.

INTRODUCTION

Anorexia nervosa (AN) is a serious illness and has the highest standardized mortality ratio of all psychiatric disorders[1]. According to the DSM-5 criteria, a person in the sick state is underweight for their for age and height, fears gaining weight or becoming fat, and persists in behaviours to avoid weight gain, and weight/shape overvaluation or body image disturbance[2]. In young people it is the third commonest chronic illness and has a mortality rate 12 times higher than the death rate from all causes in young women[3]. In addition to acute effects of self-starvation there may be severe long term medical and psychological sequalae[4].

AN is multidetermined, and as well as psychological and environmental aetiologies, there is increasing evidence for biological underpinnings of its pathophysiology[5]. These include genetic, neuroendocrinologic and metabolic pathways. Furthermore, studies of the brain structure in this mental disorder have identified structural abnormalities. Severely underweight indivi-

duals with AN have altered brain function that is considered more profound than found in any other psychiatric disorder[6]. First computed tomography and then functional magnetic resonance imaging and other neuroimaging techniques have been used to study brain alterations in AN[7]. (FMRI measures the brain activity by detecting associated changes in blood flow, on the premise that cerebral blood flow reflects neuronal activation[8]).

The mechanisms addressing brain alterations remains unclear, but changes in brain grey matter (GM) and white matter (WM) might underlie altered brain function and behaviour[9]. Both fibres are part of the central nervous system (CNS) which co-ordinates basic activities of the human body. The GM is related to processing and cognition whilst the WM co-ordinates different brain regions. The first studies found differences in the GM of AN compared to healthy people and other eating disorders. However, the peculiar architecture of WM and the limited availability of imaging methods to study the WM delayed its investigation. Now, new advances in imaging procedures such as diffusion tensor imaging (DTI) allow the study of the structure of WM and its implication in the pathology of AN.

Volumetric brain imaging studies have been used to study GM and WM volume abnormalities in underweight adolescent and adult people with AN[10]. Several have reported loss of global and regional GM and often WM volume. After 2 or 3 d of dehydration these volumes are significantly lower, whereas hyperhydration is associated with higher GM and WM volumes[11].

Functionally connected brain regions utilising WM axons are considered to modulate higher order human behaviours, cognitions and emotions[12]. Investigating WM functionality thus may elucidate the cortical networks and circuitries that underpin the clinical features of AN. Kazlouski *et al*[13] (2011), were the first to use DTI to identify microstructural WM alterations in adults with AN. They found decreased fractional anisotropy (FA) in the cingulum, fronto-occipital and fimbria-fornix WM tracts.

The normal maturation and reorganization of WM during adolescence and young adult years is thought likely to be disturbed and explain in part the onset of AN[14]. WM fibres such injured WM fibres may not recover in those with enduring illness and years of starvation[15]. Simultaneously, other studies associate nutritional status with quickly occurring GM and WM changes[11].

A variety of studies have previously used the DTI technology showing reduced FA in a multitude of regions across mood, psychotic, anxiety and substance use psychiatric disorders[16]. In AN, the limited research has just began to identify functionally related brain areas structures[13]. Therefore, added to the difficulty to identify AN prior to illness onset, it remains unclear if the structural brain abnormalities are a prerequisite or

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┌─────────────────────────────┐      ┌─────────────────────────────┐
│ Studies identified from      │      │ Studies identified from      │
│ searching                    │      │ searching                    │
│ English databases            │      │ Spanish databases (28.04.2015)│
│ (28.04.15)                   │      │ (n = 0)                      │
│ (n = 26)                     │      │ Lilacs                       │
│ PubMed (n = 10)              │      │ CSIC                         │
│ Scopus (n = 16)              │      │                              │
└─────────────────────────────┘      └─────────────────────────────┘

                    ┌─────────────────────────────┐
                    │ Studies after deleting       │
                    │ duplicates                   │
                    │ (n = 8)                      │
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                    │ Studies excluded after abstract screening      │
                    │ (n = 12)                                       │
                    │ Abstract (n = 1)                               │
                    │ Non eating disorder pathology (n = 8)          │
                    │ Review article (n = 1)                         │
                    │ Non clinical eating disorder sample (n = 1)    │
                    │ Non published in English neither in Spanish    │
                    │ (n = 1)                                        │
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Figure 1 Selection process of the retrieved studies.

consequence of the development of AN[5].

Rationale for the current review

Findings are very heterogeneous with respect to WM abnormalities regarding to brain region and behaviour-relationship. Hence, the aim of the current systematic review is to critically examine and synthesise the existing research that has investigated WM microstructural alterations in individuals with AN using DTI. Specifically, the functions of implicated brain areas and relationships with psychopathological measures, and implications for understanding the pathophysiology of AN will be described.

MATERIALS AND METHODS

Search strategy

The search strategy was designed to identify all studies of subjects with eating disorders in which microstructure of brain WM brain fibres was analysed through DTI. The literature search was conducted in November 17th 2014 in SCOPUS and Pubmed electronic databases. The following search terms were used: "DTI" AND ("AN" OR "eating disorders"). All fields were included in order to expand the quest. Trials were incorporated if they were published in English and peer-reviewed.

Secondly, we included an additional search in the Spanish language. In December 17th 2014, we intro-duced the following terms on Lilacs and CSIC electronic databases: "imagen de tensor de difusión" Y ("anorexia nerviosa" O "trastornos de alimentación"). The findings were non-existent in in both of these databases.

In order to update the search, on April 28th 2015 we conducted a third data search on SCOPUS, PubMed, Lilacs and CSIC electronic databases. We used the terms previously described.

A total of 26 papers were retrieved, from which 8 duplicates were removed. The studies were screened to determine the suitability according to this review by two authors independently. Data extraction and quality assessment were done by Beatriz Martin Monzon, checked for agreement by Nasim Foroughi and disagreements were reviewed by Phillipa Hay. According to the inclusion criteria, 7 papers were excluded based on the abstract, leaving a total of 6 articles for full review. A detailed map of the search strategy stage is shown in Figure 1.

Selection of the studies

In order to prevent selection bias, the following inclusion criteria were decided on prior to the review. The studies should be: (1) published in a peer-reviewed journal; (2) available either in English or Spanish; (3) participants were over 14 years of age and under 65 years of age; (4) the experimental group should be composed of participants who met the diagnostic criteria for current or lifetime AN according to DSM-5 or DSM-IV criteria; (5) the control group should be composed of healthy participants; and (6) correlates of brain WM fibre were assessed using DTI measures.

Papers were excluded if they were dissertations, review articles or abstracts. No restrictions were placed on year of publication, gender of participants and body mass index (BMI).

Quality assessment

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses guidelines[17].

The final retrieved articles were assessed using the

Table 1 Baseline characteristics of the included studies (all cross-sectional in design)

Ref.	AN subtype	ED subtype	Study purpose	Method DTI	Assessment
Kazlouski *et al*[13]	Current AN restricting and purging subtype	AN purging and restricting subtype	Test of the brain WM integrity alteration and its relation to heightened anxiety in AN	GE 3 Tesla whole-body MRI scanner, maximum gradient amplitude of 40 Mt/M and maximum slew rate of 150 T/m per second, 8-channel phased-array head coil	SCID-CV EDI-3 TCI STAI BDI
Frank *et al*[49]	Current AN restricting and purging subtype	AN	Analysis of GM and WM in a sample of adolescents in comparison to adults	Signa 3T Scanner, axial, 3-dimensional, T-1 weighted magnetization-prepared rapid acquisition gradient echo	C-DISC EDI-3 TCI STAI CDI SPSRQ 9 point Likert scale sweetness-pleasantness of 1 molar sucrose
Nagahara *et al*[7]	Current AN, NS subtype	AN	Exploration of WM abnormalities in AN patients compared to HC	Whole body 3 Tesla MR system with an eight-channel phased-array head coil	SCID-CV EDI-2 BDI-II
Via *et al*[53]	Current AN restricting subtype	AN	Assessment of WM microstructure in a clinical sample of AN patients	GE Signa Excite scanner at 1.5 T equipped with an 8-channel phased-array head coil	SCID-CV SCID-I/NP GHQ-28 EDI-2 HAM-D HAM-A
Frieling *et al*[5]	Current AN with NS subtype and recovered women from AN	AN and recovered women from AN	Assessment of the microstructural integrity of WM pathways through DTI in women with AN	3 T Siemens-scanner, with a gradient field strength up to 45 MT/m	None
Yau *et al*[39]	Recovered women from restricting type AN	Recovered women from restricting type AN	Examination of the microstructural alterations of WM integrity in recovered AN women	3.0 Tesla Discovery MR750 scanner with an 8-channel phase-array head coil	SCID-CV MINI TCI STAI MPS

AN: Anorexia nervosa; DTI: Diffusion tensor imaging; GM: Gray matter; HC: Healthy controls; NS: Non-specified; WM: White matter.

Quality Index from Downs and Black[18]. The "checklist for measuring study quality" is a tool for assessing the quality of randomised and non-randomised trials, which provides a profile of their methodological strengths and weaknesses. It has reported high internal (KR-20: 0.89) and external consistency (KR-20: 0.54), tested using the Kuder-Richardson formula 20[19].

RESULTS

Description of studies
The 6 studies selected are indicative of the advances in the DTI technique for investigating WM fibre in AN patients. Table 1 presents the main characteristics of the included studies and Table 2 the characteristics of their participant samples. Three of the studies were nested in United States, one in Japan, one in Germany and another in Spain.

All the studies included a group of AN people and a control or healthy group. From this total, 2 studies also included recovered AN women as one of the groups of participants investigated. As inclusion criteria, it was mandatory to be diagnosed as AN according to the DSM-IV criteria and that the BMI was measured. All of them were comprised of women with mean ages ranging from 14 to 29 years old.

All 6 papers used a cross-sectional design. Three studies used a Tesla scanner, 2 a Signa scanner and one a Siemens model scanner for the diffusion tensor templates. Five papers used self-report questionnaires to assess psychopathological characteristics and one did not report use of assessment instruments.

Table 3 presents the main outcomes of each study and author's interpretation of the findings. Following the DTI procedures, the brain areas selected by the authors as possibly shown abnormalities are the fornix (and its connection to the fimbria tract), the cingulum, the thalamus (several nuclei), the superior longitudinal fasciculus, the corona radiata, the cerebellum and the fronto-occipital fibre tracts. Their main functions are emotion processing, body perception, reward processing, anxiety and cognitive style.

Quality assessment
Overall, the studies met most of the quality criteria considered for this review (Table 4). A maximum score of 8 points based in a yes/no/partially scale response was developed to grade quality. The score range was from 4 to 7 points, with mean of 5.75 and SD of 1.57.

Specifically, all the papers described the hypothesis or main study objective. The main outcomes to be investigated were clearly described in the Introduction

Table 2 Characteristics of the participant samples in the reported studies

Ref.	AN subtype	Total size	Sample characteristics *n* Gender Mean ± SD: Age/years BMI Duration of illness/years		
			ED group (AN)	Recovered AN	Healthy group
Kazlouski *et al*[13]	Current AN restricting and purging subtype	*n* = 33	*n* = 16 (10 resAN, 6 purgAN) Female 23.9 ± 7 SD 16.5 ± 1 SD 7.5 ± 8 SD	-	*n* = 17 Female 25.1 ±4 SD 21.5 ±1 SD NA
Frank *et al*[49]	Current AN restricting and purging subtype	*n* = 41	*n* = 19 (17 resAN, 2 purgAN) NS 15.4 ± 1.4 SD 16.2 ± 1.1 SD NS	-	*n* =22 Female 14.8 ± 1.8 SD 21.3 ± 1.9 SD NA
Nagahara *et al*[7]	Current AN, NS subtype	*n* = 35	*n* = 17 Female 23.8 ± 6.68 SD 13.6 ± 1.3 SD 4.93 ± 4.9 SD	-	*n* = 18 Female 26.2 ± 5.6 SD 19.9 ± 2.0 SD NA
Via *et al*[53]	Current AN restricting subtype	*n* = 38	*n* = 19 Female 28.37 ± 9.55 SD 17.03 ± 1.09 SD 6.52 ± 6.03 SD	-	*n* = 19 Female 28.63 ± 8.58 SD 21.09 ± 1.80 SD NA
Frieling *et al*[5]	Current AN with NS subtype and recovered women from AN	*n* = 41	*n* = 12 Female 26.8 ± 6.94 SD 15.18 ± 1.39 SD NS	*n* = 9 Female 27.44 ± 5.3 SD 19.31 ± 1.39 SD NS	*n* = 20 Female 24.80 ± 2.6 SD 19.60 ± 0.94 SD NA
Yau *et al*[39]	Recovered women from restricting type AN	*n* = 22	-	*n* = 12 Female 28.7 ± 7.9 SD 21.2 ± 1.5 SD 5.6 ± 5.2	*n* = 10 Female 26.7 ± 5.4 SD 22.0 ± 1.1. SD NA

AN: Anorexia nervosa; recAN: Recovered anorexia nervosa; purgAN: Purgative subtype anorexia nervosa; resAN: Restrictive subtype anorexia nervosa; BMI: Body mass index; NA: Non-applicable; NS: Non-specified.

or Method section. The participant characteristics were clearly described, often being displayed in a chart or table. The intervention of interest wasn't applicable to the present systematic review, since none of the retrieved studies developed an interventional design. The main weakness of the studies was the description of the confounders' distribution. This was clearly described in one, partially in two and not provided in three studies. Adjustment for confounding was reported in three studies only. Also, most studies did not include another mental health disorder such as depression in participants in a control group. This was clearly described in one study, partially in two studies and not provided in three studies. The main study findings were clearly described in all the papers. All but one study, which didn't provide enough information, selected appropriate statistical tests for each research study measurement.

Measures used
DTI measures reported in the six included papers: Water diffusion direction along the WM tracts was measured with FA and MD was used as an indicator of white matter integrity, a scalar measure reflecting the magnitude of diffusion. Mean diffusivity (MD) or in other term apparent diffusion coefficient provides information about the average diffusion-freedom water molecules have in the brain tissue, and correlates with local cell breakdown[20-22].

Psychopathological measures: To confirm the diagnoses of AN, five of the 6 collected studies used the Structured Clinical Interview for DSM-IV Axis I Disorders. Specifically, four studies used the clinician version[23] and one of them included the non-patient edition for the sample group[24] and the General Health Questionnaire[25]. One of the studies chose the Computerized Diagnostic Interview for Children[26] and one The Mini-International Neuropsychiatric Interview[27].

In general, the majority of the studies used the same assessment instruments for general eating disorders symptoms, depression as well as anxiety. The presence of symptoms and psychological features

Table 3 Main diffusion tensor imaging outcomes and author interpretation

Ref.	AN subtype	Outcomes: DTI measures and areas in AN	Author interpretation
Kazlouski *et al*[13]	Current AN restricting and purging subtype	FA: Lower in the bilateral fimbria-fornix, fronto-occipital and cingulum fiber tracts	Anxiety is predicted by the fimbria-fornix FA value. Thus, reduced WM integrity could provide a mechanism for heightened anxiety
Frank *et al*[49]	Current AN restricting and purging subtype	FA: Lower in fornix, cingulum and corpus callosum (corona radiata and forceps mayor)	Abnormal fornix integrity could lead to altered feedback between limbic and higher-order brain structures. The corpus callosum could be implicated in taste processing
Nagahara *et al*[7]	Current AN, NS subtype	FA: Lower value in the left cerebellum MD: Higher value in the fornix	WM abnormalities in the fornix and the cerebellum may be neural substrates of the pathophysiology of AN. The fornix is one of the important components of the Papez circuit, which links the limbic system with other brain structures. The correlation of WM alteration with physical severity, including BMI and duration of illness may indicate that WM alteration is more relevant with regard to physical severity rather than psychological severity
Via *et al*[53]	Current AN restricting subtype	FA: Lower in the parietal part of the left SLF and the fornix. MD: Higher in the SLF and the fornix. They also reported significantly increased MD in the fornix, accompanied by decreased FA and increased RD and AD	The left SLF seem relevant to body image distortion as well as other cognitive processes like the called weak central coherence. The fornix is a key structure involved in the regulation of body-energy balance and processing of reward responses
Frieling *et al*[5]	Current AN with NS subtype and recovered women from AN	FA (AN and recAN): Lower in the posterior thalamic radiation bilaterally (which includes the of optic radiation) and the left mediodorsal thalamus	The posterior thalamic radiation fibres project to areas involved in the processing of the body image, whose alteration could explain the AN body image distortion. The left mediodorsal thalamic nucleus is connected to regions contributing to impairments in cognitive domains, especially set/shifting ability, executive control, habit learning and reward processing
Yau *et al*[39]	Recovered women from restricting type AN	FA (recAN): Insignificant alteration MD: Lower in frontal, parietal and cingulum	Lower MD was associated with harm avoidance, suggesting a possible underlying trait associated with AN. Localization of disturbances in frontal-parietal and cingulum WM suggests that these pathways, which are important for cognitive control, may be susceptible to core AN pathology. Malnutrition seems to have potentially lasting effects on WM integrity and degree of recovery

AD: Axial diffusivity; AN: Anorexia nervosa patients; HC: Healthy controls; FA: Fractional anisotropy; MD: Mean diffusivity; recAN: Recovered from AN; SLF: Superior longitudinal fasciculus; WM: White matter; RD: Radial diffusivity.

Table 4 Quality assessment of the retrieved studies

Ref.	Q1 Hypothesis clearly described	Q2 Main outcomes clearly described in the introduction or methods section	Q3 Participants characteristics clearly described	Q5 Distributions of confounders in each group of subjects clearly described	Q6 Main study findings clearly described	Q15 Study measurement blind	Q18 Appropriateness of the statistical tests	Q25 Adjustment for confounding
Kazlouski *et al*[13]	Yes	Yes	Yes	No	Yes	U	U	No
Frank *et al*[49]	Yes	Yes	Yes	Partially	Yes	U	Yes	Yes
Nagahara *et al*[7]	Yes	Yes	Yes	No	Yes	U	Yes	No
Via *et al*[53]	Yes	Yes	Yes	Yes	Yes	U	Yes	Yes
Frieling *et al*[5]	Yes	Yes	Yes	Yes	Yes	U	Yes	Yes
Yau *et al*[39]	Yes	Yes	Yes	No	Yes	U	Yes	No

Q4 regarding to interventions of interest clearly described was not applicable to this review since any of the studies undertook an interventional design. Yes scored as 1, No scored as 0, Partially scored as 0.5. U: Unable to determine.

typically presented in AN was assessed through the eating disorders inventory-3[28] in two studies whilst its second version[29] was used in other 2. Three studies complemented it with the temperament and character inventory[30]. Mood was assessed with the beck depression inventory first edition (BD I)[31] and its second edition (BD I - II)[32] in two studies. Three studies used the state-trait anxiety inventory[33] to measure anxiety levels. Other questionnaires applied the hamilton rating scale for depression[34] and the hamilton rating scale for anxiety[35,36], the children's depression inventory[36], the revised sensitivity to reward and

punishment questionnaire[37] and the multidimensional perfectionism scale[38]. In addition, in one study a molar sucrose and a control solution for sweetness and pleasantness were rated on a 9-point Likert scale.

DISCUSSION

The neurobiology of AN is still poorly understood. This systematic review reports research that investigated WM fibre tracts using advances in DTI procedures. Sample selection criteria, type of scanner and psycho-pathological measures used, as well as DTI correlates with brain functions connections were documented. Six studies were included and WM fibre changes were found in tracts relevant to emotional brain circuits as well as to neurocognitive brain structures.

Summary of findings
Four of the six included studies performed the WM fibre measurement in patients at the sick state, one included recovered AN patients and another one studied the possible WM alterations in recovered and as well AN acute patients. As a control measure, some studies included the indicator of low BMI in the healthy sample to make the groups comparable. The criteria for recovery differed in the two studies that included such participants. Specifically, in the study of Frieling *et al*[5], the participants didn't meet criteria for a diagnosis of AN at the time of inclusion, and had a BMI above 18 km/m^2. In contrast, Yau *et al*[39] defined recovery based on symptoms of no food restriction, no compulsive exercise and not engaging in any other ED behaviours as well as sustaining a stable weight (± 3 kg and 90% to 120% of mean body weight) with regular menstrual cycles.

Four of the studies found either a low FA value or a high MD correlate in the fornix. This formation is an important WM structure that originates from the hippocampus as the fimbria-fornix tract and projects superior-anteriorly toward the midline, forming the body of the fornix[40]. It is a part of the Papez Circuit, an element of the limbic system involved in the regulation of emotions by higher order frontal cortical brain regions. It is linked to the hypothalamus, the amygdala as well as to prefrontal areas like the ventral striatum, the orbitofrontal cortex and the cingulate cortex[41]. The fornix is a key connection involved in the regulation of body-energy balance and processing of reward responses. It has been associated with trait anxiety and harm avoidance and responds to leptin (feeding inhibiting hormone)[42]. The last studies suggest that fornix alterations in AN could be involved in the characteristic of food restriction, disrupted meal patterns, altered food reward processing and difficulties making behaviour changes[43]. Previous studies in rodents have identified those altered patterns[44].

Another well reported brain structure in three of the retrieved studies is the cingulum. It is composed of a WM association fibres bound from the cingulate gyrus to the entorhinal cortex in the brain. It is part of the limbic system which connects frontal lobe regions with posterior structures as the temporal lobe and hippocampus. They comprise a network that integrates the necessary behaviours for executive function and emotion recognition[45]. Alteration in this structure could be related to altered emotion identification and processing in AN, as well as disturbances in cognitive control[46].

At the same time, two studies found reduced FA in fronto-occipital association fibres. These tracts connect frontal with occipital and posterior parietal and temporal lobes. They integrate auditory and visual association cortices and may have a role in the spatial awareness and neglect, as well as emotion recognition and expression[47]. The fronto-occipital WM abnormality could be related to altered body perception shown in AN. It has been associated with abnormal recognition of emotions in others and emotion regulation within themselves[48].

Frieling *et al*[5] (2012) identified bilateral reductions of FA maps in the posterior thalamic radiation and the left mediodorsal thalamus. Alterations of the posterior thalamic radiations are of special interest because they are connected with the occipital, temporal and parietal lobes which are all involved with the cortical processing of body image perception. In contrast, the left medio-dorsal thalamus is linked to brain structures implied in cognitive domains related to the shifting ability, executive control, habit learning and reward processing.

Alterations of WM fibre were seen as well in adolescent AN samples. Frank *et al*[49] (2013) registered a low FA in the corpus callosum. This area facilitates communication between left and right sided brain structures and has been implicated in taste processing. Parent *et al*[50] (1996) found an alteration in the corona radiate and forceps major, a group of bundles that connect the cerebral cortex with the basal ganglia and spinal cord. Both structures are related to taste, whose significance could be related to altered taste and processing in AN[51].

There are several brain structures that integrate a functional network connecting the prefrontal and parietal areas which may be implicated in the body image distortion found in AN. The superior longitudinal fasciculus is the major association WM tract connecting these regions[52]. Accordingly, Via *et al*[53] (2014) reported significant FA decreases in the parietal areas of the superior longitudinal fasciculus, which sets the basis for the representation of the body self-image[54]. Proprioception, size and spatial judgment, visual imagery and the integration of visual information are properties of body image. Apart from that, the authors suggested the possible implication of the superior longitudinal fasciculus in other cognitive processes such as the "weak central coherence" reported in people with AN. This cognitive style is characterized by an enhanced attention to local details at the expense of global processing and is found in several disorders[55]. These abnormalities are thought to depend on alterations of

long-range connections between prefrontal and parieto-occipital areas, which could be also implicated in AN[56].

One of the studies[39], reported altered WM patterns in parietal areas of AN subjects. The authors found reduced diffusivity in fronto-parietal areas that was associated with cognitive control to be related to increased post-error slowing during response inhibition[57]. These results are consistent with increased cognitive control and error perseveration[58]. Thus, a low FA value may be associated with typical AN traits such as enhanced cognitive control and clinical perfectionism[6].

Moreover, it has been seen that abnormalities in the left cerebellum exist in AN patients. Nagahara *et al*[7] (2014) found a low FA value in the lateral zone of this structure. It projects to the dentate nucleus which has connections with the ventral anterior nucleus and ventral lateral nucleus of the thalamus. Added to the functions that we have specified previously, the thalamus facilitates coordinated movements and has a direct bidirectional connection with regions involved in the regulation of food intake. Specifically, the connections with several hypothalamic nuclei (the lateral hypothalamic areas, the dorsomedial hypothalamic nucleus and the ventromedial hypothalamic nucleus) could prompt abnormal food intake behaviour in AN[59]. Furthermore, the cerebellum also relates social, empathic and emotional function, including fear response[60]. Altogether, the alterations of these functions may underline the pathophysiology of AN[61].

In sum, the pathophysiology of AN is still unknown but the limbic system is undoubtedly implicated as many of the DTI studies found alterations in this network. The connections with the prefrontal areas might explain the dysfunction which appears in the cognitive style and emotion procession. Two main theories are considered in understanding these neurocognitive mechanisms in AN. The alterations in WM fibre could reflect predisposing personality traits and neurocognitive style. On the other hand, the effects of undernutrition could cause these abnormalities in WM.

Limitations and future research

Assessment instruments varied between the studies based on the analysis method. Most of the studies selected a combined method of assessment including cognitive assessment *via* questionnaires and MRI scanning for analysis of the WM fibre. Although some of the questionnaires were commonly used in most of the studies, the diverse range of used questionaries is problematic for comparing between studies. There was also diversity in the type of scanner used for imaging, the patient's position in the scanner, and the selected regions of interest.

Previous studies have analysed specified DTI measures, including FA and MD. The axial diffusion coefficient has also been used in some of the previous studies. Future studies should consider investigating the relationship between the DTI measures and indirect measures. The exposed FA and MD are broad measures that could be driven by a number of factors (*e.g.*, axonal ordering, density, degree of myelination). For this reason, axial diffusivity and radial diffusivity (RD) are thought to index more specific aspects of WM pathology, being more sensitive to changes in integrity and myelination, respectively. The combined quantification of direct and indirect measurements is recommended to better characterize any putative changes in WM structure.

All the studies used a cross-sectional design and there is a need for longitudinal studies to examine the WM alterations over time to determine if changes found in the recovered state are a persistent "scare" of illness effects such as starvation, or are innate to the disorder. Furthermore, future research should consider more comprehensive examination of myelination changes in people with AN. Changes in RD have been observed after different periods of cognitive treatment or meditation. This may reflect a brain plastic response that could provide new insights for understanding recovery in AN.

Finally, this review was limited in that only four databases were used and two of which were English language databases. However these two were the most relevant databases to the topic, and one, Scopus, is a composite of papers from many sources including several other databases such as MEDLINE and EMBASE. Insufficient funding for translations also precluded sourcing literature published in languages excepting those which the authors were fluent in.

In conclusion, the results of our review suggest that WM changes exist in brain fibres of people with AN. The research also showed that the changes appear in the brain during the sickness stage and the recovery stage in patients with AN. The main brain alterations seem to involve tracts of the limbic system, as the fornix. A prompt treatment plan for the sickness and recovery stages seems crucial for minimising the progression of brain impairments. However, there were few studies and problems of inconsistent assessment methodologies and a need for replication of findings and longitudinal study design.

COMMENTS

Background
There is evidence for biological underpinnings in anorexia nervosa (AN) that argue in favour of structural brain abnormalities. Previous research performed with Voxel Based Morphometry has found reduced grey matter (GM) volumes in people with AN compared to healthy controls. White matter (WM) is the second major component of the central nervous system (CNS) and it coordinates communication between different brain regions.

Research frontiers
Very little research has been performed on WM in AN participants due to limited technologies able to investigate its particular structure. New advances with diffusion tensor imaging (DTI) however have identified alterations in several areas of the brain WM providing new insights into the structure and related

function of WM in people with AN.

Innovations and breakthroughs

This review synthesises all the studies to date (April 2015) using DTI to assess the WM integrity in AN. Alterations in a range of WM structures of the limbic system were identified in regions associated with anxiety, body image and cognitive function. Subtle abnormalities also appeared to persist after recovery but no studies were prospective.

Applications

This systematic review synthetises high quality research evidence related to WM brain alterations in AN. It provides researchers with the latest knowledge available and directions for future research.

Terminology

WM: Component of the CNS that distributes information between different brain areas; GM: Component of the CNS related to processing and cognition activities; DTI: Magnetic resonance imaging method that allows the mapping of the diffusion of molecules in the brain, *in vivo* and non-invasively. It is able to delineate fibre tracts within the WM; Fractional anisotropy (FA): A value that describes the degree of anisotropy of a diffusion process. It is thought to reflect myelination of WM; Mean diffusivity: An opposed value to FA which reflects the isotropy of the diffusion.

Peer-review

The present systematic review aims to identify findings concerning WM fibre microstructural alterations in AN. The authors find that WM fibre is altered in diverse areas of brain, the diversity possibly reflecting the symptom complexity of the AN.

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EDITORIAL

Reporting and understanding the safety and adverse effect profile of mobile apps for psychosocial interventions: An update

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Author contributions: Naeem F conceived the idea; Gire N helped with literature search on side effects of therapy and manuscript; Xiang S and Yang M helped with search of literature on side effects of internet and app use; Syed Y helped with security issues; Shokraneh F helped with the overall script; Adams C and Farooq S provided overall support and organized multiple discussions that lead to this paper.

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Abstract

Recent years have seen a rapidly increasing trend towards the delivery of health technology through mobile devices. Smartphones and tablet devices are thus becoming increasingly popular for accessing information and a wide range of services, including health care services. Modern mobile apps can be used for a variety of reasons, ranging from education for the patients and assistance to clinicians to delivery of interventions. Mobile phone apps have also been established to benefit patients in a scope of interventions across numerous medical specialties and treatment modalities. Medical apps have their advantages and disadvantages. It is important that clinicians have access to knowledge to make decisions regarding the use of medical apps on the basis of risk-benefit ratio. Mobile apps that deliver psycho social interventions offer unique challenges and opportunities. A number of reviews have highlighted the potential use of such apps. There is a need to

describe, report and study their side effects too. The adverse effects associated with these apps can broadly be divided into: (1) those resulting from the security and safety concerns; (2) those arising from the use of a particular psycho social intervention; and (3) those due to the interaction with digital technology. There is a need to refine and reconsider the safety and adverse effects in this area. The safety profile of a mobile PSI app should describe its safety profile in: (1) privacy and security; (2) adverse effects of psychotherapy; and (3) adverse effects unique to the use of apps and the internet. This is, however, a very new area and further research and reporting is required to inform clinical decision making.

Key words: Mobile; Psycho social; Side effects; Health; Media; Security; Privacy

Core tip: Mobile apps offer unique opportunities and risks when used for delivering psychosocial interventions. While there is some evidence to inform clinicians and patients of the efficacy of these apps, only limited information is available on their risk profiles. The side effects of mobile psychosocial apps might be due to the privacy and security issues, side effects of a particular therapy that is being delivered or due to the use of excessive use of internet or the apps. There is a need for clinicians and patients to report the side effects in these areas.

INTRODUCTION

Recent advances in mobile devices and faster Internet connectivity of these devices has led to a new era in health technology. Smartphones and tablet devices are thus becoming increasingly popular for accessing information and a wide range of services, including health care services. Modern mobile phones offer stable and versatile platforms that allow delivery of a variety of services. Mobile apps can support a variety of routine medical tasks, ranging from education and assistance to clinicians to helping and supporting the patients. These apps have also been established to benefit patients by providing a range of interventions across most medical specialties. Medical apps are used by clinicians to access medical knowledge. All these mobile apps have their advantages and disadvantages. In this article, we will only focus on the mobile apps that are

used for delivering psychosocial interventions. A mobile psychosocial intervention (mPSI) app means a software used on a mobile platform to deliver a psychosocial intervention. These will include apps such as Breathe and Relax, PTSD Coach and the Big White Wall.

As with many interventions, the decision to use a mobile app in a particular clinical situation should be dependent on clinician perceived risk-benefit ratio. These decisions require health care professionals to have a good understanding of the intended benefits, limitations and risks of the medical apps in order to make an informed app usage decision. We have recently argued that providing accurate information in easy to understand language about development and initial testing should be an essential part of the mPSI app[1]. This information will help both patients and clinicians in making informed decisions. We have also suggested that the risks and adverse effects of psychosocial interventions are an important part of a description of the maps[1]. It is important that the person using these apps is fully aware of the safety profile and adverse effects of these apps. This is especially important within persons suffering from mental illness, as they may be more vulnerable to the adverse effects from these apps compared to the general population. The adverse effects associated with these apps can broadly be divided into: (1) those resulting from the security and safety concerns; (2) those arising from the use of a particular psychosocial intervention; and (3) those due to the interaction with digital technology. Most writers in this area have focused on security and privacy, an understandable concern[2-4]. We will briefly describe these here. Other adverse effects such as those resulting from the interaction with these devices have received little attention and will be described in more details[5].

SECURITY AND PRIVACY ISSUES

When conducting any form of health research, it is imperative for researchers to follow the principles set out by the World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects[6]. These guidelines ensure the safety of participants, the right by participants to withdraw from the study, recruitment of participant's security, privacy and confidentiality.

Mobile applications with a low level of security or privacy can cause serious issues, and can have severe implications for users and organizations alike. But can the mobile environment ever be considered secure? Past security incidents including vulnerabilities found in well-known mobile apps and malware attacks on mobile platforms suggest that the mobile environment is far from secure despite advances in security measures in cyberspace[4]. Rapid growth of mobile devices with position sensors has made Location-based Services readily accessible. These mobile devices send user's

location information to the third party location servers, which can be accessed by other service providers. Those aware of this, might feel continuously tracked[3]. This might have serious implications for most persons suffering from any psychiatric disorders with increased anxiety and paranoia.

Perera[7] described a number of safeguards which can be used to ensure data security on mobile devices. To ensure protection mobile devices should be accessible *via* a pin; it is recommended that rather than a four digit pin an alphanumeric passcode is used. In addition, functionality, whereby data is wiped from the device after 10 failed passcode attempts would further protect data[7]. Furthermore, encryption of mobile devices, enabling remote wiping of data held on the device and storing data in the cloud instead of the mobile device are key strategies in ensuring data security[7].

Another factor which needs considering is the number of notifications and alerts which are programmed into mPSI apps. Firstly, the notification iconography may need to be discreet/private as not to cause any distress to participants in the case of someone accidentally viewing the icon; this may infer the individual is undergoing therapy and may be stigmatizing. Individuals should be given control in the use of the mobile device, and it should not be seen as an intrusion into their daily life.

Lewis *et al*[2] suggest that these risk factors can be broken down into internal and external risk factors. Although internal risk factors may be reduced through appropriate regulation, external risk factors can only be eliminated through proper training and education. The same authors have also suggested a two-dimensional "app-space" where an app can be located depending on a variety of factors. The authors suggest that based on combined chances of harm and complexity, an app will fall into one of four categories: (1) requiring only local inspection; (2) requiring a more formal risk assessment; (3) requiring professional review of a full profile; and (4) those requiring formal regulation and review by governmental bodies such as the United States Food and Drug Administration Agency due to their high probability of causing harm". In a recent opinion paper[1], we have reported that the mPSI apps can be divided into three types: (1) type 1, intervention delivered by a human therapist through eMedia (*e.g.*, telephone-delivered problem solving by a therapist, Avatar Therapy); (2) type 2, intervention based on a manualized, well-established therapy delivered through eMedia (*e.g.*, CBT delivered from a website that is based on a manual); and (3) type 3, a new intervention that did not exist before, and is not based on previous theory or on therapeutic principles (*e.g.*, electronic dispensing). These criteria need a further definition that relates to the risks attached.

ADVERSE EFFECTS DUE TO PSYCHOSOCIAL INTERVENTIONS

Since a classic paper of Bergin[8] on the description of the possibility of a psychological treatment producing negative effects, clinicians and researchers had low interest in this area[9]. This is a re-emerging area and research has just started in this area. But it has been estimated that between 3% and 15% of the recipients experience unwanted effects. These rates are similar to those of pharmacotherapy[10]. There are only a few reported studies comparing the adverse effects of psychosocial interventions, for example, Klingberg *et al*[11] reported an RCT, which compared CBT for psychosis with Cognitive Remediation Therapy. Both groups experienced nearly the equal adverse effects. Lambert *et al*[12] has suggested that between 5% and 10% of all patients undergoing psychotherapy deteriorate.

Recently, the need for expanded monitoring of negative effects in clinical trials of psychotherapy has been discussed, resulting in different suggestions on how to define and measure the negative effects. Linden[10] presented a comprehensive checklist dividing negative effects into different categories. These include: (1) deterioration; (2) adverse events; (3) severe adverse events; (4) novel symptoms; (5) dropout; (6) nonresponse; (7) unwanted events; and (8) suicide attempts and deaths by unnatural means.

ADVERSE EFFECTS UNIQUE TO APPS AND INTERNET USE

There are a number of adverse effects that are unique to the use of mobile apps and the internet. These include reduced face to face communication which probably can result in inadequate social skills (however, it can be argued that future generations might not need social skills as we know these). This is particularly important as most psychotherapeutic interventions aim to enhance communication and social skills. The "virtual" interactions may result in reduced problem-solving skills in the real world. There are also possible adverse effects of using the internet for increased periods which can contribute to increased levels of inactivity and sedentary behaviors which have been reported to increase the risk of obesity[13].

Information overload (or worse still inappropriate information) can lead to cognitive problems. Similarly, insomnia, depression and anxiety are common among heavy net users[14]. It is important that these factors are highlighted due to the way individuals are using apps and the internet, but also due to the increasing availability of internet on mobile devices.

One of the key adverse effects of the internet is internet addiction, with a study by Boysan *et al*[15] in the United Kingdom reporting that out of 2257 university students 3.2% were addicted to the internet. Furthermore, Ko *et al*[14] suggested the heightened comorbidity of psychiatric disorders and internet addiction, with more research needed to better understand this phenomenon. Another possible adverse effect of internet usage is the potential for online sexual

grooming and exploitation of children, due to factors such as anonymity which may provide an environment for perpetrators to engage in sexually motivated behaviours[16].

More specific to the area of apps, mobile devices which run mPSI apps produce electromagnetic fields which have been suggested as being carcinogenic by the World Health Organisation (WHO) with the WHO conducting a formal risk assessment of this potential adverse reaction, due 2016[17]. Furthermore, it has also been found that another possible adverse effect of apps is high frequency usage. A study by Thomée *et al*[18] found an increased risk factor for mental health outcomes in young adults with high frequency use associated with stress, sleep disturbances and symptoms of depression at one-year follow up. In addition, there has also been research suggesting increased risk of ocular problems, with viewing mobile phone screens causing eye strain[19]. Other complications have also been found in relation to viewing mobile device screens, with Wood *et al*[20] reporting that exposure to self-luminous screens on mobile devices have the potential to increase the likelihood of sleep disorders due to factors such as melatonin suppression, particularly in the blue light spectrum. It is also important that individuals feel no pressure in replying to the mPSI app notifications and alerts, as there may be a risk of increasing paranoia and anxiety.

It is important that these adverse effects are systematically observed, and data are recorded in any psychosocial intervention studies. This will require both qualitative and quantitative studies. The qualitative studies will help us to understand patient experience, which has rarely been studied in psychosocial interventions using mobile apps. Furthermore, adverse effects should be reported to regulatory bodies such as the FDA and MHRA. Naeem *et al*[1] proposed a framework for understanding that mPSI apps use lessons learned by the pharmaceutical industry to ensure the safety of mPSI apps through rigorous testing and evaluation.

CONCLUSION

There is a need to refine and reconsider the safety and adverse effects in this area. The use of mPSI interventions offers unique opportunities and risks. The safety profile of a mobile PSI app should describe its safety profile in: (1) privacy and security; (2) adverse effects of psychotherapy; and (3) adverse effects unique to the use of apps and the internet.

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MINIREVIEWS

Bilingualism and schizophrenia

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Abstract

Although a bilingual advantage has been described for neurodegenerative disease in general, it is not known whether such an advantage could accrue to individuals suffering from schizophrenia, since language networks are known to be disrupted in this condition. The aim of this minireview was to scan the existing literature to determine: (1) whether individuals with schizophrenia are able to learn a second language as adults; (2) whether clinical assessment, both for the purpose of accurate diagnosis and for the prediction of treatment response, should be carried out in both languages in bilinguals with schizophrenia; (3) whether psychotherapy in schizophrenia is affected by bilingualism; and (4) whether speaking a second language improves outcome in schizophrenia. The literature to date is too sparse to make definitive statements, but: (1) individuals with schizophrenia appear to be capable of learning a new languages as adults; and (2) it is possible that teaching a foreign language may serve as a form of cognitive rehabilitation for this condition. This literature review recommends research into the effects of bilingualism on the outcome of schizophrenia. Included in this review is a retrospective pilot study conducted in Canada, which suggests that employment opportunities for patients with schizophrenia are improved when they speak more than one language. This is important to note because employment is generally problematic in the context of schizophrenia while, at the same time, the ability to obtain work contributes significantly to quality of life.

Key words: Schizophrenia; Bilingualism; Language; Employment; Cognitive rehabilitation; Outcome

Core tip: Even though language deficits are a core problem in schizophrenia, learning a second language may be of cognitive and social benefit. Bilingualism may contribute to cognitive reserve and may be especially valuable in increasing employment opportunities for patients with schizophrenia.

INTRODUCTION

Language and schizophrenia

Language idiosyncracies have long been observed

in patients with schizophrenia[1-4]. The deficiencies in language that have been linked to schizophrenia include problems in speaking (flat intonation, unusual voice quality, unintelligible utterances[5]), listening (inattention, distraction, failure of understanding), reading (stilted prosody, word approximation, misunderstanding of idiom and metaphor[6]), writing (erratic handwriting, unusual use of size and space[7]), grammar[8] (chaotic sentence structure and syntax, unusual order and sequence), and vocabulary (limited vocabulary, neologisms, clanging or glossomania). Alogia, or very little conversation, often accompanies schizophrenia, and, of all language disturbances, is the one most predictive of a poor quality of life[9]. Disorganization of language is almost pathognomonic of schizophrenia, is especially pronounced under conditions of stress[10], and is more pronounced in men than in women[11]. Interestingly, there is a relative absence of disorganized language in late onset schizophrenia, a condition more prevalent in women[12].

Speech in adolescent and young adult onset of schizophrenia is characterized by a loosening of associations, which takes the form of derailment, a slipping into oblique or unrelated topics, or of tangentiality, where responses to a question seem to be unrelated to the question posed. Metaphors and proverbs are characteristically difficult for individuals with schizophrenia to grasp the meaning of[13-15]. Words are used in an idiosyncratic way, suggesting a private meaning. The language of persons with schizophrenia has been referred to as a "word salad" because of the difficulty in understanding the message it conveys[16]. As linguist Chaika[17] has written, conversation in individuals with schizophrenia seems to be more influenced by the form and sound of words spoken or heard in the immediate past than by the needs of communication. The speech identifiers required to make meaning comprehensible to the listener are often missing or ambiguous[18]. Much depends on whether patients are studied when medicated or non-medicated. Medicated patients are usually more verbally communicative and show an increase in the complexity and coherence of their speech, with a decrease in pathological utterances[19-21]. This is interesting because antipsychotic drugs are antidopaminergic and research suggests that dopamine plays a role in the activation of semantic networks[22,23].

Language problems in schizophrenia have been attributed to a deficit in hemispheric lateralization[24] and to impaired executive functions such as attention and sequencing[25].

Bilingual advantage in neurodegenerative disease
While language skills form an undisputed part of the cognitive deficit seen in schizophrenia, little has been written about how bilingualism affects the course of schizophrenia. This is despite the fact that recent literature has shown potential advantages of bilingualism in other brain diseases, mainly dementia.

Bilingualism is associated with an up to five-year delay in the onset of Alzheimer's disease[26-28]. Bilingual patients diagnosed with probable Alzheimer's disease have been shown to exhibit substantially more atrophy in temporal regions than do their monolingual counterparts while still functioning at the same cognitive level[29]. fMRI studies have demonstrated greater efficiency in brain activation for bilinguals relative to monolinguals[30,31], sometimes, but not always, correlated with behaviour.

The mechanisms by which bilingualism can slow the progress of neurodegenerative disease remain speculative[32,33]. An influential model for understanding what happens in the bilingual brain is the adaptive cognitive hypothesis[34], which argues that the demands of choosing between two languages in vocabulary and in syntax mold neural networks in such a way as to benefit cognition. Particularly relevant to schizophrenia is the finding that bilingualism in the general population benefits convergent thinking while inhibiting divergent thinking[35].

Unfortunately, there is more than one definition of bilingualism[36], which probably explains the inconsistencies in the literature as to the benefit acquired through knowing more than one language[37]. It has also been suggested that there may be a bias towards reporting cognitive advantage in bilingualism studies and ignoring studies that show null or negative results[38].

AIM
In the hope of discovering whether bilingualism delays or ameliorates symptoms of schizophrenia, or improves the quality of life in schizophrenia, I did a literature search on bilingualism in psychosis.

RESEARCH
I searched the terms "foreign language", "second language", and "bilingualism" in conjunction with the terms "schizophrenia" and "psychosis" in the multidisciplinary Google Scholar database. Forty-four articles were found from the years 1955 to 2015, some theoretical, some clinical/observational, some experimental.

FINDINGS
People with schizophrenia can learn a second language
Given the cognitive deficits and negative symptoms that accompany schizophrenia, it was first necessary to find out whether people with schizophrenia were capable of learning a second language as adults. Once childhood is past, learning a second language has been considered a relatively difficult task for everyone, but it is now acknowledged that new languages can be learned at any time in life, and that adult learners have several learning advantages over children: They can rely on previously acquired language skills, their

brains are more mature, and they have more years of practice in learning how to learn. Adult learners have the disadvantage, however, of lacking the same opportunities that children have of learning a new language. They are not in school and are not always surrounded by peers speaking the new language. On the other hand, they are often more motivated to learn than they were as children[39]. Experts believe that, except for native-level pronunciation, which is difficult to attain after age ten or thereabouts, learning a new language is actually easier for adults than for children[40].

Individuals with schizophrenia are challenged by cognitive deficits, but the belief that learning a second language will exacerbate such difficulties is probably a myth[40]. Bersudsky et al[41] studied 16 Russian immigrants to Israel, eight of whom had a diagnosis of schizophrenia. They found that the two groups learned Hebrew in very much the same way and at the same speed. The investigators concluded that, despite the cognitive compromises in schizophrenia and the manifest atypicalities in the language of speakers with schizophrenia, the process of acquiring a second language was relatively unaffected by the illness[41].

Smirnova et al[42] studied Russian adults who had recently immigrated to Israel, ten of whom suffered from schizophrenia. All eight men and two women, though from varied educational backgrounds, were well able to learn Hebrew and all of them had become functionally bilingual by the time the research was conducted. Because there are so many variables influencing the speed of learning a second language (age, education, motivation, effort, exposure, and reward among others), showing that individuals with schizophrenia learn at the same speed as control subjects may require much larger samples than have heretofore been studied.

Potential benefits of bilingualism for people with schizophrenia
The cognitive benefits of bilingualism extend beyond individuals with dementia. Learning a second language has been shown to produce rapid dynamic changes in white matter tracts in all adults, changes that correspond with improved cognitive functioning[43]. This finding presents a strong argument for the general benefits of language learning for everyone. Although many types of environmental input, cognitive demand, or learning experience can result in experience-dependent neural changes, the intensity and frequency of language use appears to exert particular power in bringing about beneficial brain changes, even when languages are learned relatively late in life[44].

A second rationale for learning a second language for individuals with schizophrenia is the strong association between language skills and social functioning. Studying the language of 108 individuals in an Early Intervention Program for psychosis in Ireland, researchers found that the disorganization dimension on a formal thought

disorder scale was significantly associated with clinician-rated measures of occupational and social functioning; the higher the score on the thought disorder scale, the lower the score on functioning[45]. In addition, their measure of bizarre idiosyncratic thinking was significantly associated with a performance-based measure of functioning[45] even though, when it came to real-world measures of functioning, language disturbance was not a good predictor in this study.

Transfer of skills to real life is always problematic. Nevertheless, learning a second language can be conceptualized as a form of cognitive therapy that promises to build cognitive reserve[46]. Because cognitive reserve refers to brain plasticity, it is not an easy concept to measure, but increases in reserve may be ascertained by demonstrating changes in tests of memory/language, processing speed/executive function, and attention.

Bilingualism and assessment in schizophrenia
There is a growing literature on other aspects of bilingualism in schizophrenia, especially studies of polyglot patients with different degrees of psychotic symptoms depending on the language they use[47].

On the basis of study results, Theron[48] recommends assessment in all languages spoken by bilingual schizophrenia patients if possible, in order to determine the full range of symptoms, to gain a better indication of the severity of illness, and to be better able to track the progress of recovery.

Southwood et al[49] make the same recommendation based on oral interviews conducted with a single male patient who displayed more language disturbances in his second language than in his native language. Armon-Lotem et al[50] also describe schizophrenia patients who display more problems in their second language than in their first. Smirnova et al[42], in their study of 10 Russian Hebrew bilinguals with a diagnosis of schizophrenia, also found that some syntax and semantic impairments were more pronounced in the later-learned language.

Language idiosyncrasies in a recently learned language, however, although they may be more pronounced than in one's native language, are easily overlooked by an assessor because they are automatically attributed to imperfect language learning rather than to thought disorder[51].

It has been reported that auditory hallucinations tend to occur only in one's mother tongue[52,53], but that probably depends on the identity of the hallucinatory speaker and the content of the message[54]. To prove that diagnostic or prognostic assessment is more accurate in one language than another, the two strategies would need to be compared, which has not yet been done.

Bilingualism, schizophrenia and therapy
Psychotherapy is not considered curative in schizophrenia, but it is generally acknowledged to be an essential part of comprehensive treatment. The question often addressed in the psychotherapy literature is

whether effectiveness in bilinguals depends on the language in which therapy is delivered.

One's mother tongue is characterized in the psychoanalytic literature as the language of repressed memories or the language of the unconscious. One's second language serves more defensive purposes and is seen as the language of rationality. Given equal proficiency, the consensus is that an individual in therapy uses the language least likely to provoke anxiety, which usually means avoiding the native language whenever possible[55] because later learned languages allow more detachment when forced to speak about emotionally charged material[56].

It is reportedly typical for bilingual patients to switch back and forth between primary and secondary languages in psychotherapy, perhaps to manage anxiety or perhaps because there are some issues that can only be expressed in a specific language[57]. Some reports mention a tendency for patients in therapy to return to their mother tongue when expressing strong affects, when describing dreams, or when dealing with death or severe trauma. Memories are said to be more detailed and more emotionally-laden when told in the language in which the remembered events were originally encoded[58,59].

Bilingual patients can often express different values or even assume different identities in different languages, which can change the interpersonal relationship of patient and therapist[60-64]. Specifically for individuals with psychotic disorders, it has been suggested that avoiding the native tongue may be a defensive attempt to reduce primary process thinking and increase the strength of the healthy observing ego[65]. Such suggestions are interesting, but speculative. No empirical studies have been done.

Does a second language improve outcome in schizophrenia?

In the non-ill population, bilingualism has been shown to be associated with increased self-esteem[66], with improved communication skills, self-image, creative abilities, educational achievement, and employment opportunities[67].

Although there is no evidence of improved outcome with respect to symptoms in bilingual individuals with schizophrenia, there is a suggestion that outcome in first and second generation immigrants two years after a first episode of psychosis is superior to that of native born citizens[68]. This is the case despite the fact that immigration is a risk factor for the emergence of psychosis, and also for poor engagement in treatment[69].

In 1977, Matulis[70] gave weekly German lessons to 18 male patients in a schizophrenia ward in a Michigan hospital for almost a year, documenting language progress as well as changes in symptoms. Whenever behavioral problem emerged, instead of the culprit being removed from the language class, he was actively engaged in using the newly learned German language.

The clinical (admittedly subjective) observation was that the patients' symptoms decreased and overall well-being improved.

Quite apart from the question of the potential cognitive benefits of speaking a second language as these pertain to schizophrenia, there appear to be social advantages of second language training in psychosis.

When an individual with schizophrenia coins neologisms and speaks in "word salad" in his or her native language, communication breaks down and this results in social isolation for the speaker and an increase in stigmatizing attitudes on the part of listeners. Listeners do not react in this way when hearing disorganized language from a non-native speaker because, here, the conversational expectations are different. The use of neologisms under these circumstances is interpreted not as "madness" but as a way of new language learners to refer to objects for which they have not yet learned the correct term[51].

It has been suggested that the person with schizophrenia may even have an advantage when it comes to learning a new language. The lateral thinking ability of psychosis-prone individuals allows them to think creatively about words and automatically elaborate alternative expressions when their vocabulary is constrained[51]. In addition, the practice of listening for hallucinatory voices may sharpen one's skill for listening to interior monologue, which is a prerequisite for new language learning[51]. Such suggestions are provocative, but have not been empirically tested.

One important measure of outcome in an illness such as schizophrenia is employment. A recent study from Israel shows that, as of December 2010, only 10.6% of patients with schizophrenia with one prior hospital admission earned a minimum wage or higher. For those with multiple admissions, the percentage was 5.8%[71].

Because bilingualism is considered to be an advantage on the job market[72], and because employment rates are so low in schizophrenia[73], I compared unilingual *vs* bilingual patients in our Toronto clinic with respect to employment success.

I looked at employment outside the home of 83 consecutively admitted individuals with DSM-IV schizophrenia (33 men and 50 women) allocated to unilingual English (n = 53) and bilingual (n = 30) groups. Ages ranged from 25-55. The bilinguals were all educated in English from an early age, but had spoken another language in the parental home. In 4 out of the thirty bilingual cases, the second language was French. Six of 30 bilingual patients (all women, three French speakers) were working full or part-time. All three French speakers were teachers, two others in the group were salespersons and one was a helping professional. In the English-only group, only two of 53 patients (both women) were working, both as office assistants. The difference in employment between the monolingual and bilingual group is significant at a $P < 0.005$ level.

Other than employment, no other proxies of severity

of illness available for this sample of patients (global clinical impression, history of suicide attempts, clozapine usage, depot medication, marital status) showed any difference between the two groups.

The finding that, in schizophrenia, speaking a second language expands job opportunities, is highly relevant because, by a recent estimate, only 15.85% of individuals with schizophrenia throughout the world are gainfully employed, although 50% are judged employable[73]. Although this may be truer in Canada than elsewhere (because Canada is officially a bilingual French-English nation), bilingualism in patients with schizophrenia may prove to be an important factor in employability.

LIMITATIONS

This review has attempted to collect the literature on bilingualism and schizophrenia. The literature is sparse and conclusions remain speculative because little direct research has been done in this area.

DISCUSSION

This review has examined the literature on whether or not individuals with schizophrenia can learn a second language in adulthood and it appears that they indeed can and do. The benefits of knowing a second language are well described in the general literature, and there are theoretical reasons why bilingualism should, therefore, also benefit patients with schizophrenia. There is evidence that both native and later-learned languages are affected by the schizophrenia process, which suggests that assessment is best done in all the patient's languages whenever that is possible. A small literature on the optimal language of therapy implies that therapy in the non-native language might serve best for patients with schizophrenia. Finally, available evidence suggests that a second language might improve outcome in schizophrenia by decreasing social isolation and stigma and that it may increase the chance of employment.

CONCLUSION

The literature is sparse and overly based on small samples, making definitive statements impossible, but individuals with schizophrenia appear able to learn new languages as adults. Learning a foreign language may serve as an effective form of both cognitive and social rehabilitation. This literature review recommends research into the effects of bilingualism on various outcomes in schizophrenia.

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MINIREVIEWS

Vascular cognitive impairment, a cardiovascular complication

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Abstract

Over the past two decades, the term vascular cognitive impairment (VCI) has been used to refer to a spectrum of cognitive decline characterized by executive dysfunction, associated with vascular pathology. With 30% of stroke survivors showing cognitive impairments, it is regarded as the most common cause of cognitive impairment. This is a narrative review of available literature citing sources from PubMed, MEDLINE and Google Scholar. VCI has a high prevalence both before and after a stroke and is associated with great economic and caregiver burden. Despite this, there is no standardized diagnostic criteria for VCI. Hypertension has been identified as a risk factor for VCI and causes changes in cerebral vessel structure and function predisposing to lacuna infarcts and small vessel haemorrhages in the frontostriatal loop leading to executive dysfunction and other cognitive impairments. Current trials have shown promising results in the use of antihypertensive medications in the management of VCI and prevention of disease progression to vascular dementia. Prevention of VCI is necessary in light of the looming dementia pandemic. All patients with cardiovascular risk factors would therefore benefit from cognitive screening with screening instruments sensitive to executive dysfunction as well as prompt and adequate control of hypertension.

Key words: Vascular dementia; Leukoaraiosis; White matter hyperintensities; Cognitive screening; Neurodegeneration

Core tip: Vascular cognitive impairment (VCI) has recently been receiving more interest in the scientific world in terms of early identification, preventing as well as slowing down the rate of progression to vascular dementia. Majority of the risk factors for VCI are modifiable and thus amendable to treatment. This review aims to look at hypertension and its role in the early identification and prevention of VCI and dementia.

INTRODUCTION

Over the past two decades, the concept of vascular cognitive impairment (VCI) has been regarded as a more appropriate notion in describing the spectrum of cognitive impairment caused by or associated with vascular factors[1,2]. The concept of VCI, was proposed by Sachdev[3] in 1999 to describe the cognitive deficit of vascular origin severe enough to meet the criteria for a diagnosable disorder. It was initially ascribed to cognitive impairment of vascular origin not significant enough to impair activities of daily living (ADL), *i.e.*, cognitive impairment that does not meet the requirements for dementia[4]. The term now however refers to a broad spectrum of cognitive and behavioural changes associated with cerebral vascular pathology, characterized by attention and executive impairment ranging from early cognitive decline to dementia. VCI therefore encompasses all the cognitive disorders associated with cerebrovascular disease. It can be described more specifically as cognitive impairment affecting at least one cognitive domain, with evidence of a clinical stroke or subclinical cerebrovascular insult[5-7].

VCI is not a single disorder, rather it is a spectrum of conditions with heterogeneous clinical presentations, aetiologies and treatment[4]. VCI is characterized by deficit in executive functioning (planning, task flexibility, problem solving, *etc.*). It could however, present with a much wider range of cognitive dysfunction, non-cognitive features and behavioural changes, and currently has no universally acceptable criteria for diagnosis[5,8]. It is a concept that, in its early stage, creates an opportunity for preventive strategies aimed at preventing the onset of dementia[1].

It has been associated with the presence of white matter lesions on magnetic resonance imaging (MRI). The prevalence and degree of these lesions correlate negatively with cognitive function and positively with age[9,10]. Based on the clinical and neuroimaging features, subtypes of VCI have been proffered.

Brain at risk

Presence of cardiovascular risk factors with/without neuroimaging features of subclinical brain insult and no cognitive impairment. This is the early stage of central nervous system involvement, with the presence of cardiovascular risk factors such as hypertension and white matter hyperintensity on MRI, cognitive functioning remains within normal limits following cognitive assessment.

VCI, no dementia

Impairment in at least one cognitive domain without affectation of ADL in a patient with cardiovascular risk factors and neuroimaging features of subclinical brain insult. This follows the brain at risk but with cognitive impairment though not severe enough to affect activity of daily living.

Vascular dementia

Impairment in two or more areas of cognitive domain, severe enough to impair ADL and the presence of cardiovascular risk factors as well as neuroimaging findings of cerebral insults (white matter hyper intensities).

Mixed neurodegenerative/vascular dementia

Presence of a neurodegenerative dementia such as Alzheimer's dementia with superimposed vascular dementia[11].

This review aims to look at current concepts of VCI, its prevalence, pathophysiology and identifiable risk factors with special attention to cardiovascular risk factors as well as possible strategies aimed at preventing VCI and halting its progression to vascular dementia.

EPIDEMIOLOGY OF VCI

Community and hospital based studies on the prevalence of cognitive impairment following a stroke have consistently shown that a significant proportion of stroke patients develop cognitive impairment. Rist *et al*[12] showed that 30% of stroke survivors had cognitive impairment [as determined by a mini mental state examination (MMSE) score of < 27]. Other studies have also shown similar high prevalence of cognitive impairment ranging from 24% to 70%, three months to one-year post stroke[13-15]. Douiri *et al*[13] using the South London Stroke Register assessed the cognitive impairment of 4212 stroke patients with the MMSE and abbreviated mental test and found a prevalence of 22% at 3 mo, 22% at 5 years and 21% at 14 years post stroke. Cognitive impairment was detected within 7 d of a stroke in some of the participants but remained relatively stable after 3 mo following a stroke. The prevalence rate of cognitive impairment in this study is most likely an underestimate of the true values as the neuropsychological methods used in the assessment of cognitive function are insensitive to executive dysfunction and mild cognitive impairment[13]. Gutiérrez Pérez *et al*[16] studied cognitive function before and in the acute phase of a stroke. They found that cognitive impairment was frequent, being present in 52% of patients before the onset of stroke. In the post stroke period, 96% of the elderly were found to be cognitively impaired using a battery of neuropsychological tests while only 36% were cognitively impaired using the MMSE[16].

The Canadian study on health and ageing in a prospective cohort study of 10253 community and institution dwellers aged 65 years and over found that of the different subtypes of VCI, VCI no dementia was the

most prevalent form and it was associated with higher institutionalization and mortality rate[17]. In patients less than 74 years of age, VCI may be the commonest cause of cognitive impairment, and is associated with both an increased risk of stroke and death from stroke[17,18].

Studies done in sub-Saharan Africa have shown incidence and prevalence rates of cognitive impairment and dementia to be on the increase due to the increasing elderly population. Studies carried out in Benin, Botswana, the Central African Republic, the Congo and Nigeria determined that the prevalence of dementia ranges from 0% to 10.1% (95%CI: 8.6-11.8), and the prevalence of cognitive impairment ranged from 6.3%, in Nigeria, to 25% (95%CI: 21.2-29.0), in the Central African Republic[19]. These studies used various neuropsychological batteries in the assessment of cognitive impairment. Akinyemi *et al*[20] (2014) assessed the baseline cognitive profile and factors associated with VCI three months after stroke in the Cognitive Function after Stroke Nigerian Study. The study comprised 217 subjects of which 143 were stroke survivors. Standard neuropsychological tests including the Vascular Neuropsychological Battery, which assessed executive function/mental speed, memory, language, and visuospatial/visuoconstructive functioning were used. Among the stroke survivors, 39.9% had cognitive impairment no dementia while 8.4% had dementia at baseline. They associated pre-stroke cognitive decline as a risk factor for cognitive impairment with an odds ratio of 4.51, and educational level and dietary factors as modifiable risk factors[20]. The different prevalence values from these studies can be attributed to the different neuropsychological tests used for screening cognitive impairment, with some tests being more sensitive to the cognitive impairment profile seen in VCI than others. This buttresses the need for a standardized battery for cognitive assessment for both research and clinical use. Despite these differences in approaches to cognitive screening, these studies found high prevalence of VCI prior to and after a stroke.

Knowledge about the economic implications of VCI is valuable but insufficient. The health cost of the VCI spectrum has to be seen from a societal perspective for the true burden of disease to be appreciated as well as from the impact of cardiovascular co-morbidities on the cost and utilization of health care. The health cost of vascular dementia (VaD) (a subtype of VCI) has been found to be 23% higher than that of Alzheimer's dementia[21]. The presence of VCI superimposed on a stroke increases the economic burden as well as the burden of care. A community based study on health care utilization and cost in patients with VaD, Alzheimer's dementia, other dementias, cerebrovascular accident without dementia and control group found that the highest annual cost of health care of $14387 was in VaD compared to $7839 for Alzheimer's dementia (*P* < 0.0001). VaD had the highest cost for hospital admissions and had a three times increase in hospital days compared to cerebrovascular disease no dementia[22]. In the Sub-Saharan setting where the burden of care (physical, emotional and financial) lies heavily on the immediate and extended family, it creates and opportunity for caregiver burden/fatigue and elder abuse.

Data has shown that VCI is common irrespective of the neuropsychological test used for the screening of cognitive impairment and in the older population may be the commonest form of cognitive impairment. VCI is associated with increased mortality and higher institutionalization rates in the elderly. From an economic stand point, it carries a larger financial burden than Alzheimer's dementia and Stroke. With the looming dementia pandemic, the incidence and prevalence is expected to increase in the near future and with this increase, an increase in the financial and care giver burden.

RISK FACTORS FOR VCI
Several cardiovascular risk factors including hypertension, diabetes mellitus, dyslipidaemia, smoking and obesity have been identified as modifiable risk factors for cognitive decline. In this review, emphasis will be placed on blood pressure and its association with VCI.

Blood pressure (both lowered and elevated) has been linked with decline in cognitive function (especially in concentration). Epidemiological studies have shown an inverted "U" shaped relationship between blood pressure and cognitive performance in the elderly[23].

Zuccalà *et al*[24] studied 13635 patients without cerebrovascular disease or Alzheimer's dementia; 1583 of the subjects had heart failure. He found cognitive impairments in 26% of patients with heart failure and in 19% of the remaining subjects. Systolic blood pressure less than 130 mmHg predicted cognitive impairment among patients with heart failure. No association with specific types of heart failure was found. They concluded that systolic blood pressure was specifically associated with cognitive impairment in elderly patients with heart failure and that early treatment of low output cardiac states can reverse this cognitive state. They also buttressed the need for systematic assessment of cognitive function in the management of patients with heart failure[24]. Recent studies have supported this finding that hypotension causes reduced cognitive function (demonstrated by neuropsychological tests) and activity[25,26]. Orthostatic dysregulation has been associated with an increased tendency of white matter hyperintensities (WMH)[27]. Yamamoto *et al*[28], however disagreed with this. They found that nighttime dip in blood pressure was protective against cognitive impairment and patients who were non-dippers had a higher prevalence of cognitive impairment and vascular dementia. There is however, the need for further studies to investigate if controlling night time blood pressure would reduce the risk of cognitive impairment and dementia.

Hypertension has often been observed to be a risk

factor for vascular dementia and even Alzheimer's dementia[29]. Hypertension has been shown to cause damage to the cerebral tissues resulting in subcortical white matter lesions (leukoaraiosis), which contribute to the risk of stroke and vascular dementia. Increase in blood pressure has been associated with more severe periventricular and subcortical white matter lesions (ischaemic damage), and poorly controlled hypertension has an even higher risk of white matter lesions and thus cognitive impairment than those without hypertension, controlled hypertension or untreated hypertension[30]. In the system Europe Study, treatment of hypertensive subjects with calcium channel blockers was associated with a decrease in the incidence of dementia[31].

Leukoaraiosis has been found to be more prevalent and severe in stroke patients (both ischaemic and haemorrhagic) compared to normal people. The presence of leukoaraiosis (from its different pathophysiological processes) has more than doubles the odds of stroke and increases the odds of subsequent dementia by a factor of four[32]. Leukoaraiosis is seen on MRI as WMH in the periventricular and subcortical white matter regions of the brain. These WMH have been associated with cognitive impairment, increased risk of stroke and dementia[33]. Studies have shown that there might be a genetic basis to the development leukoaraiosis. The Genetic Epidemiology Network of Arteriopathy estimated the heritability of leukoaraiosis at 0.82 ± 0.102 (SE) *P* (< 0.0001), showing a strong genetic influence on the susceptibility of leukoaraiosis and thus the risk to cognitive impairment and dementia[34]. It is a prominent feature of Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) and is associated with increased vulnerability of the brain to ischaemic injury[35]. CADASIL is an inheritable cause of vascular dementia. Leukoaraiosis also has a sporadic form which is also associated with stroke risk factors such as hypertension and diabetes[32]. Patients with silent brain infarcts and leukoaraiosis at baseline are at an increased long-term risk for recurrent stroke, cognitive decline, and dementia[36]. A family based study design in generally healthy individuals found the heritability of WMH in individuals aged 55 years of age and over to be 0.68 (*P* < 0.0001) with heritability being higher in females than males[37]. There is a significant evidence that a gene influencing WMH is located on chromosome 4p[38]. This implies that the tendency of the cerebrovascular system to damaging changes by the cardiovascular risk factors such as hypertension may be inherited and therefore may serve as a non-modifiable risk factor or predisposing factor to VCI, with hypertension acting on this predisposition and making the brain more vulnerable to damage. Strategies that aim to prevent the onset of VCI by early identification and prompt and adequate treatment of such factors would go a long way in reducing the incidence, prevalence, economic and care giver burden of VCI.

PATHOPHYSIOLOGY OF VCI

Within the brain, there are control mechanisms that ensure adequate perfusion to meet its metabolic demands. At times of increased brain activity and metabolism, cerebral blood flow increases to meet metabolic demands and to clear metabolic waste. This is termed functional hyperaemia[39]. Cerebral autoregulation is another mechanism in which cerebral blood vessels are able to maintain a relatively stable cerebral blood flow despite changes in arterial blood pressure. These above mechanisms are able to regulate cerebral blood flow within 60 and 150 mmHg mean arterial blood pressure[40]. Aging and hypertension act independently on these mechanisms, altering the range of mean arterial pressure at which cerebral blood flow is regulated.

Aging brings with changes in cerebral blood flow, with decreased numbers of capillaries, thickened fibrotic basal membrane, some degenerative processes which contributes to decrease in cerebral perfusion, depletion of cerebrovascular reserve, and susceptibility of the brain to vascular insufficiency and ischaemic injury[41,42].

Hypertension acts on the aging brain and generates significant changes in the structure of cerebral blood vessels. It promotes atherosclerosis in cerebral arteries, promotes lipohyalinosis (a pathologic process of fibrinoid necrosis of vascular wall) of penetrating small cerebral arteries and arterioles leading to small white matter strokes (lacunas) or haemorrhages and causes adaptive remodeling of cerebral vessels leading to narrowing of the lumen and decrease cerebral blood flow with impaired cerebrovascular reactivity and functional hyperaemia. It shifts the cerebrovascular autoregulation to the right requiring higher blood pressure to maintain cerebral perfusion and increased the vulnerability of the brain to low blood pressure, predisposing to hypoperfusion during hypotension[41]. These changes may lead to cerebrovascular dysfunction through amyloid and enzyme mediated pathways. Long-term decrease in mean arterial pressure has been associated with increase tau phosphorylated at threonine 181 (p-tau 181) and thus related to decline in episodic memory[43]. Hypertension causes alteration of the blood brain barrier compromising cerebral microenvironment and increasing the vulnerability of regions of the brain critical for cognition (subcortical white matter, hippocampus and neocortex) to ischaemic hypoxic brain damage and ultimately leads to neuronal dysfunction and cognitive deficit[44]. These multiple small infarcts and small vessel disease are more often related to VaD than single major infarcts. Esiri, in his study on the neuropathological lesions seen in VaD concluded that microvascular disease, not macroscopic infarction, was the chief substrate of vascular dementia[45]. These microvascular diseases (hypoxic ischaemia and lacunar infarcts) are the processes that kick start VCI. Their appearance on neuroimaging techniques are referred to as leukoaraiosis [bilateral patchy or diffuse areas seen as hypodense areas on computer tomography (CT)

scan], and hyperintense areas on T2 weighted MRI in the subcortical regions of the brain[46].

NEUROPSYCHOLOGICAL PROFILE OF VCI

An understanding of the pattern of neurocognitive impairment in the early stages of VCI [brain at risk and vascular cognitive impairment, no dementia (VCIND)] is advantageous in the clinical detection of cognitive impairment and prevention of the progression to dementia (VaD). Cognitive function mediated by the frontostriatal loop has been shown to be susceptible to the damaging effects of subcortical disease and thus leads to impairment in frontal lobe executive function. This has been shown to occur in VCIND and VaD. The different spectrums of VCI (brain at risk, VCIND and VaD) have been proposed to have different neurocognitive patterns. While the brain at risk exhibits no clinical or functional impairment, VCIND was associated with decreased information processing speed, reduced cognitive flexibility, deficit in the ability to hold and manipulate memory, impaired verbal retrieval, and impaired verbal cognition memory and poor learning efficiency[47,48]. Therefore, in choosing a neuropsychological test for the assessment of VCI, the test in question has to be sensitive to a wide range of cognitive abilities and be especially attuned to the assessment of executive function. Using timed executive function tests may be especially sensitive to VCI (due to the slow information processing)[49].

A cross sectional study of four groups (normal control, risk of cerebrovascular disease, VCIND and VaD) was carried out to find the neuropsychological cognitive pattern using a combination of tests (trail making test, controlled oral word associated test, Boston naming test, California verbal naming test). They found that the brain at risk group was similar in their cognitive profile to the normal control as they did not fall into the cognitive impairment range of any of the tests used. VCIND group on the other hand had mild impairment in cognitive flexibility, subtle difficulty in total learning and long delay free recall. The VaD group performed uniformly well below normative expectation (moderate to severe range) in all measures of cognitive functioning[50]. Sachdev *et al*[51] in 2004 had similar findings and was able to differentiate VCI from unimpaired patients in areas of abstraction, mental flexibility, information processing speed and working memory. Cognitive impairment was also significantly correlated with deep WMH. Reed *et al*[52] however found no significant difference in memory and executive dysfunction scores in VCI and Alzheimer's disease. They concluded that cognitive effects of small vessel cerebrovascular disease were variable and not especially distinct and their use as diagnostic markers of VaD were not reliable[50]. This however is in contrast with the predominant view from research on the cognitive pattern of VCI.

The National Institute for Neurological Disorders and Stroke and the Canadian Stroke Network recommended three neuropsychological battery to diagnose VCI based on epidemiological, clinical, neuroimaging, neuropsychological and neuropathological profiles of VCI in an attempt to aid the identification of early stages of cognitive impairment, make studies comparable, and by integrating knowledge, accelerate the pace of progress[48]. They are: (1) the sixty (60) minute protocol for research studies, which assesses four cognitive domains (executive/activation, language, visuospatial, memory ± neurobehavioral changes and depression); The thirty (30) minute protocol for clinical screening of suspected VCI. This assesses Semantic Fluency (Animal Naming), Phonemic Fluency (Controlled Oral Word Association Test), Digit Symbol-Coding from the Wechsler Adult Intelligence Scale, Hopkins Verbal Learning Test, Center for Epidemiologic Studies Depression Scale, Neuropsychiatric Inventory, Questionnaire Version (NPI-Q), Supplemental: MMSE, Trail Making Test; and (3) The five (5) minute protocol for potential use by primary care physicians, nurses and other allied health professionals as well as in large epidemiological studies. This protocol contains 5-Word Memory Task (registration, recall, and recognition), 6-Item Orientation and 1-Letter Phonemic Fluency[48].

This neuropsychological battery has been validated in several countries in stroke and transient ischaemic attack patients[53]. It has also been used in studies for the identification of VCI[20]. Further validation of this neuropsychological battery for diagnosis of VCI might aid in the formulation of a diagnostic criteria for the different subtypes of VCI that would adequately capture the neuropsychological profile of VCI.

MANAGEMENT AND PREVENTION OF VCI

The role of vascular disease as a cause of cognitive impairment is evident either alone or in combination with Alzheimer's disease. The main management strategy for VCI is the symptomatic treatment of VaD, management of risk factors as well non-pharmacological approaches aimed at preventing progression to VaD. The initial assessment should include a medical history, assessment of functioning (global functioning and ADL), cognitive screening, and assessment for behavioural and psychological symptoms as well as neuroimaging techniques.

Randomized control trials for the use of cholinesterase inhibitors for the treatment of VaD have shown moderate statistical but modest clinical benefits in the treatment of VaD. Cholinesterase inhibitors (donepezil, galantamine and rivastigmine) show significant improvement in cognitive function, ADL and behaviour after 24 wk of use[49,54]. Memantine has been found to improve function and decrease care dependency when compared to controls in mild to moderately demented patients[55]. A randomized double blind placebo control

Table 1 Research studies on antihypertensive use and cognitive impairment

Ref.	Sample size	Treatment option	Primary outcome	Treatment period	Result
Tzourio *et al*[64]	6108	Participants were assigned to either active treatment (perindopril and indapamide) or matching placebo(s)	Dementia (using DSM-IV criteria) and cognitive decline (a decline of 3 or more points in the Mini-Mental State Examination score)	3.9 yr	Dementia was documented in 193 (6.3%) of the 3051 randomized participants in the actively treated group and 217 (7.1%) of the 3054 randomized participants in the placebo group [relative risk reduction, 12% (95%CI: -8% to 28%); $P = 0.2$]. Cognitive decline occurred in 9.1% of the actively treated group and 11.0% of the placebo group [risk reduction, 19% (95%CI: 4% to 32%); $P = 0.01$]. The risks of the composite outcomes of dementia with recurrent stroke and of cognitive decline with recurrent stroke were reduced by 34% (95%CI: 3% to 55%) ($P = 0.03$) and 45% (95%CI: 21% to 61%) ($P < 0.001$), respectively, with no clear effect on either dementia or cognitive decline in the absence of recurrent stroke
Dufouil *et al*[65]	192	Participants were assigned to a combination of perindopril plus indapamide or their placebos or to single therapy with perindopril or placebo	Cerebral MRI both at baseline and after a mean follow-up time of 36 mo WMHs were graded with a visual rating scale from A (no WMH) to D (severe WMH)	36 mo	Twenty-four subjects (12.5%) developed new WMHs at follow-up. The risk of new WMH was reduced by 43% (95%CI: -7% to 89%) in the active treatment group compared with the placebo group ($P = 0.17$). The mean total volume of new WMHs was significantly reduced in the active treatment group [0.4 mm^3 (SE = 0.8)] compared with the placebo group [2.0 mm^3 (SE = 0.7); $P = 0.012$)]
Hajjar *et al*[62]	53	Lisinopril, candesartan, or hydrochlorothiazide	Cerebral blood flow velocity (BFV; transcranial Doppler ultrasonography during rest, sitting, standing, hypercapnia, and hypocapnia), cognition (trail making test), and blood pressure	12 mo	There was a tendency toward an increase in BFV in the candesartan group and a decrease in the lisinopril and hydrochlorothiazide groups (between-group $P = 0.57$) that was significant in those with low BFV at baseline (< median 27.6 cm/s, between-group $P = 0.03$). The candesartan group also had the greatest improvement in executive function (Trail Making Test Part B improved by 17.1 s, *vs* hydrochlorothiazide improved by 4.2 s and lisinopril worsened by 14.4 s, $P = 0.008$). Carbon dioxide vasoreactivity and vasomotor range declined significantly in the lisinopril (within-group $P = 0.001$ for vasoreactivity and 0.02 for vasomotor range) and hydrochlorothiazide groups (within-group $P = 0.10$ and 0.009, respectively) but not in the candesartan group (within-group $P = 0.25$ and 0.38, respectively; between-group $P = 0.30$ and 0.46, respectively)
Gelber *et al*[61]	2197	Different classes of antihypertensive medication	Cognitive function was assessed at 7 standardized examinations using the CASI	5.8 yr	854 men developed cognitive impairment (median follow-up, 5.8 yr). β-blocker use as the sole antihypertensive drug at baseline was consistently associated with a lower risk of cognitive impairment (IRR 0.69; 95%CI: 0.50-0.94), as compared with men not taking any antihypertensive medications. The use of diuretics, calcium channel blockers, angiotensin-converting enzyme inhibitors, or vasodilators alone was not significantly associated with cognitive impairment

CASI: Cognitive Abilities Screening Instrument; MRI: Magnetic resonance imaging; WMH: White matter hyperintensities; IRR: Incidence rate ratio.

trial of 404 patients with moderate to severe Alzheimer's dementia was performed to compare the efficacy and safety of memantine in patients already receiving donepezil in 24 wk. Memantine resulted in significantly better outcome compared to placebo on measures of cognition, ADL, goal outcome and behavior and was well tolerated. This showed that memantine can be used in combination with cholinesterase inhibitors[56]. A Cochrane review of available studies support the benefit of donepezil in improving cognitive function, clinical global impression and ADL in patients with probable or possible mild to moderate VCI after 6 mo treatment[57].

Long standing hypertension has been continuously linked to the development of cognitive impairment and dementia in later life[58]. Population attributable risk for dementia is the highest for hypertension and therefore, should be regarded as a potential major target for the prevention of dementia[7]. Observational studies suggest that administration of antihypertensive medication in younger age group with hypertension has preventive effects on cognitive decline in later life[59,60]. β blocker use has been associated with lower risk of developing cognitive impairment (assessed using cognitive ability screening instrument) in a prospective community based cohort study examining 2197 elderly hypertensive participants. After a median follow up of 5.8 years, 854

subjects developed cognitive impairment. This study showed that β-blocker use as the sole antihypertensive medication was associated with a lower risk of VCI (incidence risk ratio of 0.69, 95%CI: 0.45-0.94) when compared to patients not taking any antihypertensive medication. Use of other antihypertensives were not significantly associated with VCI[61]. Setbacks of this study includes the fact that only hypertensive men were examined and no neuroimaging techniques were used at any point of the study to rule out subjects at early stages of cognitive impairment that may not have been captured with the tool used to screen cognitive function. Hajjar *et al*[62] conducted 12-mo double blind randomized control trial comparing candesartan, Lisinopril and hydrochlorothiazide in hypertensive patients with early executive dysfunction. The study recruited 53 participants 60 years of age and older with hypertension and executive dysfunction. Cerebral blood flow, cognition and blood pressure were measured at baseline, 6 mo and 12 mo. The candesartan group had greatest improvement in executive dysfunction (trail making test) and less improvement was seen in the hydrochlorothiazide group. The Lisinopril group showed worsened executive dysfunction. They showed that angiotensin receptor blocking group may preferentially preserve cerebral haemodynamics and executive function in individuals with executive dysfunction[62]. Various randomized controlled trials (RCT) investigated the effect of antihypertensive treatment on the incident of dementia. Majority of these RCT showed that antihypertensive treatment decrease the incidence of stroke but only a few have shown a significant positive effect on the incidence of dementia[63]. A randomized treatment trial of an angiotensin converting enzyme inhibitor, Perindopril showed that active treatment significantly decreased the risk of dementia and cognitive impairment and delayed progression of WMH on MRI. The Perindopril Protection Against Recurrent Stroke Study, was a randomized double blind placebo - control trial conducted on 6105 subjects with prior stroke or transient ischaemic attack with primary outcome of development of dementia using (DSM-IV diagnostic criteria) and cognitive decline (MMSE decline of 3 or more points). During the follow-up period (mean of 3.9 years) 193 (6.3%) of the treatment group compared to 217 (7.1%) of randomized placebo group developed dementia (Relative Risk Reduction of 12%). Cognitive decline was recorded in 9.1% of actively treated group and 11.0% of placebo (risk reduction of 19%). The risk of dementia and cognitive impairment with recurrent stroke were reduced by 35% and 45% respectively in the treatment group[64,65] (Table 1). Meta-analysis of randomized trials of antihypertensive on prevention of dementia showed an overall reduction of risk of dementia ranged from 11%-20%[7]. There is however no general consensus or standardized clinical guidelines on antihypertensive use in the prevention of VCI.

CONCLUSION

The full spectrum of VCI has been consistently linked to cardiovascular risk factors. There are constant evidences from research that hypertension is associated with the development of VCI through overt clinical stroke and small cerebral arteries and arteriole pathologies leading to subcortical infarcts (lacuna) and haemorrhages. The clinical outcome of which is cognitive impairment with predominant affectation of executive dysfunction as well as other areas of functioning.

Preventive strategies aimed at controlling hypertension have been shown to significantly reduce the incidence of vascular dementia, with antihypertensive of the angiotensin receptor blocker and angiotensin converting enzyme inhibitor to be at the forefront of research. However, more research works on preventing VCI and decreasing its progression to dementia are required as vascular dementia is one of the dementia complexes in which prevention is possible. Prevention is necessary in light of the looming dementia pandemic in order to not only decrease the burden of disease on the patient but also the economic burden on the government and care giver.

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MINIREVIEWS

Linking multiple pathogenic pathways in Alzheimer's disease

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Abstract

Alzheimer's disease (AD) is a chronic neurodegenerative disorder presenting as progressive cognitive decline with dementia that does not, to this day, benefit from any disease-modifying drug. Multiple etiologic pathways have been explored and demonstrate promising solutions. For example, iron ion chelators, such as deferoxamine, are a potential therapeutic solution around which future studies are being directed. Another promising domain is related to thrombin inhibitors. In this minireview, a common pathophysiological pathway is suggested for the pathogenesis of AD to prove that all these mechanisms converge onto the same cascade of neuroinflammatory events. This common pathway is initiated by the presence of vascular risk factors that induce brain tissue hypoxia, which leads to endothelial cell activation. However, the ensuing hypoxia stimulates the production and release of reactive oxygen species and pro-inflammatory proteins. Furthermore, the endothelial activation may become excessive and dysfunctional in predisposed individuals, leading to thrombin activation and iron ion decompartmentalization. The oxidative stress that results from these modifications in the neurovascular unit will eventually lead to neuronal and glial cell death, ultimately leading to the development of AD. Hence, future research in this field should focus on conducting trials with combinations of potentially efficient treatments, such as the combination of intranasal deferoxamine and direct thrombin inhibitors.

Key words: Alzheimer's disease; Etiologies; Iron; Oxidative stress; Thrombin; Vascular risk factors

Core tip: Patients with Alzheimer's disease (AD) have not benefited from any disease-modifying drug until now. Multiple etiologic pathways have been explored and suggest promising solutions in the

future. The iron chelator deferoxamine is one potential therapeutic solution around which future studies are being directed. Another potential therapeutic solution is related to thrombin inhibitors. In this minireview, a common pathophysiological pathway is suggested for the pathogenesis of AD that is initiated by the presence of vascular risk factors inducing brain tissue hypoxia and endothelial cell activation. In predisposed individuals, this can lead to thrombin activation and iron decompartmentalization. The resulting oxidative stress will eventually lead to neuronal and glial cell death.

INTRODUCTION

Alzheimer's disease (AD) is a chronic neurodegenerative disorder presenting as progressive cognitive decline with dementia[1-3]. In spite of considerable research, proven disease-modifying drugs are still lacking[1-3]. Currently, all phase 3 drug trials have failed to produce the desired results[1-3]. An increasing amount of literature now supports the vascular-neuronal axis hypothesis in the pathogenesis of AD owing to common risk factors for both AD and cardiovascular conditions[4]. However, it has also become widely established that disturbance in cerebral iron homeostasis also participates to the genesis of AD[5]. In this review, we will attempt to link these multiple physiopathological pathways to prove that these mechanisms converge onto the same cascade of neuroinflammatory events.

VASCULAR RISK FACTORS AND AD

Diagnostic criteria categorize dementias into vascular dementias or AD dementias even though mixed forms are frequently encountered[6]. In fact, as presented in a recent review by Hooijmans *et al*[7], there is an increasing amount of literature supporting the fact that cardiovascular risk factors (*i.e.*, diabetes, hypertension, dyslipidemias) are predisposing factors for vascular and AD dementias, and because the clinical manifestations and pathological findings are also common, the two conditions should not be seen as separate. Accordingly, there seems to be a direct increase in the risk for dementia, including AD, in the presence of these cardiovascular risk factors[8-13]. Hypertension and atherosclerotic disease both bring about vascular changes, causing alterations in the blood brain barrier and cerebral ischemia. Ultimately, this will initiate the pathological process of AD[7]. Indeed, there is *in vitro* and pathology evidence concerning chronic localized brain ischemia as playing a crucial role in the

genesis and progression of AD. The ensuing hypoxia stimulates the production and release of reactive oxygen species and pro-inflammatory proteins[14]. There also appears to be data concerning vascular risk factors and cardiovascular diseases as potentially being able to quicken Aβ 40-42 production, aggregation, and precipitation[6]. On another level, there is also evidence that endothelial dysfunction, due to cerebrovascular risk factors that include diabetes and hypoxia, precedes cognitive decline in AD and might contribute to its pathogenesis through the activation of thrombin[15].

The hypoxia inducible factor 1 (HIF-1) is a sensor for hypoxia, and its levels are increased in the cerebral circulation in both mouse and human models of AD[14]. However, an increasing amount of literature is in favor of an elevation in pro-inflammatory substances in the endothelium of the cerebral microcirculation in oxygen-deficient conditions[14]. Together, those results suggest a relationship between hypoxia and inflammation in the brain. Another phenomenon that occurs in response to hypoxia is the secretion of a strong inducer of angiogenesis known as vascular endothelial growth factor (VEGF). In AD, however, the vascular response to VEGF is deficient, resulting in an increased production of pro-inflammatory and neurotoxic substances[14].

In conclusion, recent studies are in favor of targeting vascular risk factors *via* lifestyle adjustments (physical exercise, dietary modification and abstinence of smoking) and medications (namely, cholesterol-lowering drugs) to preserve cognitive functions in the aging population and to reduce the progression toward AD. This seems to occur through the reduction of chronic focal ischemia and hypoxia in the brain, which are both harbingers of cerebral inflammation and oxidative stress[7,16].

THE EFFECT OF THROMBIN ACTIVATION IN AD

Thrombin has been demonstrated to be a key regulator of the pro-inflammatory reaction of cerebral endothelial cells in response to ischemic changes[13]. Moreover, prothrombin and thrombin are widely expressed in neurons and particularly in neurofibrillary tangles and senile plaques. This hypothesizes that thrombin could have a role in tau degradation and that a deficiency in this process could cause tau protein accumulation[17,18]. In an animal model on rats, direct intra-cerebral injection of thrombin caused in neuronal death and subsequent cognitive impairment[19]. Likewise, it was shown that thrombin can directly exert neurotoxic effects[20]. Thus, introduction of heparin, an indirect thrombin inhibitor, in the arsenal of AD medications hoped it could enhance the brain's micro-vasculature *via* its antithrombotic properties[21]. Accordingly, rats of advanced age displayed a partial but significant improvement of behavioral problems after heparin injection[22]. Moreover, an animal experiment demons-

trate protective actions of heparin after injection of amyloid peptide into the amygdala[23]. Furthermore, due to the potential side effects of long-term treatment with heparin, it has been suggested that a more specific treatment that directly inhibits thrombin would be more appropriate in terms of safety and efficacy. Indeed, direct thrombin inhibitors, such as dabigatran, would be a better choice because of their high selectivity in the inhibition of thrombin activity, thus ensuring a better side effect profile than an indirect inhibitor, such as heparin[15,24]. Dabigatran is a competitive reversible non-peptide antagonist of thrombin. Thrombin has many functions: Fibrinogen transformation into fibrin, fibrin strengthening and cross-linking, stimulation of additional thrombin production, platelet activation, and stimulation of protein C, which increases pro-thrombotic activity. Dabigatran inhibits most of these steps[25].

THE EFFECT OF VASCULAR RISK FACTORS ON ERYTHROCYTE LYSIS AND IRON DEPOSITION

Iron is crucial for the metabolic demands and functions of numerous cells, but when dysregulated, iron can become potentially harmful for these same cells. Iron circulates with the action of its transporter transferrin, which binds iron released in the blood from two sources: Enterocytes (following absorption) and the reticulo-endoplasmic cells. The iron-transferrin complex binds to the transferrin-receptor-1 so that it can be internalized within cells. Iron then enters mitochondria, where it takes part in heme synthesis. Superfluous iron is stowed and detoxified in ferritin[26].

In the central nervous system, iron, a key component for many proteins essential for brain metabolism, is predominantly concentrated in the motor system, and more specific, within glial cells[27,28]. Evidence supports the fact that iron deposition might exert a neurotoxic effect. For example, intracerebral hemorrhage causes extraversion of red blood cells (RBCs) into the parenchyma followed by hemolysis and decompartmentalization of iron, which later promotes long-term neurological deficits and brain atrophy[29-31]. In humans, iron deposition in the endothelium and vascular media is strongly associated with the progression of atherosclerotic lesions[32]. Indeed, atherosclerotic plaques with subsequent neovascularization and intra-plaque hemorrhage may constitute an important source of iron deposition in blood vessels and the brain parenchyma, but in a more localized fashion than what is observed after an intracerebral hemorrhage. Furthermore, in atherosclerotic plaques, cholesterol crystals coincide with glycophorin A (distinctive protein of red cell membranes), implying cholesterol from erythrocyte membranes could participate in the deposition of lipids and expansion of the lipid core following intra-plaque bleeding[33]. Moreover, some data support the fact that

hypercholesterolemia might enhance the crossing of iron into the brain parenchyma *via* an augmentation of endothelial permeability to iron[34]. Hence, the potential efficacy of cholesterol-lowering agents (*i.e.*, statins) in slowing the progression towards AD might be related to their indirect neuroprotective actions of preventing iron-induced neurotoxicity[34-36].

During an intra-plaque hemorrhage, red cells penetrate the oxidative environment of atherosclerotic lesions containing cytotoxic products of lipid peroxidation that can trigger the lysis of RBCs[37]. Hemoglobin released outside RBCs is oxidized and will release high-valence iron compounds with potent oxidative and inflammatory activities[38]. These activities can increase vascular endothelial activation and subsequent thrombin release. However, the known *in vitro* effect of iron ions on thrombin activity is in favor of the inhibition of its clot-forming effect[39].

EFFECT OF IRON DEPOSITION ON AD

In 1991, the first study evaluating the effect of deferoxamine (DFO) in patients suffering from AD was published[40]. DFO was evaluated because of its aluminum chelating properties and because of the evidence linking this metal ion to AD[40]. The study concluded that DFO may slow the progression of AD[40]. However, since then, additional evidence has linked the other metal ion chelated by DFO, iron, with the pathogenesis of AD. In patients with AD, iron accumulation in the cerebral cortex and hippocampus co-localizes with neurofibrillary tangles and senile plaques[41]. In their review, Peters *et al*[42] hypothesized that amyloid production is actually amplified to compensate for excessive iron levels and "patch" the subsequent vascular damage. High neuronal levels of iron stimulate amyloid protein precursor translation, and along with concomitant abnormal secretase activity, increase extracellular Aβ-42 deposition and tau protein phosphorylation. Peters *et al*[42] concluded the finding that increased iron deposition increases amyloid production emphasizes the importance of iron management in the treatment of AD.

The ability of iron to interact with oxygen is crucial for cell functioning, but it is also a source of free radicals according to Fenton's reaction: $Fe^{+n} + H_2O \rightarrow Fe^{+(n-1)} + OH^-$ (hydroxyl radicals).

Reactive oxygen species that are formed through this reaction subsequently damage intracellular structures *via* lipid peroxidation, or induction of DNA mutations[43]. In a 2015 review on vascular dysfunction in AD, Di Marco *et al*[14] fact found that high levels of lipid peroxidation and DNA oxidation were a frequent observation in AD. Furthermore, they posited that the blood-brain barrier is a central player in oxidative-stress-related tissue injury, in that it is both a source and a target of reactive oxygen species and pro-inflammatory substances. This hypothesis was based on the observation that Aβ plaques contain redox-active
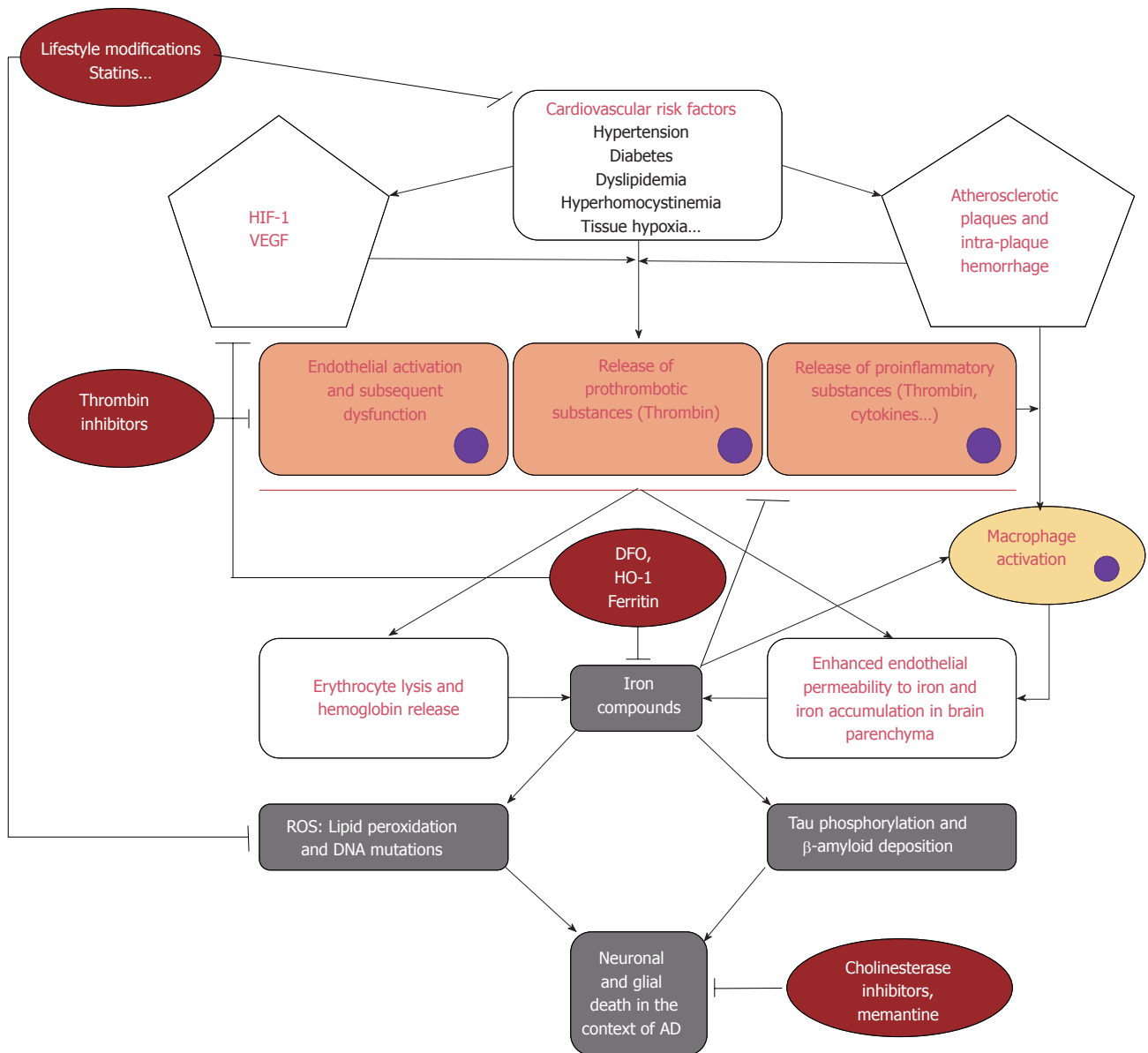
Figure 1 The physiopathological pathways linking endothelial dysfunction and thrombin activation to the neurotoxic effects of iron ions through enhancement of the oxidative stress that leads to neuronal and glial cell death. Dark red ovals: Therapeutic agents; Light orange boxes: Pathophysiological mechanisms taking place in the vascular endothelium; Gray boxes: Pathological events observed in AD; Light yellow oval: Macrophages. AD: Alzheimer's disease; DFO: Deferoxamine; HO-1: Heme oxygenase-1; ROS: Reactive oxygen species.

metals and that Aβ deposits preferentially locate in perivascular spaces[14].

β-amyloid in itself is a substrate for hydroxyl radicals[44]. Studies utilizing magnetic resonance imaging have found a positive correlation between aging and iron deposition in the brain that renders the brain more vulnerable to iron-mediated oxidative stress[45,46]. Accordingly, iron can attach to phosphorylated tau-proteins and lead to its aggregation, which causes the creation of neurofibrillary tangles[47]. Furthermore, it has been found that intranasal DFO inhibits tau phosphorylation in the brain of transgenic mice with AD[48]. It has also been demonstrated that the potential progression of senile plaques through the affinity of iron ions to β-amyloid and its precursor (*i.e.*, amyloid protein

precursor) might be prevented in transgenic mice *via* administration of intranasal DFO[49].

DISCUSSION

AD has been considered to be caused by a multitude of neuropathogenic pathways that all eventually lead to neuronal death and cognitive impairment. However, in this review, we have demonstrated in a theoretical manner that these multiple pathophysiological pathways (namely the endothelial vascular activation through thrombin activation and the neurotoxic effect of redox species through iron ion decompartmentalization) are actually interlinked (for a schematic representation of these pathophysiological pathways see Figure 1). As

a matter of fact, AD might be considered a disease of the neurovascular unit that seems to be triggered by vascular risk factors that affect the endothelium of small vessels and capillaries inside the brain. Vascular risk factors may induce atherosclerotic changes at the bifurcation of vessels, which would lead to tissue hypoxia in the brain parenchyma irrigated by the corresponding vessel. The endothelial cells lining the arterioles and capillaries in the hypoxic brain region are normally highly sensitive to hypoxic changes in the surrounding brain parenchyma and usually secrete substances that include VEGF and HIF-1 to promote neovascularization. When resources for neutralizing the oxidative stress provoked by hypoxia, such as in the elderly brain, are out-weighted by the amount and duration of oxidation, these normal responses become deleterious. A deficient vascular response to tissue hypoxia may promote intra-plaque hemorrhages. Deficient neovascularization and intra-plaque hemorrhages may be the cause of erythrocyte lysis, iron ion deposition in the brain parenchyma due to increased endothelial permeability and thrombin secretion and activation. Erythrocyte lysis liberates more iron ions that will further increase the oxidative stress. Moreover, thrombin activation may increase tissue hypoxia through increased clot formation and vasoconstriction that will also lead to increased oxidative stress. After exposure to oxidative stress compounds, many neuronal and glial cell modifications will ensue, such as lipid peroxidation, DNA mutations, cytoskeleton breakdown, *etc.* In addition, iron ions may also be responsible for the deposition of β-amyloid plaques and neurofibrillary tangles because it is implicated in enhancing amyloid protein metabolism and tau protein phosphorylation and aggregation. Accordingly, current research in AD therapeutics is focusing on one of the multiple branches of these interlinked pathophysiological pathways. However, a lack of a more global perspective may explain why no disease-modifying treatment has been discovered until now. For example, treatment with DFO may be an interesting solution for iron ion decompartmentalization but this treatment does not take into consideration the positive role that iron may play in the hypoxic tissue by reducing thrombin activation and subsequent clot formation, thus avoiding further hypoxia. Moreover, it becomes more obvious when looking at these interlinked pathways that no one molecule with a single mechanism of action can easily attenuate all the deleterious effects initiated by hypoxia secondary to vascular risk factors. Accordingly, we suggest that future research in this field should focus on testing combinations of potentially efficient treatments, such as the combination of intranasal DFO and direct thrombin inhibitors.

CONCLUSION

In this minireview, a common physiopathological pathway has been suggested for the pathogenesis of AD.

This pathway is initiated by the presence of vascular risk factors that induce brain tissue hypoxia and subsequent endothelial cell activation. The endothelial activation may become dysfunctional in predisposed individuals, leading to thrombin activation and iron ion decompartmentalization. The oxidative stress that results from these modifications in the neurovascular unit will eventually lead to neuronal and glial cell death.

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ORIGINAL ARTICLE

Basic Study

Failure of memantine to "reverse" quinpirole-induced hypomotility

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Conflict-of-interest statement: Dr. Gino Serra has applied for a patent for the use of memantine to treat bipolar disorder. No other author or immediate family member has current financial relationships with commercial entities that might represent or appear to represent potential conflicts of interest with the material presented here.

Data sharing statement: Original data are reported.

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Abstract

AIM: To evaluate antidepressant-like effect of memantine in a rat model.

METHODS: Male Wistar rats were treated intraperitoneally with either vehicle, memantine (10 mg/kg) or imipramine (20 mg/kg), for 3 wk. Twenty-four hour after the last treatment animals were challenged with quinpirole (0.3 mg/kg s.c.) and tested for motor activity. After 1 h habituation to the motility cages, the motor response was recorded for the following 45-min and the data were collected in 5-min time bins.

RESULTS: As expected, chronic treatment with imipramine potentiated the locomotor stimulant effect of quinpirole. On the contrary, chronic memantine administration failed to induce the behavioral supersensitivity to the dopamine agonist.

CONCLUSION: The results show that memantine, at variance with antidepressant treatments, fails to induce dopaminergic behavioral supersensitivity. This observation is consistent with the results of preclinical and clinical studies suggesting that memantine does not have an acute antidepressant action but does have an antimanic and mood-stabilizing effect.

Key words: Memantine; Bipolar disorder; Depression; Mood stabilizer; Imipramine; D_2 sensitization

Core tip: Memantine at variance with virtually all antidepressant treatments, fails to induce dopaminergic behavioral supersensitivity. This observation is consistent with the results of preclinical and clinical studies suggesting that memantine does not have an acute antidepressant action but does have an antimanic and mood-stabilizing effect.

INTRODUCTION

The blockade of serotonin and noradrenaline reuptake by tricyclic antidepressants or the inhibition of monoaminoxidase (MAO) by MAO inhibitors has been considered responsible for the therapeutic action of the first generation of antidepressant drugs. On the contrary, the role of dopamine in the mechanism of action of these drugs has been neglected for long time. However, in 1979[1] we first reported that chronic treatment with antidepressant drugs activates dopaminergic transmission.

In the last few decades numerous studies have confirmed that virtually all antidepressant (AD) treatments (including electroconvulsive shock and REM-sleep deprivation) increase the motor-stimulant effect of dopamine receptor agonists by sensitizing D_2 dopamine receptors in the mesolimbic system[2-9].

In these last few years, it has been hypothesized that glutamate and NMDA receptors play a role in the neurobiology of depression[10-14]. Moreover, it has been reported that the NMDA receptor blocker, memantine, reduces immobility time in the forced swimming test (FST), a widely used animal model of depression, suggesting that it could have a potential antidepressant effect[15-17].

However, it has been recently reported that memantine fails to reduce immobility time in the FST after chronic treatment[18], and to reverse anhedonia in the chronic mild stress model of depression[19], suggesting that the antidepressant-like effect observed in the FST after acute treatment should be considered a "false positive"[20].

These observations are consistent with clinical studies that failed to find an acute antidepressant effect of memantine in humans[21-29].

Moreover, we have recently reported preclinical and clinical evidence suggesting that memantine has an antimanic and mood-stabilizing action[18,30-39].

Thus, to further clarify the effect of memantine in animal models of depression, we compared the effect of chronic administration of memantine and imipramine on the locomotor response to the dopamine D_2-like receptor agonist quinpirole, as a measure of dopamine D2-like receptor sensitivity[40].

As expected, chronic administration of imipramine potentiated the locomotor stimulant effect of quinpirole, while memantine failed to affect the quinpirole action.

MATERIALS AND METHODS

Subjects

The present study was carried out in accordance with Italian law, which allows experiments on laboratory animals only after submission of a research project to the competent authorities, and in accordance with the "Guide for the Care and Use of Laboratory Animals" 8th Edition (National Research Council of Academies, The National Academies Press, Washington DC, 2011).

Male Wistar rats (Harlan, Italy), weighing initially 125-149 g, were housed in groups of 2 per cage in controlled environmental condition (temperature 22-24 ℃, humidity 50%-60%; light on at 8:00, off at 20:00), with free access to food and water.

Treatments

The animals (n = 60) were divided into three groups (n = 20) and treated with vehicle (distilled water) controls, memantine HCl (Ebixa sol. Lundbeck Italy s.p.a) and imipramine HCl (Sigma, Haldrich) for 3 wk.

They were challenged with quinpirole and tested for motor activity 24 h after the end of this treatment. Imipramine HCl and quinpirole HCl (Sigma, Haldrich) were dissolved in distilled water. Memantine and imipramine were administered intraperitoneally in daily injections, at the dose of 10 mg/kg and 20 mg/kg, respectively, in a volume of 1 mL/kg. Quinpirole was administered subcutaneously at the dose of 0.30 mg/kg in a volume of 1 mL/kg.

Motor activity

Motor activity was measured by an apparatus consisting of a mobile rack (height 180 cm, width 100 cm and depth 60 cm) with eight compartments (height 40 cm, width 45 cm, depth 50 cm), into which a transparent perspex cage (height 19 cm, floor area 23 cm^2 × 33 cm^2) was placed (Imetronic, Pessac, France). Motor activity was detected by a system of photocell infrared beams, dividing the cage area into two sectors, rear and front sector. In particular, the interruption of two photocell beams belonging to two different sectors was recorded as a "long movement" motility count. The interruption of two photocell beams belonging to the same sector was recorded as a "short movement" motility count. A "barrier" of infrared photocell beams, placed at the height of 15 cm, detected rearing activity. The apparatus was connected to a personal computer by an electronic interface. Experiments were performed between 0900 and 1500 h. After 1-h habituation to the motility cages, the rats were divided into 2 groups and treated s.c. with control vehicle (n = 30) and quinpirole (n
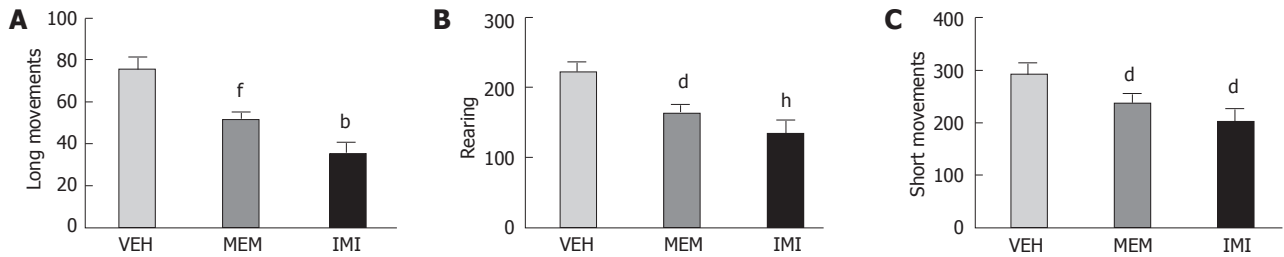
Figure 1 Spontaneous motor activity after 24 h discontinuation of chronic treatments (60 min habituation to the motility cage). Each value represents the mean ± SEM from 20 rats: Vehicle (VEH), memantine (MEM), imipramine (IMI). Number of long movements (A), rearing (B) and short movements (C) measured as indicated in the materials and methods: Motor activity. A: [f]$P < 0.001$, memantine *vs* vehicle [$F_{(1.51)} = 12.21$; $P = 0.0009$]; [b]$P < 10^{-6}$, imipramine *vs* vehicle [$F_{(1.51)} = 31.71$; $P = 0.000001$]; B: [d]$P < 0.01$, memantine *vs* vehicle [$F_{(1.51)} = 10.58$; $P = 0.0020$]; [h]$P < 10^{-4}$, imipramine *vs* vehicle [$F_{(1.51)} = 19.90$; $P = 0.000045$]; C: [d]$P < 0.01$, memantine *vs* vehicle [$F_{(1.51)} = 11.57$; $P = 0.0013$]; [d]$P < 0.01$, imipramine *vs* vehicle [$F_{(1.51)} = 10.13$; $P = 0.0024$]; ANOVA followed by Newman-Keuls-test.
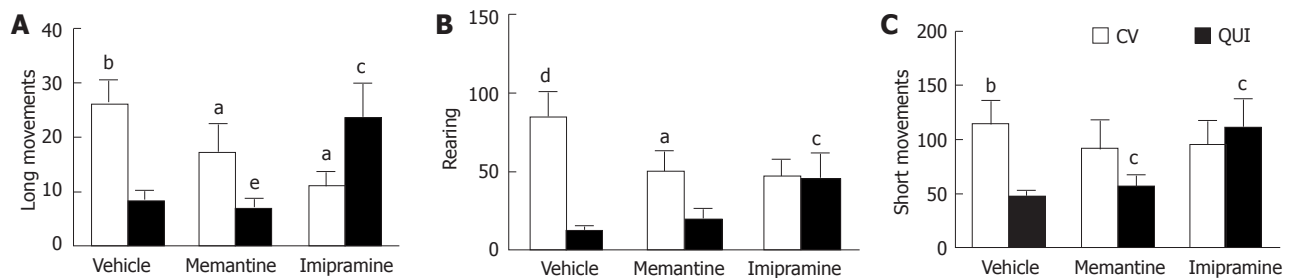
Figure 2 Motor response to quinpirole after 24 h chronic imipramine and memantine withdrawal. Number of long movements (A), rearing (B) and short movements (C) measured as indicated in the materials and methods: Motor activity. Each value represents the mean ± SEM from 10 rats (ANOVA followed by *F* test for contrast). Control vehicle (CV), quinpirole (QUI). A: [b]$P < 0.01$, Vehicle-CV *vs* vehicle-QUI [$F_{(1.48)} = 11.20$; $P = 0.0015$]; [a]$P \leqslant 0.05$, memantine-CV *vs* memantine-QUI [$F_{(1.48)} = 3.74$; $P = 0.05$]; [a]$P \leqslant 0.05$, imipramine-CV *vs* imipramine-QUI [$F_{(1.48)} = 3.74$; $P = 0.05$]. [c]$P \leqslant 0.05$, vehicle-QUI *vs* imipramine-QUI [$F_{(1.48)} = 6.39$; $P = 0.014$]; [e]$P < 0.01$, memantine-QUI *vs* imipramine-QUI [$F_{(1.48)} = 7.74$; $P = 0.007$]; vehicle-QUI *vs* memantine-QUI [$F_{(1.48)} = 0.07$; n.s]; B: [d]$P < 10^{-4}$, vehicle-CV *vs* vehicle-QUI [$F_{(1.48)} = 22.73$; $P = 0.000018$]; [a]$P \leqslant 0.05$, memantine-CV *vs* memantine-QUI [$F_{(1.48)} = 4.10$; $P = 0.048$]; imipramine-CV *vs* imipramine-QUI [$F_{(1.48)} = 0.006$; n.s.]; [c]$P \leqslant 0.05$, vehicle-QUI *vs* imipramine-QUI [$F_{(1.48)} = 3.80$; $P = 0.05$]; vehicle-QUI *vs* memantine -QUI [$F_{(1.48)} = 22.48$; n.s]; C: [b]$P \leqslant 0.01$, vehicle-CV *vs* vehicle-QUI [$F_{(1.48)} = 6.83$; $P = 0.01$]; imipramine-CV *vs* imipramine -QUI [$F_{(1.48)} = 0.24$; n.s.]. [c]$P \leqslant 0.05$, vehicle-QUI *vs* imipramine-QUI [$F_{(1.48)} = 4.83$; $P = 0.032$]; [c]$P \leqslant 0.05$ memantine-QUI *vs* imipramine-QUI [$F_{(1.48)} = 3.75$; $P = 0.05$].
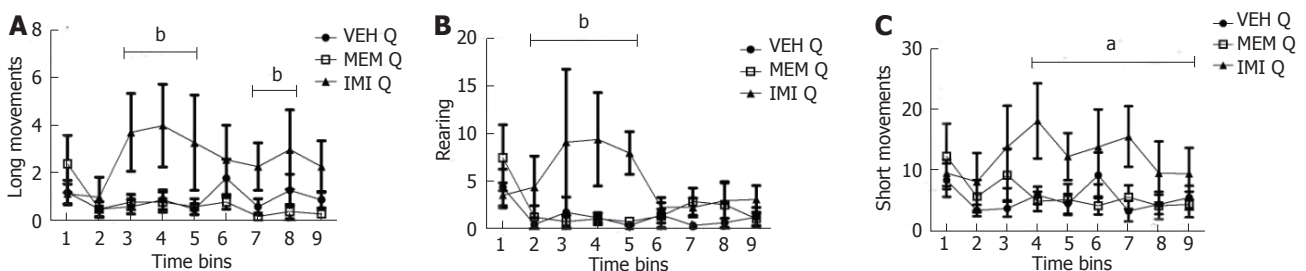
Figure 3 Time course of quinpirole effect on motor activity after 24 h chronic imipramine and memantine withdrawal. Long movements (A), rearing (B) and short movements (C) measured as indicated in the materials and methods: Motor activity. Each value represents the mean ± SEM from 10 rats: vehicle + quinpirole (VEH Q), memantine + quinpirole (MEM Q), imipramine + quinpirole (IMI Q). A, B: [b]$P < 0.01$ imipramine-quinpirole *vs* vehicle-quinpirole and memantine-quinpirole; C: [a]$P < 0.05$ imipramine-quinpirole *vs* memantine-quinpirole (ANOVA followed by *F* test for contrast; horizontal lines represent contrast involving consecutive times).

= 30).

The motor response was recorded for the following 45 min and data were collected in 5-min time bins.

Statistical analysis

The results were analysed by analysis of variance, supplemented by *F* tests for contrasts. Habituation and quinpirole challenge data were analysed separately. All data are presented as mean ± SEM; $P < 0.05$ is considered to be statistically significant.

RESULTS

Habituation

As shown in Figure 1, during 1 h of habituation to the motility cage, animals chronically treated with imipramine and memantine showed a significant reduction of motor activity, measured as long movements, rearing activity and short movements.

Quinpirole challenge

Figure 2 shows that quinpirole reduced the locomotor

activity, assessed as long movements, rearing activity and short movements, in control and memantine-treated rats, On the contrary, in imipramine-treated animals quinpirole stimulated locomotor activity (long movements and short movements) or prevented its sedative effect (rearing). Figure 3 shows the time course of quinpirole effect. Imipramine, but not memantine, stimulates locomotor activity induced by the dopamine agonist.

DISCUSSION

The present results confirm that chronic treatment with imipramine potentiated the locomotor response to the selective dopamine D2 receptor agonist quinpirole.

Quinpirole, as well as other dopamine agonists, has a biphasic effect on locomotor activity. A low doses it stimulates dopamine D2 autoreceptors mediating sedation/reduced motor activity, while at relatively high doses stimulates post-synaptic dopamine D2 receptors and and increases motor activity. In the present experiment the used dose of quinpirole reduces motor activity by stimulating dopamine autoreceptors in control animals. Imipramine, but not memantine, reverses this effect (*i.e.,* increases locomotor activity) because the stimulation of the supersensitive post-synaptic receptors overcomes the sedative effect due to the stimulation of autoreceptors (this issue has been extensively addressed in Serra *et al*[6,41,42]).

These findings are consistent with the large body of studies that strongly suggest that virtually all antidepressant treatments sensitize dopamine D_2 receptors in the mesolimbic system[2-4,6,8,9,41,42]. On the contrary, memantine fails to stimulate the locomotor response to quinpirole, suggesting that, at variance with antidepressant treatments, it does not sensitize D_2 receptors. This observation provides further support to our hypothesis[30,31,42] that the effect observed in the FST is a "false positive" and is in keeping with the failure of clinical studies to demonstrate an acute antidepressant action of memantine in depressed subjects[21-29]. Moreover, the results are consistent with our preclinical and clinical observations that strongly suggest that memantine has an antimanic and mood-stabilizing effect in patients with bipolar mood disorders. Indeed, we found that memantine prevents the dopamine D2 receptor sensitization induced by imipramine[30], that has been suggested to underly antidepressant-induced mania in humans[43,44]. Moreover, Gao *et al*[45] have reported an antimanic-like effect of memantine in two widely used animal models of mania. In addition we found that memantine prevents the bipolar-like behavior (mania followed by depression) induced by imipramine[18] suggesting that the drug may have a mood-stabilizing effect (*i.e.,* the ability to prevent mania/hypomania and depression episodes in manic depressive illness). This effect is the opposite to that observed with antidepressant drugs, which have been defined as "mood destabilizers"[46] because of their

ability to induce mania in humans suffering from mood disorders.

Finally, the results are consistent with our[34-39] and Keck *et al*[47] reports of an acute antimanic effect of memantine and our observations of a long-lasting and progressive mood-stabilizing action of memantine in severely ill patients with bipolar disorder[34-39].

COMMENTS

Background
Chronic antidepressant treatments, including electroconvulsive shock and REM sleep deprivation, potentiate the locomotor activity induced by dopamine D2 receptor agonists, suggesting that they sensitize dopamine D2 receptors in the mesolimbic system.

Research frontiers
Preclinical and clinical evidence suggests that memantine has an acute antimanic and a long-lasting mood stabilizing effect. On the contrary, while has been reported an antidepressant-like effect of the drug in the forced swimming test (FST), the administration of the compound in depressed patients appears to be ineffective.

Innovations and breakthroughs
The authors found that memantine, at variance with virtually all antidepressant treatments, fails to sensitize dopamine D_2 receptors, suggesting that the antidepressant-like effect observed in the FST should be considered a "false positive". This observation is consistent with the clinical reports of the lack of antidepressant action of memantine in depressed patients.

Applications
The authors' observation further support the suggestion to use memantine, as well as lithium, as an acute antimanic and a long-term mood stabilizing treatment.

Terminology
Memantine as a new mood stabilizer for the long-term prophylaxis of bipolar disorders.

Peer-review
A well-written paper, which adds to the evidence for the use of memantine in bipolar disorder.

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ORIGINAL ARTICLE

Case Control Study

Hippocampus and amygdala volumes in patients with vaginismus

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Abstract

AIM: To compare hippocampus and amygdala volumes of patients with vaginismus with those of healthy control subjects.

METHODS: Magnetic resonance imaging was performed on ten patients with vaginismus and ten control subjects matched for age and gender. Volumes of the hippocampus and amygdala were blindly measured.

RESULTS: We found that the mean right amygdala volume of patients with vaginismus were smaller than that of the healthy controls. With regard to hippocampus volumes, the mean left and right hippocampus volumes were smaller than those of the healthy controls.

CONCLUSION: Our present findings suggest that there have been hippocampus and amygdala structural abnormalities in patients with vaginismus. These changes provide the notion that vaginismus may be a fear-related condition.

Key words: Vaginismus; Hippocampus; Amygdala; Volumes; Patients

Core tip: Our present findings suggest that there have been hippocampus and amygdala structural abnormalities in patients with vaginismus. These changes provide the notion that vaginismus may be a fear-related condition.

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INTRODUCTION

Vaginismus is described as a condition of no permission of intercourse and vaginal examinations due to spasm of the exterior 1/3 of the vagina. The Diagnostic and Statistical Manual of Mental Disorders, 4th ed. (DSM-IV)[1] describes vaginismus as recurrent or persistent involuntary spasm of the musculature of the outer 1/3 of the vagina that interferes with sexual intercourse[2]. Although many cases of vaginismus are dealed with in sexual function disorders, when we look at its clinical picture, we can notice that vaginismus is also an anxiety related condition in daily practice.

The clinical picture of vaginismus is similar to that observed in phobic states. In usual, somatic components of anxiety accompany with other symptoms of vaginismus. Phobic states, anxiety and related clinical conditions are related to the hypothalamic-pituitary-adrenal axis (HPA). In these conditions, hypothalamic corticotropin-releasing hormone production increases, raising the pituitary release of adrenocorticotropin hormone (ACTH). This increase in ACTH levels leads to increased cortisol release by the adrenal cortex. These hormonal changes can affect many anxiety related symptoms associated with the autonomous nervous system. In this context, in our unpublished study, we performed an investigation to examine QT wave (QTd) and P wave (Pd) dispersions, which are associated with dysfunctioned autonomous nervous system, in the electrocardiogram of patients with vaginismus and healthy control subjects. We found that the mean Pmin value of the patients with vaginismus was statistically significantly lower than that of the healthy controls whereas the mean Pd value of patients with vaginismus was considerably higher than that of healthy control subjects, and that the mean QTmax value of the patients was statistically significantly higher than that of the healthy control subjects, in addition to the finding that the mean QTd value of the patients with vaginismus was considerably higher than that of healthy control subjects, considering that vaginismus patients might be vulnerable to the cardiac problems which are related to cardiac rhythm. This previous ECG study provides another support for the association between anxiety, autonomous system dysfunction and vaginismus.

The hippocampus and amygdala are important limbic and paralimbic brain areas linked to emotional regulation[3,4], with the involvement of the hippocampus in learning and verbal memory[5] and amygdala in emotional perception, possibly determining emotional and social behaviors[6,7]. On the other hand, there is an important association between hippocampal region and anxiety. First of all, the hippocampus is a glucocorticoid feedback area. Therefore, it is highly sensitive to endogenous glucocorticoid levels and is an important region affected by stress modulation which is controlled by the HPA[8-10]. In this context, although the hippocampus and amygdala are important regions in anxiety modulation, no study has examined their imaging to date. In this study, we compared hippocampus and amygdala volumes between patients with vaginismus and healthy control subjects.

MATERIALS AND METHODS

Study subjects

Ten consecutive female patients meeting DSM-IV criteria for vaginismus and seeking treatment at the Firat University School of Medicine, Department of Psychiatry, Elazig, Turkey were included in the present study. The local ethics committee approved this investigation, according to international guidelines on human research. Written informed consent was obtained from each patient. The mean age of the patients were 27.90 ± 7.25 years. Patients in the present study were those of another unpublished study in which we evaluated P and QT wave dispersions. Diagnoses were obtained by using the Structured Clinical Interview for DSM-IV Disorders-Patient Version[11,12]. In regard to comorbidity, of our patients, one had social anxiety disorder and one had depressive disorder. No other comorbidity was determined. As mentioned in our unpublished study, patients with vaginismus were on a sexual therapy program performed by a senior psychiatry assisstant. All patients and control subjects underwent a detailed physical and neurological examination and clinical assessment to exclude any neurological or comorbid conditions. The healthy control group was composed of also ten females, with a mean age of 27.40 ± 5.38 years. Exclusion criteria included: (1) a history of head trauma; (2) current or lifetime severe medical problems; (3) any current or lifetime neurologic problems; (4) the existence of mental retardation; (5) any problem that prevented them to undergo neuroimaging, particularly existence of cardiac stent; (6) and alcohol/substance abuse within the 6 mo preceding the study. Healthy control subjects were excluded if the following criteria were met: Existence of any DSM-IV axis I disorder, having any first-degree relative with a history of psychiatric disorder, a history of head trauma or seizure, existence of any current or lifetime major medical and neurologic illness, any problem that prevented them to undergo neuroimaging, particularly existence of cardiac stent.

Magnetic resonance imaging procedure

All subjects underwent magnetic resonance imaging (MRI) scans at rest. All *in vivo* data were collected on a 1.5 T General Electric scanner. In brief, the parameters used for this study were: TR = 2000 ms, TE = 15.6
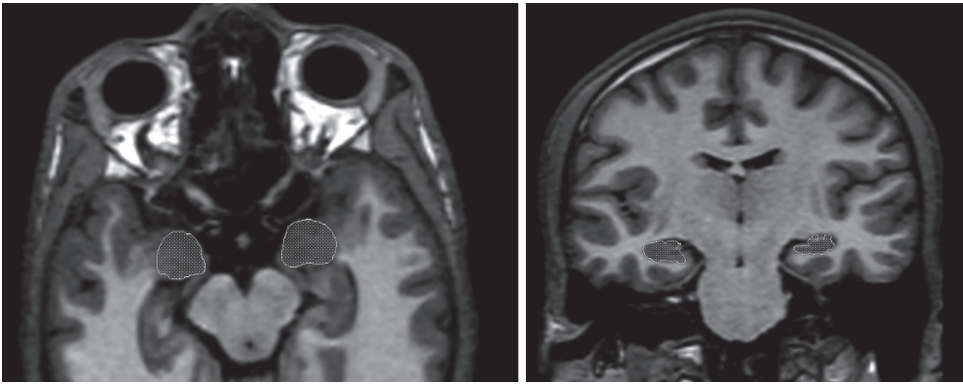
Figure 1 Delimitation of the hippocampus and amygdala according to the proposed tracings.

ms, TI = 700 ms, FOV = 240 mm, echo SPACING = 15.6 ms, 8 echoes, RESOLUTION = 0.9375 mm × 0.9375 mm × 1.328 mm, 128 contiguous slices, 8 min 36 s. The tracing and measurements were done by two neuroradiologists (HY, UA). They were blinded to identity and diagnoses of the subjects. We calculated the intra-class correlation coefficients to be 0.90 for the hippocampus and 0.90 for the amygdala.

To do manual tracing for anatomical regions, anatomic atlases were used[13-15]. On the other hand, tracings were adapted from Caetano *et al*[16] and Brambilla *et al*[17]. When tracing the hippocampus, the process was started on the coronal slice at the line that the superior colliculus was completely connected with the thalamus and was finished one slice before the mamillary bodies appeared. The superior boundary was described as the corona radiata and ambient, and the inferior border was selected as the white matter. The process was finished by tracig of lateral border which was the inferior horn of the lateral ventricle. When tracing the amygdala, the process was started at the point that the mamillary body can be observed. Temporal lobe white matter was accepted as the superior and lateral limits, and the white matter of the parahippocampal gyrus was accepted as the inferior border. The anterior border was described as the limit that the amygdala could not be observed as well. The delimitation of the hippocampus and amygdala is presented in Figure 1.

Statistical analysis

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS version 16.0, Chicago, Illinois). Independent t test was used to compare volume differences in the left and right hippo-campus and amygdala regions in addition to continous variables such as age. For the comparison of categorical variables, χ^2 test was performed. In addition, analysis of covariance (ANCOVA) was used, controlling for age. Statistical significance was accepted at $P < 0.05$.

RESULTS

As given in Table 1, we did not determine any significant differences between patients with vaginismus and healthy control subjects in regard to sex distribution (both groups were composed of female subjects), age, and educational level ($P > 0.05$).

With regard to amygdala volumes, we found that the mean right amygdala volume was smaller in patients with vaginismus than in the healthy controls (left amygdala: 1799.04 mm^3 ± 289.95 mm^3 *vs* 2006.78 mm^3 ± 425.39, $P > 0.05$; right amygdala: 1816.57 mm^3 ± 271.73 mm^3 *vs* 2055.64 mm^3 ± 284.95 mm^3, $P = 0.07$). When controlled for age, ANCOVA demonstrated that the statistically significant difference continued for the right amygdala ($P < 0.05$).

With regard to hippocampus volumes, we found that the mean left and right hippocampus volumes were smaller in patients with vaginismus than in the healthy controls (left hippocampus: 2505.22 mm^3 ± 223.03 mm^3 *vs* 2908.79 mm^3 ± 300.04 mm^3, $P < 0.05$; right hippocampus: 2501.30 mm ± 463.81 mm^3 *vs* 2907.61 mm^3 ± 247.34 mm^3, $P < 0.05$). When controlled for age, ANCOVA showed that the statistically significant difference lasted ($P < 0.05$ for both the left and right hippocampus).

No significant correlation was found between the duration of illness and age, and the volumes of both sides of the hippocampus and amygdala ($P > 0.05$).

DISCUSSION

To our knowledge, this is the first study to examine the amygdala and hippocampus volumes in patients with vaginismus, and our results provide the first MRI evidence of reduced amygdala and hippocampus volumes in patients with this disorder. The volumes of both sides of the hippocampus and the right amygdala were significantly smaller in patients with vaginismus than in healthy control subjects. Of particular note is the fact that these findings were obtained in a sample of patients with "pure" vaginismus, with one patient having social anxiety disorder and one having depressive disorder, without any other past or current major psychiatric comorbidity. On the other hand, another important feature of the present investigation was one-to-one matching between the patients and healthy

	Patients with vaginismus (n = 10)	Controls (n = 10)
Table 1 Demographic, clinical and volumetric features of healthy subjects and patients with vaginismus		
Gender (F/M)	10/0	10/0
Age	27.90 ± 7.25	27.40 ± 5.38
Handedness (right)	10	10
Amygdala		
Left	1799.04 ± 289.95	2006.78 ± 425.39
Right	1816.57 ± 271.73	2055.64 ± 284.95[1]
Hippocampus		
Left	2505.22 ± 223.03	2908.79 ± 300.04[2]
Right	2501.30 ± 463.81	2907.61 ± 247.34[2]

All volumes are in cubic mililiters (cm^3). We did not find any significant differences between groups in regard to gender, handedness and age. [1]P = 0.07, [2]P < 0. F: Female; M: Male.

control groups in regard to gender, since all subjects were females, which minimized possible confounding factors such as gender. In addition, by using ANCOVA controlling for age, we eliminated the effects of age. After this control, statistically significant differences continued for both amygdala and hippocampus regions. In fact, since there have been no previous study which evaluated the volumes of any brain region beyond the hippocampus and amygdala, we do not compare our results with those of others. However, our study grıup previously examined the volumes of the hippocampus and amygdala in patients with somatization disorder which is a psychiatric disorder strongly related to both the troubles in the regulation of emotion and copying stress. In that study, we revealed that somatization disordered patients had significantly smaller mean volumes of the left and right amygdala without any differences in regard to whole brain, total gray and white matter or hippocampus volume[18]. In addition, volumes of the hippocampus and amygdala were measured in patients with refractory obsessive-compulsive disorder (OCD)[19]. In that study we found that the mean left and right hippocampus and amygdala volumes of the patients were smaller than those of the healthy controls. Although OCD severity was not correlated with the volume of the left hippocampus, a correlation was noted between the duration of illness and the volumes of both sides of the hippocampus and the left amygdala. In that study, we commented that hippocampus and amygdala abnormalities could be considered in the occurrence of refractoriness to OCD. As can be seen in these investigations, it seems that hippocampus and amygdala structural abnormalities could be related to anxiety itself. However, it is important to perform functional neuroimaging of these regions to show functional relationship between anxiety and these regions. The hippocampus is also a glucocorticoid feedback area. Therefore, it is highly sensitive to endogenous glucocorticoid levels and is an important region affected by stress modulation which is controlled by the HPA[8-10]. In other words, there has been an important relationship between the hippocampus and anxiety. In this context, our findings revealing volumetric changes in the hippocampus and amygdala may suggest an important etiopathogenetic basis for the occurrence of vaginismus which is a fear-related clinical condition. However, these findings should be confirmed by further investigations.

It should be mentioned that our study had several limitations. First of all, although comorbid categorical diagnosis of major psychiatric conditions and substance abuse were part of the exclusion criteria, sub-categorical level of depressive or anxiety symptoms may have influenced our findings. Second, our study group was small. However, it is difficult to find vaginismus patients in such outpatient clinics which is not special for sexual function disorders. Third, we evaluated only hippocampus and amygdala regions which are associated with anxiety and fear. However, we did not examine other brain regions that can be associated with anxiety and fear. Fourth, the cross-sectional design of this study limits the interpretation of our findings. For this reason, clearer conclusions may be made about the role of the hippocampus and amygdala in the pathogenesis of vaginismus through a longitudinal design in which patients are scanned several times. Fifth, we should mention that the technique used in the present study should be compared with other possible alternative methods such as computational morphometry and multivariate approaches.

Consequently, our present findings suggest that there have been hippocampus and amygdala structural abnormalities in patients with vaginismus. These changes provide the notion that vaginismus may be a fear-related condition. Without replication studies, the possibility that findings are due to random chance and idiosyncrasies of small samples cannot be ruled out. Thus, we think that it is important to conceptualize these studies as exploratory, and place an emphasis on replication with larger sample size.

ACKNOWLEDGMENTS

COMMENTS

Background

The hippocampus and amygdala are important limbic and paralimbic brain areas linked to emotional regulation. On the other hand, there is an important association between hippocampal region and anxiety.

Research frontiers

The hippocampus is highly sensitive to endogenous glucocorticoid levels and is an important region affected by stress modulation which is controlled by the hypothalamo-pituitary-adrenal axis. In this context, although hippocampus and amygdala are important regions in anxiety modulation, no study has examined their imaging to date. In this study, the authors compared hippocampus and amygdala volumes between patients with vaginismus and healthy control subjects.

Innovations and breakthroughs

The authors for the first time found that the mean right amygdala volumes were smaller in patients with vaginismus than in healthy controls. With regard to hippocampus volumes, they also found that the mean left and right hippocampus volumes were smaller in patients with vaginismus than in healthy controls.

Applications

In vaginismus, structural brain alterations compared to healthy subjects might occur.

Peer-review

This is a good cross-sectional study in which the authors examined the volumes of the hippocampus and amygdala of patients with vaginismus.

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ORIGINAL ARTICLE

Observational Study

Peritraumatic Behavior Questionnaire - Observer Rated: Validation of the objective version of a measure for combat-related peritraumatic stress

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Abstract

AIM: To validate the first third-person-rated measure assessing combat-related peritraumatic stress symptoms and evaluate its psychometric properties and war-zone applicability.

METHODS: The valid assessment of peritraumatic symptoms in the theater of military operations represents a significant challenge in combat-related, mental health research, which mainly relies on retrospective, subjective self-report ratings. This longitudinal observational study used data from actively deployed troops to correlate third-person observer ratings of deployment peritraumatic behaviors [Peritraumatic Behavior Questionnaire - Observer Rated (PBQ-OR)] collected on a bi-monthly basis with post-deployment (1-wk follow-up) ratings of the previously validated PBQ self-rate version (PBQ-SR), and (3-mo follow-up) clinician assessed and self-report posttraumatic stress disorder (PTSD) symptoms (Clinician Administered PTSD Scale, PTSD Checklist). Cronbach's alpha (α) and correlation coefficients were calculated to assess internal reliability and concurrent validity respectively.

RESULTS: Eight hundred and sixty male Marines were included in this study after signing informed consents at pre-deployment (mean age 23.2 ± 2.6 years). Although our findings were limited by an overall sparse return rate of PBQ-OR ratings, the main results indicate satisfactory psychometric properties with good internal consistency for the PBQ-OR (α = 0.88) and high convergent and concurrent validity with 1-wk post-deployment PBQ-SR ratings and 3-mo posttraumatic stress symptoms. Overall, later PBQ-OR report date was associated with higher correlation between PBQ-OR and post-deployment measures. Kappa analysis between PBQ-OR and PBQ-SR single items, showed best agreement in questions relating of mortal peril, desire for revenge, and experience of intense physical reactions. Logistic regression demonstrated satisfactory predictive validity of PBQ-OR total score with respect to PTSD caseness (OR = 1.0513; 95%CI: 1.011-1.093; P = 0.02).

CONCLUSION: Since no comparable tools have been developed, PBQ-OR could be valuable as real-time screening tool for earlier detection of Service Members at risk.

Key words: Peritraumatic reaction; Posttraumatic stress disorder; Trauma; Military service; Combat; Assessment; Dissociation; Stress

Core tip: The assessment of combat-related peritraumatic symptoms mainly relies on retrospective, subjective self-report ratings. We have therefore developed the Peritraumatic Behavior Questionnaire - Observer Rated (PBQ-OR), a third-person-rated scale for unit-embedded medical personnel to objectively assess symptoms of combat-related peritraumatic stress in deployed troops. In this study, we validated the PBQ-OR during active deployment and longitudinally evaluated its psychometric properties and war-zone applicability. Our findings show that the PBQ-OR could be used as a screening and monitoring tool in real time and may permit earlier detection of Service Members at risk for posttraumatic stress symptoms to target prevention and early intervention efforts.

INTRODUCTION

Peritraumatic stress reactions include various behavioral, emotional, cognitive, and physiological symptoms associated with sympathetic activation during and immediately following a traumatic event[1]. Prolonged continuation of these biological and psychological responses can lead to long-term adverse biological alterations[2,3], strongly associated with the subsequent development of posttraumatic stress disorder (PTSD)[4,5]. Peritraumatic stress is, hence, a very sensitive pre-clinical risk marker and its accurate assessment could improve individual risk evaluation and provision of appropriate mental health interventions in traumatized populations[6,7].

Peritraumatic reactions are especially representative of extraordinary stress challenges and could affect the maintenance of military operational resilience in Service Members[8,9]. However, the valid assessment of peritraumatic symptoms in the theater of military operations is a significant challenge in combat-related, mental health research. To address this issue, we developed a new 15-item 5-point-Likert scale measure of combat-related peritraumatic distress symptoms: the Peritraumatic Behavior Questionnaire (PBQ). The detailed description of the development procedure of the PBQ is available in Agorastos *et al*[10]. The PBQ was designed as a military-specific, observer rated scale for unit-embedded medical personnel (UMP) for the reliable assessment of combat-related peritraumatic stress symptoms of Service Members in the theatre of operations (PBQ-OR, Table 1). The initial validation of the self-rated PBQ version [PBQ - Self Report (PBQ-SR)] confirmed the ability and reliability of PBQ-SR to assess peritraumatic reactions as a general construct unifying the major underlying peritraumatic symptom dimensions[10]. PBQ-SR demonstrated good internal consistency and convergent and discriminant validity, and showed a high correlation to various PTSD-specific/-related symptoms and PTSD caseness.

However, PBQ-SR, as well as prior research on

Table 1 The Peritraumatic Behavior Questionnaire - Observer Rated
PBQ-OR questions
For a period of time, the individual did not act like their normal self
For a period of time, the individual seemed to feel fearless and invulnerable, as if nothing could harm them
For a period of time, the individual seemed not to care about their own or others' welfare or safety
For a period of time, the individual seemed to feel no remorse for doing things that would have bothered them in the past
[1]For a period of time, the individual seemed to be determined to get revenge
For a period of time, the individual seemed unable to stop laughing, crying, or screaming
For a period of time, the individual seemed helpless and unable to look out for their own welfare
For a period of time, the individual appeared to be confused, as if having difficulty making sense of what was happening
For a period of time, the individual appeared to be disoriented, as if uncertain about where they were or what day or what time it was
[2]For a period of time, the individual appeared not to be able to move parts of their body
[2]For a period of time, the individual froze or seemed to be moving very slowly, such that they could not do everything they wanted to do
For a period of time, the individual's speech changed (such as stuttering, repeating words or phrases, or having a shaky or squeaky voice)
[2]For a period of time, the individual was not able to fully carry out their duties (during or immediately after the event)
[1]For a period of time, the individual expressed the belief that they were going to die
[1]For a period of time, the individual had an intense physical reaction such as sweating, shaking, or heart pounding

Text from PBQ-SR. [1]Questions 5, 14 and 15 have higher overall item-to-item significance of correlation to PBQ-SR; [2]Questions 10, 11 and 13 (report order 3) received no non-zero responses. PBQ-OR: Peritraumatic Behavior Questionnaire - Observer Rated; PBQ-SR: Peritraumatic Behavior Questionnaire - Self Rated.

peritraumatic stress have relied upon retrospective, subjective self-report questionnaires. Retrospective subjective assessment of peritraumatic symptoms introduces several potential biases and distortions related to cognitive barriers, adaptive denial coping, ethical concerns or current symptoms and obviates real-time case identification and intervention[11,12]. Therefore, objective third-person ratings of behavioral changes suggestive of acute peritraumatic stress for UMP, if viable, would represent a desirable approach. However, no valid instruments currently exist for objective ratings of combat-related peritraumatic symptoms, so it is yet unknown whether behavioral manifestations of acute traumatic stress are sufficiently observable or specific enough to be evaluated by third-party-UMP observers.

Thus, the primary objective of this study was the in-theater validation and psychometric evaluation of the PBQ-OR along with the assessment of its war-zone applicability through information collected in actively deployed troops. Specifically we aimed to: (1) validate and demonstrate the psychometric properties of the PBQ-OR; (2) investigate the relationship between objective, in-theater PBQ-OR ratings and self-reported peritraumatic symptoms retrospectively assessed by the PBQ-SR in Marines after deployment; (3) explore the relation of PBQ-OR ratings to post-deployment PTSD symptoms and PTSD caseness; and (4) investigate the PBQ-OR applicability as an operational clinical tool for accurate and consistent in-theater, objective assessment of peritraumatic symptoms in Marine ground combatants by especially trained UMP.

MATERIALS AND METHODS

Study design
The PBQ-OR in-theater validation study was designed as a longitudinal observational study to correlate third-person observer ratings of deployment peritraumatic behaviors (PBQ-OR) with post-deployment (1-wk follow-up) self-report measures of peritraumatic symptoms (PBQ-SR), and 3-mo post-deployment data collection of PTSD symptoms. In-theater data collection was linked to a larger IRB and VA research committee approved study entitled, "Prospective Study of the Psychological, Social and Biological Markers of Risk and Resilience for Operational Stress in Marines". Post-deployment information was assessed as part of the parent Marine Resiliency Study[13].

Collection of data
PBQ-OR data were collected by UMP on a bi-monthly basis for all consenting Marines in the enrolled deployment cohorts, beginning approximately 30 d after war zone deployment and until return to the United States approximately 7 mo later. The instructions for PBQ-OR required each symptom to be rated as present only to the extent it was a clear change from baseline behaviour for the rated individual, persisting for "a period of time" after exposure to an identifiable stressor. Throughout the confidential assessment, military operations and healthcare decision making in-theater were not directly affected and there was no direct contact between study personnel and unit members. PBQ-OR ratings were then confidentially forwarded to study investigators.

Rater training
All embedded UMP were trained at pre-deployment by mental health professionals in the administration and scoring of the PBQ-OR. UMP attended training comprised of a presentation on peritraumatic symptoms, an introduction to the PBQ-OR, presentation of videos and rating of symptoms upon completion, as well as participation in a question and answer period. UMP ratings were assessed for inter-rater reliability. UMP with a correlation of greater than 80 were certified as PBQ-OR raters or else repeated training until qualification.

Table 2 Demographic information of the included study sample

	%
Education	
Some high school	1.3
General education diploma	2.4
High school diploma	63.4
Some college	30.9
4-yr college degree	1.3
Ethnicity	
Not Hipsanic or Latino	74.0
Mexican	15.2
South/Central American	5.5
Other Spanish culture or origin	4.6
Race	
Black/African American	6.6
American Indian or Alaskan Native	9.1
Asian	2.6
Native Hawaiian or Pacific Islander	1.2
White	83.7
More than one	3.8

Demographics are given in percentage of the total available data (percentages under 1.0% are not reported).

Measures

The Clinician Administered PTSD Scale (CAPS)[14], the gold standard diagnostic interview tool for measuring PTSD in clinical research was administered by specially trained physicians or psychologists, and the PTSD Checklist (PCL)[15], a 17-item, self-report questionnaire, a validated assessment of PTSD symptom severity was filled out by each study participant at 3 mo post-deployment. A PTSD diagnosis according to DSM-IV criteria was made according to the well-established F1/I2 scoring rule[16]. All Marine participants additionally filled out the self-rate version of the PBQ (PBQ-SR)[10] 1 wk after deployment. All trauma-specific items were completed in reference to the same, indexed traumatic event.

Statistical analysis

Since PBQ-OR ratings were not completed at specific dates, Corpsmen reports were assembled into three groupings: Report order one (R1), two (R2), and three (R3) according to their submission dates with respect to post-deployment ratings. Cronbach's alpha (α) coefficients were calculated to assess internal reliability. Convergent and concurrent validity was measured by correlation coefficients between the corpsmen rated PBQ-OR and the post-deployment PBQ-SR, CAPS and PCL individual reports. To show by-symptom correlations between the corpsman's PBQ-OR report and Marine's post-deployment PBQ-SR scores, convergent validity was measured question by question using Kappa (κ) analysis. Predictive validity of PBQ-OR with respect to PTSD diagnosis at 3 mo post-deployment was calculated using logistic regression. Because PBQ-OR ratings were non-normally distributed, Spearman's rho (ρ) was used to calculate the correlations throughout the analysis. Correlations were plotted with respect to

mean days from PBQ-OR to PBQ-SR assessment. All statistical analyses of this study have been conducted and reviewed by biomedical statisticians (CJH, CVC).

RESULTS

Eight hundred and sixty male Marines were included in this study after signing informed consents at pre-deployment (mean age 23.2 ± 2.6 years). Demographic information is presented in Table 2. Twenty-nine certified male Corpsmen signed informed consent to be included as raters in the PBQ-OR study. Of those, 7 actually returned PBQ-OR ratings with dropouts occurring for different reasons (*e.g.*, non-embedment with a unit, operational schedule, serious injury, loss of data in battle). Overall, 458 PBQ-OR ratings were returned (R1: *n* = 248, R2: *n* = 128, R3: *n* = 62; Table 3). The mean number of days from PBQ-OR to PBQ-SR rating for R1, R2 and R3 was 198 ± 51.5, 170 ± 38.8 and 136 ± 24.9, respectively.

The means and standard deviations of all the rating instruments are presented in Table 3. Individual element response rates were item specific, and varied across item in both the PBQ-SR and the PBQ-OR. Marine participants who filled the PBQ-SR showed low response rates for questions relating to feelings of helplessness (#7), inability to move (#10), and inability to perform duties (#13). In regard to the PBQ-OR, Corpsmen response rates to these same items was comparably low. Corpsmen responses were low for the following additional items: Lack of remorse (#4), unstoppable laughing or crying (#6), disorientation (#9), and change in time perception (#11). Some questions (numbers 10, 11, 13), in addition to showing low ratings, received no non-zero responses from corpsmen at any of the reports (data not shown).

Psychometric properties

PBQ-OR Cronbach's α analysis showed good internal consistency (α = 0.88). R1, R2, and R3 PBQ-OR reports had Cronbach's α of 0.83, 0.90, and 0.92 respectively. PBQ-OR showed an overall significant or highly significant correlation to post-deployment PBQ-SR, PCL and CAPS total score in all three report orders (Table 4), confirming satisfactory convergent validity. A question-by-question κ analysis indicated different rates of correlations between PBQ-OR and PBQ-SR, showing best agreement between the observer and subjective ratings in questions relating to perception of mortal peril (#14) (report order 1: ρ = 0.41, *P* < 0.001; report order 2: ρ = 0.58, *P* < 0.001; report order 3: ρ = 0.50, *P* < 0.001) desire for revenge (#5) (report order 1: ρ = 0.38, *P* < 0.001; report order 2: ρ = 0.44, *P* < 0.001; report order 3: ρ = 0.61, *P* < 0.001), and experience of intense physical reactions to combat (#15) (report order 1: ρ = 0.34, *P* < 0.001; report order 2: ρ = 0.41, *P* < 0.001; report order 3: ρ = 0.53, *P* < 0.001) (cf. Table 1, Legend). No question showed significant or consistent negative correlation between

Table 3 Total scores of psychometric assessments by different instruments

Report order	*n*	PBQ				CAPS		PCL	
		PBQ-OR (Corpsman)		PBQ-SR (Marine)					
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Overall	458	2.29	4.95	6.88	8.09	21.71	20.07	28.21	13.14
1	248	1.45	3.20	6.84	8.18	21.39	20.04	28.52	13.12
2	128	3.63	6.53	7.58	8.64	22.45	20.67	28.57	13.99
3	62	3.02	6.37	6.19	7.16	22.25	20.16	26.78	12.24

Means and SD of questionnaires administered post-deployment (PBQ-SR, CAPS, PCL) by report order include only Marine reports for those with returned PBQ-OR ratings. PBQ-OR: Peritraumatic Behavior Questionnaire - Observer Rated; PBQ-SR: Peritraumatic Behavior Questionnaire - Self Rated; PCL: Posttraumatic stress disorder Checklist; CAPS: Clinician Administered posttraumatic stress disorder Scale.

Table 4 Correlations between Peritraumatic Behavior Questionnaire - Observer Rated questionnaires and Marine Resiliency Study subject measures by report order

Report order	CAPS total score		PCL total score		PBQ-SR total score	
	P	*n*	*P*	*n*	*P*	*n*
1	0.21[b]	235	0.25[b]	230	0.28[b]	249
2	0.28[a]	120	0.38[b]	113	0.41[b]	126
3	0.26[a]	63	0.46[b]	55	0.43[b]	63

Correlations plotted at mean days from report filing to PBQ-SR assessment. [a]$P < 0.05$, [b]$P < 0.001$. PCL: Posttraumatic stress disorder Checklist; CAPS: Clinician Administered Posttraumatic stress disorder scale; PBQ-SR: Peritraumatic Behavior Questionnaire - Self Rated.

PBQ-SR and PBQ-OR, suggesting overall agreement and effectiveness of the instrument (data not shown). Overall, increasing report order was associated with an improvement in correlation between PBQ-OR and post-deployment measures (Table 4). Finally, using logistic regression, PBQ-OR total score showed significant association with post-deployment PTSD caseness (OR = 1.0513; 95%CI: 1.011-1.093; *P* = 0.02), suggesting satisfactory predictive validity.

DISCUSSION

The assessment of the immediate individual response to trauma represents one of the most important challenges in traumatized populations[17]. This study contributes to the validation of the PBQ-OR, the first third-person rated, objective instrument for the assessment of peritraumatic symptoms in combat-related settings. The main findings from this study include: (1) satisfactory psychometric properties with good internal consistency for the PBQ-OR; (2) high convergent validity with respect to post-deployment PBQ-SR total score ratings; (3) high concurrent validity with respect to post-deployment PTSD symptoms as well as significant predictive validity with respect to PTSD caseness; and (4) increases in correlations between PBQ-OR and all three post-deployment measures' total scores with increasing report order. However, an overall sparse return rate of PBQ-OR ratings and a drop off in return rate of PBQ-OR ratings with increasing report order.

The sparse return rate of PBQ-OR reports is the most major limitation of this study and mirrors the well-documented key practical limitations of data assessment and documentation in military field opera-

tional research[18]. According to prior literature, the reliability and validity of in-theatre assessed psychometric measures is mostly threatened by non-response and deployment duration[19], as also seen in our study. War-zone-related research often goes hand in hand with unpredictable parameters, inconsistency in sampling practice, unit mobility, data storage, access and tracing issues, ineptness of structured interviews, time constraints, *etc.*[20], and, thus, introduces a broad spectrum of potentially quality-affecting specific features leading to sampling (*e.g.*, non-response, assessing frame bias, data access) and non-sampling error types (*e.g.*, interviewee-, rater- or scale-related errors)[21].

However, when taken together, our psychometric results suggest that PBQ-OR is a reliable and valid observer-rated measure for the global and objective assessment of combat-related peritraumatic symptoms and their underlying dimensions by UMP in currently deployed military personnel. We, thus, suggest that third-person, objectively recognized peritraumatic symptoms as measured by the PBQ-OR may constitute a valid and reliable screening for the assessment of combat-related peritraumatic reactions. There is a trend of better psychometric properties, when the PBQ-OR is administered towards the end of deployment. However, since exact time or frequency of combat-trauma exposure was not known, we cannot positively elucidate the reasons for this trend.

Because the content development of the PBQ focuses on behavioural indicators of peri-traumatic stress in the field of operations[10], the PBQ represents a uniquely appropriate peri-traumatic measure for military members.

The administration and scoring of the PBQ-

OR is easily feasible due to clear and simple rating instructions and clearly specified assessment areas. In addition, its comparability to already established self-rated peritraumatic dissociation scales (*e.g.*, Peritraumatic Dissociative Experience Questionnaire; Peritraumatic Distress Inventory)[22,23] is promoted through the 5-point-Likert scale structure applied. Such a screening tool could be used immediately after a traumatic event, but also periodically and longitudinally for monitoring. PBQ-OR represents an instrument to be used in real time, without interfering with concurrent military operations and relying on self-perception, recollection or self-report. No comparable measures have been developed so far for the assessment of acute peritraumatic-stress-related observable reactions in military Service Members, thus the PBQ may provide a template for future training of UMP. The use of PBQ for UMP training for recognition of and response to peritraumatic stress in the battlefield setting could be one of the main values of this measure.

In conclusion, PBQ-OR utilization could add up to more accurate and timely identification of peritraumatic reactions advancing the individual risk of service members for the imminent development of combat-related acute and posttraumatic stress symptoms. Regular PBQ-OR assessment could represent a focused prevention strategy through effective regular monitoring, facilitating earlier support and evidence-based treatment. The PBQ-OR ability to embody a regularly used measure with practical applicability and incremental validity in combat-related settings should, however, be prospectively validated through additional, larger-scale studies.

ACKNOWLEDGMENTS

COMMENTS

Background
Peritraumatic stress reactions include various behavioral, emotional, cognitive, and physiological symptoms associated with sympathetic activation during and immediately following a traumatic event. Prolonged continuation of these biological and psychological responses can lead to long-term adverse biological alterations, strongly associated with the subsequent development of posttraumatic stress disorder (PTSD).

Research frontiers
The assessment of the immediate individual response to trauma represents one of the most important challenges in traumatized populations.

Innovations and breakthroughs
This study contributes to the validation of the Peritraumatic Behavior Questionnaire - Observer Rated (PBQ-OR), the first third-person rated, objective instrument for the assessment of peritraumatic symptoms in combat-related settings.

Applications
The main findings from this study include: (1) satisfactory psychometric properties with good internal consistency for the PBQ-OR; (2) high convergent validity with respect to post-deployment PBQ - Self Rated (PBQ-SR) total score ratings; (3) high concurrent validity with respect to post-deployment PTSD symptoms as well as significant predictive validity with respect to PTSD caseness; and (4) increases in correlations between PBQ-OR and all three post-deployment measures' total scores with increasing report order.

Terminology
PTSD: Posttraumatic stress disorder; PBQ-OR: Peritraumatic Behavior Questionnaire - Observer Rated; PBQ-SR: Peritraumatic Behavior Questionnaire - Self Rated.

Peer-review
This is a nice article presenting a useful instrument.

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ORIGINAL ARTICLE

Observational Study

Association between recognizing dementia as a mental illness and dementia knowledge among elderly Chinese Americans

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Institutional review board statement: This study was reviewed and approved by the University of California, Los Angeles's Education and Research Institute.

Informed consent statement: All study participants, or their legal guardian, provided informed consent prior to study enrollment.

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Abstract

AIM: To investigate whether older Chinese Americans perceive dementia as a mental illness and the relationship between such perception and their general understanding of dementia remains unclear. Our study aims to understand this relationship and its future implication on improving dementia literacy among ethnic minorities.

METHODS: Elderly Chinese American participants from the Greater Los Angeles were asked to complete an 11-item dementia questionnaire, following a community health seminar. Cross-sectional survey data was analyzed using standard statistical methods.

RESULTS: The questionnaire received an 88.3% response rate. Among 316 responders, only 28.8% (n = 91) of elderly Chinese Americans identified dementia as a mental illness, and 71.2% (n = 225) did not recognize its mental disease origin. Furthermore, in comparison between these two groups, the first group demonstrated significantly higher level of baseline knowledge of the disease.

CONCLUSION: This study reveals that only approximately 1 out of 4 older Chinese Americans recognized dementia as a mental illness, consistent with previous studies on Asian Americans. Our study however showed that when dementia was being perceived as a mental illness, such perception was associated with a higher level of baseline dementia understanding. The current study suggested the potential of improving older Chinese Americans dementia literacy by increasing awareness of its mental illness origin.

Key words: Dementia literacy; Mental illness; Chinese Americans; Stigma

Core tip: This study reveals that only approximately 1 out of every 4 elderly Chinese Americans recognized dementia as a mental illness. Our study however demonstrates that when dementia was being perceived as a mental illness, such perception was associated with a higher level of baseline dementia understanding. Further research is necessary to identify any causative relationship between viewing dementia as a mental illness and improved dementia knowledge, with the ultimate goal of improving dementia management outcomes in elderly Chinese American ethnic population.

INTRODUCTION

According to the United States Census, an estimated 20% of the total United States population will be reaching age 65 or above by the year 2030[1]. With more elders living longer, age-dependent conditions like dementia have become increasingly important topics. Asians are the fastest growing ethnicity in the United States, with a 46% increase between 2000 and 2010[2]. Because Chinese Americans constitute the largest subgroup amongst Asian Americans reaching a population of 4 million in 2010, this ethnic group is particularly important when studying various conditions, including dementia[1,2].

Older Chinese Americans demonstrate profound stigma towards the term "mental illness". According to Yang *et al*[3], mental illness stigma has been described as especially pervasive and severe among Chinese population. Elderly Chinese individuals often equate "mental illness" with schizophrenia, a severe form of mental illness in which patients are generally ostracized by the Chinese society[4]. Chinese patients with mental illnesses are quite commonly unable to regain social

acceptance and thus are isolated from the society. The label of a "mental illness" may serve as cues to stereotype these patients with negative characteristics such as "crazy" or "dangerous". Several previous studies show that older Chinese Americans have a very limited level of general knowledge about mental illness, and this has further contributed to social exclusion and discrimination toward diagnosed individuals with mental illness[5-7]. Therefore, measuring whether the perception of "mental illness" will affect knowledge is particularly important.

Previous research has demonstrated that elderly Chinese American ethnic group has low baseline knowledge of dementia[8]. Studies have shown that lack of dementia knowledge in Chinese community is associated with higher care-giver burnout rate, lower overall medical resource utilization rate and reduced quality of life for dementia patients[8,9]. Unlike the western community which individualism is highly valued and appreciated, traditional values such as homogeneity forms the basis of Chinese American community. Individuals with mental illness such as dementia are perceived as outliers and thus explained the social isolation and discrimination towards those patients. As a result, Chinese Americans suffering from dementia receive less overall medical resources. Moreover, the delay in medical care exacerbates the disease progression leading to increased healthcare cost. In order to ensure a better delivery of dementia care to this ethnic group, the priority lies in the effort to increase elderly Chinese American's baseline dementia knowledge at the population level.

Dementia, widely categorized as a mental illness in the western culture, is highly stigmatized by older Chinese Americans[1,10]. Cultural beliefs, such as the view that dementia brings shame and embarrassment to the family, also play an important role in stigmatizing dementia[5,6]. Recently, DSM-5 has reclassified dementia and related disorders as neurocognitive disorders to highlight their separation from other mental illnesses and to disconnect neurocognitive disorder from the mental illness stigma associated with the term "dementia". Nonetheless, whether elderly Chinese Americans perceive dementia as a mental illness, and the association between this perception and their general baseline dementia knowledge is poorly understood. Thus in this current study, we aim to understand this relationship and its implications to improve dementia literacy among ethnic minorities.

MATERIALS AND METHODS

Design and procedure

This cross-sectional study enrolled a total of 358 participants from local Chinese American communities. Participants were recruited to attend dementia seminars hosted in the Greater Los Angeles area by means of radio, newspaper, posters, and word of mouth. Inclusion criteria included literacy in Chinese, the ability to

complete a survey questionnaire written in Chinese, and aged 55 and above in an attempt to capture the group of people entering into the age of developing cognitive impairments. Before the seminars, participants completed the questionnaire that identified their opinion on whether dementia was a mental illness, obtained their sociodemographic characteristics, and evaluated their general dementia knowledge. Sociodemographic variables included age, gender, education level, duration of residency in the United States and family history of dementia. The dementia knowledge was assessed by 11 questions that consisted of true (T) or false (F) statements regarding dementia causes, symptoms, treatment and prognosis as described in a previous study[8]. Based on the questionnaire design, the total number of correct items, ranging from a score of 0-11, was positively associated with general dementia knowledge. A higher total score indicated a better understanding of dementia and thus higher baseline dementia knowledge.

Statistical analysis

Based on self-reported yes/no response to the statement "Dementia is a chronic mental illness", participants were separated into two study groups. Sociodemographic variables such as age, gender, education level, length of residency in the United States, as well as family history of dementia between the two groups were compared. Descriptive statistical analysis was performed using SPSS v13; two-tailed t test and χ^2 test were used to compare continuous variables and categorical variables respectively. Results with a P value < 0.05 are defined to be significant. The statistical review of the study was performed by a biomedical statistician.

RESULTS

Three hundred and sixteen of the 358 enrolled participants returned valid questionnaire answers, resulting in a response rate of 88.3%. Of the 316 valid responses, 28.8% (n = 91) elderly Chinese Americans identified dementia as a mental illness similar to western culture, and 71.2% (n = 225) did not recognize its mental disease origin. The group that recognizes dementia as a mental illness had 34% (n = 31) males, 53% (n = 48) high school graduates, 47% (n = 43) had lived in the United States for less than 20 year, 26% (n = 24) with a family history of dementia and had an average age of 64. On the other hand, the group that did not identify dementia as a mental disease had 32% (n = 73) males, 58% (n = 131) graduated high school, 54% (n = 122) had resided in the United States for less than 20 years, 20% (n = 46) with a family history of dementia and had an average age of 63. No statistical significance was found when comparing these two groups based on socio-demographic variables, such as gender (P = 0.781), age (P = 0.094), education level (P = 0.374), duration of residence in the United States (P = 0.261)

and family history of dementia (P = 0.250).

We identified statistical significant difference, however, in the total score of correct items from the questionnaire, which was used to gauge participant's baseline dementia knowledge. Table 1 shows the responses to the questionnaire based on perception of dementia as a mental illness. According to the 11-item questionnaire results, the group that recognized dementia as a mental illness (n = 91) has total scores ranging from 3 to 10, while the group that failed to see dementia as a mental illness (n = 225) has total scores between 3 and 11. However, the first group has a mean total score significantly better than the latter group (6.7 ± 1.6 *vs* 6.2 ± 1.7, t(314) = 2.39, P = 0.018), indicating that the group which identifies dementia as a mental illness has higher level of baseline dementia knowledge.

Upon further analysis of individual items of the questionnaire, five (item numbers 3, 4, 5, 9 and 11) out of the 11 items were found to be significantly different between the two groups. Participants who perceived dementia as a mental illness were more likely to correctly identify "Risk of dementia increases with age" (93.4% *vs* 84.4%, P = 0.032). Among this group, 96.7% of the participants agreed that "dementia is defined as a reduction of cognitive abilities including understanding and judgment, as well as memory loss"; however, only 83.6% in the other group recognized this true statement (96.7% *vs* 83.6%, P = 0.002).

Similarly, more participants in the group that recognized dementia as a chronic mental illness correctly identify that "some types of dementia are caused by cerebrovascular diseases" (45.1% *vs* 28.0%, P = 0.004). In addition, 46.2% of participants in this group agreed that "dementia shortens the life expectancy after onset", which was significantly more than the 32.0% in the other group (46.2% *vs* 32.0%, P = 0.018).

Interestingly, more participants in the "dementia is not a mental illness" group correctly identified "senescence forgetfulness progresses with advancing age, resulting in patients being unable to recognize their families" as a false statement (81.8% *vs* 71.4%, P = 0.042). However, this one exception did not seem to change the fact that this group had a lower total score in this questionnaire.

DISCUSSION

This study reveals that only approximately 1 out of every 4 elderly Chinese Americans recognized dementia as a mental illness. This is consistent with previous study which demonstrated that Asian Americans are less likely to recognize dementia as a mental illness[9]. Our study however identifies that when dementia was being perceived as a mental illness, such perception was associated with a higher level of baseline dementia understanding.

When stigmatization of mental illness is prevalent in the society, it may further impede an individual from taking the initiative to learn more about dementia

Table 1 Association between dementia knowledge questionnaire items and perception of dementia as a mental illness

Knowledge category		Questions	True/false	Answered yes to "dementia is a chronic mental illness" (n = 91)	Answered no to "dementia is a chronic mental illness" (n = 225)	P	χ^2
General	1	Dementia is forgetfulness due to aging. Everyone will have dementia with advancing age	F	29 (31.9)	92 (40.9)	0.135	2.231
General	2	Dementia is a disease affecting the brain. Not everyone will suffer from dementia	T	88 (96.7)	207 (92.0)	0.129	2.310
General	3	Risk of dementia increases with age	T	85 (93.4)	190 (84.4)	0.032	4.609
General	4	Senescence forgetfulness progresses with advancing age, resulting in patients being unable to recognize their families	F	65 (71.4)	184 (81.8)	0.042	4.154
Symptoms	5	Dementia is defined as a reduction of cognitive abilities including understanding and judgment, as well as memory loss	T	88 (96.7)	188 (83.6)	0.002	10.131
Treatment	6	Some types of dementia are treatable	T	56 (61.5)	114 (50.7)	0.079	3.081
Symptoms	7	People suffering dementia become unable to perform familiar tasks at once	F	36 (39.6)	87 (38.7)	0.883	0.022
Symptoms	8	People suffering from dementia become unable to recognize time, place, and person at once	F	25 (27.5)	68 (30.2)	0.627	0.236
Cause	9	Some types of dementia are caused by cerebrovascular diseases	T	41 (45.1)	63 (28.0)	0.004	8.536
Cause	10	Some types of dementia are hereditary	T	57 (62.6)	136 (60.4)	0.717	0.131
Prognosis	11	Dementia shortens life expectancy after onset	T	42 (46.2)	72 (32.0)	0.018	5.629
	Total score			6.7 ± 1.61	6.2 ± 1.71	0.018	t = 2.39

T: True; F: False.

and improve level of knowledge. In fact, a previous qualitative research has highlighted that for Chinese family dementia caregivers, stigma was a common theme. They found that significantly more Asian Americans (53%) than Anglos (16%) endorsed that "Alzheimer's is a form of insanity", demonstrating a lack of dementia knowledge among Asian Americans[11]. However, our current study, surprisingly, shows quite the opposite - individuals who see dementia as a mental illness is associated with better understanding of the disease. This could be explained by a relatively non-aggressive nature of dementia compare to other mental illnesses. Indeed, Lam *et al*[12] reports that while there are different stereotypes for various mental illnesses, patients with anxiety or dementia were much better accepted than those with psychosis in Chinese communities. The term "mental illness", when associated with dementia, may not be as stigmatizing, as evidenced by higher dementia knowledge from the current study. In addition, a more recent quantitative research examining 89 Chinese American general public revealed that over three-quarter participants supported further research on dementia, suggesting their interest in learning more about this disease[13].

In general, as evidenced by questionnaire items 3, 5, 9 and 11, viewing dementia as a mental illness is associated with better understanding of dementia in the following categories: General knowledge (risk increased with advanced age), symptoms (dementia reduced cognitive ability), cause (some dementia are caused by cerebrovascular disease), and prognosis (dementia

shortens life expectancy). These specific knowledge categories are consistent with the knowledge categories found insufficient among Asian American ethnic groups in Hawaii based on a previous study[1]. Nevertheless, item 4 shows that the group who recognizes dementia as a mental illness is more likely to mistaken that senescence forgetfulness progresses with advancing age. Similarly, a number of studies have highlighted the strong tendency of Asian Americans, particularly elderly Chinese, to view dementia as a normal part of aging process in order to "buffer" the stigmatization of dementia from other aggressive mental illnesses[14,15]. Such "normalization" carries with it a soft stigma that was distinct from that of severe mental illnesses. Future intervention would be necessary to address this fundamental misbelief of dementia in this ethnic population.

Chinese Americans who do perceive dementia as a chronic mental illness generally describe dementia as a disease of the brain. In a survey of 22 Chinese Americans, all Chinese Americans (100%) tend to view dementia as an illness that affects the brain. For example, one participant stated in Chinese, "Playing mah-jongg can eliminate dementia because it activates my brain"[16]. The above finding is similar to a pilot study conducted among Vietnamese American Immigrants. Eighty percent of the participants believe that dementia is a disease affecting the brain[17]. Among 208 Chinese Americans, younger adults showed a significantly higher level of understanding that dementia could result from cardiovascular disease[18]. However, Chinese Americans

continue to associate dementia with stigma and "loss of face". In fact, family members were more likely to perceive patients with dementia to be incapable of feeling other people's worries or concerns at once[19].

Previous studies have found that older Chinese Americans with less than 20 years of residence in the United States were less likely to understand that dementia shortens life expectancy after onset[20]. Our data adds that when elderly Chinese Americans do not identify dementia as a mental illness, they are more likely to misunderstand dementia prognosis. Therefore, it appears that there are multiple factors affecting older Chinese Americans in understanding dementia prognosis knowledge. As knowledge is necessary to change health-related behaviors, a necessary step in dementia promotion involves increasing the Chinese American public's knowledge on dementia prognosis.

This study suggested the potential of improving elderly Chinese Americans dementia literacy by increasing awareness of its mental illness origin. Traditionally, programs aiming to deliver medical knowledge about dementia are done *via* classroom, pamphlets or other media outlets[21]. Such programs often have low effectiveness secondary to stigmatization/lack of interest in the general public, however they still require a sizable amount of community resources. Interestingly, our current study may point to a new direction. Simply by delivering a concise message such as "dementia is not a normal part of aging. Let's face this mental illness together", we may have challenged the popular Chinese American perception and therefore, stimulate their interest in learning more about the disease. Moreover, previous study by Ho *et al*[22] suggested that Chinese Americans are eager to learn if resources are available, despite having low level of baseline dementia knowledge. Together with Chinese Americans' willingness to seek information about this illness, future education programs that emphasize dementia's mental illness origin may have improved effectiveness in delivering dementia knowledge to this ethnic group.

This study has several limitations. First, data were based on a cross-sectional survey that relied on self-reported data, so our findings are subject to report biases inherent in these approaches. Second, the survey was brief and excluded some specific topics on dementia. It also did not directly assess the stigma of dementia. Third, due to the true/false response format, there may be lower variance among the items. Future studies should utilize scales with established psychometric properties and cut-off scores. Fourth, the sample included only participants aged 55 and older, in an attempt to capture people entering into the age group of developing cognitive impairments. Although this age group corresponds to the majority of participants who may eventually develop neurocognitive disorders, it does raise the question of whether our data are generalizable to all age groups. Fifth, the goal of our study was to explore whether viewing dementia as a mental illness is associated with overall dementia knowledge among elderly Chinese Americans. There were no differences on sociodemographic factors between the two groups. As such, we did not perform logistic regression to examine predictors. Future studies may focus on identifying additional sociodemographic characteristics with significant test of interaction and use logistic regression to examine whether perception of mental illness may be a useful predictor for high baseline dementia knowledge. Sixth, this study would benefit from using Likert scale on the question "Is dementia a chronic mental illness" to provide a way of measuring attitudes. Seventh, with the possibility that people with bias about dementia may have less interest in attending dementia seminar, this study may underestimate the bias by collecting data from seminar attendees. Lastly, we recognized that the term, "chronic mental illness", carries significant stigma as well. Future studies may consider using the term, "severe mental illness" to prevent potential confounding bias.

This study reveals that only approximately 1 out of every 4 elderly Chinese Americans recognized dementia as a mental illness. Our study however demonstrates that when dementia was being perceived as a mental illness, such perception was associated with a higher level of baseline dementia understanding. Further research is necessary to identify any causative relationship between viewing dementia as a mental illness and improved dementia knowledge, with the ultimate goal of improving dementia management outcomes in elderly Chinese American ethnic population.

ACKNOWLEDGMENTS

COMMENTS

Background
As one of the fastest growing ethnic groups in the United States population, elderly Chinese Americans are known to have a profound cultural stigma towards mental illnesses and poor knowledge about these diseases. Dementia, widely categorized as a mental illness based on a biomedical model in the Western World, is highly stigmatized in this ethnic group. Nonetheless, whether older Chinese Americans perceive dementia as a mental illness and the relationship between such perception and their general understanding of dementia remains unclear. This study aims to understand this relationship and its future implication on improving dementia literacy among ethnic minorities.

Research frontiers
Research focusing on the Chinese American population has been scarce in general, especially in the field of mental illness. The research hotspot is to gain more understanding in this topic *via* a variety of research modalities and approaches.

Innovations and breakthroughs
Since people's perception of mental illness is a very subjective opinion, a few of research studies have tried to detour from investigating this subject. The study, however, confronted this perception of dementia as a mental illness, and attempted to find its association with dementia knowledge.

Applications

As knowledge is necessary to change health-related behaviors, a necessary step in dementia promotion involves increasing the Chinese American public's knowledge on dementia. Together with Chinese Americans' willingness to seek information about this illness, future education programs that emphasize dementia's mental illness origin may have improved effectiveness in delivering dementia knowledge to this ethnic group.

Terminology

Dementia is a broad category of disease that causes a long term and often gradual decrease in the ability to think and remember that is great enough to affect a person's daily functioning.

Peer-review

The work is interesting and it has a significant degree of originality since it focuses on dementia as a mental illness among elderly Chinese Americans, which represents a rapidly growing population in the United States.

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ORIGINAL ARTICLE

Observational Study

Effectiveness of an intervention for reducing social stigma towards mental illness in adolescents

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Abstract

AIM: To evaluate the effectiveness of an intervention for reducing social stigma towards mental illness in adolescents. The effect of gender and knowledge of someone with mental illness was measured.

METHODS: Two hundred and eighty secondary school students were evaluated using the Community Attitudes towards Mental Illness (CAMI) questionnaire. The schools were randomized and some received the intervention and others acted as the control group. The programme consisted of providing information *via* a documentary film and of contact with healthcare staff in order to reduce the social stigma within the school environment.

RESULTS: The intervention was effective in reducing the CAMI authoritarianism and social restrictiveness subscales. The intervention showed significant changes

in girls in terms of authoritarianism and social restrictiveness, while boys only showed significant changes in authoritarianism. Following the intervention, a significant reduction was found in authoritarianism and social restrictiveness in those who knew someone with mental illness, and only in authoritarianism in those who did not know anyone with mental illness.

CONCLUSION: The intervention was effective to reduce social stigma towards people with mental illness, especially in the area of authoritarianism. Some differences were found depending on gender and whether or not the subjects knew someone with mental illness.

Key words: Social stigma; Adolescent; Mental illness; Intervention studies; Prevention

Core tip: The intervention was effective to reduce social stigma towards people with mental illness in schools. Authoritarianism was the area that improves more after the intervention. Women and people with knowledge of someone with mental illness were the collective were the intervention was more effective.

INTRODUCTION

Stigma is a social construct that includes negative attitudes, feelings, beliefs, and behaviours that are configured as prejudice and which has negative consequences for the stigmatized person, making them feel like a lower class[1,2].

People who suffer from mental illness, particularly people with schizophrenia, are one of the most stigmatized groups[3]. Serafini *et al*[4] (2011) found that the fact that schizophrenia is perceived as a genetic disorder and not environmental disorder, increase the stigma towards this mental illness. Different studies show that this group is subject to prejudice, discrimination, and the greatest impact of stigma leading to social isolation, loss of social roles, lowering of self-esteem, increases the possibility of depression, shame and fear of exclusion[5-9]. Sartorius *et al*[10] note that stigma is very harmful, and despite the advances and improvements in psychiatry and medicine, stigma continues to grow.

Lack of knowledge and false ideas about mental illness produce an increase in discriminatory behaviour in society towards this group, lower their quality of life, lower rates of help-seeking and service use[11-13]. It is important to try to change society's attitudes towards people with mental disorders and to reduce stigmatizing behaviours and attitudes. Griffiths *et al*[14] showed that educational interventions addressed to reducing social stigma were effective. Additionally, different studies describe the best way of reducing social stigma as being through intervention and social integration programmes, specifically, in children and adolescents[15-18]. Negative attitudes toward mental illness are commonly supported by teens[19]. Education is fundamental to improve understanding of mental health, reduce stigma and improve access to care[20]. Therefore, it is a good idea to create intervention programmes in schools, with the objective of counteracting these stereotypes before they arise. In the same line, Roeser[21] and Weist[22] said that in the schools should carry out programs to promote mental health. He explained that it is necessary include training on interdisciplinary collaboration, working closely with schools and community personal health, and on understanding systems issues (for example, community mental health). The study by Pinfold *et al*[23] revealed that young people do not have a clear idea regarding what mental illness is and the cultural stereotypes associated with it are not fully developed until adolescence or early adulthood and could be modify[18]. So projects aimed at children and adolescents appear to be promising as they allow the modification of ideas relating to mental illness more easily than in adults.

Regarding the type of intervention, it is not clear which elements are most effective. Clement *et al*[24] showed, with student nurses, that there were no differences in effectiveness between the DVD and direct contact groups. However, greater improvement was produced when both techniques were used together. In the same way, Penn *et al*[25] found that a film regarding people with schizophrenia could reduce stigmatizing beliefs. The use of filmed material has many advantages. It is more easily extended to the wider population and it has the potential to reach large audiences, it can be made available on the internet, and more cost-effective solution. Also it allows the participation of more presenters, the material is more consistent, there is greater control through editing, and it is less stressful for users compared to live exposure.

Other variables such as gender and knowledge have been studied in relation to the reduction of stigmatizing attitudes. Martínez-Zambrano *et al*[26] found that women showed significant changes after the intervention in the authoritarianism and social restrictiveness subscales of OMI questionnaire, while men showed changes in negativism and interpersonal etiology. Regarding the question of knowing someone with a mental disorder, those people who knew someone showed significant changes in the authoritarianism, interpersonal etiology, and negativism subscales, while those people who

did not know anyone with mental illness improved in restrictiveness and authoritarianism. Högberg *et al*[9] described how people who are in contact with those who suffer from a mental disorder presented lower levels of social stigma. In the same line, Graves *et al*[27], in a study of students (aged 17-27 years), considered that those having a higher level of contact with someone with a mental disorder showed lower negative affect towards these people and less social distance, compared to those students with a lower level of contact. Despite this, the high contact group attributed significantly higher levels of dangerousness to the people with a mental disorder; this may be because interactions are more difficult and complex rather than a perception of dangerousness in the strictest sense. On the other hand, Angermeyer *et al*[28] found that students who were familiar with people with a mental disorder were less likely to believe that people with schizophrenia or major depression were dangerous. Furthermore, another study by Högberg *et al*[29] revealed that it is not obvious that those individuals with considerable knowledge about mental disorders always have positive attitudes towards persons with a serious mental disorder. So it seems that knowledge of someone with a mental disorder is related to lower levels of social stigma, although some attitudes concerning danger and living in proximity are not clearly defined.

In summary, there are few studies that seek to reduce social stigma in adolescents. Few studies have used filmed material as a tool for intervention to reduce stigma and few of them have been carried out on adolescents. Furthermore, the evaluation of stigma varies widely and does not facilitate the comparability of different studies.

Therefore, the principal aim of the present study was to evaluate the effectiveness of an intervention with professionals and documentary film in the reduction of social stigma towards a mental disorder in adolescents regarding stigma domains (authoritarianism, benevolence, social restrictiveness, and community mental health ideology). Secondary aims were to analyse whether there were differences regarding gender and knowing someone with a mental disorder.

MATERIALS AND METHODS

Design

The schools that participated in the study were randomised in two groups. In some schools the assessments and intervention was applied while in the other school only the assessments were provided. The intervention group included $n = 128$ while the control group included $n = 152$. The study consisted in measuring the effect of reduction in stigma before and after the intervention, controlling for the effect of intervention. Our sample size of 280 people, would allow to detect an effect size of at least 0.3 between the groups, with a significance level of 5% and 80% power, with an unilateral Student's *t* test.

Subjects

The subjects evaluated were 280 adolescents aged between 14 and 18 years old from four secondary schools located near Barcelona, Spain. Based in a previous study we need a sample of minimum of 100 students in each condition (Martínez-Zambrano *et al*[26] 2013). Specifically, these four schools, of a middle-class socioeconomic level and roughly the same ethnicity, that participate in the Escola Amiga Program from the Parc Sanitari Sant Joan de Déu (PSSJD). The program offers young people the opportunity to learn firsthand stories of groups at risk of social exclusion and engage to change situations of injustice. The schools that participated in the program are interested in receiving information about several issues and one of this is mental health.

Evaluation instrument

The Community Attitudes towards Mental Illness (CAMI)[30] was chosen for the assessment of social stigma because it is the only scale validated in our context[31]. Originally this questionnaire was devised to predict and explain the reactions of the general population towards local mental health services. The CAMI report social stigma in general population regarding mental disorders. The CAMI is a scale consisting of 40 items with a 5 Likert-scale that ranges from totally agree to totally disagree. Accordingly, to test the Spanish validity and reliability of the CAMI, it was translated and back-translated by our group. The psychometric properties of the instrument showed adequate data, a Cronbach alpha of 0.867, and a temporal stability measured with a difference of one week varied between 0.324 and 0.775. The validation of CAMI showed four factors originally called authoritarianism, benevolence, social restrictiveness, and community mental health ideology[30]. The authoritarianism scale reflects the view of people with a mental disorder as an inferior class. The benevolence scale represents attitudes that are encouraging of people with mental disorders but which exhibit a paternalistic attitude. The social restrictiveness scale assesses danger to society and suggests that people with a mental disorder should be restricted both during and after hospitalization. And, lastly, the community mental health ideology subscale evaluates attitudes and beliefs related to the integration of people with a mental disorder into the community and into society in general. Higher scores in authoritarianism and social restrictiveness indicate greater stigma, while lower scores in benevolence and community mental health ideology indicate greater stigma.

Data were also collected on the gender of the students, their age, and whether or not they personally knew someone with a mental disorder.

The intervention procedure

The first contact was to introduce ourselves and collected the informed consents. The informed consents

Table 1 Mean scores of the Community Attitudes towards Mental Illness subscales and comparison of the differences in the averages of the control and intervention groups

	Baseline		Post-intervention		Difference between baseline and post-intervention		*T*-students	*P* value
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)		
	Control	Intervention	Control	Intervention	Control	Intervention		
Authoritarianism	26.61 (3.23)	27.26 (3.75)	26.79 (3.98)	25.32 (4.09)	-0.30 (2.83)	1.94 (4.03)	5.186	0.000
Benevolence	21.74 (4.00)	21.98 (4.08)	22.14 (4.28)	22.14 (4.13)	-0.27 (2.91)	-0.14 (3.55)	0.330	0.742
Social restrictiveness	20.89 (4.51)	20.07 (4.10)	22.14 (5.16)	20.34 (4.33)	-1.25 (3.79)	-0.20 (3.49)	2.355	0.019
Community mental health ideology	24.43 (5.20)	23.00 (5.22)	24.79 (5.60)	22.53 (5.52)	-0.37 (3.55)	0.40 (4.40)	1.572	0.117

were delivered to the teachers before the study started in order to be signed by their parents and to be brought by the students before the first assessment. The questionnaires were anonymous, but the students were asked to use a pseudonym in order to identify their second questionnaire. A total of 12 students were not assessed in the second evaluation, because they did not assist to the school the second day therefore these cases were lost.

Regarding the intervention group, two activities were carried out. In one, the subjects were shown a documentary film related to mental disorder, featuring adolescent characters, in order to produce a greater level of connection and empathy with the students. The film used for intervention lasts approximately 20 min and it was developed for actors coordinated by professionals of the PSSJD. In the film, three adolescents do a school project about mental disorders. At this point, they discuss the choice of this issue, raising many questions and bringing out stereotypes (for example, if they are dangerous, if they can work, the relationship with others, etiology of the illness...). The film is presented as a conversation with different professionals and users with mental disorders, reaching conclusions, in addition to answering the questions initially posed and responding questions about this social group. Once the film was finished, a brainstorming session of questions with the two specialised mental healthcare staff from the rehabilitation services (psychologists, social workers, and occupational therapists) and from the mental health centres (nurse and social workers) was held.

All participants responded to the CAMI questionnaire at two different time points. In the intervention group, the intervention was carried out one week after completing the first questionnaire, and in the week following the intervention the questionnaire was administered for a second time. In the control group, two assessments were performed in a week.

The study was approved by the PSSJD Research and Ethical Committee.

Statistical analysis

The data analysis was carried out using the statistics package SPSS version 19. The score difference of each factor at the two administration times was calculated in order to analyze the difference between the responses of the students at the two points. The difference in scores for each group was analysed with a Student *t* test for independent samples. The same test was repeated to assess these differences in terms of gender and whether or not the students knew someone with a mental disorder. Once all the variables were obtained, a general linear model for repeated measures was carried out controlling for the variables of gender and whether or not the subjects knew someone with a mental disorder.

RESULTS

Differences between the means in the two groups, both at baseline and post-intervention were shown in Table 1. The differences of each intervention group were significant in the authoritarianism and social restrictiveness subscales ($P < 0.001$ and $P = 0.019$, respectively). Conversely, the benevolence and community mental health ideology subscales did not show significant changes.

Regarding gender, at baseline girls showed scores significantly lower in all of the CAMI subscales: authoritarianism ($P < 0.001$), benevolence ($P = 0.002$), social restrictiveness ($P = 0.019$), and community mental health ideology ($P = 0.013$) (Table 2). Regarding whether or not they personally knew someone with a mental disorder at baseline, people who did had significantly lower scores on authoritarianism ($P = 0.005$) and social restrictiveness ($P < 0.001$) at baseline than those who did not (Table 3).

Tables 4 and 5 show the means of each subscale, according to the group at baseline and post-intervention, taking into account the covariates of gender and whether or not the subjects knew someone with a mental disorder, respectively. Significant differences in the authoritarianism subscale were found in the case of boys ($P < 0.001$), and in authoritarianism and social restrictiveness in the case of the girls ($P = 0.010$ and $P = 0.037$, respectively) (Table 5). Lastly, in Table 5, a significant difference in the authoritarianism and social restrictiveness subscales in those people who knew someone with a mental disorder was found ($P < 0.001$ and $P = 0.018$, respectively), and only a change in authoritarianism ($P = 0.010$) in those people who did not know anyone with a mental disorder was significant.

Regarding the linear regression models, controlling for gender and whether or not the subject knew

Table 2 Mean scores of the Community Attitudes towards Mental Illness subscales comparing the differences between boys and girls at baseline moment				
	Baseline		*T*-students	*P* value
	Mean (SD)	**Mean (SD)**		
	Girls	**Boys**		
Authoritarianism	25.96 (3.26)	27.92 (3.45)	4.824	0.000
Benevolence	21.11 (3.45)	22.62 (4.46)	3.121	0.002
Social restrictiveness	19.91 (4.11)	21.14 (4.50)	2.352	0.019
Community mental health ideology	22.97 (4.72)	24.56 (5.65)	2.504	0.013

Table 3 Mean scores of the Community Attitudes towards Mental Illness subscales comparing the differences between knew someone or not at baseline moment				
	Baseline		*T*-students	*P* value
	Mean (SD)	**Mean (SD)**		
	Knows someone	**Does not know anyone**		
Authoritarianism	26.48 (3.33)	27.74 (3.66)	-2.847	0.005
Benevolence	21.53 (4.06)	22.50 (3.91)	-1.887	0.060
Social restrictiveness	19.86 (4.21)	21.81 (4.32)	-3.594	0.000
Community mental health ideology	23.37 (5.27)	24.60 (5.14)	-1.811	0.071

someone with a mental disorder, the authoritarianism and social restrictiveness subscales presented significant differences during the intervention in both groups ($P <$ 0.001 and $P = 0.017$, respectively). In the benevolence and community mental health ideology subscales there were no significant differences controlling for these variables. Figure 1 show the results of the linear regression, comparing the scores between the control group and the intervention group, controlling for gender and whether or not they knew someone with a mental disorder. Authoritarianism showed significant improvement in the adolescents in the intervention group compared to those in the control group. In terms of the social restrictiveness scale, changes were only observed in the control group, with some significantly higher scores and therefore more negative attitudes towards social stigma in mental disorder. Regarding the benevolence and community mental health ideology scales, no significant changes were produced.

DISCUSSION

The results show that the intervention was effective, with significant changes produced in the intervention group for the most stigmatized factors such as authoritarianism and social restrictiveness. Moreover, in women and people who knew someone with a mental illness the intervention was more effective.

This type of intervention carried out with adolescents was effective, coinciding with other studies[15-18,23,32]. In this line, Penn *et al*[25] found that a film could reduce beliefs about guilt and responsibility; however it is not useful for reducing behaviour intentions. In addition suggest educational strategies probably do not affect all aspects of psychiatric stigma.

The results of our study indicate that using documentary film is useful for reducing social stigma. The use of documentary film provide to the adolescent information and experiences from different professionals and users. Few studies have used this intervention method in adolescents. Our data are consistent with other studies[24,33]. In the study by Pinfold *et al*[33] an intervention was carried out in adolescents of the same age as in the present study, although the authors used other actions (workshops and didactic experiences), and it is not clear whether the reduction corresponded to the

workshops or to the filmed material, or rather occurred at a general level.

Girls showed fewer stigmatizing patterns than boys at baseline. Boys show higher scores in authoritarianism and social restrictiveness subscale, and girls show higher scores in benevolence and community mental health ideology. Regarding the intervention, both the girls and the boys lowered their social stigma scores with respect to authoritarianism. In the case of the girls, there was also a significant reduction in the social restrictiveness subscale. Therefore, in general, girls have fewer stigmatizing attitudes than boys, both at baseline and post-intervention. These results are consistent with those previously found by the group in Martínez-Zambrano *et al*[26] and with those found by Morrison[32]. In the same way, the study by Savrun *et al*[34] showed that female university students were less inclined than men to hold prejudices against people with psychiatric disorders.

Furthermore, differences in the perception of stigma taking into account the variable of knowing someone with a mental disorder were also found. Our results show that when the students knew someone with a mental illness they scored significantly lower in authoritarianism and in social restrictiveness, and higher, although not significantly so, in benevolence, compared to those students that did not know anyone with a mental disorder. In this way, the fact of knowing someone with a mental illness engenders fewer stigmatizing attitudes in adolescents. Our results are consistent with the previous study of the group[26] and with others studies[28,32,35]. The intervention was effective in reducing authoritarianism whether or not the subjects personally knew someone who had a mental disorder.

The results show the attitudes or frames of mind that are less stigmatized in adolescents. Future interventions should take into account differences in terms of gender and whether or not the subjects know someone with a mental disorder. It would be a good idea to carry out an intervention in which, in addition to documentary film and contact with healthcare staff, there was also a certain amount of contact with mental healthcare users. In this way, for those people who do not know anyone with a mental illness, the contact produced would probably allow for a further reduction in the social

Table 4 Mean scores of the Community Attitudes towards Mental Illness subscales and comparison of the differences in the averages of the control and intervention groups by gender

		Baseline		Post-intervention		Difference between baseline and post-intervention		*T*-students	*P* value
		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)		
		Control	Intervention	Control	Intervention	Control	Intervention		
Boys	Authoritarianism	27.44 (3.12)	28.50 (3.77)	28.04 (4.31)	26.18 (4.16)	-0.74 (3.12)	2.27 (3.93)	4.771	0.000
	Benevolence	22.33 (4.40)	22.95 (4.54)	22.93 (4.95)	22.76 (4.51)	-0.34 (3.12)	0.19 (3.61)	0.901	0.369
	Social restrictiveness	21.45 (4.61)	20.77 (4.39)	23.06 (5.78)	21.65 (4.34)	-1.64 (4.06)	-0.63 (3.97)	1.432	0.154
	Community mental health ideology	25.04 (5.82)	23.98 (5.43)	25.99 (6.20)	23.89 (5.57)	-0.87 (3.81)	0.32 (5.40)	1.477	0.142
Girls	Authoritarianism	25.80 (3.14)	26.14 (3.41)	25.58 (3.23)	24.51 (3.92)	0.12 (2.47)	1.67 (4.14)	2.617	0.010
	Benevolence	21.19 (3.53)	21.02 (3.38)	21.40 (3.41)	21.51 (3.69)	-0.21 (2.63)	-0.45 (3.52)	-0.473	0.637
	Social restrictiveness	20.35 (4.37)	19.36 (3.73)	21.26 (4.36)	19.08 (3.99)	-0.88 (3.50)	0.30 (2.91)	2.106	0.037
	Community mental health ideology	23.82 (4.45)	21.97 (4.86)	23.65 (4.74)	21.17 (5.17)	0.13 (3.22)	0.49 (3.23)	0.649	0.518

Table 5 Mean scores of the Community Attitudes towards Mental Illness subscales and comparison of the differences in the averages of the control and intervention groups according to whether or not they knew someone with mental illness

		Baseline		Post-intervention		Difference between baseline and post-intervention		*T*-students	*P* value
		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)		
		Control	Intervention	Control	Intervention	Control	Intervention		
Knows someone	Authoritarianism	25.97 (3.27)	26.88 (3.39)	26.43 (4.06)	24.98 (4.14)	-0.47 (3.01)	1.905 (4.01)	4.511	0.000
	Benevolence	21.53 (4.31)	21.62 (3.92)	21.89 (4.3)	21.61 (4.28)	-0.36 (2.98)	0.01 (3.51)	0.571	0.569
	Social restrictiveness	20.19 (4.42)	19.57 (3.96)	21.81 (5.44)	19.81 (4.52)	-1.62 (3.92)	-0.24 (3.57)	2.388	0.018
	Community mental health ideology	24.24 (5.48)	22.49 (4.9)	24.5 (5.80)	22.03 (5.57)	-0.26 (3.35)	0.46 (4.37)	1.039	0.300
Does not know anyone	Authoritarianism	27.35 (3.13)	28.52 (4.38)	27.3 (3.80)	26.61 (4.07)	0.05 (2.68)	1.91 (4.24)	2.679	0.010
	Benevolence	22.2 (3.61)	23.06 (4.36)	22.48 (4.24)	23.67 (3.28)	-0.28 (2.99)	-0.61 (3.63)	-0.437	0.663
	Social restrictiveness	21.93 (4.47)	21.52 (4.03)	22.83 (4.75)	21.82 (3.26)	-0.9 (3.7)	-0.30 (3.39)	0.783	0.435
	Community mental health ideology	24.7 (4.6)	24.27 (5.82)	25.32 (5.16)	24 (5.29)	-0.62 (3.72)	0.27 (4.81)	1.114	0.268

stigma associated with a mental disorder.

The study has some limitations. Firstly, the intervention shows significant changes in the reduction of social stigma in adolescents. However, although differences have been found in the questionnaire it is difficult to assess if the adolescent have changed their attitude regarding mental health in real life. Moreover, for a future study it would be helpful to carry out another evaluation after a longer period of time, around 6 mo, in order to learn whether the change produced by the intervention is stable. Furthermore, the randomisation was done by the schools in order to control the possibility of the students from the class that did not receive the intervention scoring better after conversation with those who had undergone the intervention. However, it would be better to have a sample from the intervention group and a sample from the control group selected from each school in order to control for the possible effect of the educational methodology of the school and its geographical location, among other factors. Another limitation is the doubt as to the nature of the contact the students have with an individual with mental illness, given the opportunity to respond merely yes or no. Perhaps the answer to this question should be to clarify the relationship with this contact. Moreover better assessment between attitude

regarding mental health and behavior addressed to people with a mental illness should be included in next studies. Also, in any future study, the documentary film and the contact with professionals could be combined with some kind of contact with people with a mental disorder, in form of discussions, visits, workshops, or games, among other possibilities, in order to increase the effect of the intervention[24,36-38]. Although Schomerus *et al*[39] found that providing information on a mental health-mental illness could modify attitudes towards people who suffer of a mental disorder. Therefore, by combining two different types of effective interventions, the results of the stigma reduction would be enhanced, although the cost-effectiveness of this would have to be assessed. The concept of "mental disorder" used in the CAMI questionnaire reflects a period of time in which people with serious mental disorder were considered abnormal and were excluded and isolated. Maybe it is not the most appropriate instrument, but it is the only one that is validated in Spanish. Furthermore, mental disorder is a very broad and imprecise concept, due to a great range of psychiatric disorders included in it, such as depression, anxiety, alcoholism, schizophrenia… This concept has in itself now changed, and a mental disorder is not viewed as unnatural or abnormal and the former isolation no
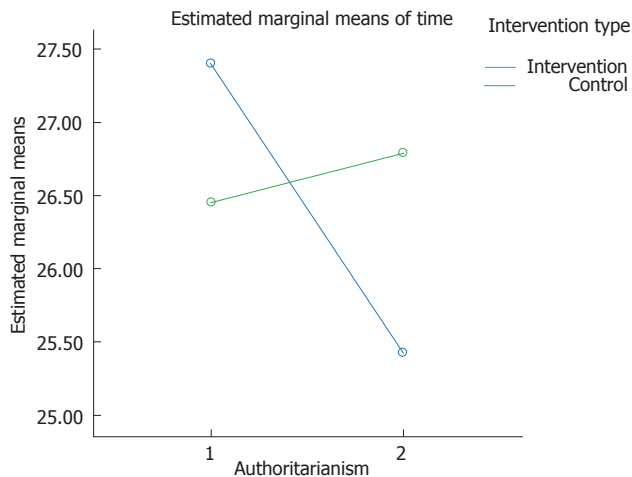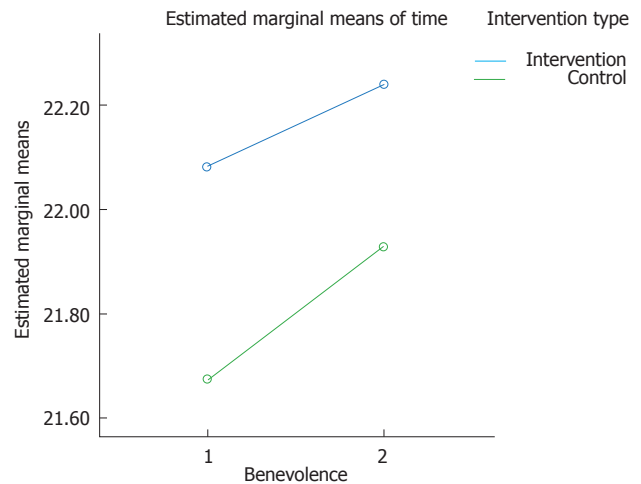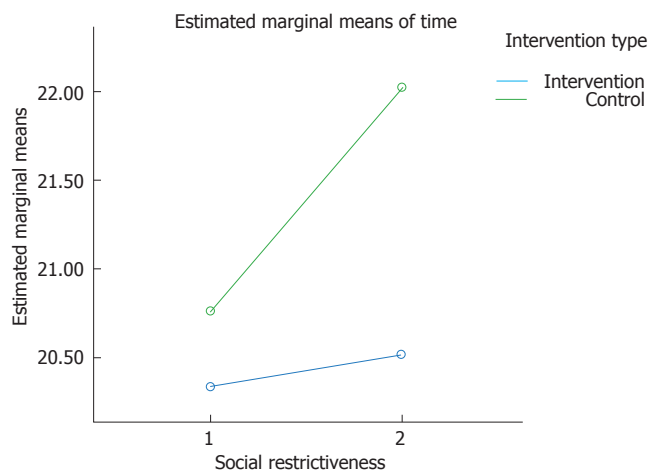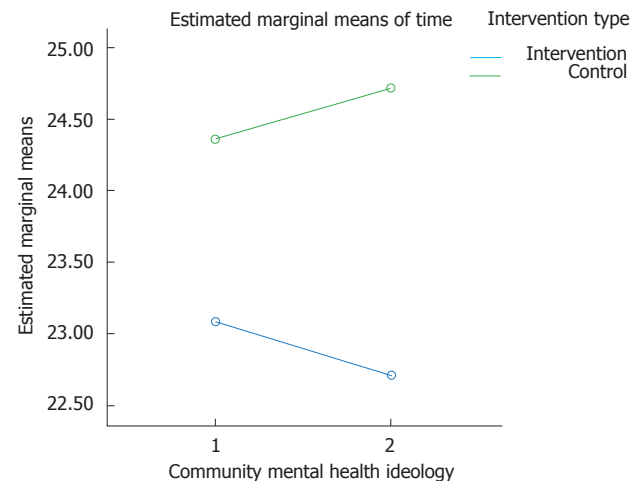
Figure 1 Differences between each subscale of the Community Attitudes towards Mental Illness at two time points by group controlling for gender and knowledge of someone with a mental disorder.

longer exists.

To conclude, the study has demonstrated that interventions with adolescents carried out with documentary film and with healthcare professionals are effective in reducing social stigma, especially in authoritarianism and social restrictiveness. Furthermore, interventions carried out in a school environment should take both gender and the fact of knowing, or not, someone with a mental disorder into account, since these are important differential variables.

COMMENTS

Background

The study shows the effectiveness of an intervention to reduce social stigma towards mental illness in the adolescents. This intervention consists in a documentary film about mental illness and a talk with healthcare professionals.

Research frontiers

It is important to try to change the attitudes of our society towards people with mental illness, in order to increase their autoestigma and consequently increase their self-esteem and quality of life as well as their families.

Innovations and breakthroughs

There have been few studies addressed in reducing stigma towards mental illness, much less using visual material. Is not clear the role of gender and the fact of know someone with a mental illness or not in reducing the stigma.

Applications

This study provides information on the effectiveness of interventions in schools, made with visual and informative material, to reduce stigma towards mental illness. It is important to take into account gender differences and the fact of know someone with a mental illness or not. Adolescents are a good collective to carry out talks to raise awareness of mental illness.

Terminology

Stigma is a social construct that includes negative attitudes, feelings, beliefs, and behaviours that are configured as prejudice.

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ORIGINAL ARTICLE

Observational Study

Path analysis of relationship among personality, perceived stress, coping, social support, and psychological outcomes

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Abstract

AIM: To provide a structural model of the relationship between personality traits, perceived stress, coping strategies, social support, and psychological outcomes in the general population.

METHODS: This is a cross sectional study in which the study group was selected using multistage cluster and convenience sampling among a population of 4 million. For data collection, a total of 4763 individuals were asked to complete a questionnaire on demographics, personality traits, life events, coping with stress, social support, and psychological outcomes such as anxiety and depression. To evaluate the comprehensive relation-

ship between the variables, a path model was fitted.

RESULTS: The standard electronic modules showed that personality traits and perceived stress are important determinants of psychological outcomes. Social support and coping strategies were demonstrated to reduce the increasing cumulative positive effects of neuroticism and perceived stress on the psychological outcomes and enhance the protective effect of extraversion through decreasing the positive effect of perceived stress on the psychological outcomes.

CONCLUSION: Personal resources play an important role in reduction and prevention of anxiety and depression. In order to improve the psychological health, it is necessary to train and reinforce the adaptive coping strategies and social support, and thus, to moderate negative personality traits.

Key words: Structural equations model; Personality traits; Stressful life events; Social support; Coping strategies; Depression and anxiety

Core tip: Personality traits, stressful life events and personal resources (coping strategies and social support) are among the factors that can influence psychological outcomes. Personality traits have an important role as the basis for coping skills and social support. Stressful life events and personal resources can modulate psychological outcomes. There is a vital role for holistic medicine which addresses the whole aspects of personality, perceived stress, and personal resource for mental health. The presence of a model that includes all the factors influencing the mental health allows planning for improvements with reality-based interventions.

INTRODUCTION

In order to appreciate the depression and the anxiety status as the stress-related negative outcomes, we need to assess the interaction between coping strategies and social support acceptance with perceived stress in the context of personality.

Stress as an inevitable life experience, develops when an individual fails to cope with the external physiological and cognitive distress in daily life[1,2]. Perceived stress is defined as an individual understands the amount of stress he or she is exposed to in a period of time. It incorporates the feeling of uncertainty and instability in life, and depends upon the confidence in one's ability in dealing with difficulties[3]. Personality is a significant factor in stressful events and is considered the basis for not having the required resources to cope with an unexpected situation[4,5]. It can influence the perception of stress upon the exposure to the stressful event or in reaction to it[6]. As a result, maladaptive personality traits are related to greater distress, while more positive and sociable personalities experience more favorable psychological well-being[7]. Studies have suggested an interaction between personality traits that are independently related to depression and anxiety[8]. Furthermore, different personal and social factors can also influence the reaction to the stressful situations as well as the level of stress[9]. Two types of personal resources that affect adaptation and psychological well-being include coping strategies and social support as the internal and external resources, respectively[10]. The perceived stress has a considerable impact on the coping process which in turn plays an important role in adaptation to stressful life events[11,12]. Coping is an ongoing process that changes in response to variations of the situation[13]. Coping strategies can be categorized into the active and avoidant[14]. Active coping manages the problem cognitively by taking action to mitigate the enfeebling effects of stress[14], while the avoidant coping regulates the negative emotional state activated by the stressors[15]. Coping mechanism can take on various roles in the stressor-symptom relationship, the context that varies by the type of coping[16]. Moreover, personality traits can affect coping in the daily life[17]. Active coping is a protective factor in the stressor-symptom model[16]. On the contrary the avoidance coping is considered a maladaptive response to stressful life events[18]. There is a relationship between psychological distress and different coping strategies[19]. While the problem-focused coping is negatively related to anxiety, stress and depressive symptoms, the avoidant coping is shown to be positively associated with these symptoms[19]. Depression, as the outcome of a defective stress management, may be related to certain coping strategies[20]. Particular types of coping strategies are linked to positive psychological outcomes[21]. For instance, cognitive reinterpretation and social support are associated with lower perceived strain[22]. In general, active coping results in a more effective adjustment to chronically stressful events than the avoidant[23]. Social support is another factor that can moderate the effect of stress[24]. The buffering effect of social support is either by prevention of potential stressful situations to be perceived as the stressor, or by reducing the intensity of the reaction to these events[24]. Social support is related to productive psychological responses, and its absence can be a cause of stress[25,26]. The lack of social

support is associated with psychological problems such as depression and anxiety[27]. On the other hand, the presence of resources such as family and friends is associated with a reduction in psychological distress[24]. Little is known about the structural equations through which the stress influences the psychological health. In this study we intended to examine the interaction between personality, perceived stress, coping and social support in stressful situations and to determine their effect on negative psychological outcomes such as anxiety or depression.

MATERIALS AND METHODS

Study population and data collection
This cross-sectional study is a part of the "Study of the Epidemiology of Psychological, Alimentary Health and Nutrition" (SEPAHAN)[28]. Multistage cluster and convenience sampling was used to select the group of interest among 4 million people residing in Isfahan province. SEPAHAN study was designed in such a way to enhance the accuracy and response rates by executing the data collection in two separate phases. During the first phase, participants completed a self-administered questionnaire on demographics and lifestyle such as nutritional habits and dietary regimens. In the second phase, different questionnaires provided information on various aspects of psychological variables (response rate: 86.16%). In total, 4763 individuals participated in our study and data were collected on demographics, personality traits, life events, coping with stress, social support, and psychological outcomes such as anxiety and depression. Written informed consent was obtained after clarifying the study protocol and study process. The study was approved by the institutional review board and ethics committee of the Isfahan University of Medical Sciences.

Measures
Demographic factors included sex, age, marital status of married or unmarried (single, divorced, widowed) and educational level of graduate and undergraduate.

Big Five Personality Inventory Short Form (NEO FFI): This 60-item scale comprises five personality traits of extraversion, neuroticism, agreeableness, openness, and conscientiousness with 12 items for each. These items are scored from 1 to 5 (strongly disagree to totally agree) with higher scores highlighting a particular personality trait[29]. The reliability of the entire scale (α = 0.70) and subscales (α_s > 0.68) has been confirmed[30].

Stressful life events questionnaire: This questionnaire measures the frequency and significance of perceived stress in daily life. It consists of 46 items with 11 domains including home life, financial problems, social relation, personal conflict, job-related stress, educational concerns, job security, loss and separation, sexual life, daily life and health concerns. This scale is rated base on the presence of an stressful life

event over the last year from 0 (never) to 5 (very severe)[31,32].

Hospital Anxiety and Depression Scale: The questionnaire consists of 14 items under two scales of depression (α = 0.84) and anxiety (α = 0.82). Each scale has 7 items with a score of 0 to 21. The clinical definition of anxiety or depression is set at a score $\geqslant$ 11[33].

Coping Strategies Scale: A multi-component questionnaire to assess the coping with stressful life events. It includes 23 items categorized into five subscales of positive re-interpretation and growth, problem engagement, acceptance, seeking support, and avoidance. Scores are reported separately for each scale with a 3 point Likert type score of 0 (never), 1 (sometimes) or 2 (often)[34]. Its reliability is determined using Cronbach's alpha coefficient (α = 0.84).

Multidimensional Scale of Perceived Social Support (MSPSS): The questionnaire consists of 12 items with 5-point Likert-scale which evaluate 3 sources of social support including family, friends, and significant other. Adequate psychometric properties have been found with the MSPSS[35].

Statistical analysis
All data analyses were performed with SPSS version 15.0 (SPSS Inc., Chicago, Illinois, United States). A *P* value < 0.05 was considered statistically significant. Continuous variables are shown as mean ± SD and Pearson correlation coefficient was used to test the relationship between personality traits, perceived stress, personal resources (social support and coping strategies), and psychological outcomes (anxiety and depression). To examine the simultaneous comprehensive relationship between studied variables, a path model was fitted. Path analysis as a generalization of the regression model estimates direct, indirect, and total effects of each variable on dependent variable to describe the observed correlation among them[36]. In path analysis some variables are exogenous or endogenous depending on hypothesized pathways. Two separate path models were fitted to evaluate the relationship between personality traits as exogenous variables and perceived stress, and personal resources (social supports and coping strategies) as mediators and psychological outcomes (*i.e.*, anxiety and depression) as endogenous variables. In both fitted models, one of the mediators was considered as a latent variable (coping strategies) and was extracted based on three observed indicators including problem focus coping, emotional focus copping and avoidance. Even though some consider values of 4 and even 5 to indicate a good fit, the χ^2 to degree of freedom index (χ^2/df) less than 3 is preferred in relation to fitness indices of models in path analysis[37]. Other indices for fitting the model include Normed Fit Index (NFI), Comparative Fit Index (CFI), and Goodness Fit Index (GFI), with preferred values over 0.9. In the Root Mean Square Error of

Table 1 Mean, standard deviation and range of study variables (*n* = 4763)			
Variable		**Mean (SD)**	**Range**
Personality traits	Neuroticism	18.72 (7.87)	0-45
	Extraversion	29.03 (7.08)	0-48
	Openness	24.04 (5.28)	0-41
	Agreeableness	31.00 (6.37)	0-48
	Conscientiousness	36.20 (7.22)	0-48
Social support		7.63 (3.64)	0-12
Perceived stress		28.57 (19.86)	0-132
Coping strategies	Problem focused coping	9.65 (2.12)	0-12
	Emotional focused coping	6.44 (1.49)	0-8
	Avoidance	3.41 (1.76)	0-8
Psychological	Anxiety	3.55 (3.72)	0-21
outcomes	Depression	6.14 (3.37)	0-21

Approximation (RMSEA) criteria, values up to 0.08 are acceptable, and values equal to or less than 0.05 indicate a good fit.

RESULTS

A total of 4763 respondents with an age of 36.58 ± 8.09 (mean ± SD) years were included in the study; 2106 (44.2%) were male; 2650 (57.2%) were university graduates; and 3776 (81.2%) were married. The study variables are presented in Table 1.

The correlations between personality traits, perceived stress, coping strategies, social support and psychological outcomes are demonstrated in Table 2. Among personality traits, extraversion had the most negative correlation ($P < 0.001$), whereas neuroticism had the most positive correlation ($P < 0.001$) with psychological outcomes. The perceived stress had a positive correlation with psychological outcomes as well as with neuroticism among personality traits ($P < 0.001$). Low levels of social support were related to higher levels of anxiety and depression ($P < 0.001$). Among personality traits, only neuroticism showed a negative correlation with social support ($P < 0.001$). Furthermore, neuroticism had a negative correlation with problem-focused and emotional-focused coping ($P < 0.001$). Meanwhile, a positive correlation was observed between neuroticism and avoidance. Extraversion had the most positive correlation with emotional focused coping, with a non-significant correlation with avoidance.

The pathways of personality traits, perceived stress, coping strategies, and social support were analyzed to evaluate their direct, indirect, and total effects on psychological outcomes of anxiety or depression (Figures 1, 2 and Table 3). As is seen from path coefficients, neuroticism had the most direct (0.38), indirect (0.15) and total positive effects (0.52) and extraversion showed the most direct (-0.21), indirect (0.14) and total negative effects (-0.28) on depression. Similar results on the direct, indirect and total effects of neuroticism regarding anxiety were observed, however, extraversion (-0.19) and agreeableness (-0.04) had direct negative

effects on anxiety. Moreover, the perceived stress had direct positive effects on depression (0.28) and anxiety (0.33). Personal resources including social support (-0.09) and coping strategies (-0.08) had direct negative effects on depression. Social support and coping also showed a direct negative effect on anxiety (-0.05). The mediating effects of perceived stress, social support and coping strategies were also examined. Personality traits were shown to be influential on perceived stress and personal resources. Out of analyzed personality traits, neuroticism (0.35) and openness (0.09) had positive effects on perceived stress, agreeableness, on the other hand, had a negative effect (-0.14). Among personal resources, agreeableness (-0.15) and neuroticism (-0.29) had negative effects on coping strategies. Other traits of extraversion (0.28) and conscientiousness (0.23) had positive effects. Neuroticism was the sole factor with a negative effect on social support (-0.19). Extraversion was found to have a positive effect on social support (0.33). Analyses of the total effects showed a significant indirect effect of neuroticism, extraversion, conscientiousness, agreeableness, and openness on depression and anxiety. Perceived stress positively mediated the positive effects of neuroticism and negative effects of extraversion on psychological outcomes. Whereas copping strategies and social support, in part, negatively mediated positive effects of neuroticism and negative effects of extraversion. The perceived stress positively mediated the negative effects of other personality traits on psychological outcomes, while coping strategies and social support enhanced the negative effects. On one hand, social support and coping strategies reduced the increasing cumulative positive effects of neuroticism and perceived stress on psychological outcomes, on the other hand, they strengthened the protective effect of extraversion through decreasing the positive effect of perceived stress on psychological outcomes. Table 4 shows the model fit indices for the final models. The model chi square divided by degree of freedom was less than 3 for both fitted models. The fit indices of GFI, NFI, TLI, and CFI in the models were all above 0.9, and RMSEAs were in an acceptable range.

DISCUSSION

In this study, we examined the relationship between personality traits, perceived stress, coping strategies, and social support with psychological outcomes such as depression and anxiety. Our results showed that among personality traits, neuroticism and extraversion exert the strongest direct and indirect effects on psychological outcomes. In other words, neuroticism had the most positive effect by increasing the depression and anxiety, and extraversion had the most negative effect by decreasing these psychological outcomes. The results are consistent with findings of similar studies[38,39]. It is discussed that neuroticism is characterized by disordered
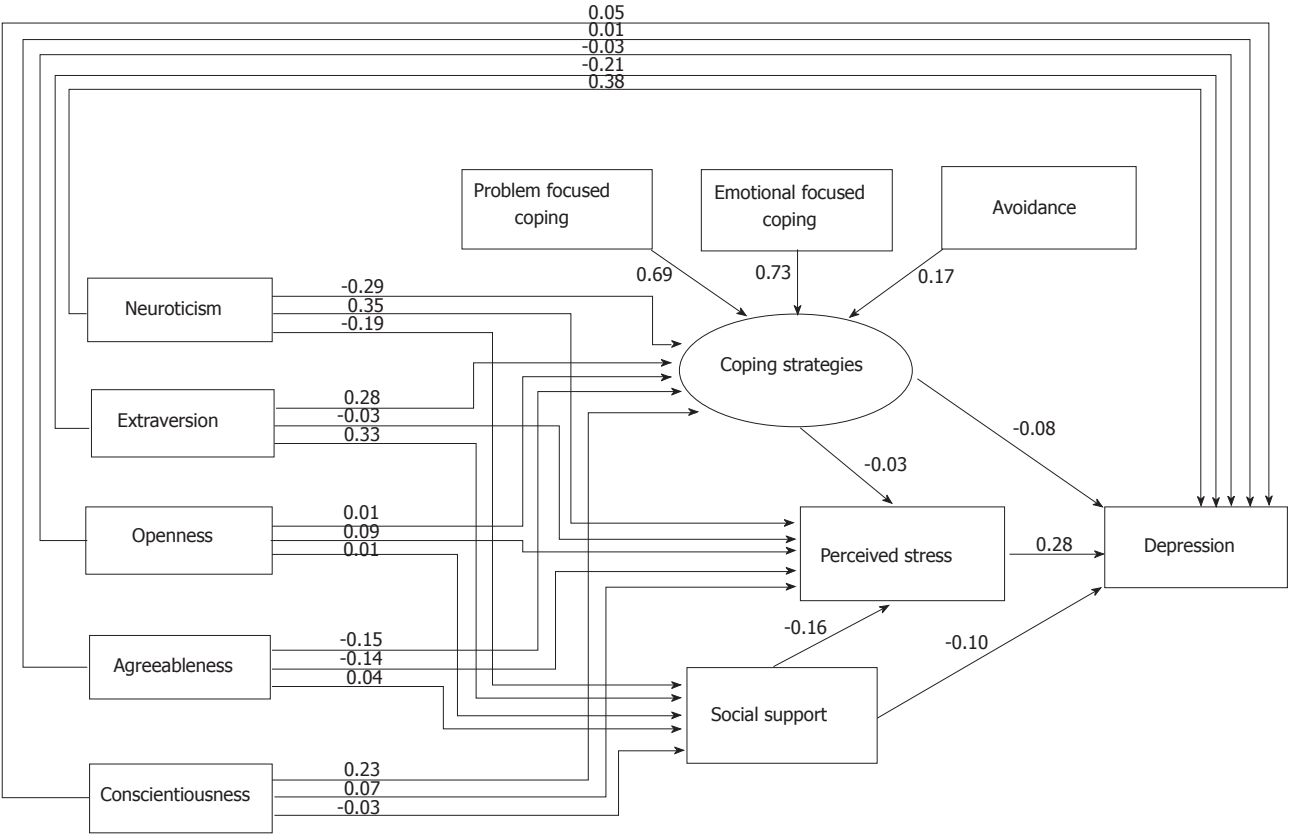
Figure 1 Path coefficients showing direct and indirect effects of personality traits, stressful events, social support and coping strategies on depression.
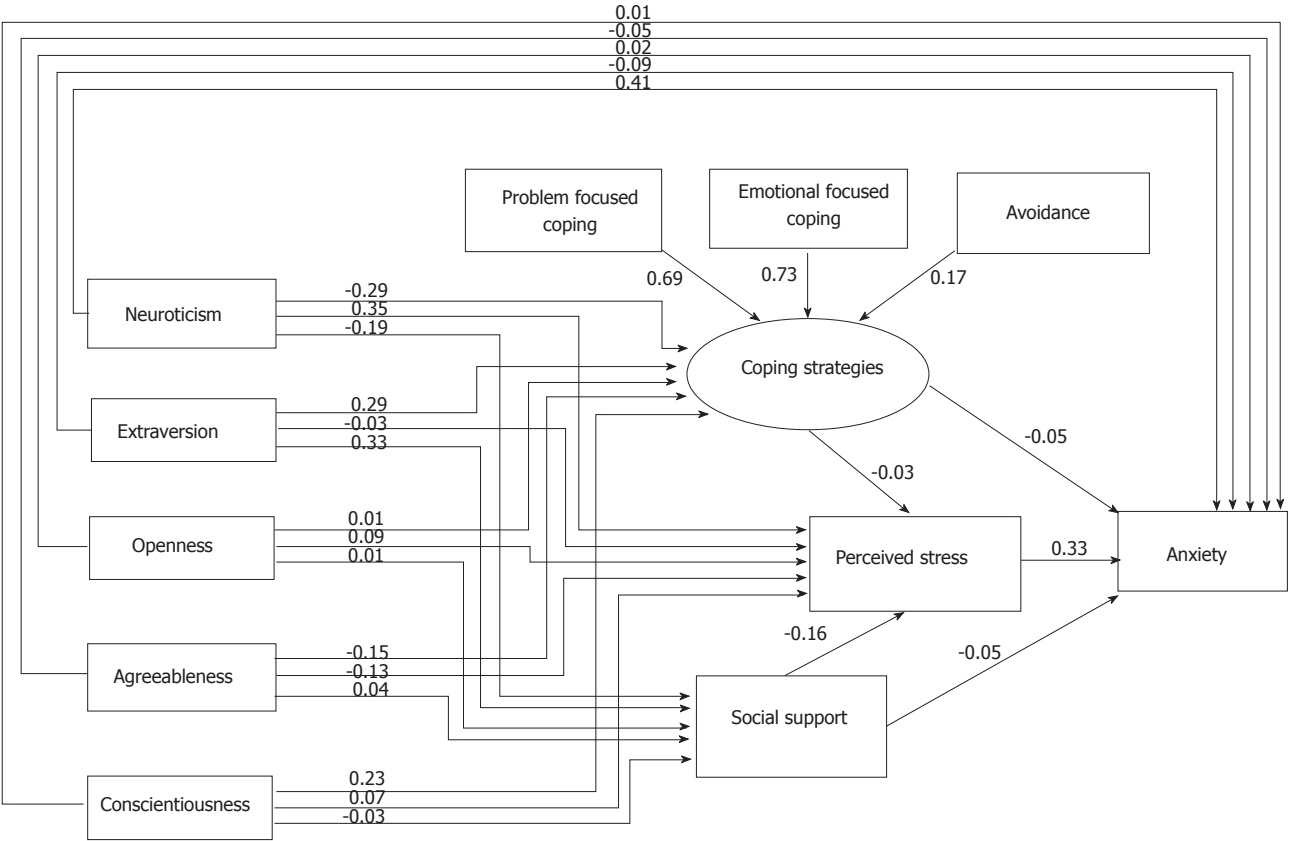

Figure 2 Path coefficients showing direct and indirect effects of personality traits, stressful events, social support and coping strategies on anxiety.

Table 2 Correlation coefficients between research variables

Variable		N	E	O	A	C	Anxiety	Depression
Social support		-0.34[b]	0.41[b]	0.13[a]	0.24[b]	0.24[b]	-0.33[b]	-0.39[b]
Perceived stress		0.42[b]	-0.23[a]	0.01	-0.21[a]	-0.12[a]	-0.55[b]	0.51[b]
Coping	Problem focused coping	-0.30[b]	0.30[b]	0.13[a]	0.14[a]	0.31[b]	-0.23[b]	-0.27[b]
strategies	Emotional focused coping	-0.33[b]	0.34[b]	0.08	0.17[a]	0.25[b]	-0.26[b]	-0.31[b]
	Avoidance	0.12[a]	-0.01	-0.03	-0.16[a]	-0.11[a]	0.07	0.09
Psychological	Anxiety	0.62[b]	-0.40[b]	-0.07	-0.29[b]	-0.26[b]	-	0.76[b]
outcomes	Depression	0.63[b]	-0.50[b]	-0.14[a]	-0.30[b]	-0.29[b]	0.76[b]	-

[a]$P \leqslant 0.05$; [b]$P \leqslant 0.01$. N: Neuroticism; E: Extraversion; O: Openness; A: Agreeableness; C: Conscientiousness.

Table 3 Regression coefficients for structural equations model

	Depression			Anxiety		
	Direct	Indirect	Total	Direct	Indirect	Total
Neuroticism	0.378[a]	0.145[a]	0.523[a]	0.406[a]	0.155[a]	0.561[a]
Extraversion	-0.208[a]	-0.145[a]	-0.283[a]	-0.085[a]	-0.090[a]	-0.175[a]
Openness	-0.037[a]	0.026	-0.011	0.015	0.025[a]	0.040[a]
Agreeableness	0.014	-0.033	-0.019	-0.043[a]	-0.037[a]	-0.080[a]
Conscientiousness	0.045[a]	0.006	0.051[a]	0.010	0.006	0.016
Social support	-0.098[a]	-0.044	-0.142[a]	-0.047[a]	-0.021	-0.068[a]
Perceived stress	0.279[a]	0.000	0.279[a]	0.330[a]	0.010	0.340[a]
Coping strategies	-0.080[a]	0.006	-0.074[a]	-0.053[a]	0.005	-0.048[a]

[a]$P \leqslant 0.05$.

Table 4 Model fit indices for the final modified models

	Goodness of fit indices 8k				
	χ^2/d.f	NFI	CFI	GFI	RMSEA (Lower-upper)
Depression	1.9	0.96	0.96	0.97	0.091 (0.085-0.097)
Anxiety	2.1	0.95	0.96	0.96	0.089 (0.084-0.095)

NFI: Normed fit index; CFI: Comparative fit index; GFI: Goodness of fit index; RMSEA: Root mean square error of approximation.

emotional regulation, motivation, and interpersonal skills leading to a negative mood experience[40]. Consequently, those with neuroticism may present with psychological desperateness and failing process of thought[41]. On the contrary, extraversion is usually positively associated with more interpersonal interactions, capacity for having a joyful and active appreciation of stressful situations[42]. Therefore individuals scoring high on this trait are more sociable, person-oriented, fun-loving and affectionate. In addition, we showed that perceived stress had strong negative effects on psychological outcomes. It is estimated that approximately 70% of initial depressive episodes are preceded by a stressful life event which plays a causal role in about 20%-50% of cases[43,44]. The relationship between various forms of stressful events such as work stressors and housing problems and various psychological outcomes has been shown in several studies[16,24]. It could be concluded that the inability to properly manage the emotional responses upon exposure to stressful situations can lead to longer and more severe periods of emotional difficulties.

With regards to personal resources, our results showed that social support and coping strategies generally decrease psychological outcomes. Problem-focused coping strategies and some types of emotional-focused coping strategies are associated with better health outcomes[45,46]. Problem-focused coping helps to manage the stress causing problem[47], and emotional-focused coping diminishes the negative emotions associated with stressor. However, avoidance coping as a type of passive coping is highly related to psychological outcomes due to minimizing, denying or ignoring to deal with a stressful situation[16,48].

Meanwhile, social support resources such as family and friends are associated with diminishing psychological distress[24]. Social support prevents a situation to be perceived as distress and also promotes healthy behavior at the time of stress[49]. It can also exert its influence by positive thinking and cognitive restructuring[50]. In general, it can be concluded that factors such as personality traits, stressful life events and personal resources have various effects on psychological outcomes. Personal resources can negatively moderate the effects of neuroticism and

stressors on psychological outcomes. Contrary to our expectations, the role of personal resources in reducing psychological outcomes was demonstrated to be weak. This finding might be due to the high level of daily stressful events in our community in which personal resources seem to be insufficient or ineffective. Previous studies have shown that coping strategies appear to be functioning differently based on the nature of stressor, the social context of stressful event, and individual's personality[51]. In a highly frequent stressful context, the individual's ability to respond to future stressors can also be impaired[24]. Having said that, the relation between the type and severity of stressor with particular coping strategies appear to be the most important predictor of psychological outcomes.

Severe emotional reactions to stressors can exacerbate maladaptive and neuroticistic behaviors. Moreover, individuals with neuroticism negatively evaluate and interpret events and ambiguous stimuli as threatening and tend to remember these unpleasant events more than emotionally stable individuals[52]. Therefore, these individuals mostly get involved in maladaptive coping strategies like avoidance[53]. Personality characteristics influence the degree to which an individual seeks social support when confronted by an stressful event[54]. Neuroticism interferes with seeking of social support and has a negative effect on outcomes. Extraversion, on the contrary, acts as a protective factor in the stress and coping process[11]. Even though there is a growing trend in our community for learning of coping process and gathering information on social support system, lack of proper and sufficient training early in childhood makes individuals incapable of using these resources as a continual skill. All in all, although this study demonstrates personality traits and perceived stress as the most important determinants of psychological outcomes, the presence and accessibility of personal resources in reducing and prevention of anxiety and depression need to be highlighted. An improved social support system is a necessity to better psychological health and well-being. It is imperative to start training for personal resources and to reinforce appropriate behavioral reactions early in childhood through the educational system and family training sessions.

Large sample size and validated instruments are among the strengths of this study. The main limitations are self-report questionnaires and no control over biasing factors affecting the level of stress. In addition, due to the complexity of the model, the relationship of each coping strategy with other research variables was not evaluated.

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COMMENTS

Background

Personality traits and stressful life events lead to psychological distress. Coping strategies and social support can predict the occurrence of depression and anxiety, considering their role as internal and external stress-controlling resources, respectively. It is important to determine the interaction between coping strategies and social support with the stress in the context of personality.

Research frontiers

This is a path analysis to determine the interaction between coping strategies and acceptance of social support with perceived stress in the context of personality in the normal population.

Innovations and breakthroughs

This work deals with the interaction between these four factors in such a large scale that can be considered a basis for designing social studies involving these variables.

Applications

This model demonstrates the efficacy of multi-pronged interventions in promoting the psychological well-being.

Terminology

Perceived stress: The understanding of an individual of the amount of stress he or she is exposed to in a given point of time or specific period. Personality traits: Five major traits underling personality. Social support: The perception of being cared for, availability of assistance and being a part of supportive social network. Coping strategies: The internal effort that seeks to minimize the distress to solve personal and interpersonal conflicts.

Peer-review

The authors examine a hot and interesting topic.

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SYSTEMATIC REVIEWS

Facial emotion perception in schizophrenia: Does sex matter?

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Abstract

AIM: To review the literature on sex differences in facial emotion perception (FEP) across the schizophrenia spectrum.

METHODS: We conducted a systematic review of empirical articles that were included in five separate meta-analyses of FEP across the schizophrenia spectrum, including meta-analyses that predominantly examined adults with chronic schizophrenia, people with early (onset prior to age 18) or recent-onset (experiencing their first or second psychotic episode or illness duration less than 2 years) schizophrenia, and unaffected first-degree relatives of people with schizophrenia. We also examined articles written in English (from November 2011 through June 2015) that were not included in the aforementioned meta-analyses through a literature search in the PubMed database. All relevant articles were accessed in full text. We examined all studies to determine the sample sizes, diagnostic characteristics, demographic information, methodologies, results, and whether each individual study reported on sex differences. The results from the meta-analyses themselves as well as the individual studies are reported in tables and text.

RESULTS: We retrieved 134 articles included in five separate meta-analyses and the PubMed database that examined FEP across the schizophrenia spectrum. Of these articles, 38 examined sex differences in FEP. Thirty of these studies did not find sex differences in FEP in either chronically ill adults with schizophrenia, early-onset or recently diagnosed people with schizophrenia, or first-degree relatives of people with schizophrenia. Of the eight studies that found sex differences in FEP, three found that chronically ill women outperformed men, one study found that girls with early-onset schizophrenia outperformed boys, and two studies found that women (including first-degree relatives, adults with schizophrenia, and the healthy control group) outperformed men on FEP tasks. In total, six of the eight studies that examined sex differences in FEP found that women outperformed men across the

schizophrenia spectrum.

CONCLUSION: Evidence to date suggests few sex differences in FEP in schizophrenia; both men and women across the schizophrenia spectrum have deficits in FEP.

Key words: Clinical high risk; Emotion; Facial emotion perception; Gender; Recent-onset schizophrenia; Schizophrenia; Sex differences

Core tip: People with schizophrenia exhibit deficits in facial emotion perception (FEP) compared to healthy controls. These deficits are associated with poorer functioning and more severe symptoms. Although the literature to date suggests that there are few sex differences in FEP in schizophrenia, continued assessment of sex differences in FEP can help researchers and clinicians better understand other sex differences in the disorder and assist in treatment development aimed at improving functioning in people with schizophrenia. This review summarizes and critically evaluates the literature on FEP across the schizophrenia spectrum, focusing on the evidence related to sex differences in FEP.

INTRODUCTION

"The movements of expression in the face and body, whatever their origin may have been, are in themselves of much importance for our welfare" (Charles Darwin, p. 229, The Expression of the Emotions in Man and Animals[1]).

Facial emotion perception (FEP), or the ability to accurately identify the emotion on the face of another person, is an integral part of our everyday lives. FEP is a crucial component of our ability to function interpersonally in the world[2]. Whether it is identifying happiness from a smiling infant or disappointment from an employer during a performance review, our perception of the emotions of others influences our thoughts ("She looks so peaceful in my arms", "I hope I don't get fired") and behaviors (smiling back, promising to improve performance) towards them. Without this ability, many of the interpersonal skills required to function in daily life - engaging in socially appropriate behaviors, empathy, prosocial behavior, conflict resolution - would suffer.

Due to its importance related to interpersonal skills, FEP has been studied extensively in schizophrenia, a mental illness characterized by (among other features) poor social functioning, social cognition deficits, and emotion response deficits[3,4]. People with schizophrenia are less accurate at FEP compared to people without schizophrenia[5-9], and poorer accuracy on FEP tasks is positively correlated with poorer functioning, poorer social skills, and more severe symptoms, particularly negative symptoms, in schizophrenia[3,8,10]. To illustrate, imagine a person with schizophrenia who consistently perceives the smiling face of his landlord as anger (inaccurate FEP). This may lead to confusion, where the person may not understand why his landlord is angry, which may further lead the person with schizophrenia to perceive his landlord's expressions as unjustified or cruel. These thoughts may enhance this person's feelings of mistrust regarding those around him and lead him to socially isolate himself more from others (increased symptom severity). This person may begin to feel so confused and frustrated by his landlord's unjustified anger that he shouts at his landlord, which may ultimately lead to eviction and temporary homelessness (impairment of functioning). While this is just a fictitious example, it is clear that poor FEP can impact a person with schizophrenia's capacity to function in the world day-to-day.

The relationship between FEP and functioning in schizophrenia has led to the development of cognitive remediation interventions that target emotion perception abilities, including FEP (*e.g.*, teaching people with schizophrenia that a scowling face represents anger, a smiling face represents happiness, *etc.*). These interventions have been found to improve functioning (*e.g.*, social skills, community functioning) in people with schizophrenia[11]. Interestingly, a recent meta-analysis found that this relationship was moderated by sex: There was a stronger positive correlation between functional outcome and FEP in men compared to women with schizophrenia[10]. In other words, men with schizophrenia appeared to benefit more (show improved functioning) from interventions targeting FEP compared to women.

Sex differences in schizophrenia have been studied extensively in many domains of the illness[12-15]. For example, reviews and meta-analyses have shown that men have a 1.15-1.4:1 higher incidence of schizophrenia compared to women[16-19]. Men also have an earlier age of onset compared to women across varying definitions of onset (*e.g.*, first psychotic symptoms, first diagnosis, first hospitalization)[20,21]. Women on average have a better treatment response to antipsychotic medications compared to men, including a more rapid treatment response and lower required dose to achieve a response[12,22]. Further, women with schizophrenia tend to have a less severe course of the illness, including a lower frequency of hospitalization, less severe negative symptoms, better social skills, and better overall functioning[23,24]. However, no review to date has examined sex differences in FEP.

One possibility is that women with schizophrenia are better at FEP compared to men. Women with schizophrenia (when compared to men) exhibit clinical features (less severe illness course, less severe negative symptoms, better overall functioning, better social skills) that are typically associated with better FEP performance. Further, healthy women are more accurate at FEP than healthy men[25-27].

This review will examine the literature on FEP in schizophrenia and summarize relevant findings related to sex differences in chronically ill adults with schizophrenia, people with early or recent-onset schizophrenia, and people at clinical high risk for developing schizophrenia (specifically, unaffected first-degree relatives of people with schizophrenia). We will summarize relevant meta-analyses and studies included within the meta-analyses, focusing on results with regards to sex differences in FEP. We will also review the findings from the recent studies (2011-2015) that examined sex differences in FEP in schizophrenia but were not included in the summarized meta-analyses. Finally, we will offer a critical analysis of previous research on understanding sex differences in FEP and considerations for future research.

MATERIALS AND METHODS

Tasks that assess FEP can be divided broadly into two categories: Identification tasks and discrimination tasks. In identification tasks, participants view photographs or images of emotional faces that have been previously tested to categorically portray one emotion (*e.g.*, Ekman stimuli)[28] and are instructed to choose among a list of multiple emotion terms (forced-choice) the word that best describes the emotion portrayed on the face. Similarly, on identification tasks participants may be asked to rate on an anchored scale the valence (pleasant/unpleasant) the face is portraying or decide whether or not a face is portraying one emotion (*e.g.*, happiness) *vs* another (*e.g.*, sadness). Alternatively, on discrimination tasks participants are typically asked to view two emotional faces and state whether or not the stimuli match on emotion (label) or valence, or they may be asked to match a target face with the face among an array of facial stimuli that "matches" the target's emotion. Studies will be identified throughout the review as to whether they include an identification task or a discrimination task.

Within this review, we summarize recent meta-analyses of FEP in schizophrenia across the schizophrenia spectrum, including meta-analyses that predominantly examined adults with chronic schizophrenia (illness duration greater than 2 years)[5,7,9], people with early (onset before age 18) or recent-onset (experiencing their first or second psychotic episode or illness duration less than 2 years) schizophrenia[29], and unaffected first-degree relatives of people with schizophrenia[30]. While meta-analytic results related to sex differences in FEP

will be discussed, an examination of all studies included in the meta-analyses will be conducted to determine the quantity, samples sizes, methodologies, and results of studies that examined sex differences in FEP to better compare studies that examined sex differences with those that did not.

In order to examine recent studies on sex differences in FEP that were not included in the meta-analyses, we conducted a literature review in the PubMed database. We searched for studies in English from November 2011 (the cut-off for studies included in Savla *et al*[9]) through June 2015. Keywords included in the search were "schizophrenia" or "psychosis" in the article title or abstract, and "gender difference" or "sex difference" and "emotion recognition" or "emotion percept*" in the body of studies. The original search resulted in 319 studies. Exclusion criteria included studies that: (1) did not examine people on the schizophrenia spectrum or a group of first-degree relatives of people with schizophrenia; (2) did not include at least one FEP task; (3) did not include a relevant nonclinical comparison group; (4) examined unconscious processing of facial stimuli; or (5) used dynamic stimuli (*e.g.*, films). Following application of exclusion criteria, 22 relevant empirical articles were identified, including 20 studies with chronically ill adults with schizophrenia, one study with early-onset schizophrenia, and one study with people at clinical high risk for schizophrenia, including a group of unaffected first-degree family members (see Table 1 for summary of all studies that examined sex differences in FEP in schizophrenia).

RESULTS

Adults with chronic schizophrenia

Three recent meta-analyses have examined FEP in schizophrenia and have included sex as a potential moderating factor in their analyses[5,7,9]. Predominantly, these meta-analyses included studies with chronically ill adults with schizophrenia, although they also included a smaller number of studies with early or recent-onset samples of people with schizophrenia as well as first-degree relatives of people with schizophrenia.

Kohler *et al*[7] analyzed 53 studies on FEP in people with schizophrenia published from 1970-2007. They found that irrespective of task type (identification or discrimination), people with schizophrenia showed impairments in FEP compared to healthy controls, with poorer performance correlated with both positive and negative symptoms, later age of onset, and inpatient status. There were no significant differences in the effect sizes of FEP performance between task types (identification *vs* discrimination). The authors investigated the effects of sex on FEP by examining whether the percentage of men with schizophrenia or men without schizophrenia moderated the effect sizes of the study results. They found that the percentage of male controls, but not the percentage of men with

Table 1 Description of studies across meta-analyses that examine sex differences in facial emotion perception in schizophrenia

Ref.	Cited from	SZ group(s)	SZ group description			CT group description		CHR group description		FEP task	Stimuli emotion description	Sex difference results
			n	% women	In/out	*n*	% women	*n*	% women			
Addington *et al*[44]	Chan, Kohler	Chronic	40	33	IN	40	43			ID and DISC	Anger, disgust, fear, happy, neutral, sad, surprise	None
Alfimova *et al*[84]	Lavoie	CHR, chronic	103	59	IN	99	67	55	49	ID	Anger, contempt, disgust, fear, happy, interest/ excitement, neutral, sad, shame, surprise	Women > men
Amminger *et al*[73]	Barkl	CHR, first-episode	30	40	OUT	30	50	79	67	ID	Anger, disgust, fear, happy, neutral, sad, surprise	None
Bellack *et al*[45]	Chan	Chronic	35	49	IN	19	58			ID and DISC	Anger, disgust, fear, happy, sad, surprise	None
Bölte *et al*[76]	Kohler	CHR, early-onset	21	29	OUT	22	50			ID	Anger, disgust, fear, happy, neutral, sad, surprise	None
Borod *et al*[42]	Kohler, Savla	Chronic	20	5	OUT	21	48			DISC	Anger, disgust, fear, happy, neutral, sad, surprise	None
Castagna *et al*[69]	PubMed	Chronic	94	30	OUT	51	62			ID	Anger, disgust, fear, happy, neutral, sad, surprise	None
de Achával *et al*[31]	Savla	CHR, chronic	20	35	OUT	40	45	20	55	ID	Afraid, anger, disgust, distress, happy, sad, surprise	None
Donohoe *et al*[70]	PubMed	Chronic	487	28	OUT	163	60			ID	Not listed	None
Erol *et al*[80]	Lavoie	CHR, chronic	57	39	OUT	58	40	58	41	ID and DISC	Anger, fear, happy, sad, shame, surprise	None
Erol *et al*[72]	PubMed	Chronic	70	50	OUT	70	50			ID and DISC	Anger, fear, happy, sad, shame, surprise	Women with SZ = women and men CT
Gessler *et al*[50]	Chan	Chronic, recent-onset	60	38	Not described	20	50			ID	Happy, sad	Men > women
Habel *et al*[77]	Chan, Kohler	Early-onset, recent-onset	20	50	IN	20	50			ID	Happy, neutral, sad	Men > women
Kington *et al*[32]	Savla	Chronic	16	19	IN and OUT	16	19			ID	Afraid, anger, disgust, distress, happy, sad, surprise	None

Study		Sample								Task	Emotions	Sex
Kohler et al[51]	Chan, Kohler, Savla	Chronic	35	43	OUT	45	44			ID	Happy, neutral, sad	Women > men
Kohler et al[33]	Kohler	Chronic	28	32	OUT	61	52			ID	Anger, disgust, fear, happy, neutral, sad	None
[1]Kohler et al[86]	PubMed	CHR, chronic	91	33	OUT	90	49	52	48	ID and DISC	Anger, fear, happy, neutral, sad	None
Kucharska-Pietura et al[58]	Chan	Chronic	50	48	IN	50	52			ID	Interest-excitement, enjoyment-joy, surprise-startle, distress-anguish, disgust, contempt, anger-rage, shame-humiliation, fear-terror	None
[2]Kucharska-Pietura et al[35]	Chan, Kohler, Savla	Chronic, recent-onset	100	49	IN	50	52			ID	Interest-excitement, enjoyment-joy, surprise-startle, distress-anguish, disgust, contempt, anger-rage, shame-humiliation, fear-terror	Women > men
Kucharska-Pietura et al[34]	PubMed	Chronic	84	48	IN	50	50			ID	Anger, disgust, fear, happy, neutral, sad, surprise	None
Leitman et al[46]	Kohler, Savla	Chronic	43	23	IN and OUT	34	59			ID and DISC	Anger, fear, happy, sad, shame, surprise	None
Leppänen et al[85]	Lavoie	CHR, chronic	36	28	OUT	22	50	23	65	ID	Anger, happy, neutral	Women > men
[2]Leung et al[74]	Barkl	Chronic, recent-onset	101	46	OUT	54	46			ID	Anger, disgust, fear, happy, sad, surprise	None
McCown et al[81]	Lavoie	CHR				50	50	50	50	ID	Disgust, fear, happy, neutral, sad, surprise	None
Mendoza et al[82]	Lavoie	CHR, chronic	93	33	OUT	109	63	110	41	ID	Anger, disgust, fear, happy, neutral, sad, surprise	None
Mueser et al[47]	Chan, Kohler, Savla	Chronic	28	53	IN	15	67			ID and DISC	Anger, disgust, happy, sad, shame, surprise	None
Muzekari et al[36]	Kohler	Chronic	32	50	IN	32	50			ID	Anger, fear, happy, sad	None
Novic et al[37]	Chan, Kohler, Savla	Chronic	17	24	IN	17	59			DISC	Not listed	None

Penn et al[43]	Chan, Kohler	Chronic	74	30	IN	40	53			DISC	Anger, disgust, happy, sad, shame, surprise	None
Reske et al[75]	Barkl, Savla	Recent-onset	18	44	OUT	18	44			ID	Happy, neutral, sad	None
Rubin et al[38]	Savla	Chronic	48	46	IN and OUT	57	54			ID	Happy, neutral, sad	None
Sachs et al[48]	Kohler, Savla	Chronic	40	38	IN	43	44			ID and DISC	Happy, sad	None
Schneider et al[39]	Chan, Kohler	Chronic	40	48	IN and OUT	40	48			ID	Happy, sad	None
Schneider et al[40]	Chan, Kohler, Savla	Chronic	20	50	IN	20	50			ID	Anger, fear, happy, neutral, sad	None
Scholten et al[52]	Chan, Kohler, Savla	Chronic	53	47	IN and OUT	42	50			ID	Anger, disgust, fear, happy, sad, surprise	Women > men
Walker et al[41]	Kohler	Chronic, recent-onset	48	48	IN	48	48			ID	Afraid, ashamed, curious, disgust, joy, mad, sad, surprise	None
Weniger et al[49]	Chan, Kohler, Savla	Chronic	45	38	IN and OUT	30	50			ID and DISC	Anger, disgust, fear, joy, neutral, surprise	None
Wolf et al[83]	Lavoie	CHR				25	52	20	55	ID	Anger, fear, happy, neutral, sad	None

[1]Only first-degree relatives of people with schizophrenia included in "CHR group description"; [2]Chronic and recent-onset groups combined in "SZ group description". SZ: Schizophrenia; CT: Control; CHR: Clinical high risk; IN: Inpatient; OUT: Outpatient; ID: Identification task; DISC: Discrimination task.

schizophrenia, significantly moderated the effect sizes of FEP results. Specifically, the higher the percentage of male controls included in studies, the smaller the magnitude of group differences in FEP between controls and people with schizophrenia. The authors suggested that because healthy men perform worse on FEP than healthy women[27], having more women in the control group may have boosted the overall control group mean, thus amplifying group differences between people with and without schizophrenia. Furthermore, because the percentage of men with schizophrenia did not significantly moderate the effect sizes in FEP, the authors concluded that men and women with schizophrenia performed similarly on FEP tasks.

Chan et al[5] analyzed 28 FEP studies in schizophrenia published from 1980-2008. This meta-analysis differed from Kohler et al[7] in that their study inclusion criteria required that the study not only include an identification and/or discrimination FEP task, but also a "control" facial processing task (e.g., identifying the age or sex of the facial stimuli) in addition to an FEP task. In examining this smaller pool of studies, they found that people with schizophrenia showed impairments in both identification and discrimination FEP tasks compared to healthy controls. However, unlike Kohler et al[7], their results showed that poorer FEP performance was only correlated with negative, not positive, symptoms. Furthermore, through meta-regression analyses, the authors found that sex did not have a significant effect on FEP performance in schizophrenia. That is, men and women with schizophrenia performed similarly on FEP tasks in the studies included in their meta-analysis.

Savla et al[9] conducted a meta-analysis on 112 studies published from 1980-2011 to examine seven aspects of social cognition in schizophrenia. They included 62 studies that examined "emotion perception" broadly in schizophrenia, including studies that asked participants to identify the emotion portrayed on facial stimuli, through the vocal prosody of audio stimuli, and/or as portrayed by actors in film stimuli. Of these 62 studies, 54 included an FEP task. They found that people with schizophrenia were significantly worse at identifying emotions across different emotion perception tasks compared to healthy controls. They found that inpatient status (compared to outpatient status) significantly contributed to the heterogeneity of effect sizes across studies, suggesting that inpatients showed greater deficits in emotion perception. The percentage of male participants did not significantly account for variability in study effect sizes.

In sum, across 81 non-overlapping studies of FEP in three meta-analyses, sex was not a significant

moderator of effective sizes for the schizophrenia group. In other words, it does not appear as if men and women with schizophrenia differ in FEP, and that both men and women with schizophrenia perform worse than healthy controls. However, the meta-analyses may have been underpowered to detect sex differences as not every study within the meta-analyses included a substantial number of women with schizophrenia. Indeed, only 22 of the 81 studies included a sample of at least 39%-40% women. Further, these meta-analyses examined studies that included samples other than only chronically ill adults with schizophrenia. To more systematically examine sex differences in FEP in adults with chronic schizophrenia, we reviewed the specific studies from the meta-analyses.

Of the 81 non-overlapping studies from Kohler et al[7], Chan et al[5] and Savla et al[9], 59 studies either failed to report on sex differences in FEP or statistically controlled for sex in their analyses. Twenty-two studies examined sex differences in FEP in adult samples of chronically ill people with schizophrenia. The majority of these studies (n = 19) did not find sex differences in FEP, either on identification tasks[31-41], discrimination tasks[42,43], or in studies that included both task types[44-49].

Three studies included in Kohler et al[7], Chan et al[5], and Savla et al[9] found sex differences in FEP performance on identification tasks in adults with chronic schizophrenia. One study found that men outperformed women overall (i.e., in both the control and schizophrenia groups)[50]. Two studies found that women, regardless of diagnostic status, performed better than men[51,52]. Specifically, Kohler et al[51] found that sex was a moderator in participant performance on a task where participants were asked to rate on a seven-point scale (varying from very sad, to neutral, to very happy) the emotion of putatively happy, sad, and neutral faces: Women with and without schizophrenia made significantly fewer errors in identifying emotion compared to men. Scholten et al[52] found that overall, women performed better than men on an FEP identification task only for negative faces (including facial stimuli portraying fear, sadness, and disgust). To summarize, when comparing chronically ill people with schizophrenia to healthy controls, only two of the 22 studies that examined sex differences provided evidence that women with schizophrenia outperform men on FEP tasks.

From our PubMed search of more recent studies not included in the meta-analyses, a total of 16 of the 20 studies that examined adults with chronic schizophrenia either did not report on sex differences or controlled for sex in their FEP analyses[53-67], with one study including an all-male sample[68]. Of the remaining four studies, three studies did not find sex differences on an FEP identification task[69-71]. By contrast, Erol et al[72] found sex differences in FEP: Chronically ill women with schizophrenia performed equivalently to men and

women without schizophrenia on both identification and discrimination FEP tasks, while men with schizophrenia performed worse on both tasks. In sum, out of four recent studies, only one found that women outperformed men with schizophrenia in FEP[72].

In total, out of 101 studies that examined FEP in chronically ill adults with schizophrenia, 26 examined sex differences. Twenty-two out of 26 studies did not find sex differences in FEP performance. One study found that men outperformed women regardless of diagnostic status on an identification task[50] and three studies found that women outperformed men on an identification task[51,52,72] and discrimination task[72]. Taken together, the evidence suggests that men and women with chronic schizophrenia perform comparably on FEP.

Early and recent-onset schizophrenia

To ascertain whether there are sex differences in FEP early in the course of the illness, we examined studies in people with early or recent-onset schizophrenia. Barkl et al[30] conducted a meta-analysis of 12 FEP studies in people with early-onset schizophrenia (2 studies) or in people who recently had their first psychotic episode (10 studies) published from 1806-2013. Similar to studies with chronically ill adults with schizophrenia, they found that people during the early stages of the illness showed impairments in FEP compared to healthy controls. Of the 12 studies included in Barkl et al[30], only three examined sex differences in FEP. None of the three studies found sex differences in FEP on identification tasks in recent-onset samples[73-75].

Of the 81 non-overlapping studies that examined sex differences in FEP in schizophrenia included in Kohler et al[7], Chan et al[5], and Savla et al[9], four studies examined sex differences in FEP in samples of either recent-onset schizophrenia or children and adolescents diagnosed with schizophrenia-spectrum disorders (these studies were not included in Barkl et al[30]). Two studies did not find sex differences in FEP in children or adolescents diagnosed with schizophrenia[41,76]. One study found that men outperformed women regardless of diagnostic group in a sample of people ages 11-20 with and without schizophrenia-spectrum disorders[77]. Kucharska-Pietura et al[35] compared inpatients who were either: (1) experiencing their first or second psychotic episode; or (2) chronically ill with schizophrenia with a healthy control group and found that women, regardless of diagnostic status, were more accurate at identifying emotions in a variety of affective facial stimuli compared to men. When the authors examined whether sex differences existed within each diagnostic group separately (examining sex differences in the recent-onset group vs the chronically ill group), they found that sex differences only existed within the recent-onset group: Women who were experiencing their first or second psychotic episode outperformed men on FEP tasks[35]. We found one study that

examined FEP in an early onset group in our PubMed search, but the authors did not report sex differences[78].

In summary, out of seven studies of FEP in early or recent-onset schizophrenia that examined sex differences, five did not find sex differences[41,73-76]. One study found that boys outperformed girls[77] and one study found that recently diagnosed women outperformed men[35].

Unaffected first-degree relatives

In order to examine sex differences in a clinical high risk group, we examined studies in unaffected first-degree relatives of people with schizophrenia. Lavoie *et al*[29] conducted a meta-analysis examining 29 studies published from 1985-2011 on five aspects of social cognition in unaffected first-degree relatives. Within this meta-analysis, they examined 20 studies examining "emotion processing", including one study that used affective facial stimuli but was not an FEP task (thus, is not included in this review)[79] and 19 studies that included an FEP task. They found that unaffected, first-degree relatives of people with schizophrenia performed significantly worse on these tasks compared to control groups, although the effect sizes were smaller than effect sizes found in studies that compare people with schizophrenia to heathy controls. They also found a significant difference in task type: First-degree family members performed significantly worse on identification tasks *vs* "differentiation" tasks (tasks where participants state whether a face is emotional *vs* neutral or positive *vs* negative).

Of the 29 studies included in Lavoie *et al*[29], eight examined sex differences on an FEP task. Six studies did not find differences between men and women on an identification task[31,76,80-83] or on a discrimination task[80]. Two studies found that women, across diagnostic groups of first-degree relatives, outperformed men on FEP identification tasks[84,85].

Across the meta-analyses of Kohler *et al*[7], Chan *et al*[5], Savla *et al*[9], and Barkl *et al*[30], one study did not find sex differences on an identification FEP task in a clinical high risk group, a first-episode group, and a healthy control group[73]. We found one study through our PubMed literature search that examined FEP in two clinical high risk groups (one group with prodromal symptoms and another group of unaffected first-degree relatives); results indicated no sex differences in performance on either an identification or discrimination FEP task[86].

In sum, of 10 studies that examined sex differences in samples that included a group of unaffected first-degree relatives of people with schizophrenia, eight did not find sex differences on either identification or discrimination tasks[31,73,76,80-83,86]. Two studies found that women, including first-degree relatives, people with schizophrenia, and healthy controls, outperformed men on an identification FEP task[84,85].

DISCUSSION

Across five separate meta-analyses including people with chronic schizophrenia, early or recent-onset schizophrenia, and a clinical high risk group of unaffected first-degree relatives of people with schizophrenia, the evidence suggests that men and women across the schizophrenia spectrum show equivalent performance in FEP, and they both show poorer performance compared to healthy controls. Women with schizophrenia do not appear to retain the superiority in FEP that is found in their healthy counterparts. Thus, it does not appear likely that FEP ability contributes to women with schizophrenia having less severe negative symptoms, better social skills, and better functioning compared to men with schizophrenia. Women may exhibit strengths in other skills besides FEP that they rely on to navigate the social world. It would be important to understand why women with schizophrenia have better social functioning despite showing equivalent FEP ability compared to men, and whether there are other treatment targets outside of FEP that may better benefit both men and women with schizophrenia in improving functioning.

It also remains unclear why men with schizophrenia would benefit more from cognitive remediation treatments that target FEP compared to women[10] when they both show equivalent deficits in FEP. Again, women may have other social or emotional skills that may help them function in the world despite their deficits in FEP, thus improving this one skill may not benefit them as much as it benefits men. Future studies should continue to examine whether FEP is an effective treatment target in improving functioning in women as well as men with schizophrenia.

Despite the seemingly overwhelming evidence that there are no sex differences in FEP in schizophrenia, there are limitations to the existing body of literature worth noting. First, the studies that do examine sex differences vary widely in the percentage of female participants, ranging from 5% to over 50% of women in the schizophrenia group. Indeed, only 22 studies included 39%-40% or more women in both their schizophrenia and control samples. These percentages of female participants are unrepresentative of the incidence rate of schizophrenia among women in the general population, which is closer to 40%-50% of people with schizophrenia[17]. A recent review found that across studies of schizophrenia, men outnumber women almost 2:1, suggesting that the majority of knowledge we have about schizophrenia, including FEP, comes from studies that include more (or sometimes, only) men[87]. Further, the majority of treatment intervention studies that target FEP have included predominantly male samples[11]. While some researchers examine sex differences within or across their study groups, others either control for sex during their analyses prior to examining main effects or interactions of sex on their outcome variable or fail to examine sex differences at

all. In short, while there are not many studies that have adequately examined sex differences in FEP, those that have done so with reasonably balanced and sufficiently large sample sizes do not find sex differences in FEP.

A second limitation is that among the studies that examine sex differences, the majority do not examine differences in clinical features - such as symptom severity - that differ between men and women and that are also related to performance on FEP tasks. Men with schizophrenia tend to have more severe negative symptoms, are more likely to be inpatients, and have a younger age of onset compared to women. Negative symptoms and inpatient status are associated with poorer performance on FEP tasks. On the other hand, Kohler *et al*[7] found that older age of onset was associated with more impairment in FEP, a clinical feature associated more with women than men with schizophrenia[21]. It may be the case that the relationships between FEP and symptoms may differ between men and women; for example, negative symptoms might only be associated with poorer FEP ability in men. Examining sex differences in these clinical features in addition to FEP performance would illuminate whether FEP performance is partially related to clinical features alone, sex, or both.

Of the 38 studies included in this review that examined sex differences in FEP, only four reported on the relationship between sex and symptoms. Taken together, the evidence from these studies is mixed: When symptoms and/or other clinical features (*e.g.*, duration of illness, number of hospitalizations) do not differ between men and women with schizophrenia, two studies do not find sex differences in FEP performance[38,48] and two studies find that women outperform men[52,72]. The studies that found that women outperformed men examined positive and negative symptoms separately, while the studies that did not find sex differences either did not examine symptoms at all[48] or only examined total symptom scores as measured by the PANSS[38]. In other words, women outperformed men in FEP performance in two studies where they did not differ in either negative or positive symptoms. It remains unclear how or whether symptoms and other clinical features affect sex difference findings (or the lack thereof) in FEP studies in schizophrenia as the majority of studies do not examine sex differences in symptoms. Future studies should examine both facets - sex and clinical features - to better understand how these factors may or may not be interacting and influencing performance on FEP tasks.

While the majority of studies on FEP do not find sex differences in schizophrenia, there are similarities across studies that do find sex differences. First, the majority of these studies find that, regardless of diagnostic status, women outperform men[35,51,52,72,84,85]. Second, the majority of studies that find sex differences do so using FEP identification tasks[35,51,52,84,85]. Third, all but one study[85] have included at least 40% women with

schizophrenia in their samples. Fourth, the natures of samples are such that sex differences have been found in inpatients[35,84], outpatients[51,72,85], or both[52]. Fifth, of the few studies that report better FEP performance for women than men, this appears to be found across the schizophrenia spectrum, including in chronically ill adults[51,52,72], recent-onset samples[35], and in unaffected first-degree relatives[84,85].

While it may seem non-critical to focus on sex differences in FEP in schizophrenia when the majority of existing evidence suggests that none exist, the above noted limitations to the pre-existing literature suggest that it is worth additional study. Future studies should include more women with schizophrenia in their samples, examine the relationship between symptoms and clinical features and sex, and continue to study early or recent-onset populations in addition to clinical high risk populations. Further clarification on sex differences in FEP would also help us understand why men with schizophrenia appear to benefit more from interventions that target FEP when compared to women and whether women with schizophrenia show strengths in other social skills to compensate for their deficits in FEP. Controlling for sex differences or ignoring potential sex differences in FEP tasks, as well as other tasks that may be related to functioning and symptoms in schizophrenia, is limiting our ability to uncover potentially important differences between men and women with schizophrenia, such as why women with schizophrenia overall show better functioning and less severe negative symptoms when compared to men. Finally, while the magnitude of sex differences in FEP may be small, a recent meta-synthesis of 106 meta-analyses of sex differences in nonclinical populations found that the effect sizes for all sorts of sex differences are typically relatively small, suggesting that men and women are more similar than dissimilar on a variety of psychological outcomes[88]. However, the authors cautioned that although such differences "are typically small, they should not be regarded as trivial, as even small effects can have important everyday consequences" (p. 17)[88]. Thus, it may well prove fruitful to continue to study the relationship between FEP and sex across the schizophrenia spectrum.

In conclusion, men and women across the schizophrenia spectrum - including chronically ill adults, people with early and recent-onset schizophrenia, and unaffected first degree relatives of people with schizophrenia - do not exhibit large differences in FEP. Both men and women across the schizophrenia spectrum perform more poorly on FEP tasks compared to people not on the spectrum. However, there are noteworthy limitations in the existing literature that can be addressed, including the inclusion of more women in studies and understanding the role of symptoms and sex differences in FEP in schizophrenia. The continued assessment of sex differences in FEP remains important to help researchers and clinicians further understand

other sex differences in the disorder as well as develop future treatment targets to improve functioning in both men and women with schizophrenia.

COMMENTS

Background
Facial emotion perception (FEP) refers to the ability to identify the emotion on the face of another person and is typically assessed with laboratory tasks.

Research frontiers
To the best of our knowledge, no systematic review of sex differences in FEP across the schizophrenia spectrum has previously been published. The objective of this study was to systematically review all studies on sex differences in FEP across the schizophrenia spectrum and critically evaluate the available literature.

Innovations and breakthroughs
The majority of studies examined in this review suggest that men and women across the schizophrenia spectrum do not differ in FEP ability. However, the limitations of the available literature warrant further investigation.

Applications
Future studies on FEP in schizophrenia should include more women in their studies, continue to examine the relationship between symptoms and clinical features and sex, and continue to study early and recent-onset populations in addition to clinical high risk populations. Future studies should also attempt to understand whether men might benefit more from interventions that target FEP than women with schizophrenia.

Terminology
Schizophrenia is characterized by positive symptoms (hallucinations and delusions), negative symptoms (blunted affect, alogia, anhedonia, asociality, and avolition), and disorganization symptoms. Approximately 1% of the general population has schizophrenia and the prevalence of the disorder is approximately the same between men and women.

Peer-review
This is a nice and complete study. It is nicely written, readable and documented.

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SYSTEMATIC REVIEWS

Review of key telepsychiatry outcomes

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Abstract

AIM: To conduct a review of the telepsychiatry literature.

METHODS: We conducted a systematic search of the literature on telepsychiatry using the search terms, "telepsychiatry", "telemental health", "telecare", "telemedicine", "e-health", and "videoconferencing". To meet criteria for inclusion, studies had to: (1) be published in a peer-reviewed journal after the year 2000; (2) be written in English; (3) use videoconferencing technology for the provision of mental health assessment or treatment services; and (4) use an adequately-powered randomized controlled trial design in the case of treatment outcome studies. Out of 1976 studies identified by searches in PubMed (Medline database), Ovid medline, PsychInfo, Embase, and EBSCO PSYCH, 452 met inclusion criteria. Studies that met all inclusion criteria were organized into one of six categories: (1) satisfaction; (2) reliability; (3) treatment outcomes; (4) implementation outcomes; (5) cost effectiveness; and (6) and legal issues. All disagreements were resolved by reassessing study characteristics and discussion.

RESULTS: Overall, patients and providers are generally satisfied with telepsychiatry services. Providers, however, tend to express more concerns about the potentially adverse of effects of telepsychiatry on therapeutic rapport. Patients are less likely to endorse such concerns about impaired rapport with their provider. Although few studies appropriately employ non-inferiority designs, the evidence taken together suggests that telepsychiatry is comparable to face-to-face services in terms of reliability of clinical assessments and treatment outcomes. When non-inferiority designs were appropriately used, telepsychiatry performed as well as, if not better than face-to-face delivery of mental health services. Studies using both rudimentary and more sophisticated methods for evaluating cost-effectiveness indicate that telepsychiatry is not more expensive than face-to-face delivery of mental health services and that telepsychiatry is actually more cost-effective in the majority of studies reviewed. Notwithstanding legal concerns about loss of confidentiality and limited capacity to respond to psychiatric emergencies, we uncovered no published reports of these adverse events

in the use of telepsychiatry.

CONCLUSION: A large evidence base supports telepsychiatry as a delivery method for mental health services. Future studies will inform optimal approaches to implementing and sustaining telepsychiatry services.

Key words: Telepsychiatry; Telemental health; Videoconferencing; Treatment access; Implementation

Core tip: Telepsychiatry represents a highly promising approach to reducing the treatment gap by making it easier for patients, especially those in isolated contexts, to access expert mental health care. There is a robust evidence base for the use of telepsychiatry as a delivery method for mental health services. Given sufficient empirical justification for telepsychiatry in routine clinical settings, future research studies should focus on clarifying best practices for implementing and sustaining telepsychiatry services.

INTRODUCTION

Innovative approaches to delivering mental health services are urgently needed to increase access to evidence-based care. Telepsychiatry, which in its contemporary use refers to the delivery of mental health services *via* video-based conferencing, has great potential to address mental health disparities by extending the reach of mental health care to those living in rural areas or to those who otherwise have limited access to care. Rapid changes in technology and the medical landscape have undoubtedly accelerated the growth and reach of telepsychiatry. The enthusiasm for the delivery of mental health services *via* telepsychiatry is evident in its adoption in large health care organizations such as the United States Department of Veterans Affairs[1] and establishment of national practice guidelines[2].

Prior reviews have focused on different domains of the evidence based for telepsychiatry[3,4]. We sought to build on prior reviews by systematically reviewing and critically summarizing the evidence base for telepsychiatry. For the purposes of this review, telepsychiatry is defined as the provision of mental health services by a mental health professional *via* videoconferencing technology.

MATERIALS AND METHODS

We conducted a review of the telepsychiatry literature in PubMed (Medline database), Ovid medline, PsychInfo, Embase, and EBSCO PSYCH. We used the keywords, "telepsychiatry", "telemental health", "telecare", "telemedicine", "e-health", and "videoconferencing". To meet criteria for inclusion, studies had to: (1) be published in a peer-reviewed journal after the year 2000; (2) be written in English; (3) use videoconferencing technology for the provision of mental health assessment or treatment services; and (4) use an adequately-powered randomized controlled trial (RCT) design in the case of treatment outcome studies. Additionally, we searched reference lists of included studies to identify additional publications not captured by our literature search. The last search was conducted in June 2015.

The first two authors (Sam Hubley and Sarah B Lynch) reviewed all abstracts to identify eligible studies. Studies that met all inclusion criteria were organized into one of six categories: (1) satisfaction; (2) reliability; (3) treatment outcomes; (4) implementation outcomes; (5) cost effectiveness; and (6) and legal issues. All disagreements were resolved by reassessing study characteristics and discussion. When consensus was not reached between the first two authors, the last author (Jay Shore) made the final decision.

RESULTS

We identified a total of 1668 full-text articles based on our literature search and excluded 1534 based on the inclusion criteria (Figure 1). Of the remaining 134, 86 reported on satisfaction with telepsychiatry, 38 evaluated reliability of clinical assessments conducted *via* telepsychiatry, 32 were RCTs, 43 reported on implementation outcomes, 29 estimated cost-effectiveness, and 23 evaluated legal issues associated with telepsychiatry mental health services. Note that some studies reported on more than one outcome ($n = 117$) and were thus included in each relevant section.

Satisfaction

Adequate patient and provider satisfaction is a prerequisite for wide scale implementation of telepsychiatry. This is especially true in light of the emphasis on patients' experience of care as a key component of the Triple Aim Framework[5], by which many healthcare innovations are evaluated. There is a substantial body of literature focusing on patient and provider satisfaction with telepsychiatry. Studies employed a range of descriptive, qualitative, experimental, and mixed-methods designs to assess satisfaction outcomes.

Patient satisfaction: The majority of studies summarize patients' responses to quantitative self-report questionnaires with descriptive statistics and report high satisfaction with telepsychiatry services. The Client Satisfaction Questionnaire[6] is a commonly used measure in these studies and consists of 8 items rated on a 5-point Likert scale. Many investigators also developed their own satisfaction measures that
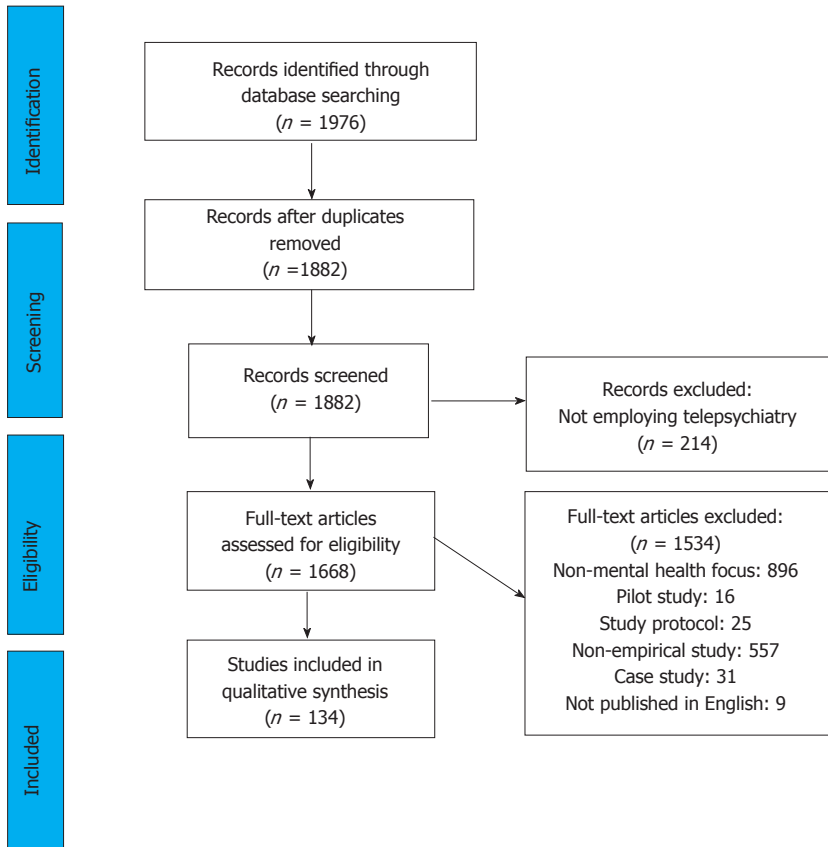
Figure 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram.

consist of similar items rated on a Likert-scale. Of the 31 studies reviewed, 23 concluded that patients rated their experience with telepsychiatry services as "good" to "excellent"[7-28], while the remaining 7 studies reported mixed reactions among participants[29-38]. For example, Hilty et al[33] reported generally high satisfaction among outpatients seeking specialty mental health care but satisfaction scores were statistically higher for rural patients compared to suburban patients. Studies that used qualitative ($n = 6$) qualitative methods to better understand patients' experiences with telepsychiatry services suggest a less uniform pattern of findings compared to results from studies using quantitative methods only[32,34,37,39,40]. Participants' responses to individual interviews and focus groups follow themes characterized by both positive and negative reactions to telepsychiatry. Prominent positive themes include ease of use and decreased burden of transportation to and from appointments; whereas prominent negative themes include privacy concerns, challenging to establish patient-doctor relationship, and technical challenges. Finally, some studies ($n = 7$) used an experimental design to assess patient satisfaction[10,21,26,36,41-43]. In a study that compared reactions of 48 outpatients randomized to telepsychiatry or face-to-face (FTF) psychiatric consultation, telepsychiatry patients reported comfort in disclosing the same information they would disclose in FTF consultation but reported slightly lower levels of satisfaction regarding feeling supported and

encouraged than did FTF patients[36].

Provider satisfaction: Based on 11 studies that use qualitative self-report methods, providers tend to have mixed reactions to telepsychiatry[25,33,34,44-52]. Some studies have shown that adult and child psychiatrists reported adequate to high satisfaction with telepsychiatry[53] and one study demonstrated that mental health providers prefer telepsychiatry over telephone-based consultation[51]. Allied providers such as primary care providers (PCPs)[49] and emergency room providers[25,44] have also expressed satisfaction with telepsychiatry. Other studies have yielded mixed results as rural PCPs are more satisfied with telepsychiatry than are PCPs based in suburban locations[33], providers perceive patients to be less satisfied with telepsychiatry services than actual patient report[41] and provider concerns that their lack of experience delivering telepsychiatry may result in lower levels of care[54]. Finally, some investigators have documented negative reactions to telepsychiatry as providers are resistant to use telepsychiatry[55] and concern that perceived technological challenges associated with telepsychiatry may hinder doctor-patient interactions[56].

Qualitative interviews yield similarly mixed results as some allied health providers report satisfaction with telepsychiatry services[44], while others[45,46] express concerns about the potential adverse impacts of telepsychiatry on the therapeutic alliance and cited barriers

such as difficulties incorporating telepsychiatry into their practice, including difficulty accessing trainings for telepsychiatry, and lack of resources. Experimental studies have confirmed the finding that providers are not uniformly satisfied with telepsychiatry services, based in large part about concerns over therapeutic rapport. For example, in an RCT comparing cognitive behavioral therapy (CBT) for bulimia nervosa delivered FTF and *via* telepsychiatry, patients and psychotherapists completed ratings of the therapeutic alliance[57]. Results showed that psychotherapists generally reported lower alliance scores with telepsychiatry participants than FTF participants whereas there no differences between alliance scores among FTF and telepsychiatry participants' own evaluations of the therapeutic alliance.

Summary of patient and provider satisfaction: The evidence to date on patient and provider satisfaction is generally positive as most studies demonstrate that providers and patients find telepsychiatry acceptable. In general, (1) patients tend to report higher satisfaction than providers, and this appears to be especially true among parents seeking services for their children; (2) patients acknowledge potential changes to the therapeutic alliance inherent in providing services remotely but are less concerned than providers; (3) allied health professionals (*e.g.,* PCPs and ED providers) report high satisfaction with telepsychiatry provided the technology works well and does not majorly interfere with workflows; and (4) study design appears to influence outcome as studies that used purely descriptive methods tend to report more positive outcomes than studies using qualitative and experimental methods.

Reliability

The reliability of assessments conducted *via* telepsychiatry compared to assessments conducted *via* the gold-standard of FTF interviews is a key area of research. The primary variables of interest are inter-rater reliability and inter-method reliability. Inter-rater reliability refers to the degree of agreement between two raters assessing the same patient with the same assessment tools, whereas inter-method reliability refers to the degree of agreement between one rater using one assessment tool with two different methods (*i.e.,* telepsychiatry *vs* FTF). This area of research represents a hybrid between inter-rater and inter-method reliability.

Several statistical approaches are used to quantify the degree of agreement between two different ratings. Most relevant to evaluating reliability in telepsychiatry assessments are the kappa statistic, correlation coefficients, and the intra-class correlation coefficient (ICC). The kappa statistic assesses agreement on categorical outcomes (*i.e.,* whether or not a patient receives a diagnosis of bipolar disorder) and Pearson's r is a correlation coefficient that assesses agreement on continuous outcomes such as total scores on the

Hamilton Depression Rating Scale (HAMD)[58]. Finally, the ICCs is a more sophisticated version of Pearson's r because it accounts for variance in assessments due to true between-subject variability in addition to variance due to disagreement between the raters.

We identified 21 studies that evaluated reliability of clinical assessments conducted *via* telepsychiatry. Two studies compared the accuracy of diagnoses made using the SCID[59,60], two studies evaluated reliability of child assessments[42,61], four studies evaluated reliability of neuropsychological assessments[62-65], and three studies evaluated reliability of measures of depressive symptom severity[66-68]. The remaining studies evaluated a range of targets such as alcohol use severity[69], diagnostic accuracy[70-72], competency to stand trial[73], psychosis[26], and adult autism[21]. The majority of studies report moderate to high level of agreement between raters using telepsychiatry and FTF regardless of instrument, provider, or setting type.

Several authors used clever study designs to evaluate reliability at a more nuanced level. For example, two studies assessed reliability estimates between telepsychiatry conducted with varying levels of bandwidth to determine if connection quality impacted reliability. One report observed ICCs greater than 0.95 across all conditions of varying bandwidth quality among two psychiatrists rating eight psychiatric outpatients and nine health controls using the BPRS[74]. On the other hand, Yoshino *et al*[75] observed statistically significantly lower ICC scores in the narrow bandwidth condition compared to the broad bandwidth in for BPRS scores. In a rigorous comparison of inter-rater reliability that reduced artificial inflation of reliability estimates by requiring interviewers to generate their own clarifying questions and probes, Kobak *et al*[66-68] observed very high reliability estimates between four psychiatrists at three different locations using the HAMD *via* telepsychiatry or FTF[67]. And although diagnostic assessments *via* telepsychiatry appear to be reliable even when using interpreters[60], there are mixed results of the reliability of telepsychiatry assessments when visual information is required. For example, Jones *et al*[76] found that raters assessing a geriatric population on a psychiatric inpatient unit achieved lower reliability on the observational items based on participants' behavior than the subjective items based on participants' self-report. This difference in Kappa scores was statistically significant for assessments conducted *via* telepsychiatry, but not for assessments conducted FTF[76]. Conversely, Amarendran *et al*[62] found that assessment of abnormal movements among patients with at least 10 years of antipsychotic medication exposure was not significantly less reliable when using telepsychiatry compared to FTF.

Summary of reliability estimates in telepsychiatry: The studies included in this review suggest that, in general, assessments made *via* telepsychiatry are comparable to FTF assessments in terms of reliability. No studies provided strong evidence that telepsychiatry

assessments are significantly less reliable than FTF assessments. Studies that have not reported uniformly high reliability estimates for telepsychiatry assessments represent important caveats. First, adequate reliability may be contingent on bandwidth quality as the quality of observations required for a given assessment decreases as video and audio quality deteriorate. Second, the use of interpreters does not appear to reduce the reliability of telepsychiatry assessments but more studies that attempt to replicate this finding are needed. Third, assessments that require objective observations (*i.e.,* the Brief Psychiatric Rating Scale) may be more difficult to conduct *via* telepsychiatry.

Treatment outcomes

The crucial question in telepsychiatry research is whether or not mental health interventions delivered *via* telepsychiatry can achieve similar outcomes compared to interventions delivered FTF. We identified 13 RCTs that evaluated treatment outcomes for mental health interventions delivered *via* telepsychiatry (Table 1). Seven studies targeted depression[77-83], two targeted symptoms of PTSD[11,84,85], two targeted ADHD[86], one targeted bulimia nervosa[87], and two targeted common psychiatric disorders presented in an outpatient medical and mental health settings.

Telepsychiatry compared to usual care: RCTs are required to determine if telepsychiatry interventions are comparable to FTF interventions in terms of outperforming usual care. Of the seven studies that made this comparison, six studies compared psychotropic medication management *via* telepsychiatry to FTF delivery of usual care[78-81,83,88]. One study included delivery of psychotherapy *via* telepsychiatry[11]. Four studies demonstrated superiority of telepsychiatry over FTF usual care[11,78,79,86], while telepsychiatry failed to achieve superior outcomes in three studies[77,80,81]. Fortney *et al*[11] revealed that although cognitive processing therapy (CPT) for PTSD was available in both the collaborative care delivered *via* telepsychiatry and FTF, participants randomized to telepsychiatry were 18 times more likely to initiate CPT and suggested that long travel distances discouraged weekly psychotherapy.

Telepsychiatry compared to FTF: A second and distinct question is whether or not mental health services delivered *via* telepsychiatry generate outcomes that are equivalent to FTF services. Of the seven studies making this comparison[82-85,88-90], only Mitchell *et al*[87] - who evaluated CBT for Bulimia Nervosa - found FTF to be superior to telepsychiatry. It is important to note that this finding held despite a high attrition rate of over 33% in both arms that reduced statistical power. The remaining six studies[81-83,88,90,91] reported that telepsychiatry was equivalent to FTF but only three conducted a non-inferiority trial that was explicitly designed to determine equivalency between telepsychiatry and FTF[84,85,89].

Summary: Overall, mental health interventions delivered *via* telepsychiatry and FTF resulted in similar treatment outcomes. To summarize: (1) telepsychiatry appears to be better than usual care, except possibly in the case of depression treatment in primary care where telepsychiatry has failed to show superior treatment outcomes to usual care in multiple studies; (2) There were no differences in the patterns of findings for the delivery of pharmacotherapy or psychotherapy delivered *via* telepsychiatry; (3) With the exception of one study, current data suggest telepsychiatry interventions produce outcomes that are statistically equivalent to outcomes produced by FTF interventions; and (4) The treatment outcome data on telepsychiatry is strongest for the treatment of depression, whereas the compelling data for psychiatric disorders other than depression is strongest in publications that focus on program descriptions and retrospective single cohort designs.

Implementation

There are two primary approaches to developing frameworks that guide the implementation of telepsychiatry. First, "purist" approaches use a comprehensive review of theory, basic science, and expert consensus to develop theoretically- and empirically-derived implementation frameworks (CFIR, RE-AIM, PRISM). Second, "pragmatic" approaches emerge from a practical need to organize implementation efforts for a given type of intervention or intervention delivery method for a defined population (*i.e.,* pharmacotherapy *via* telepsychiatry for primary care patients with mental illness). For the scope of this review, pragmatic approaches such as the "Lexicon of Assessment and Outcome Measures for Telemental Health" are most appropriate[92]. This lexicon, derived by consensus from 26 established telepsychiatry experts, contains 36 implementation variables to consider. We review access, utilization, and the impact on clinical skill and workflows as particularly relevant variables in the implementation of telepsychiatry services.

Studies from the implementation of telepsychiatry in the VA provide data documenting the power of telepsychiatry to reach great numbers of people. Since 2003, the VA has documented nearly 500000 telepsychiatry encounters[93]. In an assessment of telepsychiatry services for over 100000 patients between 2006 and 2010, researchers found that hospitalization utilization decreased by approximately 25%[94]. However, patients in other settings do not always use telepsychiatry services, even when they are freely available. For example, in the Fortney *et al*[79] trial reviewed above in which free psychotherapy was available *via* telepsychiatry, Deen *et al*[30] demonstrated 76% of patients reported that psychotherapy was acceptable, 38% scheduled a telepsychiatry psychotherapy session, 17% attended a session and 8% attended at least 8 session. Out of a range of possible patient sociodemographic and clinical factors, perceptions depression would remit on its own and low treatment efficacy predicted treatment utilization. On the other hand,

Table 1 Review of telepsychiatry randomized controlled trials

Ref.	Participants				Interventions			Results	
	n	Recruitment source	Mean age (SD)	Target disorder	Conditions	Duration (# of visits)	Provider	Attrition	Findings
Nelson *et al*[82]	38	Urban schools	10.3 (2.0)	Childhood depression	CBT TP / CBT FTF	8 / 8	dnr / dnr	26% / 26%	Child Depression Inventory scores reduced from 14.36 (SD = 9.85) at baseline to 6.71 (SD = 4.78) at post-treatment for CBT TP and from 13.57 (SD = 8.75) to 11.64 (SD = 4.78) for CBT FTF [Wilks' L $_{(1, 26)}$ = 0.83; Eta^2 = 0.17]
Ruskin *et al*[83]	119	VA outpatient mental health clinics	49.6 (12.8)	Depression	Pharmacotherapy TP / Pharmacotherapy FTF	8 / 8	Psychiatrist / Psychiatrist	27% / 30%	Mean scores not reported. Differences between response rates according to the Hamilton Rating Scale for Depression for TP (49%) and FTF (43%) were not statistically significant (χ^2 = 0.4, $P > 0.05$)
Fortney *et al*[79]	395	VA community-based outpatient clinics	59.2 (12.2)	Depression	Stepped collaborative care TP / Usual care in primary care setting	Flexible number of visits up to 12 mo / Flexible number of visits up to 12 mo	On-site PCP + Off-site psychiatrist, care manager, PharmD / PCP	10% / 9%	At 12 mo, TP participants had greater odds of qualifying for remission than usual care participants (OR = 2.4, $P = 0.04$) but were not more likely to qualify for treatment response (OR = 1.4, $P = 0.18$) using the Hopkins Symptom Checklist
Hilty *et al*[80]	94	Rural primary care clinics	46	Depression	Psychiatric consultation TP, PCP training, disease management modules / Disease management modules, usual care in primary care setting	5 with psychiatrist 5 with PCP / 5 with PCP	Psychiatrist, PCP / PCP	dnr / dnr	Mean scores not reported. Differences between response rates according to the Beck Depression Inventory-13 for TP (42%) and augmented usual care (42%) were equivalent and not analyzed with odds ratios. Similarly, response rates according to the Hopkins Symptom Checklist-90 for TP (53%) and augmented usual care (42%) were not analyzed with odds ratios
Chong *et al*[77]	167	Community health center	43.0 (12.0)	Depression	Pharmacotherapy *via* TP + integrated primary care / Integrated primary care	7 with psychiatrist, no limit on other visits / No limit	Psychiatrist, PCP, mental health specialist / PCP, mental health specialist	13.8% / 10.3%	Patient Health Questionnaire-9 scores reduced from 17.3 (SD = 4.9) at baseline to 6.8 (SD = 6.0) at post-treatment for TP and from 18.3 (SD = 4.5) to 4.7 (SD = 5.1) for FTF (F =1.1, $P > 0.05$. Eta^2 = 0.17)
Moreno *et al*[81]	167	Community health center	43.2 (11.9)	Depression	Pharmacotherapy *via* TP + integrated primary care / Integrated primary care	7 with psychiatrist, no limit on other visits / No limit	Psychiatrist, PCP, mental health specialist / PCP, mental health specialist	dnr / dnr	Patient Health Questionnaire-9 scores reduced from 17.6 (SD = 7.6) at baseline to 5.1 (SD = 6.8) at post-treatment for TP and from 18.4 (SD = 4.9) to 4.5 (SD = 5.3) for FTF (t =2.30, $P < 0.05$. Eta^2 =0.11)
Fortney *et al*[79]	364	Federally qualified health centers	47.2 (12.6)	Depression	Enhanced collaborative care TP / Collaborative care in primary care setting	Flexible number of visits in 12 mo / Flexible number of visits in 12 mo	On-site PCP + Off-site psychiatrist, care manager, behavioral health, PharmD / PCP, care manager	23% / 19%	At 12 mo, TP participants had greater odds of qualifying for remission than usual care participants (25.8% *vs* 9.9%; OR = 3.2, $P < 0.001$) and were more likely to qualify for treatment response (47.7% *vs* 21.9%; OR=3.3, $P < 0.001$) using the Hopkins Symptom Checklist-20

Ref	N	Setting	Age	Disorder	Condition	Visits	Provider	Dropout	Results
Fortney *et al*[11]	265	VA community-based outpatient clinics	52.2 (13.8)	PTSD	Enhanced collaborative care TP	Flexible number of visits in 12 mo	On-site PCP + Off-site psychiatrist, care manager, psychologist, PharmD	16%	At 12 mo, Posttraumatic Diagnostic Scale scores decreased 4.17 (SD = 9.8) for TP and 1.32 (SD = 8.8) for FTF (t = 2.30, $P < 0.05$. Cohen's $d = 0.31$)
					Collaborative care in primary care setting	Flexible number of visits in 12 mo	PCP, care manager, social worker	11%	
Morland *et al*[84]	125	VA clinical sites and VA Vet Centers	54.7 (9.6)	PTSD	Group CBT TP	12	Clinical psychologist	10%	In a non-inferiority trial, State-Trait Anger Expression scores reduced from 56.7 (SD = 12.0) to 46.6 (SD = 12.2) in TP and from 55.0 (SD = 10.3) at baseline to 46.6 (SD = 12.2) at post-treatment for FTF. Using CIs and a priori cut-offs, criteria for non-inferiority met (Cohen $d = 0.44$ in favor of CBT TP)
					Group CBT FTF	12	Clinical psychologist	11%	
Morland *et al*[85]	125	VA clinical sites and VA Vet Centers	55.3 (12.5)	PTSD	CPT-C TP	12	Clinical psychologist or master's level social worker	18%	In a non-inferiority trial, Clinician-Administered PTSD Scale scores reduced from 72.0 (SD = 14.6) to 55.6 (SD = 18.8) in CPT-C TP and from 68.9 (SD = 13.0) at baseline to 58.7 (SD = 21.0) at post-treatment for CPT-C FTF. Using CIs and a priori cut-offs, criteria for non-inferiority met (Cohen $d = 0.27$ in favor of CBT TP)
					CPT-C FTF	12	Clinical psychologist or master's level social worker	14%	
Myers *et al*[86]	233	Primary care	9.2 (2)	ADHD	Pharmacotherapy *via* TP + caregiver training	6	Psychiatrist, master's level therapist	13%	At 12 mo, TP participants had greater odds of no longer meeting diagnostic criteria for ADHD-inattentive subtype according to Vanderbilt ADHD Rating Scale at post-treatment (12% *vs* 26%; OR = 0.149, $P < 0.001$)
					Psychiatric consultation with PCP + caregiver training	1	Psychiatrist, PCP	5%	
Mitchell *et al*[87]	128	Patient panels of rural physicians and therapists	29.0 (10.7)	Bulimia nervosa	CBT TP	20	Clinical psychologist	34%	At post-treatment, abstinence from binge-eating episodes, purging episodes, and combined episodes ranged from 27%-50% for TP CBT and 29%-50% for FTF CBT with non-significant trend in favor of FTF. TP participants reported significantly more binge episodes (M = 6.2, SD = 12.3) than FTF participants (M = 3.7, SD = 11.2) at post-treatment (F = 6.76; $P < 0.05$)
					CBT FTF	20	Clinical psychologist	41%	
De Las Cuevas *et al*[88]	140	Community mental health center	Adults	Psychiatric disorders	Pharmacotherapy, CBT TP	8	Psychiatrist	6%	Differences between improvement rates according to the Clinical Global Impressions scale for TP (67.2%) and FTF (62.5%) were not statistically significant ($P > 0.05$)
					Pharmacotherapy, CBT FTF	8	Psychiatrist	7%	
O'Reilly *et al*[89]	495	Rural hospital and primary care clinics	Adults	Psychiatric disorders	Psychiatric consultation TP	Flexible number of visits in 4 mo	Psychiatrist	7%	In a non-inferiority trial, 22% of TP participants and 20% of FTF participants returned to functional status at post-treatment according to the Brief Symptom Inventory. Using CIs and a priori cut-offs, criteria for non-inferiority met
					Psychiatric consultation FTF	Flexible number of visits in 4 mo	Psychiatrist	3%	

ADHD: Attention deficit hyperactivity disorder; CBT FTF: Cognitive behavioral therapy delivered face to face; CBT TP: Cognitive behavioral therapy delivered *via* telepsychiatry; CPT-C: Cognitive processing therapy-cognitive version only; dnr: Did not report; PCP: Primary care provider; PTSD: Post-traumatic stress disorder.

there is some evidence that telepsychiatry may be more efficient that FTF. In a clinic that compared utilization data for 7523 telepsychiatry appointments and 115148 FTF appointments, patients kept more telepsychiatry appointments than FTF appointments (92% telepsychiatry *vs* 87% FTF). Also, patients were less likely to cancel telepsychiatry appointments (3.5% *vs* 4.8%) and were significantly less likely to be no-shows (4.2% *vs* 7.8%)[95]. Finally, it appears that telepsychiatry delivered in primary care does not increase PCP or mental health provider burden[96]. Hilty *et al*[97] demonstrated successful uptake of skills among PCPs treating anxiety and depression following consultations with telepsychiatrists. In this study, PCPs' ability to appropriately dose medications for depression and anxiety improved from 47% to 64% (*P* < 0.001).

Cost-effectiveness

The cost of telepsychiatry is widely debated and discussed. Several methods to estimate the cost of telepsychiatry compared to FTF mental health services in traditional clinic settings as well as specialty environments (*e.g.,* emergency departments) exist in the literature. One of the simplest assessments of cost data compares the collection of direct and indirect costs associated with patient encounters in telepsychiatry and FTF psychiatry visits. Studies that assessed the cost effectiveness of telepsychiatry have evaluated the direct costs of provider time[98], medical supplies[96], technology[53], and reimbursement[99]. Measurement of indirect costs include resources that facilitate patient encounters, such as clinic space[9], administrative support[96], and transportation[53,100-103]. Other studies compare healthcare utilization, such as visits to the emergency department[101] or primary care encounters[96] as a proxy measure of cost associated with telepsychiatry of FTF treatment. Return on investment (ROI) is another approach to calculating cost comparisons between telepsychiatry and FTF consolations. Simply stated, ROI is a cost-benefit ratio that assesses the cost of the service relative to an outcome measure, such as quality adjusted life years (*e.g.,* QALY) or disability adjusted life years (*e.g.,* DALY). Typically, QALY and DALY are measured by taking the difference between number of days symptom free days and days with clinical symptoms over a set period (12-18 mo). That difference is then divided by 365 d to create a range of time spent fully symptomatic to symptom-free.

Assessment of direct and indirect costs: Several studies (*n* = 18) compared the direct or indirect costs associated with providing telepsychiatry services. The majority of these studies (*n* = 13) found that telepsychiatry was associated with less direct and indirect costs than FTF services. A handful of studies utilized expenses associated with patient travel time as a cost outcome measure and found telepsychiatry reduced costs associated with travel compared to

FTF[53,99,102,104-107]. The literature reviewed suggests that telepsychiatry may have greater up-front costs associated with service delivery when compared to FTF; however, there appears to be "tipping points" at which telepsychiatry begins to eclipse the cost-effectiveness of FTF interventions. Several studies identified the number of consultations at which telepsychiatry became more cost effective than FTF, with the number of consultations delivered ranging from 131[99] to 249[98,100] to 379[103]. For example, Butler *et al*[98] found that the cost savings of telepsychiatry occur after the health center or provider delivers 249 consultations. In more rural settings, the costs saving effects of telepsychiatry for the provider and patient could be established in as little as 6 consultation sessions[102]. Of note, two studies found that telepsychiatry was more expensive than FTF[108,109]. Pyne *et al*[109] hypothesized that telepsychiatry may prompt patients to seek additional specialty care because the telepsychiatry intervention delivered promotes a more integrated approach to care than traditional FTF delivery. Modai *et al*[108] suggested that telepsychiatry leaded to more hospitalizations than FTF; however, it should be noted that Modai *et al*[108] study was limited by a small sample size (*n* = 49). When excluding hospitalization from the analysis, Modai *et al*[108] found telepsychiatry was associated with decreased travel costs when compared to FTF.

Return on investment: A smaller number of studies (*n* = 4) utilized ROI methodology to determine cost effectiveness of telepsychiatry compared to FTF. Three of these studies utilized the QALY as an outcome measure[109-111] and the remaining study utilized the DALY as an outcome measure[112]. Results using these more sophisticated methods to evaluate cost-effectiveness found telepsychiatry to be more cost effective than FTF in underserved primary care populations[111], management of pain and depression in cancer populations[110], and in depressed populations[112]. However, Pyne *et al*[109] found that telepsychiatry was not cost effective compared to FTF in treating depression in rural primary care settings; while telepsychiatry was effective in treating depression in rural populations, there were greater costs associated with the delivery of services and greater utilization of outpatient services among patients receiving telepsychiatry compared to FTF consultations.

Summary: The evidence to date on the cost effectiveness of telepsychiatry is generally positive as most studies demonstrate that telepsychiatry reduces direct and indirect costs and increases quality of life adjusted years when compared to FTF. For example, in one study the authors estimated that utilizing telepsychiatry to deliver 16 sessions of CBT for over 20 wk could save a clinic approximately $2025 per patient compared to FTF services[104]. In general, (1) telepsychiatry reduces costs associated with patient travel; (2) there are likely more upfront costs associated with telepsychiatry but

the costs are recovered after 6-379 sessions depending in the population being served; (3) cost effectiveness of telepsychiatry may differ depending on setting (*e.g.,* rural or urban). Significant differences emerged specific to operationalizing cost effectiveness and the method of assessing cost effectiveness. It appears that studies that utilized a simple approach to cost effectiveness by comparing direct and indirect costs had similar cost saving findings, whereas the handful of studies that utilized a more sophisticated approach suggest telepsychiatry is more cost-effective than FTF.

Legal issues

Despite the promise of telepsychiatry to reduce barriers associated with accessing mental health services and increase access to services, legal issues specific to telepsychiatry remain an extant and significant barrier. We identified two primary themes concerning legal issues associated with providing mental health services *via* telepsychiatry - licensing regulations and risks to patient confidentiality.

Several publications highlighted the legal challenges that telepsychiatry presents concerning provider licensure. Telepsychiatry increases providers' catchment area such that patients living outside of the state where a provider is licensed to practice may request their services *via* telepsychiatry. However, each state has its own licensing boards that establish practice jurisdictions for providers licensed in the state, and some have specific regulations related to telepsychiatry[113]. As the field progresses, the topic of licensure jurisdictions within the United States will continue to be discussed on the National level[114]. Until a resolution is reached, telepsychiatry providers must have separate licenses for each state in which they provide services and are advised to be familiar with state-specific regulations. For example, Shore *et al*[115] suggests that a priori arrangements should be made with local law enforcement and social services that are responsible for initiating involuntary commitments.

Patient confidentiality is also a debated topic specific to telepsychiatry. Baker *et al*[116] outlined several considerations for patient confidentiality when utilizing telepsychiatry: Verification of the patient's identity, establishing and ensuring privacy on both the provider and patient's location, disruption of technology, involving others in treatment, and storage of information collected or recorded during the telepsychiatry consultation. Several studies encourage telepsychiatry providers to go above and beyond normal methods of informing patients about limits of confidentiality such that they are aware of possible privacy breaches that are less likely to occur in traditional FTF consultations[116-119].

DISCUSSION

There is a large evidence base for the use of telepsychiatry as a delivery method for mental health services. Contemporary healthcare innovations such

as telepsychiatry are commonly evaluated according to the Triple Aim Framework which addresses patient satisfaction, care quality, and cost effectiveness[5]. We reviewed 569 studies that focus on Triple Aim domains and the evidence indicates that patients are satisfied, telepsychiatry is comparable to FTF delivery of mental health interventions, and telepsychiatry can be a cost-effective approach to increasing access to mental health care.

A socioeconomically and clinically diverse patient and provider population has reported on their experience with telepsychiatry. Their responses to self-report questionnaires, qualitative interviews, and mixed-methods assessments suggest that they are comfortable using this technology, appreciate the practical benefit of avoiding travel, and are less concerned than providers about potentially adverse impacts of telepsychiatry on the therapeutic alliance. In terms of care quality, the evidence reviewed suggest that telepsychiatry is comparable to FTF in the reliability of assessment and effective treatment of a range of behavioral and mental health disorders. Importantly, a small number of high quality studies have used non-inferiority designs to demonstrate statistically equivalence in treatment outcomes between telepsychiatry and FTF.

The remaining literature we reviewed focus on practical factors related to the implementation of telepsychiatry such as adaptability of telepsychiatry to routine care settings, cost-effectiveness, and legal issues. Several program descriptions discuss the actual usage of telepsychiatry services in routine clinical settings. Although it is not a foregone conclusion that patients will use telepsychiatry services, even when they are freely available, telepsychiatry is comparable to FTF in terms of service utilization patterns and can help allied health providers develop clinical skill in treating mental illnesses. Using both face-valid and sophisticated approaches to evaluating cost-effectiveness, telepsychiatry can be as cost-effective as FTF services and more studies are needed to determine how cost-effectiveness is affected by rurality, patient sociodemographic and clinical characteristics, provider type, and organizational characteristics. Notwithstanding legal concerns about loss of confidentiality and limited capacity to respond to psychiatric emergencies, we uncovered no published reports of these adverse events in the use of telepsychiatry.

Limitations and future directions

Despite many strengths evident in the evidence base for telepsychiatry, there are important limitations to consider. First, it is important to acknowledge the limitations inherent in assessing consumer attitudes with healthcare services. As reported over a decade earlier[119] there are several factors that continue to limit the quality and generalizability of research on patient satisfaction with telepsychiatry services such as: (1) over-reliance on self-report methodologies; (2) selection biases that over-represent patients amenable

to telepsychiatry; (3) insufficient sample sizes; and (4) omission direct comparison of preferences for telepsychiatry *vs* FTF. Quantitative designs that rely solely on participant self-report are sufficient for demonstrating minimum standards of acceptability, but to obtain a more nuanced understanding of reactions to telepsychiatry, mixed-methods and experimental designs are strongly recommended. In light of the consistent finding that patients are satisfied with telepsychiatry services, we recommend that future studies focus less on assessing satisfaction, and more on clarifying the actual effects of telepsychiatry on therapeutic rapport. For instance, comparing ratings of therapeutic rapport by blind assessors listening to audio recordings of telepsychiatry and FTF mental health services would provide a more objective test of the notion that telepsychiatry impairs rapport. A more thorough understanding of reactions to telepsychiatry, especially as they pertain to provider skepticism based on the assumption that telepsychiatry impairs rapport and intervention quality, has the potential to increase acceptability and uptake of telepsychiatry services.

Second, although the studies reviewed suggest that telepsychiatry is a reliable method for assessment of mental health constructs, it is inappropriate to interpret a null hypothesis without using a non-inferiority design. In this case it is particularly problematic because there is a consistent pattern of higher kappa statistics for FTF assessments than for telepsychiatry assessments, and some authors interpret a lack of statistically significant differences between assessment modality as equivalence in their reliability. To claim that assessments conducted *via* telepsychiatry are as reliable as assessments conducted FTF, researchers must use study designs that either explicitly test for equivalence, or are adequately powered to detect clinically significant differences between the reliability of telepsychiatry and FTF assessments. We recommend that future studies shift focus from establishing equivalent reliability between telepsychiatry and FTF assessments to identifying which types of assessments are most amenable to the telepsychiatry modality, which types of assessments are most difficult to administer *via* telepsychiatry, and which types of adaptations help improve the accuracy, efficiency, and consumer experience of telepsychiatry assessments.

Third, we did not perform an intensive quality appraisal of the treatment outcome studies reviewed. Even rigorously designed and executed RCTs are subject to several sources of biases such as selection bias, performance bias, attrition bias, and reporting bias. Furthermore, several studies claim equivalency between telepsychiatry and FTF with using non-inferiority designs. However, we do not recommend more equivalency studies; rather, it is clear that telepsychiatry is comparable to FTF and the field now needs more research focusing on factors that increase telepsychiatry adoption, moderators to determine for which patients

in which settings telepsychiatry is most effective, and strategies for integrating telepsychiatry services within the broach context of healthcare service delivery.

Finally, assessing costs associated with the delivery of telepsychiatry is an important, yet often overlooked, factor when evaluating telepsychiatry outcomes. There is room for improvement in cost evaluations of telepsychiatry in order to generate high-quality generalizable findings. Only four of the studies reviewed used sophisticated methods of cost effectiveness. Additionally, cost effectiveness has only been evaluated in a narrow range of patient populations. Future research would benefit from using methodologies that incorporate DALYs or QALYs to examine cost-effectiveness among diverse psychiatric populations, as there is evidence to suggest that telepsychiatry may be more economical than FTF with patients with specific demographic characteristics (*e.g.,* patients seeking services in primary care *vs* specialty mental health, rural *vs* urban patients).

The gap between the need for mental health treatment and availability of mental health providers has prompted national and international organizations such as the National Institute of Mental Health (NIMH; 2013) and the World Health Organization (WHO; 2013) to prioritize the development and evaluation of novel service delivery methods. Telepsychiatry represents a highly promising approach to reducing the treatment gap by making it easier for patients, especially those in isolated contexts, to access expert mental health care. Just as the NIMH and WHO have shifted emphasis from efficacy to effectiveness testing, it also time for telepsychiatry researchers to focus less on pure outcome studies to document patient acceptability and high care quality, and more on studies that inform evidence-based approaches to implementing and sustaining telepsychiatry services.

COMMENTS

Background
Innovative approaches to delivering mental health services are urgently needed to increase access to evidence-based care. Telepsychiatry, which in its contemporary use refers to the delivery of mental health services *via* video-based conferencing, has great potential to address mental health disparities by extending the reach of mental health care to those living in rural areas or to those who otherwise have limited access to care.

Research frontiers
For many years, an important question for telepsychiatry researchers was, "Is telepsychiatry a *via* ble delivery method for mental health services relative to face-to-face delivery"? As experts in mental health service delivery have shifted emphasis from efficacy to effectiveness testing, so too is it time for telepsychiatry researchers to focus less on pure outcome studies to document patient acceptability and high care quality, and more on studies that inform evidence-based approaches to implementing and sustaining telepsychiatry services.

Innovations and breakthroughs
Telepsychiatry represents a highly promising approach to reducing the treatment gap by making it easier for patients, especially those in isolated contexts, to access expert mental health care.

Applications

This review suggests that telepsychiatry is a *via*ble and cost-effective method to increase access to mental health services.

Terminology

Telepsychiatry, in its contemporary use, refers to the delivery of mental health services *via* video-based conferencing.

Peer-review

In this review, the authors have provided a comprehensive commentary on the state of the research for key outcomes in telepsychiatry.

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REVIEW

Neuroinflammation and cytokine abnormality in major depression: Cause or consequence in that illness?

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Abstract

Depression results from changes in the central nervous system (CNS) that may result from immunological abnormalities. The immune system affects the CNS through cytokines, which regulate brain activities and emotions. Cytokines affect two biological systems that are most associated with the pathophysiology of depression: The hypothalamic-pituitary-adrenal axis and the catecholamine/sympathetic nervous system. Neuroinflammation and cytokines affect the brain signal patterns involved in the psychopathology of depression and the mechanisms of antidepressants, and they are associated with neurogenesis and neural plasticity. These observations suggest that neuroinflammation and cytokines might cause and/or maintain depression, and that they might be useful in the diagnosis and prognosis of depression. This psychoneuroimmunologic perspective might compensate for some of the limitations of the monoamine theory by suggesting that depression is a result of a failure to adapt to stress and that inflammatory responses and cytokines are involved in this process. In this review, the interactions of cytokines with the CNS, neuroendocrine system, neurotransmitters, neurodegeneration/neurogenesis, and antidepressants are discussed. The roles of cytokines in the etiology and psychopathology of depression are examined. The use of cytokine inhibitors or anti-inflammatory drugs in depression treatment is explored. Finally, the significance and limitations of the cytokine hypothesis are discussed.

Key words: Depression; Neurogenesis; Antidepressant; Cytokine; Neuroinflammation; Psychoneuroimmunology; Neuroendocrine; Neurotransmitter

Core tip: We investigated the etiology and the pathogenesis of depression regarding the cytokine network. It was concluded that depression may be caused by neuroinflammation and cytokine imbalances, which are closely connected with the central nerve system, hypothalamic-pituitary-adrenal axis, neurotransmitter, autonomic nerve system, neural plasticity, and antidepressants.

INTRODUCTION

Severe psychological and/or physical stress can result in homeostatic imbalances and abnormal immune responses. Several hypotheses have proposed that immunologic imbalances affect the central nervous system (CNS) and result in psychopathology. Depression is a disease that is associated with changes in the CNS that might be caused by immunological abnormalities. Recent clinical and experimental studies have confirmed that internal and external stress significantly affects the expression of depressive symptoms and their persistence in vulnerable individuals with immunological abnormalities[1]. Moreover, cytokines affect the activity of the two biological systems that are most associated with the pathophysiology of depression: The hypothalamic-pituitary-adrenal (HPA) axis and the catecholamine/sympathetic nervous system[2].

The CNS affects the immune system through the autonomic nervous system and the neuroendocrine system. Reciprocally, the immune system affects the CNS through cytokines secreted by immune cells that regulate brain activities and emotions[3]. Thus, the immune system can be regarded as a sensory organ that recognizes internal or external stress. Stress can trigger overall changes in the immune system, neurotransmitters, neuroendocrine system, and CNS, and their interactions contribute to the expression, continuation, and termination of depressive symptoms.

This psychoneuroimmunologic perspective suggests that depression is mediated by inflammatory responses and cytokines, and that the disease results from a failure to adapt to stress. This view might compensate for some of the limitations of the monoamine theory, which is an important psychopathologic model of depression.

In this review, the interactions of cytokines with the CNS, neuroendocrine system, neurotransmitters, neurogenesis, and antidepressants are investigated. The roles of cytokines in the etiology and psychopathology of depression are examined. In addition, the use of cytokine inhibitors and anti-inflammatory drugs in the treatment of depression are explored, and the significance and limitations of the cytokine hypothesis are discussed.

CYTOKINE SYSTEM

Peripheral and central cytokines and neuroimmune circuits

Cytokines mediate signaling among immune cells. They activate or inhibit other immune cells, which results in a complicated circuit. Cytokines act on cell membrane receptors like neurotransmitters or on intracellular receptors like hormones to transmit information to cells. They are mainly secreted from monocytes (or macrophages) or lymphocytes as well as from brain cells, such as neurons, endothelial cells, astrocytes, and microglia. Cytokines are divided into various types, including interleukins (ILs), chemokines, tumor necrosis factors (TNFs), interferons (IFNs), and transforming growth factors (TGFs).

Proinflammatory cytokines include IL-1, IL-2, IL-6, IFN-γ, and TNF-α. Anti-inflammatory cytokines include IL-4, IL-10, IL-11, IL-13, and TGF-β[4]. The proinflammatory cytokines activate cyclo-oxygenase-2 (COX-2), increase the levels of prostaglandin E2 (PGE2), activate inflammatory cells, and induce inflammatory reactions. They interact with each other to maintain balance. For example, IL-10 reduces TNF production, and the IL-1 receptor antagonist (IL-1ra) antagonizes the IL-1 receptor. In chronic inflammation, the proinflammatory cytokines are increased and the anti-inflammatory cytokines are decreased, which results in the onset of various diseases[5].

The production of the peripheral cytokines that are secreted from monocytes or macrophages is determined by the level of immune activity. In pathologic states, such as acute or chronic inflammation or tissue damage, immune function and macrophages are activated to increase the levels of proinflammatory cytokines. Cortisol secreted from the adrenal cortex as a result of HPA-axis activation is most important in peripheral cytokine production. When cortisol levels are low, the production of proinflammatory cytokines increases, while their production is inhibited when cortisol levels are high[6]. Neurotransmitters regulate peripheral cytokines through cortisol levels. For example, acetylcholine (Ach), dopamine (DA), and noradrenaline (NA) promote the secretion of corticotropin-releasing hormone (CRH) in the hypothalamus, and serotonin (5-HT) inhibits the secretion of CRH in the hypothalamus and adrenocorticotropic hormone (ACTH) in the pituitary[7]. In addition, the autonomic nervous system regulates peripheral cytokine production. Parasympathetic nerves directly affect the immune system, while sympathetic nerves affect the immune system through NA secretion from the peripheral sympathetic ganglia. The vagus nucleus, which is located in the pons, inhibits immune functions and cytokine production through the secretion of Ach from the vagus nerve[8] (Figure 1).

Because peripheral cytokines are hydrophilic and have large molecular weights, they are unable to pass through the blood-brain barrier (BBB) in their normal state. However, they can pass through the BBB in pathological states that involve increased BBB permeability. Moreover, cytokines are also able to affect the CNS through mediators, such as nitric oxide or prostaglandins released in response to cytokines[9,10]. IL-1 receptors are densely distributed in glial cells near arterioles or the plexus choroideus[11]. This suggests that the IL-1 receptors in the CNS and IL-1 in the peripheral blood actively communicate with each other. Additional channels through which peripheral cytokines
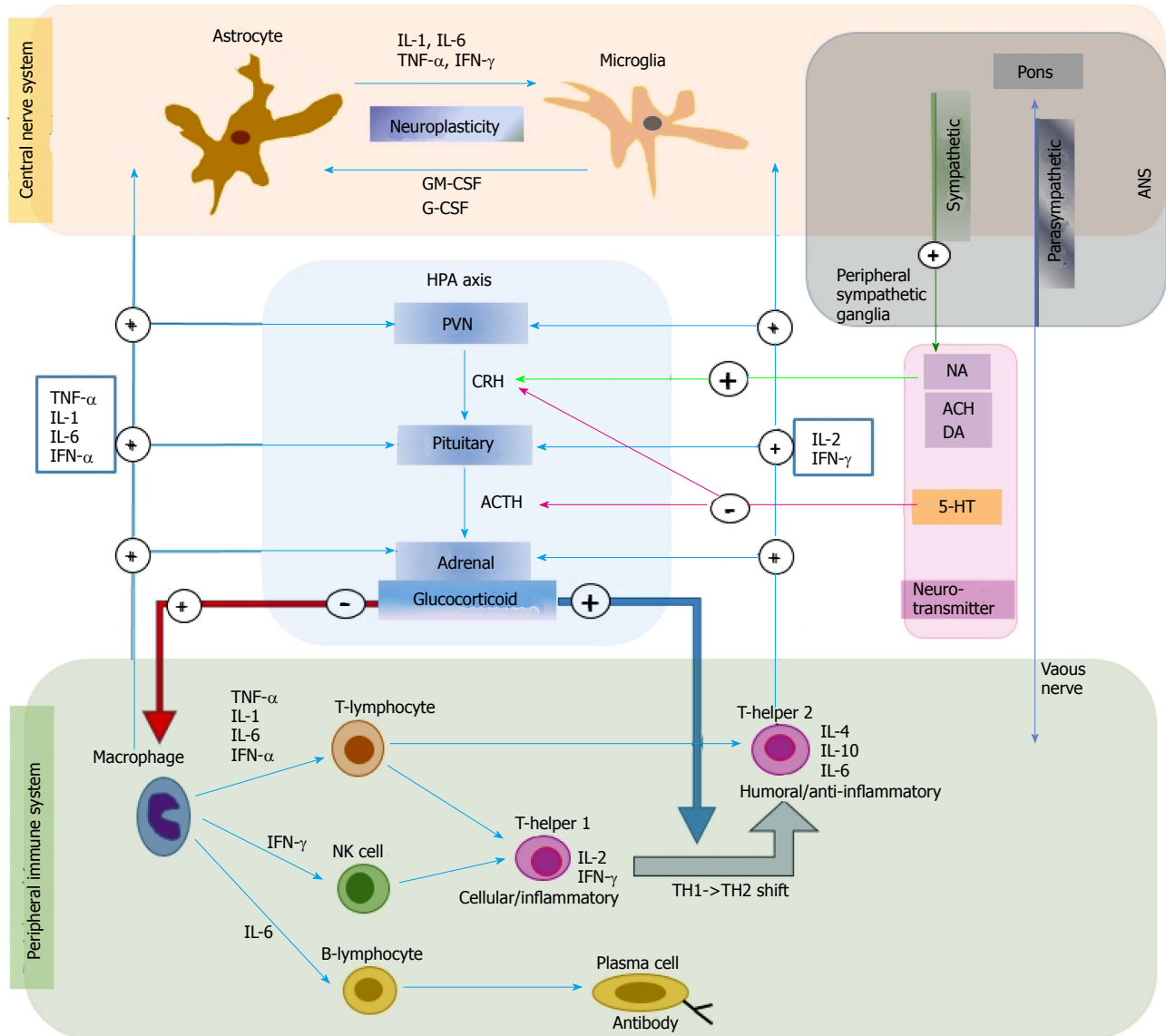
Figure 1 The role of cytokine network in depression in connection with immune system, hypothalamic-pituitary-adrenal axis, neurotransmitter, and autonomic nerve system. The figure shows communication between peripheral and central cytokine system. Early innate proinflammatory cytokines released by macrophage (TNF-α, IL-1, IL-6 and INF-α), and late acquired T cell cytokines (IL-2 and INF-γ) stimulate glucocorticoid secretion by acting at all three levels of the HPA axis. Glucocorticoids are negatively feedback on the peripheral immune system to suppress the production of proinflammatory cytokines. Glucocorticoids also play an important role in causing a shift from cellular (T-helper 1) to humoral (T-helper 2) immune responses. The central cytokines are usually secreted from the astrocyte or microglia. Central cytokines (IL-1, IL-6, TNF-α, and IFN-γ) are considered to be involved in neuroplasticity in brain. The neurotransmitters (NA, ACH, and 5-HT) regulate the peripheral cytokines by changing the cortisol concentration level. The Ach, DA, and NA promote the secretion of the CRH in hypothalamus, and 5-HT inhibits the secretion of the CRH in hypothalamus and the ACTH in pituitary. The ANS also regulates the peripheral cytokine production. The parasympathetic nerve directly reaches the immune system while the sympathetic nerve affects the immune system through the NA secretion from the peripheral sympathetic ganglia. 5-HT: Serotonin; ACH: Acetylcholine; ACTH: Adrenocorticotropic hormone; ANS: Autonomic nerve system; CRH: Corticotropin-releasing factor; DA: Dopamine; HPA: Hypothalamic-pituitary-adrenal; NA: Noradrenalin; PVN: Paraventricular nucleus of the hypothalamus; TH: Helper T cell; IL: Interleukins; TNF: Tumor necrosis factor; IFN: Interferons.

transmit immune signals to the CNS include passive diffusion through the circumventricular organs (brain regions that do not have a BBB), active transport to the CNS, and nerve conduction pathways through the vagus nerve[6].

Central cytokines are usually secreted from astrocytes or microglia, but neurons can secrete them in certain conditions[12]. Central cytokines are produced in a number of brain regions, including the circumventricular region, hypothalamus, hippocampus, cerebellum, forebrain,

basal ganglia, and brain stem nuclei[13]. IL-1, which is secreted from the brain, is found in the hypothalamus and hippocampus[14]. The roles of central cytokines in the brain are not fully understood. However, the proinflammatory cytokines IL-1, IL-6, TNF-α, and IFN-γ have been implicated in neuronal development, neuroplasticity, synaptogenesis, and tissue repair[15]. Proinflammatory cytokines promote neuronal necrosis after traumatic brain injuries[16].

Cytokine receptors are located in the immune

system and various tissues, including the peripheral nervous system and CNS. For example, IL-1, IL-2, IL-6, and TNF-α receptors are densely distributed in the hippocampus and hypothalamus[17]. IL-1 has two receptor types: Type I and type II. The nuclear transcription factor nuclear factor kappa B (NFκB) is activated and intracellular signals can be transmitted through the type I receptor. A role of cytokines in specific mental functions and/or mental diseases has been suggested because of the locations of their receptors in the CNS and not because of the specific functions of the cytokines. The important CNS structures that are affected by central cytokines include the locus coeruleus, hippocampus, prefrontal cortex, and hypothalamus. These CNS structures are associated with the biological processes that underlie psychological changes[18].

THE CYTOKINE HYPOTHESIS OF DEPRESSION

Stress-cytokine-inflammation-depression

According to the cytokine hypothesis (Figure 2), internal or external stress induces cytokine imbalances that play important roles in the expression and continuity of depressive symptoms in vulnerable individuals[19]. A number of major research findings support the cytokine hypothesis.

First, the injection of cytokines into animals and humans induces depression-like symptoms. Depression occurs frequently in patients with hepatitis C undergoing INF treatment. Of note in one study, 23% of patients during INF treatment satisfied the diagnostic criteria for major depressive disorders; in 74% of them depression occurred within 2 mo after the start of INF treatment[20]. The levels of IL-6 and TNF-α, which increase after IFN-α administration, are significantly associated with the severity of depression[21]. Polymorphisms in the 5-hydroxytryptamine (5-HT) transporter and *IL-6* genes contribute to the fatigue and depressive symptoms that are observed after IFN-α administration[22].

Second, increases in the levels of proinflammatory cytokines, such as IL-1, IL-6, IL-12, TNF-α, prostaglandin E2 (PGE2), and negative immunoregulatory cytokines have been observed in patients with depression[23,24].

Third, cytokines trigger activity in the HPA axis and the catecholamine/sympathetic nervous system, two biological systems that are closely associated with the pathophysiology of depression[2]. Cytokines stimulate corticotrophin-releasing hormone (CRH) and adreno-corticotropic hormone (ACTH), and activate the HPA axis[25]. In addition, cytokines activate indoleamine-2,3-dioxygenase (IDO), which catalyzes the metabolism of the 5-HT precursor tryptophan to kynurenine, and inhibits 5-HT synthesis in the brain[26]. The proinflammatory cytokine, NA, and DA promote CRF secretion, activate the sympathetic nerve system, and promote immune reactions. During this process, the temperature of the CNS increases and sickness behaviors may be induced[27].

Sickness behaviors refer to behavioral changes that are observed during an infection period. These include feelings of helplessness, depressive mood, anxiety, hypersomnia, loss of appetite, and inattention. Based on findings that patients with depression exhibit increased levels of proinflammatory cytokines in the plasma[23,24], decreased levels of anti-inflammatory cytokines[28], and increased levels of PGE2 in the cerebrospinal fluid[29], depression is considered a sickness behavior.

Fourth, antidepressants improve depressive symptoms by inhibiting cytokine secretion from immune cells or by acting as an antagonist of cytokine receptors. Antidepressants inhibit proinflammatory cytokine secretion from monocytes or macrophages, act as chemotaxis inhibitors, and increase the production of anti-inflammatory cytokines[30]. An *in vitro* study reported anti-inflammatory reactions with therapeutic doses of antidepressants that involved the inhibition of IFN-γ and increased IL-10[31]. In addition, antidepressants significantly inhibit the lipopolysaccharide-induced production of IL-1β, IL-6, and TNF-α, as well as the secretion of IL-2 and IFN-γ in T cells[32].

In summary, neuroinflammation and cytokines, which affect patterns of brain signal transmission, are important in the psychopathology of depression and mechanism of antidepressants. Furthermore, they are associated with neurogenesis and neural plasticity in the brain. Thus, neuroinflammation and cytokines appear to cause or continue depression and might be useful for determining the diagnosis and prognosis of depression. Epidemiological studies support the view that increased levels of IL-6, IL-1ra, and C-reactive protein (CRP) can be harnessed to predict the occurrence of depression[33]. A recent meta-analysis demonstrated that the markers of inflammation with relatively consistent increases in patients with depression are IL-6, TNF-α, TNF-β1, IFN, and CRP[34].

ARE CYTOKINES A CAUSE OF DEPRESSION?

Cytokine, HPA-axis activation, and glucocorticoid receptor resistance

HPA-axis activation is one of the most important biological findings in depression research. The activation results in increased cortisol concentrations in the plasma, urine, and cerebrospinal fluid, and exaggerated cortisol responses against ACTH[35]. HPA-axis activation has been suggested to result from excessive secretion of CRF, which triggers depressive mood, loss of appetite, and sleep disturbance[36]. These suggestions are supported by findings of increased CRF levels in the cerebrospinal fluid, increased levels of CRF mRNA in the paraventricular nucleus of the hypothalamus, blunted ACTH responses in CRH tests due to the down-regulation of CRF receptors in the pituitary gland in patients with depression, and also by the down-regulation of CRF receptors in the frontal cortex to compensate for CRF oversecretion in patients
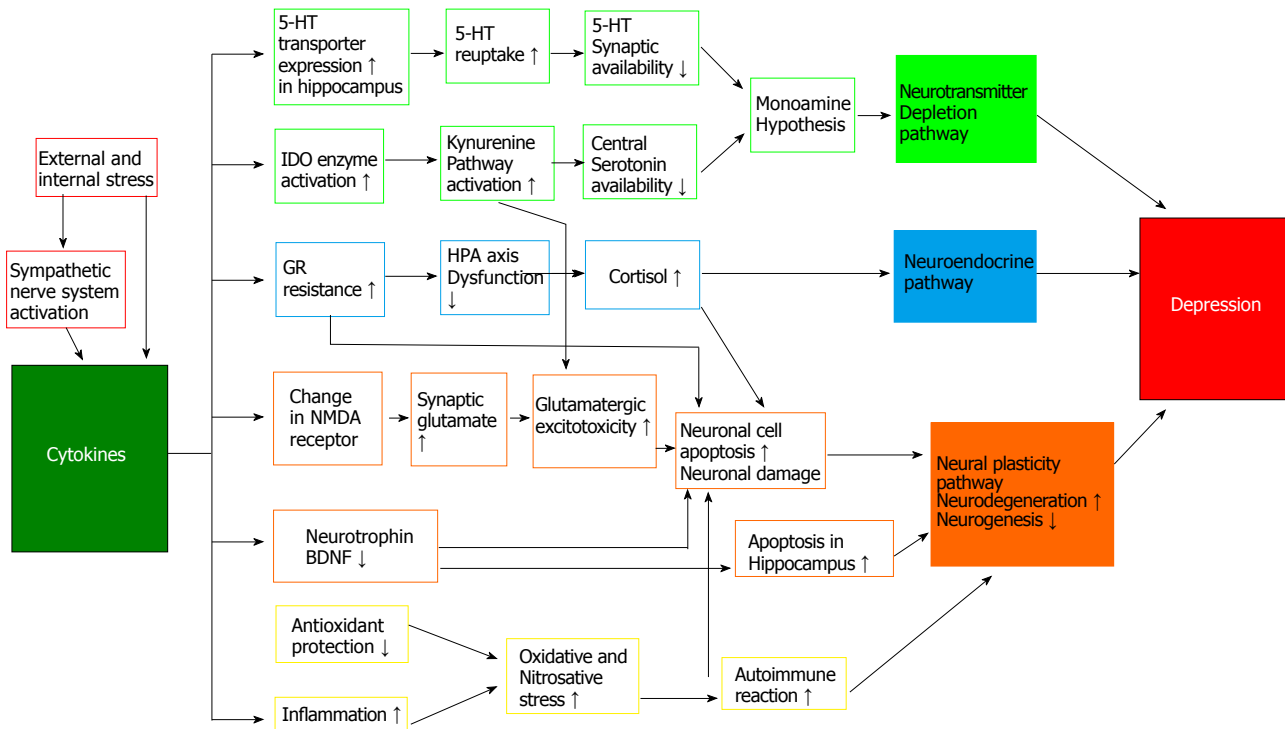
Figure 2 Schematic representation of neuroinflammatory pathways in the pathogenesis of depression. Cytokine production is initially activated by stress and sympathetic nerve system activation. In turn, cytokines have an important role by acting *via* neurotransmitter depletion pathway, neuroendocrine pathway, and neural plasticity pathway. There are multiple interactions between these pathways suggesting existence of a complex model for pathogenesis of depression. 5-HT: Serotonin; BDNF: Brain derived neurotrophic factor; GR: Glucocorticoid receptor; HPA: Hypothalamic-pituitary-adrenal; IDO: Indoleamine-2,3-dioxygenase; NMDA: N-methyl-D-aspartate.

who committed suicide[35,36]. In patients with depression, CRF oversecretion can appear due to dysfunction of the negative feedback of glucocorticoids. Accordingly, cortisol is not inhibited in dexamethasone suppression tests in these patients. This might be due to deterioration in glucocorticoid receptor (GR) sensitivity. Glucocorticoid resistance results in absence of inhibition in the dexamethasone suppression test and CRF oversecretion.

Cytokines can cause HPA-axis activation, increased CRF, and glucocorticoid resistance. Proinflammatory cytokines, such as IL-1β and IL-6, stimulate CRH secretion from the paraventricular nucleus of the hypothalamus, activate the HPA axis, and promote ACTH and glucocorticoid secretion[37] (Figure 1). In early depression research, the cortisol oversecretion from HPA-axis activation was thought to inhibit immune function[32]. However, more recently, immune cells are thought to be unaffected by cortisol in chronic stress and depression because of the inhibition of GR function in immune cells[38]. This may be a result of the oversecretion of proinflammatory cytokines from increased cell-mediated immunity[38]. In support of this, IL-1 inhibits the translocation of the GR from the cytoplasm into the cell nucleus and GR-mediated gene transcription[39]. These findings suggest that cytokines directly affect GR function and induce glucocorticoid resistance. Moreover, antidepressants like desipramine stimulate GR translocation from the cytoplasm to the nucleus and increase GR-mediated gene transcription, which eventually promotes the feedback inhibition that

is mediated by glucocorticoids in the HPA axis[40].

Theoretically, the glucocorticoid increase that is induced by HPA-axis activation and the cytokine increase that results from immune activation are not likely to occur at the same time in depression, but an inverse correlation should be observed because the synthetic glucocorticoids that are used to treat inflammatory diseases inhibit the release of proinflammatory cytokines and their synthesis, which results in anti-inflammatory effects[41]. However, no inverse correlations between the concentrations of plasma glucocorticoids and cytokines have been observed in patients with depression. Because the negative inhibitory mechanism of cortisol that prevents increased levels of CRF is impaired in patients with depression, the negative inhibitory mechanism of cytokine secretion in immune cells against increased levels of cortisol is also impaired in these patients. In other words, depression is characterized as a dysfunction of the cortisol feedback inhibitory mechanism to the GR, which is the mechanism for inhibiting CRF oversecretion, and to immune cell receptors, which is the mechanism for inhibiting cytokine oversecretion. These impairments may be directly related to the etiology of depression.

Cytokines and central neurotransmission
Stress simultaneously activates the HPA axis and the sympathoadrenal system (sympathetic nervous system and adrenal medulla). The most important stress response is activation of the noradrenergic (NA) neurons,

which show stress responses through pathways from the locus coeruleus to the cortex, hippocampus, and cerebellum, and from the nucleus tractus solitarius to the hypothalamus. Dopaminergic (DA) neurons display stress responses through the nigrostriatal, mesolimbic, and mesocortical pathways. The mesocortical system, which connects the prefrontal cortex and cingulate, is the most important. The stress responses of the serotonergic (5-HT) system result in significant increases in tryptophan in all brain regions. These increases are not localized to the specific brain areas where 5-HT neurons are present. During stress responses, changes in the metabolism and secretion of neurotransmitters, such as Ach and γ-aminobutyric acid (GABA), are observed. Neuropeptides of the peptidergic system, which include CRF, are also involved in the stress response.

According to the monoamine depletion hypothesis, depression develops as a result of decreased availability of monoamine neurotransmitters, especially 5-HT and NA, in the synapse. Proinflammatory cytokines significantly affect the peripheral and central 5-HT systems. Peripheral injections of IL-1β and TNF-α increase the extracellular levels of 5-hydroxyindoleacetic acid (5-HIAA) in the nucleus raphe dorsalis, and central injection (intracerebroventricular application) of IL-1β, IFN-γ, and TNF-α stimulates the 5-HT transmission in the nucleus raphe dorsalis[42]. Peripheral injections of IL-1 increase NA turnover in the hypothalamus and hippocampus, 5-HT turnover in the hippocampus and prefrontal cortex, and DA turnover in the prefrontal cortex[43]. In an *in vitro* study, IL-1β increased the activity of the 5-HT transporter[44], which has a critical role in 5-HT transmission because it facilitates 5-HT reuptake. If the 5-HT transporter is activated in central 5-HT neurons, the amount of 5-HT removed from the synapses increases, which results in a deterioration of 5-HT-mediated functions. In addition, IL-1β receptors are expressed in 5-HT neurons and IL-1β is synthesized in neurons and glia[45]. IL-1 and IFN-γ increase the activity of IDO, which promotes the metabolism of tryptophan and decreases 5-HT synthesis in the brain[26]. IL-1β acts on 5-HT transporters to increase 5-HT reuptake in the synapse, and the decreased concentrations of serum tryptophan decrease the usefulness of the 5-HT system, which eventually induces depression (Figure 2).

The neurodegeneration hypothesis of depression: Cytokine-5-HT interaction

According to the monoamine hypothesis, patients with depression have a vulnerable 5-HT system. Their 5-HT turnover is increased and then becomes depleted, inducing 5-HT2 receptor up-regulation. The levels of tryptophan, a precursor of 5-HT, in the blood of patients with depression are decreased compared with those of healthy people[46]. Eating tryptophan-deficient foods worsens mood, while the administration of tryptophan improves depressive symptoms[47].

Depression is significantly associated with old age, chronic medical diseases (*e.g.*, coronary heart diseases,

diabetes, Parkinson's disease, stroke, and cancer), and chronic stress. Nevertheless, not all elderly or chronically ill people experience depression. How can these individual differences be explained? One hypothesis that can explain these differences is the neurodegeneration hypothesis: Cytokine-5-HT interaction[47,48].

According to this hypothesis, acute psychological stress triggers tryptophan defects and mood swings. To correct the 5-HT imbalance, 5-HT synthesis and receptor expression are modified. This is the first stage of coping with psychological stress. If the psychological stress is chronic, the levels of proinflammatory cytokines increase. The levels of proinflammatory cytokines also increase in cases of physical stress or chronic diseases. These increases in proinflammatory cytokines trigger an increase in the levels of anti-inflammatory cytokines as a compensatory mechanism in order to maintain balance. This is the second stage. If the balance is not maintained and the levels of proinflammatory cytokines increase excessively, animals show sickness behaviors, while humans show depressive symptoms. The increased levels of proinflammatory cytokines activate the IDO enzyme and accelerate the metabolism of tryptophan to kynurenine. The level of 5-HT in the brain decreases, which further aggravates the symptoms of depression in individuals vulnerable to depression. Through the complicated tryptophan metabolism process, the neurodegenerative quinolinate and the neuroprotective kynurenate are formed in the brain. This is the third stage, which is important for maintaining balance between neurodegeneration and neuroprotection (Figure 2).

Minor neurodegeneration can occur in all people. However, in the elderly population or in individuals with severe stress or chronic diseases, the balance between the proinflammatory cytokines and anti-inflammatory cytokines is lost, and the process of metabolizing tryptophan to kynurenine is accelerated, which lowers the concentration of 5-HT in the brain. If the balance between neurodegeneration and neuroprotection is also lost, neurodegeneration begins. Neurodegeneration in brain regions including the hippocampus and frontal lobe results in cognitive and memory impairments. As a result, the neurodegeneration process inhibits all of the brain strategies that might cope with the stress, which induces depression or treatment-resistant depression. The neurodegeneration process is further deteriorated due to neurotoxicity from cortisol oversecretion from stress-induced HPA-axis activation[49].

The neurodegeneration hypothesis of depression can explain the development of depression in the elderly and chronically ill. In addition, it suggests methods for coping with various stresses in various stages according to the stress intensity and period. In a recent study[49], patients with depression showed a significantly higher tryptophan breakdown index and lower kynurenic acid concentration level compared with those in the normal population. These findings imply that patients with depression exhibit decreased levels of neuroprotection markers, which

supports the neurodegeneration hypothesis.

Cytokines, microglia, and neurogenesis

Cytokines have been reported to promote neuronal differentiation and remodeling in the brain. Accordingly, their roles in neurodegenerative diseases are of interest. The brains of patients with chronic depression show increased cell apoptosis with decreased volumes of the hippocampus, prefrontal cortex, and amygdala and increased ventricular volume. The chances for developing dementia increase accordingly in these patients, and chronic inflammatory responses are thought to be involved in this process[50]. Proinflammatory cytokines reduce neuroplasticity by increasing the levels of quinolinic acid, which is a strong agonist of the N-methyl-D-aspartate (NMDA) receptor[51] (Figure 2).

Stress induces inflammatory responses through cytokine secretion. Cytokines are secreted from peripheral immune cells and central immune cells. Chronic stress activates brain microglia, which secrete cytokines and in turn affect neurogenesis. Neurogenesis is either inhibited or stimulated according to the level of microglia activation[52]. This means that various microglia perform various functions, such as stimulating or inhibiting neurons[52]. Inflammation and cytokines usually directly inhibit neurogenesis. Proinflammatory cytokines, such as TNF-α and INF-α, inhibit neurogenesis through IL-1 regulation[53]. The decline of neurogenesis is prevented by inhibiting IL-1β activity[54], confirming the important role of cytokines in inhibiting neurogenesis in the brain. In contrast, the administration of drugs that inhibit inflammation recovered or increased neurogenesis[55]. In summary, chronic stress promotes cytokine secretion in the peripheral blood and brain microglia, and cytokines affect neurogenesis.

CYTOKINES AND ANTIDEPRESSANTS

The mechanisms of antidepressants relative to cytokines

Stress induces proinflammatory cytokine oversecretion, which results in depressive symptoms. Antidepressants may inhibit the production and function of peripheral and brain cytokines. As mentioned above, the use of antidepressants decreases the levels of proinflammatory cytokines and increases the levels of anti-inflammatory cytokines[30]. These findings imply that antidepressants inhibit cytokine secretion in immune cells and/or antagonize cytokine receptors to improve the depressive symptoms.

How do antidepressants regulate cytokine secretion and improve depressive symptoms? Several hypotheses have been suggested[19,56-58]. First, the changes in peripheral and central cytokines after antidepressant treatment might be secondary results of the neurotransmitter changes that are induced by antidepressants. Stress-induced increases in IL-6 levels are inhibited by pretreatment with propranolol, a β-adrenoceptor antagonist, which

suggests that the IL-6 increases could be mediated by the sympathetic nervous system and increased adrenalin in the adrenal medulla. Immune cells have neurotransmitter receptors, and antidepressants act on these receptors to regulate immune cell activity. T lymphocytes express 5-HT receptors (5-HT1A and 5-HT2A/2C) and high-affinity 5-HT transporters. Macrophages have a 5-HT uptake system that is similar to the system in platelets. Antidepressants can have negative immunoregulatory effects by causing deficiencies in intracellular 5-HT storage, increases in extracellular 5-HT, and blocking 5-HT2A/2C receptors; second, antidepressants restore the cytokine-induced GR resistance. In addition, they restore the inhibition of the negative feedback of the HPA axis and normalize HPA axis function; third, antidepressants inhibit the nitric oxide and PGE2 production that is increased by the cytokines; fourth, antidepressants inhibit IDO activity; fifth, antidepressants directly act on macrophages and lymphocytes to stimulate the production of anti-inflammatory cytokines.

In meta-analyses of 22 types of antidepressants, treatments with antidepressants, especially selective serotonin reuptake inhibitors (SSRIs), result in decreased levels of IL-1β and IL-6[58]. Inflammation increases the activities of the microglial cells and induce astroglial loss, which consequently induces glutamate release and an upregulation of NMDA receptors[59]. The anti-inflammatory effects of riluzole and ketamine, which are glutamatergic modulators, are being studied. Riluzole and ketamine prevent neurotoxicity and relieve inflammation by inhibiting glutamate secretion and modulating NMDA receptors[60].

Promising cytokine-related antidepressants

If cytokines are associated with the pathophysiology of depression, then receptor antagonists that can regulate inflammatory cytokines, anti-cytokine antibodies, and anti-inflammatory cytokines might improve depressive symptoms. Although the therapeutic usefulness of cytokine inhibitors in depression treatment has not been fully investigated, the possibility has been suggested by the results of experimental studies.

The long-term administration of antidepressants in mice results in significant increases in the mRNA levels of IL-1ra in the hypothalamus, hippocampus, frontal lobe, and diencephalon. The learned helplessness that is induced by inescapable shocks is inhibited in mice that were pretreated with IL-1ra[61]. These results suggest that stress-induced IL-1 secretion is a primary cause of the behavioral disturbances shown in the learned helplessness model of depression. CRF receptor antagonists could prevent learned helplessness[62] because the behavioral changes induced by IL-1 occur through central CRF secretion. The cytokine antagonists with a broad action range, such as IL-4 and IL-10, might be more effective than cytokine antagonists, such as IL-1ra, which inhibit specific cytokines, in the treatment of depression. In one study, seven severely depressed patients given low-dose lipopolysaccharide showed

improved symptoms the next day, when the levels of anti-inflammatory cytokines were expected to peak[63]. These mood changes were transient, and the patients' previous conditions returned after several days. TNF-α has most recently drawn attention as a treatment that can change the course of bipolar disorder (disease-modifying treatment)[64]. Current evidence suggests that TNF-α regulates apoptotic cascades that may be associated with neuronal and glial loss in bipolar disorder. TNF-α antagonists, such as adalimumab, etanercept, and infliximab, have been used as therapeutic agents for rheumatic diseases, and are currently being used in clinical trials to treat the depressive episodes of patients with bipolar disorder[64].

The antidepressant effects of anti-inflammatory drugs
If inflammatory responses contribute to the pathogenesis of depression, anti-inflammatory agents are expected to be effective for treating depression. The use of celecoxib, a COX-2 inhibitor, to augment SSRI treatment resulted in better treatment effects compared with the use of the SSRI alone[65]. Celecoxib augmentation therapy can accelerate the treatment responses in depressive episodes of patients with bipolar disorder[66]. In addition, acetylsalicylic acid (aspirin) improves the effects of SSRIs[67]. However, the combination of SSRIs and non-steroidal anti-inflammatory drugs inhibits the effects of the antidepressant. The effects of citalopram, which regulates TNF-α and IFN-γ in mouse frontal lobe, are inhibited by ibuprofen[68]. These laboratory results have been confirmed in clinical trials; the combined use of citalopram and anti-inflammatory drugs resulted in more treatment failures than the use of citalopram alone. These results were not found in subsequent studies[69]. These discrepancies suggest that inflammatory drug reactions vary according to depression subtype.

Eicosapentaenoic acid and docosahexaenoic acid, which are omega-3 fatty acids, can be used to treat rheumatoid arthritis, psoriasis, asthma, and inflammatory bowel diseases because they reduce proinflammatory cytokines. In addition, they can be used as a supplemental agent of antidepressants[70]. Angiotensin receptor blockers, which are hypertension agents, are thought to have anti-inflammatory effects in the CNS. When their mechanism is fully understood, they can be used in depression treatment.

LIMITATIONS

Limitations of the cytokine hypothesis of depression
Many clinical studies have suggested that the neuro-immune activation that results from the production of proinflammatory cytokines is significantly involved in the etiology and pathogenesis of depression. However, the cytokine hypothesis of depression is still controversial due to the following limitations.

First, the cytokine hypothesis states that increased levels of proinflammatory cytokines cause secondary changes, such as neurotransmitter depletion, HPA-axis activation, and the expression of depressive symptoms. Nevertheless, it has not been clarified if the increased levels of proinflammatory cytokines are the cause of depression or are a concomitant phenomenon that maintains the depressive symptoms regardless of the depression etiology. When IFN-α was used in cancer patients as an immunotherapy, the patients developed depression. When the immunotherapy was discontinued or antidepressants were administered, the depressive symptoms improved[20,21]. Accordingly, the proinflammatory cytokines probably function as a causative factor in patients with depression from medical diseases or immunotherapies. However, it is still unclear how the proinflammatory cytokines function as a causative factor in patients with depression that is caused by etiologies other than diseases or immunotherapies[71].

Second, the effects of antidepressant treatments are not always associated with decreased levels of proinflammatory cytokines[72,73]. The effects of antidepressant treatment may not be caused by decreased cytokine release or synthesis but rather by disturbance of the actions of peripheral-released cytokines on the CNS regardless of the concentration of released cytokines. Thus, antidepressants may not directly inhibit immune activation but, rather, indirectly regulate immune functions.

Third, previous studies have shown that the increased levels of cytokines in depression are within a low range compared with those in systemic infection and inflammation. In acute infection, the huge amounts of cytokines are produced act on the brain functions, which often results in the progression of depression. However, in most clinical conditions, such as chronic infection and inflammation, only low amounts of cytokines circulate. Thus, there is a question whether and how low amounts of peripheral cytokines act on the brain and develop depression, even under baseline conditions. The difference in brain function affected by low and high levels of cytokines is another issue. Low levels of peripheral cytokines have a similar effect on sleep-awake behaviors compared with a high dose of peripheral cytokines[74]. On the other hand, low levels of cytokines promote non-REM sleep, but this stage of sleep is suppressed by high level of cytokines[75]. It is still unclear, if the doses have similar effects on depression[74]. Because insufficient research for depression and brain functions in various levels of cytokines, the answer to these questions depend on further experimental studies.

It is unclear whether depression is caused by increased neuroinflammation or vice versa because depression diagnoses are made by examining patients' histories of perceptible symptoms and the intrinsic heterogeneity and various environmental factors of the patients are not controlled. We may consider various aspects of the cytokine hypothesis of depression. These include genetic aspects, role of early life stress and trauma, information on modulators of cytokine activity in depression (diet, obesity, gut health, physical condition, sleep deprivation,

vitamin D deficiency), medical illness, differences of cytokine activities between animal models and human, inflammatory markers in suicide, and the influence of treatments like antidepressant drugs, psychotherapy, and electroconvulsive therapy.

CONCLUSION

Depression is considered a syndrome that includes diverse symptoms and a mental disorder with various causes. No single mechanism that explains every aspect of depression exists. Some depressive symptoms appear in association with the cell proteins that are produced by the complicated intracellular signal transmission of neurotransmitters, such as 5-HT and NE. However, in some cases, childhood stress sustains CRF hyperactivity and increases stress responses in adulthood, which then results in the oversecretion of cerebral CRF and eventually leads to depression[75,76]. In other cases, increases in the levels of cytokines from immune system activation activate the HPA axis and increase neurotransmitter turnover, thus leading to depression. These findings suggest that either various etiologies can be observed at the same time in a single patient with depression or that a specific etiology can be dominant.

From a psychoneuroimmunological point of view, the immune, endocrine, and neurotransmission systems closely interact with each other, and inflammation acts as an allostatic load to disconnect them. Depression can be caused by these functional impairments. The sickness behaviors that are observed under inflammatory conditions are similar to depressive symptoms, and some cytokine treatments lead to depression. These results confirm the association between inflammation and depression. Cytokines including IL-1, IL-2, IL-6, IFN-γ, and TNF-α, and hormones like CRF and glucocorticoid have been suggested as inflammation markers. Inflammatory responses that are thought to affect the synthesis and transmission of neurotransmitters, glucocorticoid resistance, and neurodegeneration/neurogenesis contribute to the onset of depression and inhibit recovery. Although no definitive markers of inflammation have been established, they could at least be used in vulnerable patients with depression. Future studies on the mechanisms of neuroinflammation are expected to help overcome the limitations of the monoamine theory and contribute to finding new solutions for the diagnosis and treatment of depression.

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Gene environment interaction in periphery and brain converge to modulate behavioral outcomes: Insights from the SP1 transient early in life interference rat model

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Abstract

It is generally assumed that behavior results from an interaction between susceptible genes and environmental stimuli during critical life stages. The present article reviews the main theoretical and practical concepts in the research of gene environment interaction, emphasizing the need for models simulating real life complexity. We review a novel approach to study gene environment interaction in which a brief post-natal interference with the expression of multiple genes, by hindering the activity of the ubiquitous transcription factor specificity protein 1 (Sp1) is followed by later-in-life exposure of rats to stress. Finally, this review discusses the role of peripheral processes in behavioral responses, with the Sp1 model as one example demonstrating how specific behavioral patterns are linked to modulations in both peripheral and central physiological processes. We suggest that models, which take into account the tripartite reciprocal interaction between the central nervous system, peripheral systems and environmental stimuli will advance our understanding of the complexity of behavior.

Key words: Gene-environmental interaction; Specificity protein 1; Mithramycin; Stress; Animal-model; Essential amino acids; Tryptophan; Insulin

Core tip: We review the main theoretical and practical concepts in the research of gene environment interaction. We present a novel approach to study gene environment interaction in which a brief post-natal interference with the expression of multiple genes by inhibiting the activity of the ubiquitous transcription factor specificity protein 1 is followed by later-in-life exposure of rats to stress. Finally, we discuss the role of peripheral processes in behavioral responses, demonstrating how specific behavioral patterns are linked to modulations in interwoven brain and body physiological processes due to gene and environmental changes.

INTRODUCTION

The role of nature *vs* nurture in shaping complex behavior and in mental disorders is a matter of long running dispute and creates a split between psychobiology, which emphasizes the dominancy of the being's innate predispositions and psychotherapy supporters, which point out the surrounding influence. Evidence from decades of heredity research has suggested that complex behaviors and psychiatric disorders have a solid genetic basis[1,2]. It has been reported by many studies that consistent differences in behavioral traits between subjects, such as stress responsivity and temperament, show a familial pattern[3]. On the other hand, the impact of environmental factors on physical illness is well established[4,5] and well recognized in behavioral disturbance[6,7]. Numerous studies reported a correlation between candidate genes, and behavioral phenotype[8,9], yet with significantly lower rate of replication and a clear tendency toward positive results[10]. Environmental aspects are formulated in the vast psychoanalytical literature and in models that use a scientific platform, such as the impact of different nursing abilities of female rats, on stress responsivity of their pups[11]. Environmental physical factors such as intrauterine inflammatory reaction induced by Lipopolysaccharide, which simulates the impact of prenatal infection on behavior[12,13] were studied as well.

The stress diathesis theory, which suggests that disorders induced by the combination of varying degrees of predisposition with invers degrees of stressful stimuli, has become an accepted conceptual research framework for studying complex behaviors. Following this hypothesis numerous studies over the last decade assessed the relationship between candidate genes and behavior in the form of genome-wide association studies (GWAS)[14], most of them focusing on the pathologic consequences of genetic alteration. Despite the remarkable advances in genetic tools and techniques, very few direct genetic effects on mental health have been identified and replicated[10].

An alternative paradigm to the stress diathesis theory is the differential susceptibility theory, which assumes that individuals react differently to environmental stimulus depending on the plasticity of gene rather than their susceptibility. Thus showing higher responsiveness to both positive and negative external cues, frequently with opposing outcomes[15,16]. For example, high frequency of antisocial behavior was correlated with childhood maltreatment the in low activity MAO-A allele carriers[17]. Interestingly, low activity MAO-A allele carriers, who

were not exposed to childhood maltreatment showed the lowest anti-social behavior scores compared with normal activity MAO-A carriers[16]. An additional extensively studied genetic variant in psychiatry is the short allelic form of the serotonin transporter-linked polymorphic region (5-HTTLPR), which is associated with a reduction in the transporter activity[18] and with high risk for major depression in individuals exposed to stressful life events[19]. Yet, 5HTT s-allele carriers were shown to be more responsive to the Attention Bias Modification training than long-allele carriers, supporting Belsky's hypothesis that the s-allele may be considered as a plasticity gene rather than a susceptible gene[20]. Taken together, these examples support the differential responsiveness theory of sensitive genes in the etiology of complex behaviors[16].

Unfortunately, the current gene environment interaction models have substantial limitations, ranging from weak validation to poor predictive power and although genome studies have expanded our understanding of complex phenotypes and many human diseases, they hardly explain a small proportion of their heritability in the population[21]. Genetic variants are usually considered as having additive, suppressive or neutral effects on the phenotype, but the effect size for a single genetic variant is minor[22]. A more comprehensive model of real life interaction between multiple genes that influence the expression of each other and thereby the manifestation of a particular phenotype, is needed.

One possible gate for modelling real life gene environment interaction are through manipulation of key point genes, genes that are essential for the modulation of multiple genes' activity, such as the ubiquitous transcription factor specificity protein 1 (Sp1). Sp1 is a member of the SP proteins family, which constitutes a group of highly conserved transcription factors present in a wide range of organisms. Their structure is defined by the presence of three highly conserved DNA-binding zinc finger domains which bind to similar, yet distinct, GC-rich target sequences. Members of the SP family function either as activators or repressors in cell *via* a promoter-dependent manner[23]. Sp1 is essential for the regulation of various physiological functions, maintains organ homeostasis, regulates tissue repair, and possibly serves as an anti-inflammatory mechanism that protects against organ inflammation and injury[24]. Sp1 regulates the expression of numerous genes in early developmental stages[25] and the expression of most growth factors and their receptors depend on Sp1[26-28]. Sp1 activity can be modulated by various environment-dependent factors including metabolic factors such as glucose and insulin, immunologic factors such as tumor necrosis factor-alpha (TNF-α), glucocorticoid receptors and several major kinases including CDK2 and ERK1/2[29-32]. Sp1 also modulates the expression of many genes implicated in psychiatry research, including neuregulin-1[33], reelin[34], GAD67[35], MAO A and B[36,37], NMDA receptor subunits (NR1 and NR2)[38,39], GABA A receptor[40], DA receptors[41] and genes of the oxidative phosphorylation

system (OXPHOS)[42]. In this article we will describe how simulating real life by minor manipulation of multiple gene expression, based on Sp1 unique characteristics, models more accurately gene environment interaction in behavioral sciences.

SP1 IN SCHIZOPHRENIA AND OTHER NEUROPSYCHIATRIC DISORDERS

The alterations in different genetic trajectories in schizophrenia reported by numerous studies[43], can result from transcriptional dysregulation in the disorder[44]. Our group showed that the expression of Sp1 is disrupted in brain samples and peripheral blood lymphocytes of schizophrenia patients as compared with those of healthy subjects. Specifically, downregulation in Sp1 mRNA expression was prominent in the prefrontal cortex and the striatum, while upregulation was observed in the parieto-occipital cortex and in blood lymphocytes of schizophrenic patients. Sp1 levels were highly and significantly correlated with two subunits (NDUFV2 and NDUFV1) of the first complex of the OXPHOS in lymphocytes and brain specimens of normal subjects, while abolished in schizophrenic patients[45]. We have shown that Sp1 is a transcription factor of both subunits, which have been repeatedly implicated in schizophrenia[46,47]. A defect in Sp1 transcriptional activity, which leads to abnormal expression of complex I subunits, can be one of the causes for reduced complex I activity associated with mitochondrial dysfunction and reduced energy metabolism observed in schizophrenia brains by numerous imaging studies[48]. Such distortion in brain energy production can affect synaptic plasticity and connectivity of neuronal networks and thereby cognitive and emotional behaviors[49]. Additional studies by other groups substantiated the role of Sp1 in mental and neurological disorders by showing a reduction of Sp1 protein and mRNA in the postmortem prefrontal cortex brain of chronic schizophrenia patients[50], increased Sp1 mRNA levels in the hippocampus[51] and a reduction in Sp1 and Sp4 protein levels in lymphocytes of first-episode psychosis patient[52]. These reports are in line with other studies demonstrating arole of Sp1 in the regulation of many genes associated with neuropsychiatric psychiatric disorders. Thus, elevation in Sp1 protein levels was observed in autistic brains, which was associated with altered expression of autism candidate genes such as OXTR and PTEN[53]. Sp1 mRNA and protein was also found to be up-regulated in Alzheimer's disease (AD) brains and in a transgenic mice model of the disease[54]. In Huntington disease, Sp1-regulated huntingtin transcription is dysregulated. In adrenal medulla-derived PC12 cell cultures it was shown that Sp1 is involve in the regulation of epinephrine biosynthesis in response to acute and chronic stress[55]. The, dual characteristic of Sp1, having specific environmental and internal signal regulated transcriptional activities, together with its role in the regulation of multiple genes, coincide with the multi-gene alteration and the heterogeneous symptomatology of mental disorders.

SP1 MANIPULATION MODELS

Complete inhibition of Sp1 is incompatible with life and Sp1 knockout mice die *in utero* with multiple phenotypic aberrations[56]. However, Sp1 transcriptional activity can be inhibited by Mithramycin (MTR)[57]. MTR is an antineoplastic antibiotic, which binds to GC-rich regions on the DNA displacing the Sp family transcription proteins from their binding sites[58]. MTR is a clinically approved antibiotic that is effective for the treatment many cancers such as testicular cancer[59] and also for cancer induced hypercalcemia[60]. Our group has reported that MTR induced a time dependent decrease in the expression levels of complex I subunits NDUFV1, NDUFV2 and NDUFS1, as well as of reelin, all regulated by Sp1 and implicated in schizophrenia[45]. The availability of a simple pharmacologic agent that modulates the transcription of different genes, turns it into an attractive tool for modelling multiple gene dysregulation. Indeed, neonatal rats treated for a few days (7-10 postnatal days) with MTR, showed three months later cognitive and behavioral deficits such as spatial working memory impairment and anxious behavior, without any impact on their bodily well-being[61]. The effect of MTR treatment was also studied in AD experimental models. Thus, MTR injections to AD transgenic adult mice for several months, resulted in greater memory impairment in these mice and increased amyloid β peptide levels[62], with no additional behavioral differences. These data suggest that manipulation of Sp1 transcriptional activity at adulthood has long lasting effects on behavior depending on predisposing genetic aberration earlier in life. In contrast to these results chronic MTR administration to AD transgenic mice by another group resulted in cognitive improvement[63], emphasizing the need for better understanding the role of Sp1 transcriptional activity in the pathophysiology of AD. In a mouse model for Huntington disease chronic MTR treatment from PND 20 throughout life extended survival, enhanced motor performance, and improved brain histopathology[64]. The neuroprotective effect of MTR was also demonstrated in adult rats exposed to repeated administration of methamphetamine[65], an accepted model for schizophrenia[66].

STRESS EXPOSURE MODEL

Studying the additive effect of environmental variables on top of the predisposing susceptibility is complex and may have many bias pitfalls. Stress is commonly used to mimic environmental insults in models of mental disorders and complex behaviors. We have used peripubertal mild unpredictable stress protocol. One major parameter in modeling environmental effects is the timing of exposure to insult. However, timing and duration of exposure to stress differ between studies. There are early in-life stress models, mainly maternal separation, which increase

stress reactivity in the offspring[67], while there are adult stress models, including the unpredictable chronic mild stress model, which differ in chronicity, protocol elements and actual age of stress exposure, adolescence or adulthood[68-70]. Both prenatal period and postnatal mid to late adolescence were shown to be particularly vulnerable to stress in rats[61]. Chronic adolescence stress was repeatedly shown to be associated with HPA dysfunction[71], hippocampal volume reduction and impairments in spatial learning[72] later in life. To elaborate our view on the impact of environment we compared two stress regimens differing only in duration, chronic and sub-chronic regimens, in adolescence. Interestingly, high serum corticosterone levels and higher anxiety index were related to the sub-chronic stress regimen, while rats exposed to chronic stress did not differ significantly from the controls, which implies adaptation to stress[61]. Although chronic mild stress is an accepted paradigm for induction of depressive-like symptoms in rats[73], several studies show resilience effects of long-term stress[74,75] which is in line with the adaptation to the chronic stress regimen.

MANIPULATION OF GENE EXPRESSION AND THE ENVIRONMENT

Studies modeling genetic predisposition for behavioral alterations, induce predisposition in one or more of the four following paradigms: Manipulation of a candidate gene, interference with a candidate system/pathway, intrauterine insults or exposure to early post-natal stressors that induce epigenetic changes.

Numerous studies using candidate gene knockout mice and chronic stress were published. Candidate system interference studies mostly involve HPA axis manipulation either pharmacologically by glucocorticoids administration[76] or induced by early life stress[77]. Examples for intrauterine insult models include the prenatal protein malnutrition, which affects development of the brain in utero and induces cognitive impairment and severe widespread morphological abnormalities similar to schizophrenia[78,79]. Other models are based on the intrauterine infection theory for schizophrenia[80]. These models include prenatal exposure of mice to viruses, such as the influenza virus[81] which cause brain developmental damages similar to those observed in schizophrenia brain, or maternal immune activation by lipopolysaccharide or polyinosinic:polycytidylic acid (Poly I:C) during pregnancy, which model schizophrenia and autism in the offspring[82,83]. The best studied model for epigenetic changes induced by early life stressors is the maternal separation model, which enhances behavioral changes[84,85], and causes epigenetic modifications that can be transmitted through generations[11]. We hypothesize that a transient interference with the expression of many various genes, by MTR for example, at a critical developmental stage of the brain together with an exposure of the animal to stressful environment later

in life, will provide an animal model to study the role of gene environment interaction in long lasting complex behavior relevant to mental disorders. Although it may be argued that modification of the expression of numerus genes is inaccurate and difficult to monitor, we believe that it is a closer model to real life complexity. Indeed, we found that MTR treated rats exposed to sub-chronic stress demonstrated higher anxiety index, anhedonia and indifference to novel objects. However, MTR treated rats exposed to the chronic stress paradigm demonstrated normal sucrose preference, low anxiety index and high novelty seeking behavior. These findings support the differential sensitivity theory, claiming increased reactivity to environmental stimuli in genetically sensitive individuals, with differential responses to various stimuli[16].

INTERTWINED PERIPHERAL AND BRAIN INTERACTION

The molecular and biochemical pathways that contribute to behavioral phenotypes are still a mystery and it is almost impossible to differentiate between genetic and environmental impacts. The currently common dominate hypothesis is that changes in brain cellular pathways are responsible for alterations in behavioral responses. We and others suggest that peripheral factors are essential for formulating behavioral responses. In our rat model for example, we showed that exposing MTR treated rats to chronic stress (MTR + stress) caused a significant reduction in tryptophan brain levels, which in part stems from peripheral changes. Alteration in peripheral tryptophan levels was found to be associated with behavioral and cognitive phenotypes. For example, aggression tendencies associated with a low serum tryptophan levels[86] and impulsivity[87] was observed in the course of manic episodes[88], while increased serum tryptophan levels were observed during the recovery periods in bipolar manic patients[89]. Tryptophan depletion studies have reported association with worsening of depressive symptoms in human, yet the data are inconclusive[90,91]. In addition, it was reported that a reduction in tryptophan levels interrupts memory consolidation yet improves attention[92]. Dietary tryptophan depletion is also used in modeling major depression in rats[93] and dietary prenatal protein deprivation is used to model cognitive impairment observed in schizophrenia[78]. In our model, the reduction in brain tryptophan in the MTR + stress rats was probably not due to its extensive metabolism in brain, as no change was observed in its two major metabolic pathways the serotonin and kynurenine pathways[94]. However, being an essential amino acid tryptophan level in brain depends also on its availability, which can be modulated by several variables including its serum level and its BBB transporter (LAT1) activity[95]. Serum level of amino acids, which compete with tryptophan on its transporter, the branched chain amino acids (BCAA) for example[96,97], can affect tryptophan availability to the brain. Indeed, serum tryptophan/BCAA ratio is an established measure to
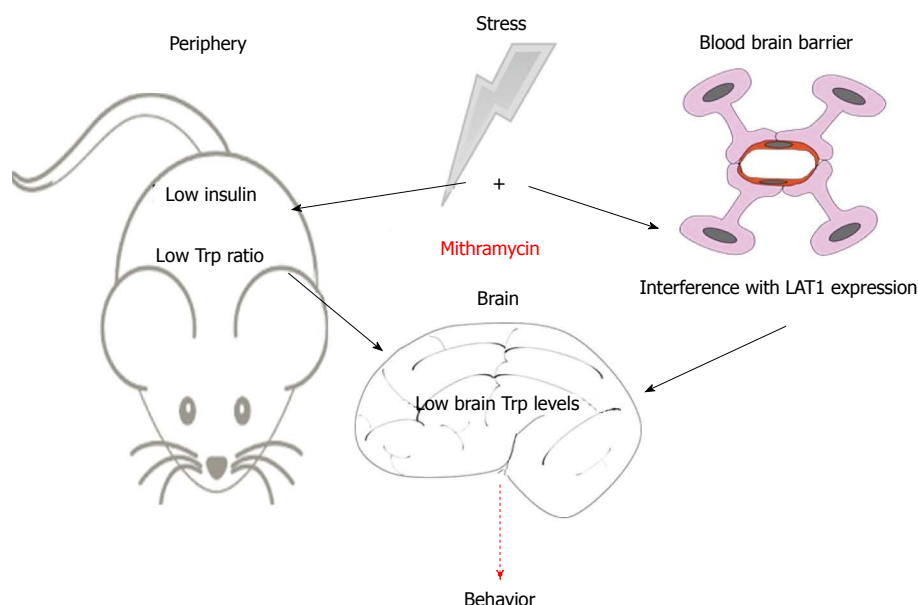
Figure 1 Brain and periphery combined effects modulate behavior in the specificity protein 1 rat model. Early in life transient interference with specificity protein 1 activity by mithramycin and later in life exposure to chronic stress, affect availability of tryptophan (Trp) to the brain, both by reducing serum Trp ratio and brain LAT1 expression. Deficits in brain Trp levels may affect behavior. LAT1: BBB transporter.

estimate brain tryptophan levels[98]. In the MTR + Stress rats, reduced tryptophan brain levels were associated with reduced LAT1 protein levels and its light chain SLC3A2 transcript levels. In addition, we observed a reduction in serum tryptophan/BCCA ratio, implying a peripheral contribution to reduced brain tryptophan levels. We further suggest that tryptophan/BCCA reduction is due to a failure of these MTR treated rats to respond to stress by increasing serum glucose and insulin, a known regulator of serum BCAA[99], as did rats exposed to chronic stress only. Taken together, these data suggest that interference with brain tryptophan homeostasis is due to joint brain and peripheral physiological processes. In line with the latter is the finding that brain tryptophan levels were only affected in rats receiving the combined treatment of MTR + stress, while serum tryptophan/BCAA ratio or brain LAT1 were affected by either Stress or MTR, respectively[94]. Our data suggest that a mild modulation of both peripheral and central processes, which converge and mutually interact, can influence behavioral phenotype. A similar interaction can be seen in circuits of energy balance regulation in the body. Thus, adipose tissues secrete leptin as an afferent signal, which influences the activity of the hypothalamus. The hypothalamus signals decrease food intake by inhibiting anabolic circuits, and enhance energy expenditure through the activation of catabolic circuits[100]. It is quite intuitive, but sometimes neglected, that the brain collects both central and peripheral internal inputs, as well as external inputs and executes reaction based on the sum of predisposition and experience. The recent increasing interest in the link between microbiome and brain function and its role in mental disorders[101] further substantiates a role for peripheral inputs in behavior.

CONCLUSION

The ubiquitous transcription factor Sp1 plays a role in the regulation of many genes in response to internal and environmental signals and is suggested to have implication in neuropsychiatric disorders and complex behaviors. Using simple manipulation of Sp1 we showed that a wide and transient interference with gene expression in inbred rats at a critical developmental stage, can induce a long lasting impact on metabolic and behavioral response to environmental stimuli, with different and even opposite outcomes, depending on the characteristics of the environmental stimuli/insult. Already at 1963, Bleuler[102] wrote that "unfavourable nature and environment develop together and influence each other. They are interwoven from babyhood. The environment influencing the manifestation of the hereditary disposition is already a reflected image of this disposition"[102]. Peripheral and central physiological processes, which are both subjected to genetic and environmental changes, interact reciprocally to induce specific behavioral patterns (Figure 1). Further studies could shed light on the importance of these brain-periphery reciprocal interactions for whole body homeostasis and its influence on behavior. In addition, new targets may emerge from such a perspective of behavioral modulators for future clinical intervention.

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ORIGINAL ARTICLE

Basic Study

Ecological Momentary Assessment with smartphones for measuring mental health problems in adolescents

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Abstract

AIM
To analyze the viability of Ecological Momentary Assessment (EMA) for measuring the mental states associated with psychopathological problems in adolescents.

METHODS
In a sample of 110 adolescents, a sociodemographic data survey and an EMA Smartphone application over a one-week period (five times each day), was developed to explore symptom profiles, everyday problems, coping strategies, and the contexts in which the events take place.

RESULTS
The positive response was 68.6%. Over 2250 prompts about mental states were recorded. In 53% of situations the smartphone was answered at home, 25.5% of cases

they were with their parents or with peers (20.3%). Associations were found with attention, affective and anxiety problems ($P < 0.001$) in the participants who took longer to respond to the EMA app. Anxious and depressive states were highly interrelated (rho = 0.51, $P < 0.001$), as well as oppositional defiant problems and conduct problems (rho = 0.56, $P < 0.001$). Only in 6.2% of the situations the subjects perceived they had problems, mainly associated with inter-relational aspects with family, peers, boyfriends or girlfriends (31.2%). We also found moderate-high reliability on scales of satisfaction level on the context, on positive emotionality, and on the discomfort index associated with mental health problems.

CONCLUSION
EMA methodology using smartphones is a useful tool for understanding adolescents' daily dynamics. It achieved moderate-high reliability and accurately identified psychopathological manifestations experienced by community adolescents in their natural context.

Key words: Ecological Momentary Assessment; Mental health problems; Smartphone; Coping; Adolescents

Core tip: Adolescence is a stage of life characterized by a great many changes. If they are not coped with effectively, these changes may trigger mental health problems. Among the range of methodologies used to assess the impact of daily problems, Ecological Momentary Assessment allows the recording of mental microprocesses and fluctuations as they happen. We found anxious and depressive states were highly interrelated, as well as oppositional defiant problems and conduct problems in daily life. This methodology based on mobile technology using smartphones is a useful tool with high viability for measuring psychopathological mental states in adolescents in their natural context.

INTRODUCTION

Adolescence is a stage of life characterized by a great many changes which, if not addressed effectively, may trigger problems of mental health[1-5]. There is a substantial body of literature assessing the possible impact of individual everyday problems on the development of mental health disorders[6].

One of the formats used to assess the impact of everyday problems is Ecological Momentary Assessment (EMA). This methodology allows the recording of the expression of mental microprocesses and their fluctuations in several situational contexts as they happen[7]. Its applicability has been demonstrated in a variety of populations in studies of general health[8].

Specifically, in adolescent samples, EMA has been used to assess or predict health needs[9], mental status[10] emotional instability[11], drug use[12], stress associated with traumatic events[13], and anxiety problems[14].

In fact EMA has been used for decades[7], but reports of its use in the field of mental health are relatively recent. At present there is no evidence of its use in adolescent community populations to measure broad areas of psychopathological symptoms (sadness, anxiety, somatic, thought, behavioral and attentional problems) in real time in their natural setting, focusing on the coping strategies used and their relation to their situational and personal contexts.

The aim of this study is to explore the viability of EMA for measuring the mental states associated with psychopathological problems in adolescents, taking into account the situational context and the coping strategies they apply.

MATERIALS AND METHODS

Participants
The sample was constituted initially by 110 adolescents from the province of Barcelona, of whom 101 successfully completed the EMA study. Following the recommendations of previous researchers regarding the quality of information[6,15], only subjects who completed at least 12 of the 35 possible recordings (roughly 33%) were considered.

Instruments
Sociodemographic data: A survey sheet was created *ad hoc* to gather basic data on age, gender, school year, citizenship, family and employment status.

EMA
Each participant received a smartphone with an Android-based EMA application already installed. This application was programmed to give a series of alarms associated with the task of answering mini-questionnaires at five semi-random moments over the course of the day between 9 am and 9 pm for a complete week. The mini-questionnaires comprised 21 items (five with multiple choice answers, two with yes/no answers, 14 to be rated on a 5-point Likert scale) which participants were required to answer within three minutes of hearing the first alarm. If the user did not start to answer within this period of time the application stopped the smartphone alarm and blocked the unit.

Users could not delay their answers beyond five minutes after their last interaction with the application, or take longer than ten minutes to answer the entire mini-questionnaire, in order to ensure that the information was instantaneous and not retrospective. If users were unable to answer within these time limits the application
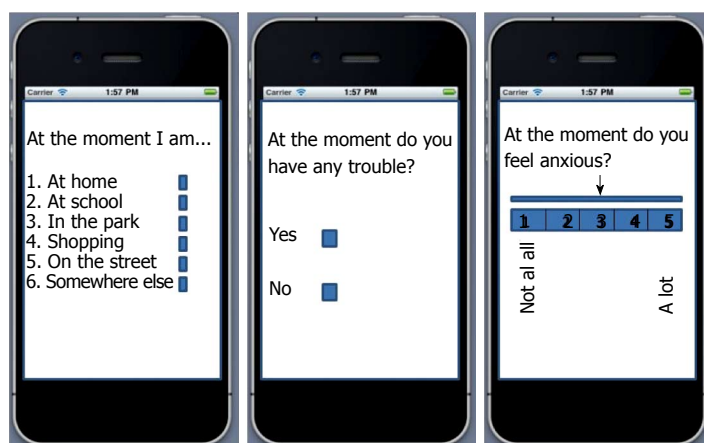
Figure 1 Example of questions asked within Ecological Momentary Assessment app in this study.

blocked the smartphone and they were told to wait until the next random alarm; this reply attempt was considered empty.

These mini-questionnaires comprised questions regarding situational context and broad areas associated with psychopathological problems such as behavior problems, anxiety, sadness, lack of concentration, and so on. It also covered everyday problems and how to cope with them. Figure 1 shows an example of the questions asked.

The application stored the data collected for a week (7 d). Once the evaluation period finished, the data were downloaded by the research team. As a result, a maximum of 35 moments of semi-random evaluation were obtained from each subject. Figure 2 displays the EMA cycle used in this study.

Procedure

Several social service centers and schools in the metropolitan Barcelona area and its surroundings were contacted. Two centers agreed to participate after a full explanation of the project and its logistic implications with regard to the distribution and application of EMA methodology with smartphones. After obtaining the centers' approval, 30-min information sessions were arranged for students from eighth to eleventh grade. In these sessions, students received explanations of their role in the study and the implications of their participation. Informed consent forms were then distributed among the participants for authorization, either by the participants themselves or by their parents and/or tutors. All the procedure and assessment protocols had been previously authorized by the research ethical committee of the University, and complied with the guidelines of the Declaration of Helsinki and legislation regarding confidential data protection.

After obtaining written consent, meetings were arranged for explaining the instruments and the workings of the smartphone devices and EMA app functioning. On receiving the smartphone, participants were assigned an individual alphanumeric code to protect their identity in case of loss or theft of the device. They were informed that they would have the phone for a whole week and that it would ring five times a day at semi-random times,

and that they should answer as often as possible. They were also asked as well to sign a commitment to take good care of the smartphone and received an information sheet explaining how the smartphone functioned and how to answer and containing contact data in case of any technical problem during the experiment.

Statistical analysis

The χ^2 test was used to calculate differences between proportions in frequencies, the *t*-student test to calculate differences of means between two groups, ANOVA for comparisons between several subgroups with Bonferroni correction, and Spearman correlations to calculate associations between variables.

RESULTS

Socio-demographic data

The sample comprised 101 adolescents (age M = 14.68 ± 1.64; 61% women). Sixty percent were Spanish natives and 40% were foreigners (28% were Latin Americans). Regarding family structure, between 67% and 71% of participants' parents were married and between 21% and 23% separated or divorced. As for parents' employment, 77% of fathers were employed and 70% of mothers.

EMA answer rates

In all, 68.6% of questionnaires were completed, while in 31.4% data were missing: Ignored alarms accounted for 23.9% of the missing data (due to a lack of time to answer or not paying attention), rejected answer attempts for 4.5%, and incomplete records not quantified in the analysis for 3.0%. The difference between the types of answer was significant (χ^2 = 3712.60; $P < 0.001$). The mean time taken to answer the questionnaires was 80.6 s ± 56.5 s. These data suggest a mean answer time of between one and three minutes.

Contextual variables

Variables about the context (Where, who with, and what were they doing?). In 53% of situations the smartphone was answered at home, followed by 24.3% at school and 15.5% outside in the street. There were
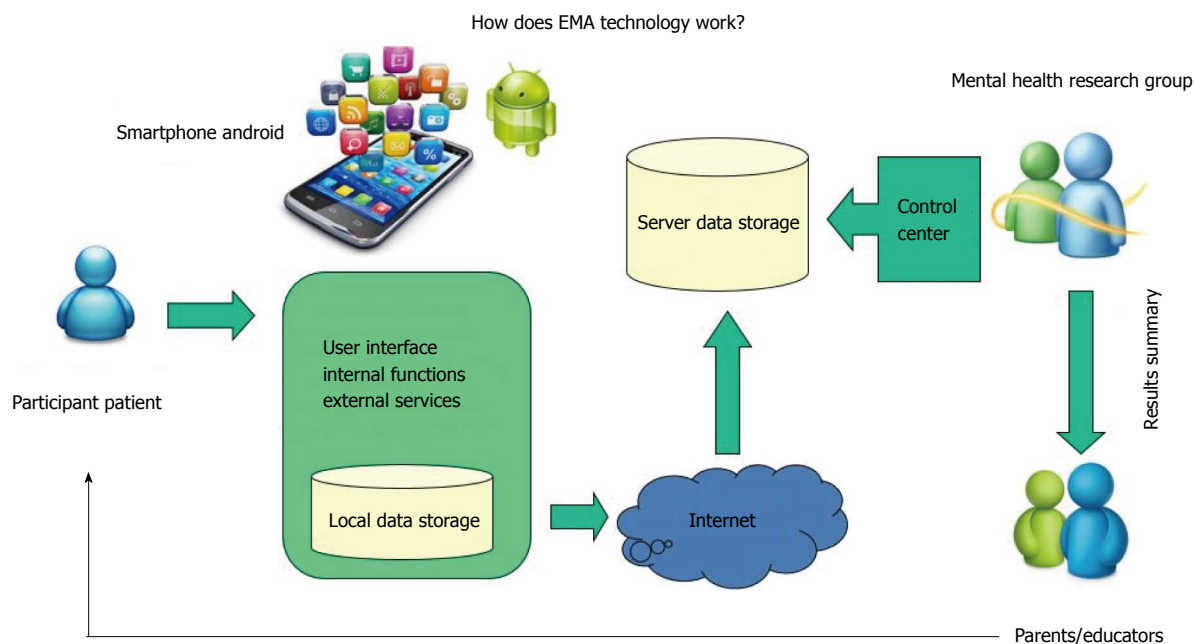
Figure 2 Assessment cycle used in this study with Ecological Momentary Assessment methodology.

significant differences between these three response contexts ($\chi^2 = 1072.38$; df = 2; $P < 0.001$). On a 1-5 point Likert scale, in 69% of cases the situational context was assessed as pleasant (a score of 4) or very pleasant (a score of 5). Only in 11.2% of the situations was the context unpleasant or very unpleasant (scores of 1 or 2).

Regarding the people being with the adolescents at the time of assessments, in 25.5% of cases they were with their parents, in 20.3% with peers and in 19.1% alone. There were significant differences between these three groups ($\chi^2 = 25.28$; df = 2; $P < 0.001$). The company was regarded as pleasant or very pleasant in 77.9% of situations and unpleasant or very unpleasant in only 7.1%.

The three activities that participants were most frequently engaged in at the time of answering the EMA questionnaires were school tasks or activities (26.7%), talking to somebody (19.7%), and watching TV or the computer (16.8%). There were significant differences between these three activities ($\chi^2 = 58.11$; df = 2; $P < 0.001$). The adolescents regarded the activities they were engaged in when they answered the smartphone as pleasant or very pleasant in 64% of cases and as unpleasant or very unpleasant in only 8.9% ($\chi^2 = 113.49$; df = 3; $P < 0.001$). The momentary level of satisfaction associated with the context (physical, relational and activity-related) composed by three items, obtained a Cronbach's alpha of 0.75.

Momentary emotional status and behaviors
In a collection of around 2250 responses obtained from the participants during the week, it was found that in most of situations, these people reported the absence of problems associated with the following

symptoms: Affective (79%), anxious (78.2%), somatic (84.3%), inattentive (81%), oppositional (93.7%), aggressive (95%), obsessive-compulsive (82.6%) or traumatic (93.6%). All these situational problems were significantly absent ($P < 0.001$). Regarding the intensity of problems according to gender, girls presented more affective ($t = -9.077$; $P < 0.001$), anxious ($t = -4.808$; $P < 0.001$), somatic ($t = -6.603$; $P < 0.001$) and post-traumatic symptoms ($t = -4.040$; $P < 0.001$) than boys. Boys presented slightly more inattentive-hyperactive problems ($t = 2.046$; $P = 0.041$), but there were no significant differences in other disruptive behaviors or obsessive-compulsive problems in daily life. All eight items constituting the general discomfort index associated with momentary mental health problems obtained a Cronbach's alpha of 0.76.

It was also observed that subjects who presented strong situational problems of inattention, sadness or anxiety, they also took longer to respond (120 s ± 78 s for inattention; 111 s ± 57 s for sadness; and 122 s ± 79 s for anxiety) than subjects who did not present these problems (75 s ± 52 s in absence of inattention, 76 ± 53 s in absence of sadness and 75 s ± 53 s in absence of anxiety). The difference was significant ($F = 37.15$; df = 4; $P < 0.001$ for inattention; $F = 28.31$; df = 4; $P < 0.001$ for sadness; and $F = 31.33$; df = 4; $P < 0.001$ for anxiety). Bonferroni's post-hoc results showed that significant differences appeared essentially between subjects who did not have these problems and subjects who had them with a certain degree of intensity.

With regard to situations associated with positive emotionality, in particular to feeling happy or loved, subjects reported being happy or very happy (64.2% of cases) and feeling loved or loved very much (66% of cases). There were significant differences in comparison

Table 1 Mental health symptomatology areas measured by Ecological Momentary Assessment in boys and girls								
Variables	1	2	3	4	5	6	7	8
Affective problems	--	0.38	0.26	0.33	0.15	0.11	0.2	0.32
Anxiety problems	0.57	--	0.23	0.35	0.18	0.14	0.27	0.18
Somatic problems	0.22	0.23	--	0.14	0.12	0.09	0.20	0.10
Hyperactivity-Inattention problems	0.39	0.38	0.18	--	0.22	0.21	0.34	0.28
Oppositional-defiant problems	0.27	0.30	0.19	0.29	--	**0.58**	0.29	0.31
Conduct Behavior problems	0.27	0.31	0.20	0.27	**0.56**	--	0.16	0.30
Obsessive compulsive problems	0.25	0.34	0.22	0.31	0.31	0.32	--	0.31
Posttraumatic stress problems	0.34	0.36	0.19	0.34	0.47	0.46	0.39	--

Above the diagonal, boys' results, below the diagonal, girls' results. All the results are significant ($P < 0.001$). In bold-face, the correlations with high-moderate index.

with unhappy situations ($\chi^2 = 978.15$; df = 4; $P < 0.001$) or situations in which one does not feel loved ($\chi^2 = 1101.58$; df = 4; $P < 0.001$). The intensity of positive emotionality of these two items obtained a Cronbach's alpha of 0.81 in this study.

Anxious and depressive states were interrelated in the whole sample (rho = 0.51; $P < 0.001$), and oppositional/defiant problems were associated with conduct problems (rho = 0.56; $P < 0.001$). Table 1 displays the relationship between problems associated with mental health symptoms by gender. Specifically, girls presented strong relationships between anxiety and affective problems, oppositional/defiant problems and conduct problems, posttraumatic stress problems and disruptive behaviors, while in boys the only strong relationship observed was between oppositional/defiant problems and conduct problems.

Everyday problems and coping strategies
Only in 6.2% of the situations in which they were asked to answer the mini-questionnaire with the smartphone did the subjects perceive they had a problem. The most common problems, in order of frequency, were those associated with inter-relational aspects with family, peers, boyfriends or girlfriends (31.2%), followed by schoolwork or worrying about exams (29.1%), and arguments or behavior problems (9.9%). Significant differences were found between these three types of everyday problems in adolescents ($\chi^2 = 14.80$; df = 2; $P = 0.001$).

The most frequently used kind of coping strategy when a problem appeared was seeking relaxing diversions (21.5%), followed by trying to see the positive side of the situation (20.1%) and then seeking help from peers (16%). The least used strategies were looking for help in the family (6.3%) and seeking spiritual support (1.4%). Significant differences were found in the types of coping ($\chi^2 = 38.23$; df = 7; $P < 0.001$).

Regarding their satisfaction with their coping strategies, on more than a third of occasions (35%) adolescents felt neither happy nor unhappy with the strategy chosen to face a specific problem. In 11.2% of cases the subjects were discontent with their choice

of strategy, but in 28% they felt very satisfied. There were differences in the appreciation of the application of coping strategies ($\chi^2 = 35.92$; df = 4; $P < 0.001$), in that case the majority believed them to be effective.

DISCUSSION
The most relevant result in this study is the finding that in most situations in daily life (between 78% and 93%) adolescents do not present problems that trigger mental health symptoms. When these problems appear, they tend to be closely related to states of sadness or anxiety. Similarly, oppositional defiant behaviors were associated with conduct problems, a finding that corroborates the syndromic daily patterns associated with the two broad areas of symptoms (internalizing/externalizing) regularly found in the field of child and teenager psychopathology[16-18]. It was also observed that the positive response rate of the EMA app (68.6%), and the time taken (about 1-2 min) reflect an accurate measurement of associated context, the broad areas of clinical symptomatology and coping strategies in real time. We also found moderate-high reliability on scales of satisfaction level on the context, on positive emotionality, and on the discomfort index associated with mental health problems. These results show that EMA methodology based on mobile technology offers high viability for measuring mental health states in adolescents.

Among the contextual variables, we stress that during the week-long EMA, the adolescents were regularly at home when answering the smartphone application. This result should be borne in mind, due to the contextual relation of family dynamics in the promotion and development of symptomatology or solutions to everyday problems.

For their part, the results regarding satisfaction in the immediate context show that in most cases adolescents feel satisfied with the surrounding environment (in this case their home). Moreover, in relation to variables of interpersonal contact, it is interesting that the people with whom they have the most contact are their parents, followed by their colleagues or peers: This finding may challenge the concept of adolescence as a stage in which

subjects prefer to be with their peers or alone[4,5].

Nevertheless, this does not mean that they prefer to be with their parents, because the adolescents can share a great deal of time together. Perhaps this result reflects the fact that our adolescents regularly answered the application at home (53%), where they are more likely to record direct contact with their parents. However, this shows us that the parents may play a significant role in their children's development of functional patterns of psychological adaptation *via* this continuous contact during the adolescent stage, as stated previously by other authors[19]. In adolescent-parent relationships related to parenting styles, it is highlighted the importance of authoritative homes (where parents are both demanding and responsive) with more psychosocial and academic competence[5], that prevent some potential risk situations such as a problematic drug use[3], internalizing/externalizing symptoms[4] or victimization problems[1].

When studying daily patterns it is important to bear in mind that the context and the people with whom we are in contact have a strong effect on the activities we perform. Being in tune with the evolutive stage and the main function to be developed by adolescents at these ages. The adolescents reported that when they were had to answer the questionnaire, in more than a quarter of the cases they were doing school homework, followed by talking and leisure activities (TV-PC). Here the level of pleasure was influenced considerably by their interaction with the place and the people present[4]. Being the homework an activity regularly imposed, they reflect their perception of not being completely satisfied with this activity development, not necessarily meaning extreme dissatisfaction level, in this contextual activity.

In relation to emotional states, momentary symptomatology was broadly absent in the different areas studied (affective, anxious, somatic, inattentive, oppositionist, aggressive behavior, obsessive-compulsive problems, and trauma situations).

In general, the low results for impairment obtained reflect the type of population studied (*i.e.*, a community population). Higher reports of impairment would be likely if the assessment had been applied in a clinical sample[6,20]. The intensity of impairments may also depend on the level of clinical assistance received (outpatient, day hospital or inpatient). These hypotheses should be verified in further studies of clinical samples of adolescents with psychiatric disorders.

The perception of everyday problems and the use of coping strategies is an interesting result. Only rarely did the adolescents record the presence of problems in the assessment, the most frequent being inter-relational and academic performance problems. This reflects the main worries of adolescents at this development stage, characterized by openness to new experiences in the field of social relations[5] and an increase in academic pressure prior to entry to university or to a vocational training center. Similar results has been obtained by other others using traditional methodology[21]. On the

other hand, it is important to promote a high parental self-efficacy, which would be highly related to ecological variables and parenting competence, in such a way that environmental conditions and ecological contexts may influence and undermine parent's confidence and parenting competence[22].

This study has a number of limitations that may affect the interpretation of the results. The first is the sample size. The application of the EMA methodology for seven consecutive days raises a series of logistical issues that complicate the recruitment and maintenance of participants. Compared with the pencil-paper assessment at a specific moment, EMA offers a considerable number of benefits (assessment in real time, decrease in memory bias, contextual association) and drawbacks (time-limited evaluation, sampling, loss of important events and overload) as noted by Shiffman *et al*[7] 2008, van Os *et al*[20] 2014, and Stone[23] 2007. All these points should be taken into account when applying EMA. In fact, the sample size in this study is within the range of most other studies in clinical[12-14,24] or community[9-11,25] adolescent populations.

Another issue to be considered is the type of population studied. As our data are from a community sample, they cannot be directly extrapolated to the clinical population or to a specific subgroup with psychiatric disorders. Nevertheless, one should bear in mind that the clinical population is a particular and acute subgroup of the community population. This study may represent a first step in the advancement of knowledge of daily patterns associated with mental health problems in adolescence and the assessment of contextual variables and coping strategies. Third, as this assessment study was carried out over a week, it would be interesting to compare these results with those from wider populations with specific psychiatric disorders, over different time periods, and with a longitudinal design. Despite these limitations, this is, to our knowledge, the first study in adolescents to apply the smartphone-based EMA methodology to measure the triad of contextual variables, symptoms associated with mental health problems and coping strategies.

EMA methodology using smartphones is a useful tool for assessing daily dynamics. It provides a sufficiently accurate measure of the psychopathological manifestations experienced by community adolescents in their natural context. In the study of momentary states associated with mental health symptomatology over a one-week period, we found that in most cases adolescents do not present emotional alterations or problems in their daily life. Girls were slightly more affected in their momentary emotional status and behaviors in daily life than boys. And among the situations in which a conflict is generated - on the one hand, anxious-depressed states, and on the other the oppositional-aggressive behavior are closely inter-related. Our results show that the family and home context could be crucial for the potential development of training interactions, both positive and negative, in the

mental health field, and they also stress the importance of individuals' coping resources in relation with their formative, relational, and physical context.

COMMENTS

Background

Adolescence is a stage of life characterized by a great many changes which, if not addressed effectively, may trigger problems of mental health. There is a substantial body of literature assessing the possible impact of individual everyday problems on the development of mental health disorders.

Research frontiers

Ecological Momentary Assessment (EMA) is a methodology allows to assess the impact of everyday problems in several situational contexts as they happen. Its applicability has been demonstrated in a variety of populations in studies of general health.

Innovations and breakthroughs

This study may represent a first step in the advancement of knowledge of daily patterns associated with mental health problems in adolescence and the assessment of contextual variables and coping strategies. The results show that EMA methodology based on mobile technology offers high viability for measuring mental health states in adolescents.

Applications

EMA methodology using smartphones is a useful tool for assessing daily dynamics. It provides a sufficiently accurate measure of the psychopathological manifestations experienced by community adolescents in their natural context.

Terminology

EMA is a methodology that allows the recording of the expression of mental microprocesses and their fluctuations in several situational contexts as they happen. Compared with the pencil-paper assessment at a specific moment, EMA offers a considerable number of benefits (assessment in real time, decrease in memory bias, contextual association) and also drawbacks (time-limited evaluation, sampling, loss of important events and overload).

Peer-review

The authors have reported their findings based on EMA with Smartphones for measuring mental health problems in adolescents. The study was well taken and the results indicate that such an evaluation is helpful to assess whether using smartphones is a useful tool for assessing daily dynamics or sufficiently accurate measure of the psychopathological manifestations experienced by community adolescents.

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ORIGINAL ARTICLE

Case Control Study

Voxel-based magnetic resonance imaging investigation of poor and preserved clinical insight in people with schizophrenia

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Institutional review board statement: The study procedures had the approval (reference number 209/02) of the ethics committee of the Institute of Psychiatry and South London and Maudsley Foundation NHS Trust, London.

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Conflict-of-interest statement: None of the authors declare any conflict of interest in this study.

Data sharing statement: The anonymised dataset is available from the first author and the corresponding author.

Abstract

AIM

To define regional grey-matter abnormalities in schizophrenia patients with poor insight (Insight⁻), relative to patients with preserved clinical insight (Insight⁺), and healthy controls.

METHODS

Forty stable schizophrenia outpatients (20 Insight[-] and 20 Insight[+]) and 20 healthy controls underwent whole brain magnetic resonance imaging (MRI). Insight in all patients was assessed using the Birchwood Insight Scale (BIS; a self-report measure). The two patient groups were pre-selected to match on most clinical and demographic parameters but, by design, they had markedly distinct BIS scores. Voxel-based morphometry employed in SPM8 was used to examine group differences in grey matter volumes across the whole brain.

RESULTS

The three participant groups were comparable in age [$F(2,57) = 0.34$, $P = 0.71$] and the patient groups did not differ in age at illness onset [$t(38) = 0.87$, $P = 0.39$]. Insight[-] and Insight[+] patient groups also did not differ in symptoms on the Positive and Negative Syndromes scale (PANSS): Positive symptoms [$t(38) = 0.58$, $P = 0.57$], negative symptoms [$t(38) = 0.61$, $P = 0.55$], general psychopathology [$t(38) = 1.30$, $P = 0.20$] and total PANSS scores [$t(38) = 0.21$, $P = 0.84$]. The two patient groups, as expected, varied significantly in the level of BIS-assessed insight [$t(38) = 12.11$, $P < 0.001$]. MRI results revealed lower fronto-temporal, parahippocampal, occipital and cerebellar grey matter volumes in Insight[-] patients, relative to Insight[+] patients and healthy controls (for all clusters, family-wise error corrected $P < 0.05$). Insight[+] patient and healthy controls did not differ significantly ($P > 0.20$) from each other.

CONCLUSION

Our findings demonstrate a clear association between poor clinical insight and smaller fronto-temporal, occipital and cerebellar grey matter volumes in stable long-term schizophrenia patients.

Key words: Psychosis; Insight; Grey matter volumes; Fronto-temporal; Neural networks; Birchwood insight scale

Core tip: Poor clinical insight is the most prevalent symptom in patients with schizophrenia and is of growing importance due to its direct association with poor clinical outcomes, such as frequent relapses and hospital admissions. This study identified significantly reduced fronto-temporal, parahippocampal, occipital and cerebellar grey matter volumes in Insight[-] patients relative to both Insight[+] patients and healthy controls. The involvement of multiple brain areas and corresponding neural networks supports the theory that clinical insight, as a neurological function, is not confined to specific neuroanatomical regions but probably a function of a complex neurocognitive interplay with contributions from multiple neural networks.

INTRODUCTION

Nearly a century ago, Kraepelin (1919) observed that schizophrenia patients often had "no real understanding of the gravity of their disorder" and regularly disputed that they suffer from a mental illness[1]. In the 1930s, Lewis described clinical insight as having "a correct attitude to a morbid change in one's self"[2,3] and low clinical insight is the most prevalent symptom occurring in about 97% of schizophrenia patients[2,4]. Impaired insight in schizophrenia is of growing importance due to its direct correlation with poor clinical outcomes, such as frequent relapses and hospital admissions[5], poor compliance with medication and treatment plans[6-8], severe psychopathology[9], greater suicidal tendencies and self-injurious behaviour[9-12]. Some studies reporting positive correlations between improvement in clinical insight and better global clinical impression and clinical outcome scores[13] have further suggested the adoption of clinical insight as a possible therapeutic target in schizophrenia patients[14].

Similarities between impaired insight in schizophrenia and unawareness of neurological deficits such as anosognosia, first described in patients with acute brain lesions with left-sided hemiplegia who were unaware of the impairments in their paralysed limbs[15,16], led to the notion that both phenomena share a common neurological basis[17-19] and prompted investigations of neuroanatomical abnormalities in relation to impaired clinical insight in schizophrenia. Earliest studies, using computerized tomography (CT) scan, reported significant and direct associations between impaired clinical insight and ventricular enlargement[20], total insight scores and total brain volumes[21] and a linear relationship between global cortical atrophy and impaired clinical insight[22]. These studies all concluded that there is a significant association between whole brain volume loss and impaired clinical insight in schizophrenia. Structural magnetic resonance imaging (MRI) studies also reported correlations between impaired clinical insight and smaller regional grey matter volumes, including the frontal lobe, anterior cingulate cortex (ACC), posterior cingulate, temporal and parietal lobes[23-28]. More recently, correlations have been reported between impaired insight and smaller right posterior insula volumes[29], smaller grey matter volumes of the right ventro-lateral prefrontal cortex (PFC)[30], left ventrolateral PFC, right dorsolateral PFC, insula, bilateral premotor area and the putamen; and reduced white matter volumes of the right superior longitudinal fasciculus, left corona radiata, left forceps minor and bilateral cingulum[31].

Although most studies have reported a correlation between brain volume loss and impaired insight, some studies failed to find any correlation between clinical insight and either ventricular or total/regional brain volumes[3,32,33], while others reported associations between impaired clinical insight and increased (rather than decreased) right medial orbitofrontal cortex grey matter volumes[28], and between symptom misattribution and increased grey matter volumes in bilateral caudate regions, right thalamus, left insula, putamen and cerebellum[34]. Bassitt *et al*[35] found no significant inverse correlation between total or regional grey matter volumes and clinical insight but, contrary to their expectations, observed a positive correlation between degree of insight impairment and the left medial PFC and ACC grey matter volumes, which they attributed to higher doses of antipsychotics given to patients with impaired clinical insight in their sample. The marked variation in findings may be due to the use of different brain volumetric assessment techniques, the heterogeneity of clinical insight measures and varying clinical characteristics of schizophrenia patients studied[25,35,36].

The aim of the present study was to characterise grey matter alterations in stable long-term schizophrenia outpatients with impaired clinical insight by directly comparing them, for the first time to our knowledge, with schizophrenia outpatients with preserved clinical insight, matched on average for age, sex and relevant demographic and clinical characteristics. Our approach of utilising the two extremes of the insight distribution should yield the largest structural difference in relation to insight. We also compared how these distinct groups of patients might differ from healthy controls, matched on average on age and sex of the patient groups. Based on the findings (where positive) of existing studies involving solely or predominantly chronic patient samples, we hypothesised that, patients with impaired insight (Insight⁻) will show smaller frontal and temporal regional grey matter volumes compared to patients with preserved insight (Insight⁺) and healthy controls. This hypothesis also has support from previous studies showing, on average, poor cognitive function in patients with impaired insight[25,37,38] and a positive association between grey matter volumes of these regions and a range of cognitive functions in schizophrenia[39].

MATERIALS AND METHODS

Participants and study design
This study included 60 right-handed participants. Forty of these were patients with a diagnosis of schizophrenia, confirmed using the Structured Clinical Interview for DSM-IV (SCID)[40]. The patients formed two groups of 20 patients each, pre-selected to have preserved and impaired insight, out of a larger pool of 70 stable community patients. The assessment of insight and differentiating criteria are described in detail under "clinical assessment". All included patients were required to be: (1) on well established antipsychotic medication doses for ≥ 3 mo; (2) in the stable (chronic) phase of the illness; and (3) ≥ 2 years from illness onset. Twenty healthy controls screened to exclude neuropsychiatric conditions and matched, on average, for age and sex of the patients were studied for comparison purposes. Ethics approval was granted by the ethics committee of the Institute of Psychiatry and South London and Maudsley Foundation NHS Trust, London. All participants provided written informed consent.

Clinical assessment
Birchwood Insight Scale (BIS)[41], a self-rated questionnaire, was used to assess insight in all patients. The BIS measures three different aspects of clinical insight[2]: (1) the awareness of the presence of a mental disorder (2nd and 7th statement); (2) the awareness of the need for treatment (3rd, 6th statement); and (3) the ability to label symptoms as abnormal (1st and 8th statement). Each individual BIS statement (8 in total) is rated and given a score of one for unsure, and either 0 or 2 for agree and disagree, depending on whether agreeing with the statement depicts preserved clinical insight (all statements are corrected for response valence). As we did not include any inpatients, Item 4 "My stay in hospital is necessary" was deleted, thus yielding a maximum possible score of 14, compared with a maximum possible score of 16 in the full scale BIS. In operationalising the BIS, Birchwood *et al*[41] classified preserved insight as having a minimum score of 9 (out of 14). In this study, we defined "preserved insight" as a minimum score of 13 (out of 14) and "impaired insight" as a score of 8 or below. This rather conservative method was designed to ensure that the two groups had distinct levels of insight and also to eliminate those with partial clinical insight levels. All patients were supervised during the completion of the BIS. The BIS has acceptable internal consistency ($\alpha = 0.75$) and one week test-retest reliability ($r = 0.90$ for the total score[41]), and insight assessed on the BIS correlates positively with scores on other measures of insight[10,26,42]. For sample characterization purposes, symptoms in patients were assessed using the Positive and Negative Syndrome Scales (PANSS[43]). In addition, predicted IQ of all study participants was measured using the National Adult Reading Test (NART[44]).

Image acquisition and processing
Whole brain MRI scans were acquired for all study participants using a 1.5 Tesla GE NV/I Signa system (General Electric, Milwaukee WI, United States) at the Maudsley Hospital, London. A series of sagittal fast gradient echo scout images were obtained to correct for head tilt and to orient subsequent images relative to the anterior-commissure/posterior-commissure line and the interhemispheric fissure. A 3-D inversion recovery prepared fast spoiled GRASS sequence was applied to acquire T1-weighted images in the axial plane with 1.5 mm contiguous sections (TR = 18 ms, T1 = 450 ms, TE = 5.1 ms, flip angle = 20° with one data average and a 256 × 256 × 128 voxel matrix). Acquisition

parameters were selected employing a sophisticated image simulation[45]. All MRI images were converted into ANALYZE format (ANALYZE software, BRU, Mayo Foundation, Rochester, MN) and pre-processed using Statistical Parametric Mapping (SPM8; http://wwwfil.ion. ucl.ac.uk/spm) running in MATLAB 2006a (MathWorks, Natick, MA). Customised T1-weighted templates of the whole brain, grey matter, white matter and cerebro-spinal fluid (CSF) were created for patient and healthy participant groups separately, and also for the whole study sample combined.

Statistical analysis
Demographic and clinical measures: Possible group differences in age, education and NART IQ were examined using analyses of variance (ANOVAs), and significant Group effects were followed by independent sample *t*-tests. Possible differences between the two patient groups in clinical variables (age at illness onset, PANSS symptom scores and medication) were examined using independent sample *t*-tests. All statistical analyses were conducted using SPSS 22, with alpha level for significance testing maintained at $P \leqslant 0.05$ (two-tailed), unless stated otherwise.

MRI: Group differences (healthy controls *vs* Insight⁻ patients, health controls *vs* Insight⁺ paitents, and Insight⁺ *vs* Insight⁻ patients) in grey matter volumes, across the whole brain, were examined using ANOVA in SPM8 (height threshold $P < 0.005$; familywise-error (FWE)-corrected at the cluster level $P < 0.05$). To rule out the possibility that any observed group differences were due to trend-level Group differences in education and IQ (see RESULTS, demographic and clinical measures), group differences in grey matter volumes were re-evaluated using analysis of co-variance, with education and IQ entered as co-variates.

RESULTS

Demographic and clinical characteristics
The three participant groups did not differ in age [$F_{(2,57)} = 0.34$, $P = 0.71$]. There were trend level effects of Group in years of education [$F_{(2,57)} = 2.60$, $P = 0.08$] and NART IQ [$F_{(2,57)} = 2.67$, $P = 0.08$]. Healthy controls spent more years in education than Insight⁻ patients [$t_{(38)} = 2.11$, $P = 0.04$] but differed only at a trend level when compared with Insight⁺ patients [$t_{(38)} = 1.77$, $P = 0.08$]. Healthy controls also had higher NART IQ than Insight⁻ patients [$t_{(38)} = 2.47$, $P = 0.02$] but did not differ from Insight⁺ patients [$t_{(38)} = 1.19$, $P = 0.24$]. There were no significant differences the Insight⁻ and Insight⁺ patient groups in education [$t_{(38)} = 0.06$, $P = 0.95$] and NART IQ [$t_{(38)} = 1.04$, $P = 0.31$] (Table 1). The two patient groups were similar in age at illness onset [$t_{(38)} = 0.87$, $P = 0.39$], positive symptoms [$t_{(38)} = 0.58$, $P = 0.57$], negative symptoms [$t_{(38)} = 0.61$, $P = 0.55$], general

psychopathology [$t_{(38)} = 1.30$, $P = 0.20$] and total PANSS symptoms [$t_{(38)} = 0.21$, $P = 0.84$]. Patients in the two groups were on a range of typical and atypical antipsychotics (Table 1) but received, on average, similar doses of antipsychotic medication [$t_{(38)} = 0.86$, $P = 0.40$]. The Insight⁺ patient group, confirming our insight-based pre-selection, had significantly higher BIS score than the Insight⁻ group [$t_{(38)} = 12.11$, $P < 0.001$].

MRI: Group effects in regional grey matter volumes
Group differences in brain MRI grey matter volumes are presented in Table 2, and described below.

Insight⁻ *vs* Insight⁺ patients: Compared to Insight⁻ patients, Insight⁺ patients had larger grey matter volumes in the inferior frontal and precentral gyri, superior and middle temporal gyri, parahippocampus, cuneus and cerebellum of both cerebral hemispheres (Figure 1).

Healthy controls *vs* Insight⁻ patients: Compared to Insight⁻ patients, healthy controls had larger grey matter volumes in the left inferior and middle frontal gyri, left superior, middle and inferior temporal gyri, left parahippocampus, right cerebellum, and bilateral superior, middle and inferior occipital gyri (Figure 1).

Healthy controls *vs* Insight⁺ patients: There were no significant differences between healthy controls and Insight⁺ patients.

Group differences after co-varying for education and predicted IQ
Differences in grey matter volumes (noted earlier) between healthy controls and Insight⁻ patients remained present but with reduced significance when we co-varied for education and IQ (Table 3). Group differences between Insight⁻ and Insight⁺ patients, however, were not affected.

DISCUSSION

In this study, we directly compared two matched groups of schizophrenia patients but with distinct levels of clinical insight (Insight⁻ *vs* Insight⁺) and investigated how they differ from each other and also from healthy controls in regional grey matter volumes examined using voxel-based morphometry (VBM) technique. We tested the hypothesis that Insight⁻ patients will show smaller frontal and temporal grey matter volumes compared to Insight⁺ patients. All three participant groups were comparable in age and the two patient groups were similar in all demographic and clinical parameters, including age at illness onset, years of education, NART IQ, symptoms (PANSS scores) and doses of medication prescribed. Insight⁻ patients, however, had lower IQ and fewer years in education than healthy controls. Although, on average, lower IQ as well as deficits in many specific cognitive

Table 1 Demographics and clinical characteristics of the study groups

	Healthy controls (n = 20; 15 male, 5 female)		Patients insight+ group (n = 20; 16 male, 4 female)		Patients insight- group (n = 20; 16 male, 4 female)	
	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
Demographics						
Age (yr)	35.25 (10.93)	20-59	36.15 (10.54)	19-54	37.80 (7.85)	22-49
Education (yr)	15.05 (2.86)	10-20	13.45 (2.86)	9-20	13.40 (2.01)	11-19
Predicted IQ (NART)	113.10 (9.91)	91-128	109.20 (10.80)	86-122	106.10 (7.87)	90-119
Clinical characteristics						
BIS			11.65 (0.57)	13-14	5.88 (2.05)	1-8
Age at illness onset (yr)			25.90 (8.72)	13-48	23.85 (5.84)	10-37
PANSS positive symptoms			16.15 (5.38)	8-25	17.05 (4.43)	8-23
PANSS negative symptoms			17.20 (4.38)	7-27	18.15 (5.46)	8-27
PANSS general psychopathology			34.35 (7.36)	24-56	31.55 (6.27)	21-40
PANSS total symptoms			67.70 (14.90)	43-108	66.75 (14.02)	37-86
Medication (chlorpromazine equivalent in mg)			461.21 (333.95)	100-1600	556.63 (366.49)	200-1367
Medication type						
Atypical antipsychotics			18 (9 olanzapine, 5 risperidone, 3 clozapine, 1 quetiapine)		13 (7 olanzapine, 3 clozapine, 1 aripiprazole, 1 amisulpride, 1 risperidone)	
Typical antipsychotics			2 (1 sulpiride, 1 haloperidol)		5 (2 flupenthixol, 1 fluphenazine, 1 sulpiride, 1 haloperidol)	
Both			--		2 (1 on clozapine + levomepromazine, 1 zuclopenthixol + aripiprazole)	

NART: National Adult Reading Test[44]; BIS: Birchwood insight scale[41]; PANSS: Positive and negative syndrome scale[43].

domains in patients with schizophrenia, relative to the healthy population, are commonly reported[46], our study suggests that this may be particularly true for those with impaired insight[37] and in turn may also explain the finding of significantly fewer years in education in the Insight- (but not Insight+) patient group, compared with the healthy controls. The patient groups scored at opposing ends of the BIS scale; this allows for the interpretation of observed neuroanatomical differences in relation to clinical insight levels of the respective patient group.

As hypothesized, we found that Insight- patients had smaller grey matter volumes than Insight+ patients, bilaterally in the frontal and temporal lobes (mainly in the inferior frontal and precentral gyri and superior and middle temporal gyri), as well as in the parahippocamal gyrus, occipital lobes (including the cuneus) and the cerebellum. Insight- patients also showed similar grey matter deficits, particularly on the left, when compared to healthy controls (Figure 1).

Our findings of smaller fronto-temporal regional grey matter volumes are in accordance with previous imaging studies, that used the "Region of Interest" (ROI) approach and found a significant and direct correlation between smaller frontal areas, including the dorsolateral PFC, inferior frontal and middle frontal gyri[22,26-28,47,48] and impaired clinical insight. Early reports of poor executive functioning in schizophrenia patients with impaired insight, similar to those with frontal lobe lesions, initiated the interest in the integrity of the frontal lobe in schizophrenia. Since then, several studies[26,30,31,47],

including this one, have reported frontal neuroanatomical abnormalities in relation to impaired clinical insight in schizophrenia. Some functional imaging studies have further associated aberrant frontal functional MRI activity with impaired clinical insight during working memory[49], self-reflection[50], self-monitoring[51] and self-awareness tasks[52] in schizophrenia. In addition, earlier correlational VBM studies have also reported associations between smaller superior and middle temporal lobe grey matter volumes and impaired clinical insight[23,48].

Our other finding of smaller cuneus and occipital grey matter volumes in Insight- patients is also broadly in agreement with the earlier reported association between poor symptom relabelling dimension of clinical insight and smaller grey matter volumes of the precuneus, cuneus and medial occipital gyrus by Morgan *et al*[25]. Unlike Morgan *et al*[25], we did not investigate preferential or predominant contribution of particular insight dimensions because the BIS subscale scores in our sample were highly positively correlated with each other (rho = 0.50-0.882; *P* < 0.001). This might be due to our sampling methods that ensured that our Insight- and Insight+ patient groups had markedly different insight levels, possibly in all domains. Other VBM studies have also reported an association between the smaller precuneus grey matter volumes and lower insight in schizophrenia[23]. The role of the precuneus has been described in the facilitation of increased awareness into one's mental states[23,53] and has also been implicated, in conjunction with other midline structures, in the self-appraisal processes[54,55]. Compared to anterior cortical

Table 2 Group differences in grey matter volumes (height threshold *P* < 0.005)

Groups	BA	Size	Side	MNI			*T* value	Cluster *P* FWE-corrected unless in italics	Voxel *P* FWE-corrected
				X	Y	Z			
Insight+ > Insight- patients									
Superior temporal gyrus	22	46555	R	63	-3	5	4.91	0.001	0.020
				45	20	-33	4.74		0.034
				66	-8	4	4.68		0.040
Precentral gyrus	4			66	-5	22	4.55		0.057
Inferior frontal gyrus	47			54	19	0	4.52		0.063
Precentral gyrus	6			64	0	26	4.40		0.088
Postcentral gyrus	43			66	-8	16	4.33		0.106
parahippocampus	28			14	0	-27	4.07		0.406
Inferior frontal gyrus	47	103898	L	-41	15	-6	4.81	< 0.001	0.027
Middle frontal gyrus	9			-37	19	35	4.74		0.034
Inferior frontal gyrus	47			-37	15	-10	4.73		0.035
				-35	20	-10	4.54		0.059
Precentral gyrus	44			-59	8	7	4.39		0.091
Superior temporal gyrus	22			-62	-4	8	4.36		0.097
Precentral gyrus	6			-60	4	6	4.33		0.107
Middle temporal gyrus	21			-35	-3	-23	4.27		0.126
parahippocampal gyrus	20			-34	-5	-28	4.16		0.166
Cuneus	18	35993	L	-5	-83	5	4.43	0.003	0.082
Cerebellum	-		R	35	-90	-17	4.26		0.129
Cuneus	18		R	26	-93	-18	3.90		0.305
Cerebellum	-		R	4	-61	2	3.88		0.317
Cuneus	18		R	5	-98	10	3.50		0.630
			R	5	-96	3	3.44		0.674
Cerebellum	-		L	-36	-82	-15	3.38		0.730
Insight- > Insight+ patients									
Nil significant									
Healthy controls > Insight- patients									
Inferior frontal gyrus	47	35300	L	-49	19	-3	4.63	0.004	0.046
Superior temporal gyrus	22			-60	1	3	4.30		0.115
Inferior frontal gyrus	47			-41	18	-5	4.21		0.144
				-38	22	-8	4.19		0.153
				-36	-1	-14	3.86		0.333
Inferior temporal gyrus	20			-28	-14	-41	3.61		0.530
Parahippocamal gyrus	34			-13	4	-23	3.58		0.552
Middle frontal gyrus	11			-42	40	-19	3.39		0.722
Inferior occipital gyrus	18	11168	L	-38	-92	-2	4.51	0.034	0.065
Middle occipital gyrus	19			-52	-76	-10	4.29		0.117
				-48	-80	-14	3.96		0.266
				-49	-81	7	3.90		0.302
Middle temporal gyrus	39/			-53	-72	22	3.37		0.740
	19			-52	-74	18	3.33		0.768
				-49	-76	20	3.29		0.797
Cerebellum	-	25235	R	35	-90	-17	4.46	0.016	0.074
(posterior lobe)				11	-90	-37	4.21		0.146
Occipital lobe	18			23	-94	-18	4.01		0.238
Cerebellum				34	-85	-40	3.93		0.355
(posterior lobe)				38	-82	-41	3.91		0.489
Insight- patients > healthy controls									
Nil significant									
Healthy control > Insight+ patients									
Nil significant									
Insight+ patients > healthy controls									
Nil significant									

BA: Brodmann area; L: Left; R: Right; MNI: Montreal Neurological Institute.

regions, much less is known about the involvement of posterior medial cortices due to the dearth of research into the contributions of these brain regions to various aspects of psychotic disorders[25]. In our recent study, we found further evidence of functional contributions from the precuneus, as well as the cerebellum, in supporting neural activities sub-serving the preservation of insight in schizophrenia patients[49].

There have been previous reports of cerebellar atrophy, on average, in schizophrenia patients[56]. A previous study[48] also observed a significant association between impaired clinical insight and reduced bilateral

Table 3 Group differences in grey matter volumes after co-varying for education and National Adult Reading Test IQ (height threshold $P < 0.005$)

Groups	BA	Size	Side	MNI			T value	Cluster P FWE-corrected unless shown in italics	Voxel P FWE-corrected
				X	Y	Z			
Insight+ > Insight- patients									
Superior Temporal gyrus	22	37261	R	63	-3	5	4.70	0.002	0.044
				45	20	-33	4.56		0.066
				66	-8	4	4.45		0.088
Precentral gyrus	4			66	-5	22	4.44		0.092
Inferior frontal gyrus	47			54	19	0	4.39		0.103
Precentral gyrus	6			64	0	26	4.28		0.137
Postcentral gyrus	43			66	-8	16	4.15		0.192
Inferior frontal gyrus	47	65047	L	-42	16	-4	4.65	< 0.001	0.050
				-38	14	-8	4.65		0.052
				-36	18	-10	4.52		0.073
Middle frontal gyrus	9			-37	19	35	4.52		0.073
Superior temporal gyrus	22			-61	-2	7	4.28		0.139
Precentral gyrus	44			-59	9	9	4.17		0.184
Parahippocampual gyrus	21			-34	-3	-36	4.10		0.213
Cuneus	18	24291	L	-5	-83	5	4.32	0.014	0.125
Cerebellum	-		R	35	-90	-17	4.17		0.181
Cuneus	18		R	26	-93	-18	3.73		0.466
Cerebellum	-		R	4	-61	2	3.80		0.409
Cuneus	18		R	5	-98	10	3.35		0.787
Medial frontal gyrus	10	16854	L	0	60	3	3.98	0.050	0.285
Superior frontal gyrus	9			0	51	26	3.64		0.544
Insight- > Insight+ patients									
Nil significant									
Healthy controls > Insight- patients									
Inferior frontal gyrus	47	9770	L	-51	19	-2	3.68	0.036	0.511
Superior temporal gyrus	38			-21	5	-24	3.35		0.786
Inferior frontal gyrus	47			-26	18	-7	3.34		0.796
Parahippocampal gyrus	34			-16	4	-23	3.29		0.827
Inferior occipital gyrus	18	4935	L	-38	-92	-2	3.92	0.122	0.323
Middle occipital gyrus	19			-52	-76	-10	3.70		0.494
				-44	-83	8	3.37		0.775
Middle temporal gyrus	18			-43	-81	13	3.22		0.873
Cerebellum (posterior lobe)	-	6085	R	35	-90	-17	3.68	0.089	0.304
				11	-90	-37	3.60		0.378
Occipital lobe	18			28	-94	-16	3.32		0.656
				23	-94	-18	3.26		0.713
Insight- patients > healthy controls									
Nil significant									
Healthy controls > Insight+ patients									
Nil significant									
Insight+ patients > healthy controls									
Nil significant									

BA: Brodmann area; L: Left; R: Right; MNI: Montreal Neurological Institute.

cerebellar grey matter volumes in schizophrenia, and that this relationship was not associated with any specific dimension of clinical insight. Other studies have described the involvement of the cerebellum in higher cognitive functioning, with its extensive connectivity with limbic structures, including the parahippocampal gyrus, and associated cortical areas involved in cognition and executive function[57,58], and this has been implicated in the neuropathology of schizophrenia and poor clinical insight[48,59]. Our recent finding of increased cerebellar activity, detected using fMRI, in Insight+ patients compared to Insight- patients, during a working memory task, also indicated cerebellar involvement in the preservation of clinical insight in schizophrenia[49].

In accordance with the observations made by other studies, we also found grey matter reductions in many areas in Insight- patients, compared to healthy controls[48]. These differences remained, but became less significant, after we co-varied for education and NART IQ. Co-varying for education and NART IQ had no effects on grey matter volume differences between preserved and Insight- patient groups, most likely because these two groups were comparable on these parameters.

Strengths and limitations
We employed a direct comparison method between distinct groups of schizophrenia patients (Insight- and Insight+) with closely matched demographic and clinical
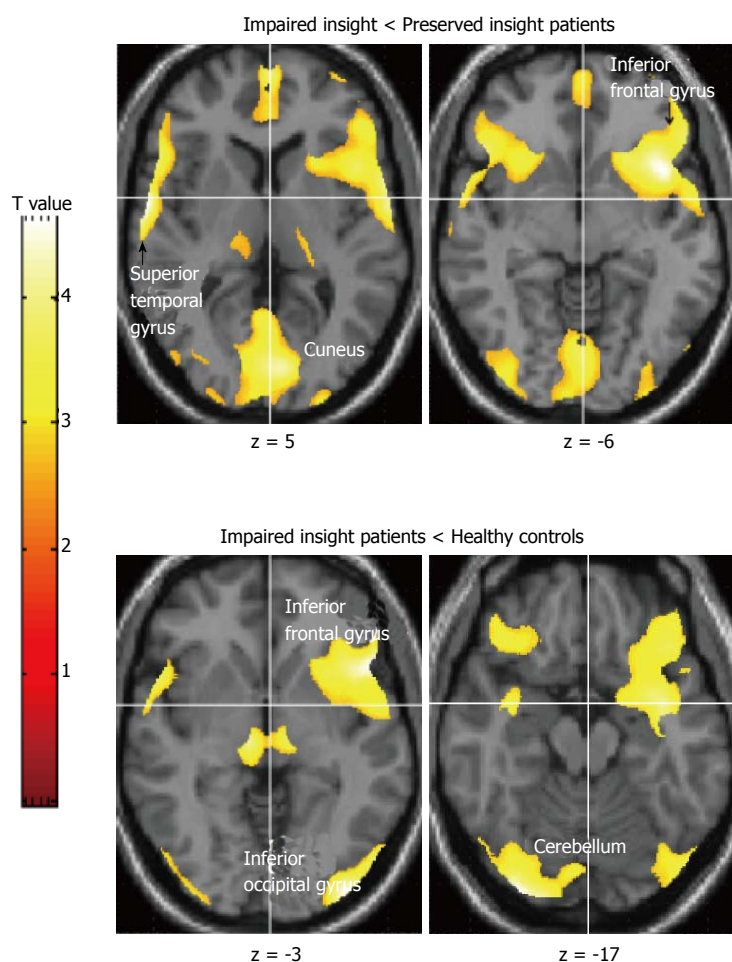
Figure 1 Images showing regions of decreased grey matter volume in the impaired insight patient group, relative to the preserved insight patient and healthy controls (maps thresholded at *P* = 0.005; left = right).

qualities, thereby facilitating valid comparisons and inferences. The study also had 60 participants (*n* = 20 per group) and thus was adequately powered for the observations made. We were, however, limited in our ability to explore the effects of sex on brain volumes and in the observed group differences, as our sample was predominantly male. Nonetheless, male:female ratios were similar and any possible effect is expected to be uniform in all groups. Also, although the patient groups were comparable in all relevant areas, our healthy controls had more education than our patient groups, and had higher IQ scores than Insight⁻ patient group, although co-varying for these differences did not change the pattern of observed group differences. By adopting a direct comparison method between matched patient groups at the extremes of insight measures, we minimised confounding effects of partial insight levels and were able to exclude overall effects of schizophrenia on brain volumes. However, in as much as we endeavoured that our two patient groups are highly comparable but for their insight levels, there are possibilities of other differential properties, such as brain functional properties, which could possibly contribute to our findings. Lastly, patients in both the Insight⁺ and Insight⁻ groups were on a range of atypical and typical antipsychotics (Table 1) which vary in their pharmacological profiles[60,61] as well as in their effects on brain volumes[62]. This may have

influenced the results we observed in this study.

In conclusion, schizophrenia patients with impaired insight patients have smaller fronto-temporal, parahippocampal, occipital and cerebellar grey matter volumes, compared with preserved insight schizophrenia patients and healthy controls. The involvement of multiple brain areas and corresponding neural networks supports the theory that clinical insight, as a neurological function, is not confined to specific neuroanatomical regions in the brain but probably a function of a complex neurocognitive interplay with contributions from neural networks, including working memory and executive functioning, self-monitoring and awareness and others[19,23,49,63,64].

COMMENTS

Background

Impaired insight in schizophrenia is found to have a direct correlation with poor clinical outcomes, such as frequent relapses and hospital admissions, poor compliance with medication, greater suicidal tendencies and self-injurious behaviour. Some studies reporting positive correlations between improvement in clinical insight and better clinical outcomes have further suggested the adoption of clinical insight as a possible therapeutic target in schizophrenia

patients.

Research frontiers

The ability to target insight therapeutically is highly complex and remains elusive to most methods trialled so far. The identification of the underpinning neural correlates of clinical insight will aid the development of specific treatment strategies aimed at improving insight in schizophrenia.

Innovations and breakthroughs

The study reported in this manuscript is distinct from all previous studies in this area (mostly correlational) in that it identifies regional grey matter abnormalities in stable schizophrenia outpatients with impaired clinical insight, relative to those with preserved clinical insight (impaired and preserved insight groups scoring at extreme ends of a multidimensional insight scale but matched on age, sex and other symptoms) as well healthy controls, using a categorical approach. The authors found a clear association between impaired clinical insight and smaller fronto-temporal, occipital and cerebellar grey matter volumes in stable long-term schizophrenia patients.

Applications

Clinical insight, as a neurological function, is likely to be dependent on complex neurocognitive interplay with contributions from multiple neural networks.

Terminology

Voxel-based-morphometry is a neuroimaging analysis technique in which structural brain properties are examined on a voxel-by-voxel basis and reported in standardized coordinates. Clinical insight refers to a patient's complex state of awareness of his or her own mental disorder.

Peer-review

The study is well designed and the manuscript is clearly written and easy to read all throughout.

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ORIGINAL ARTICLE

Case Control Study

Stressful life events and psychosocial correlates of pediatric inflammatory bowel disease activity

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Abstract

AIM

To investigate the association of psychiatric and psychosocial correlates with inflammatory bowel disease (IBD) activity in children and adolescents.

METHODS

A total of 85 pediatric IBD patients (in remission or active state of the disease) and their parents completed a series of questionnaires and semi-structured interviews measuring life events, depression, anxiety, family dysfunction, and parent mental health. Differences between the remission and the IBD active group and the association of any significant variable with the disease activity state were examined.

RESULTS

Parents of children being in active state of the disease reported more life events ($P = 0.005$) and stressful life events ($P = 0.048$) during the past year and more mental health symptoms ($P < 0.001$), while the children

themselves reported higher levels of anxiety symptoms (P = 0.017) compared to the remission group. In the logistic regression multivariate analysis, the only predictor which had a significant positive effect on the probability of the patients being in active state was parent mental health symptoms (OR = 4.8; 95%CI: 1.2-25.8).

CONCLUSION
Life events, child anxiety and parent mental health symptoms may be important correlates of pediatric IBD activity and targets of thorough assessment and treatment.

Key words: Inflammatory bowel disease; Children and adolescents; Stressful events; Anxiety; Depression

Core tip: The present study examined the associations of several psychosocial factors and outcomes with pediatric inflammatory bowel disease (IBD) activity. Second, it shed some light on the relationship of the disease activity (*i.e.*, IBD remission or active state) with preceding life events. Addressing simultaneously psychosocial needs of both children and parents in the course of pediatric IBD seem to be of importance in any effective preventive and therapeutic intervention. Moreover, the role of stressful events in the course of pediatric IBD although being mediated or moderated by individual factors seem to be a possible target for future research and psychosocial treatment modalities.

INTRODUCTION

Epidemiological studies indicate that the incidence of pediatric inflammatory bowel disease (IBD), consisting of Crohn's disease (CD), ulcerative colitis (UC) and IBD unclassified (IBDU), has been increasing over time[1]. Elevated levels of depression, anxiety, low self-esteem, disrupted social functioning, family dysfunction, and parental distress are among the most common findings from studies comparing pediatric IBD patients with other chronic disease patients or healthy controls[2,3].

However, only few studies have investigated the association of psychiatric and psychosocial correlates with IBD activity in children and adolescents. Specific depressive symptoms (*e.g.,* lack of interest and energy, decreased appetite) have been shown to be related with moderate/severe disease activity[4]. Higher levels of depressive symptoms have been also related to poorer subjective health in IBD pediatric patients[5]. A recent

study[6] found that the disease activity was associated with adolescents' general well-being, emotional functioning, social functioning, and body image. In another sample of young adults with IBD, poor college adjustment and physical quality of life were correlated with increased disease activity[7]. However, no significant correlations were reported elsewhere[5,8-11].

Moreover, impaired parent mental health and physical functioning have been correlated significantly with pediatric IBD symptom exacerbation[12]. Similarly, family general dysfunction has been related with more symptomatic IBD among adolescents, and maternal positive affect (*e.g.,* mothers describing themselves as more active, and interested) has been related with less IBD symptoms[13]. Finally, although the role of stressful life events has been studied in adult IBD patients with mixed findings[14], there are no published reports examining the relationship of stressful events with the disease activity in pediatric populations. Only two studies comparing IBD patients to healthy and clinical controls[15,16] and one study comparing depressed to non-depressed IBD pediatric patients[17] supported the association of retrospectively reported stressful life events with the onset of pediatric IBD.

The present study investigates the relationship of several psychosocial factors and outcomes with pediatric IBD activity. More specifically, we try to provide a more comprehensive examination than currently available evidence by assessing differences in the often neglected life events among other possibly significant psychosocial problems, such as depressive and anxiety symptoms, family dysfunction, and parent mental health between an IBD remission group and an IBD active group of children and adolescents. It was hypothesized that the active group would show more stressful life events the year prior the present assessment and higher levels of psychosocial problems. Furthermore, we examine the association of any psychosocial variable that is shown to be correlated with the disease activity state by entering these variables in the same model as covariates. The aim of the latter examination is to clarify interactions and the possible moderating role of any of the abovementioned psychosocial correlates in their association with pediatric IBD activity.

MATERIALS AND METHODS

The study was reviewed and approved by the Aghia Sophia Children's Hospital Institutional Review Board. All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Participants

This cross-sectional study was conducted at the Gastroenterology Unit of the First Department of Pediatrics in collaboration with the Department of Child Psychiatry of the National and Kapodistrian University of Athens, School of Medicine, Aghia Sophia Children's Hospital. Eligible for inclusion were all children and their parents,

Table 1 Demographic information by inflammatory bowel disease activity and type of disease

	IBD active group (*n* = 43)		Remission group (*n* = 42)	
	CD (*n* = 30)	UC (*n* = 13)	CD (*n* = 27)	UC (*n* = 15)
Age, mean (yr)	12.8 ± 2.2	12.9 ± 2.0	13.9 ± 2.0	13.1 ± 1.4
Child gender (female), %	56.7	61.5	66.7	46.7
Parent gender (female)[1], %	80.8	77.8	86.9	75

[1]Gender of the parent who was present during the assessment. IBD: Inflammatory bowel disease; CD: Crohn's disease; UC: Ulcerative colitis.

diagnosed with IBD according to the ESPGHAN Porto-criteria[18,19] who were either admitted in the First Department of Pediatrics or followed as outpatients. The period of recruitment lasted for 24 mo. Inclusion criteria were age between 8-18 years and the ability to read Greek and complete the questionnaires themselves. Exclusion criteria were diagnosis of developmental pervasive disorder or mental retardation, and a co-morbid chronic illness. No patient was on psychiatric medication. One hundred subjects fulfilling the inclusion criteria were asked to participate. A total of 85 children and adolescents (50 females) with a mean age of 13.2 years ± 2.4 years and their parents agreed to participate in the study (85% response rate) and they were included in the analysis. The cases that refused to participate did not differ from the study group according to age, sex and disease activity. The characteristics of the study sample are shown in Table 1. Regarding steroid medication, 44.9% (*n* = 38) of the study population was on steroids at the time of evaluation, 14.7% (*n* = 6) of those in remission and 74.9% (*n* = 32) of those with a relapse. Three participants were of non-Greek ethnicity.

Materials and procedures

All patients were classified at the time of the evaluation, as being in remission or in active state of IBD according to the Pediatric Crohn's Disease Activity Index (PCDAI)[20] or the Pediatric Ulcerative Colitis Activity Index (PUCAI)[21] depending on the diagnosis. For both scales, remission is defined by a total score < 10 and active state if the total score was ≥ 10. Disease activity was assessed both as activity index and as disease state (remission or relapse). Both children and their parents completed the questionnaires in the outpatient or inpatient clinics. Socio-demographic details and past medical and psychiatric history were recorded.

The parents completed a semi-structured psycho-social interview evaluating recent life events for their children[22,23], which is based on Coddington's Life Events Questionnaire[24]. The onset of each event that had occurred over the previous 12 mo was dated to within 4 wk. Life events that are measured through this interview include: (1) danger to self (child's illness or accidents, involvement in a household or community disaster or being the subject of a personal attack; (2) danger to others (expectation or occurrence of physical threat where the person exposed is a parent, sibling, friend or

significant other; (3) disappointments (*e.g.,* to the self includes breakdown of boyfriend/ girlfriend relationship, examination failure; to another includes loss of job, new financial difficulties, extramarital affair); and (4) loss (includes only death and permanent separations). Life events data were collected only from parents in order not to cause survey fatigue to the patients. Both the stressful events (those carrying a moderate to severe degree of negative impact on the child according to parents' subjective perception) and the total number of events (the objective number of reported events) were computed in the present analysis, so as to take into consideration the possible impact of events that parents may underestimate.

Child and adolescent anxiety symptoms were measured by the Revised Children's Manifest Anxiety Scale (RCMAS)[25]. The RCMAS is a widely used self-report 37-item questionnaire, with higher values corresponding to higher levels of anxiety. The psychometric properties of the RCMAS have been found acceptable in previous research[26].

The Children's Depression Inventory (CDI)[27], a self-report 27-item scale, was used to screen for depressive symptoms. The total score ranges from 0 to 54, with higher values corresponding to higher levels of depression. Previous research has shown that the CDI is adequately reliable and valid with respect to depressive symptoms[28].

In order to assess parent mental health symptoms, the Symptom Checklist-90-Revised (SCL-90-R) was administered[29]. The SCL-90-R is a 90-item multidim-ensional questionnaire designed to screen for a broad range of psychological problems. Each of the 90 items is rated on a five-point Likert scale of distress. The Global Severity Index (GSI), the mean rating across all items, was used for assessing parent mental health symptoms. The psychometric properties of the SCL-90-R GSI have been found acceptable in previous research[30] and support its use as a self-report measure.

Family dysfunction was assessed through the Mc-Master Family Assessment Device (FAD)[31]. It is a 60-item instrument that assesses 6 domains of family functioning as well as general family dysfunction. The 12-item General Functioning scale, which assesses overall family pathology or dysfunction, was analyzed in the present study. Higher mean values indicate greater general dysfunction. The reliability and validity of the FAD General Functioning scale have been demonstrated in clinical and non-clinical samples[32].

Table 2 Comparisons of self-reported child emotional problems, parent mental health symptoms, family dysfunction and life events by inflammatory bowel disease activity group

Variable	IBD active group (*n* = 43)	IBD remission group (*n* = 42)	*P*
Life events	3.8 ± 2.5	2.4 ± 2.8	0.005[2]
Stressful life events	2.9 ± 2.1	2.0 ± 2.3	0.048[2]
FAD GFS family dysfunction	22.6 ± 5.5	20.1 ± 4.9	0.061[1]
SCL-90-R parent mental health symptoms	1.11 ± 0.72	0.52 ± 0.39	< 0.001[1]
RCMAS anxiety symptoms	54.3 ± 12.7	46.9 ± 13.1	0.017[1]
CDI depression symptoms	8.9 ± 5.6	7.2 ± 5.5	0.2[1]

[1]Student's *t* test; [2]Mann-Whitney test. IBD: Inflammatory bowel disease; FAD GFS: Family Assessment Device General Functioning Scale; SCL90-R: Symptom Checklist-90-Revised; RCMAS: Revised Children's Manifest Anxiety Scale; CDI: Children's Depression Inventory.

Statistical analysis

Continuous variables are presented using mean ± standard deviation (SD) while categorical variables are presented by absolute (*n*) and relative (%) frequencies. In order to test our hypothesis, Student's *t*-test was used for comparisons of means between IBD remission group and IBD active group, with the exception of continuous variables with a small number of distinct values in the dataset where the non-parametric Mann-Whitney statistic was used. Categorical variables were compared between the two groups by Fisher's exact test. Exact logistic regression analysis was applied in order to evaluate to what extent the explanatory variables that were already found to be associated with IBD activity in univariate analysis had a multivariate effect on the latter. The estimate of the relative risks of being in IBD active state was performed by calculating the odds ratios (OR) and the corresponding 95%CI. All tests were two-sided and a level of ≤ 0.05 was considered statistically significant. Data were analyzed using STATA 11.0 (Stata Corporation, College Station, TX 77845, United States).

RESULTS

Disease state was not related to age or gender (*P* = 0.17 and *P* = 0.55 respectively).

Comparisons of psychosocial variables by IBD activity group

The results of the univariate analysis, that is the differences in each psychosocial factor tested individually by IBD activity group, are presented in Table 2. Parents of children being in active state of the disease reported more life events (*P* = 0.005) and stressful life events (*P* = 0.048) during the past year, and more mental health symptoms (*P* < 0.001), while the children themselves reported higher levels of anxiety symptoms (*P* = 0.017), compared to the remission group. Similarly, when the disease activity was assessed through activity index, it was positively associated with life events (*P* = 0.04) during the past year, parent mental health symptoms (*P* = 0.0081) and children's anxiety symptoms (*P* = 0.0028). Evaluation of the abovementioned differences

after stratifying according to the diagnosis (CD or UC) had not sufficient statistical power.

Multivariate effects of psychosocial variables on IBD activity in the logistic regression analysis

Since no change in the direction of the observed relations was detected in the stratified analysis, data were combined for all the subsequent analyses. When the factors which were proven to be significant in the univariate analysis (*i.e.*, life events and stressful life events during the past year, self-reported anxiety symptoms, and parent mental health symptoms) were included in the logistic regression multivariate analysis, the only predictor variable which had a significant positive effect on the probability of the patients being in IBD active state was parent mental health symptoms (OR = 4.8; 95%CI: 1.2-25.8). Since steroid medication was related to self-reported anxiety symptoms (*P* = 0.006) and parent gender was related to parent mental health symptoms (*P* = 0.038), were both considered confounding variables and included in the final model. Steroid medication had a significant association with disease state with patients on steroids being more likely to be in relapse (OR = 19.8; 95%CI: 3.2-120.8), whereas parent gender was statistically not significant and therefore removed from the regression equation.

DISCUSSION

This study was an effort to examine differences in the often neglected life events among other possibly significant psychosocial variables, such as depression and anxiety symptoms, family dysfunction, and parent mental health between an IBD remission group and an IBD active group of children and adolescents. Results indicate that parent-reported life events during the past year, self-reported anxiety symptoms, and parent mental health symptoms are related with the disease activity. Moreover, parent mental health symptoms seem to be a strong correlate of IBD activity when all significant variables are entered into a model simultaneously.

Self-reported emotional problems (*i.e.*, depressive and anxiety symptoms) tended to be higher in the IBD active group than in the remission group, with self-reported

anxiety symptoms, in particular, differing significantly between the 2 groups. This finding agrees with previous research showing that internalizing problems are correlated with IBD symptom exacerbations[4]. Family dysfunction also did not differ between children and adolescents with or without active state of the disease, although the difference was close to statistical significance.

Parents reported significantly more mental health symptoms in the IBD active state than in the remission group, consistent with previous studies[12,13]. Interestingly, they also reported significantly more life events during the year prior the present assessment. This finding is the first to our knowledge to support the relationship of preceding life stress with IBD activity in pediatric populations, although 2 previous studies have supported the association of stressful life events with the onset of pediatric IBD[15,16]. Moreover, in the present study, while the parents in the active group compared to the remission group reported significantly more life events in total, this difference between the two groups was not significant regarding the events that the parents perceived as stressful for their children. It could be hypothesized that this discrepancy might suggest that parents could underestimate the potential negative impact of some life events on their children wellbeing and functioning and consequently not report them as stressful. In general, parents could ignore some aspects of the life of the patients, and underestimate or overestimate other known stressors. At the same time, one could argue that young patients couldn't estimate correctly the power of different events when using a questionnaire to detect them. The combination of reports from multiple informants (*i.e.*, parents and children) could possibly yield more reliable estimations.

Parent mental health symptoms were shown to be the only strong independent psychosocial correlate of IBD activity when all significant variables were entered into the same model of regression analysis. It is interesting that the associations of childrens' anxiety symptoms and life events with the disease activity were not significant anymore in the final model. These findings could be interpreted in different ways. With regard to anxiety symptoms, although some of the effect seen at the univariate analysis was probably due to the confounding effect of steroid medication (which was strongly related to the probability of relapse, as most patients experiencing a flare of the disease receive steroids) there is a possibility that anxiety symptoms may have an important impact on disease activity and a larger sample would provide enough power to detect it. With regard to life events, the significant relationship with disease activity that was initially found in the univariate analysis may have been subsequently moderated by the effect of parent mental health symptoms in the model of regression analysis since parents themselves reported the events and this reporting may have been possibly influenced by their mental health state. Alternatively, there may be a mediating factor such as parent coping strategies that was not examined in the present analysis

and was related to parent mental health symptoms on the one hand and the effect of life events on the other hand. This unexamined factor could have weakened the effect of life events on IBD activity in the final model, although it did not manage to weaken the relative effect of parent symptomatology. Moreover, the disease activity is a momentary state that can change within days or weeks, so that it may be difficult to detect any associations with the number of life events. In any case, these preliminary findings deserve further examination in future research.

The present study extends previous research mainly in two ways. First, it examined the associations of several psychosocial factors and outcomes with pediatric IBD activity both in univariate and regression analyses providing a more comprehensive picture of these complex relations; second, it shed some light on the relationship of the disease activity (*i.e.*, IBD remission or active state) with preceding life events, an issue that was missing in pediatric IBD literature. The findings reported here can offer some useful implications. Addressing simultaneously psychosocial needs of both children and parents in the course of pediatric IBD seem to be of importance in any effective preventive and therapeutic intervention; moreover, the role of stressful events in the course of pediatric IBD although being mediated or moderated by individual factors seem to be a possible target for future research and psychosocial treatment modalities.

The present findings should be interpreted in the context of some limitations. First, to diminish the burden of the examination on the patients we did not use adequate diagnostic interviews to screen for co-morbid psychiatric disorders; second, the study was based only on parent-reported life events that limit the interpretation of the results; moreover, the study did not examine the effect of socioeconomic status on the reported differences, although this limitation is common in pediatric IBD studies, with high socioeconomic status threatening generalizability of results. Similarly, factors such as hospital stay and parent perceived social support that have been found to be associated with impaired mental health outcomes in children and parents, respectively, were not examined in the present analysis. In addition, the sample size was rather small, leading to a low statistical power. The deviance from normality of the continuous variables of our sample led to the use of non-parametric statistics that had less statistical power than parametric ones if there was a normal distribution. The cross-sectional design of the study did not allow us to examine variations over time or make causal inferences; last, it would be interesting to include a control group not affected by IBD, since even inactive IBD patients may have a higher rate of self-reported psychosocial problems than age-matched controls. Regardless of these limitations, the present study clearly suggests that several psychosocial factors and outcomes (*i.e.*, life events, child anxiety and parent mental health symptoms) may be important correlates of pediatric IBD

activity and they may be targets of thorough assessment and treatment.

COMMENTS

Background
Epidemiological studies indicate that the incidence of pediatric inflammatory bowel disease (IBD) has been increasing over time. Elevated levels of depression, anxiety, low self-esteem, disrupted social functioning, family dysfunction, and parental distress are among the most common findings from studies comparing pediatric IBD patients with other chronic disease patients or healthy controls.

Research frontiers
Only few studies have investigated the association of psychiatric and psychosocial correlates with IBD activity in children and adolescents. Moreover, although the role of stressful life events has been studied in adult IBD patients with mixed findings, there are no published reports examining the relationship of stressful events with the disease activity in pediatric populations.

Innovations and breakthroughs
The authors provide a more comprehensive examination than currently available evidence by assessing differences in the often neglected life events among other possibly significant psychosocial problems, such as depressive and anxiety symptoms, family dysfunction, and parent mental health between an IBD remission group and an IBD active group of children and adolescents. Furthermore, they examine the association of any psychosocial variable that is shown to be correlated with the disease activity state by entering these variables in the same model as covariates.

Applications
Several psychosocial factors and outcomes (*i.e.*, life events, child anxiety and parent mental health symptoms) may be important correlates of pediatric IBD activity and they may be targets of thorough assessment and treatment.

Peer-review
The paper is well-written, easy to read and give some new considerations on the treated issue. The authors indicate correctly all the limitations.

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ORIGINAL ARTICLE

Case Control Study

Self-worth and psychological adjustment of obese children: An analysis through the Draw-A-Person

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Abstract

AIM

To investigate psychopathological correlates of child obesity *via* the Draw-A-Person test (DAP).

METHODS

The participants were 50 children with a mean age of 9.74 years. Body mass index (BMI) was used as a measure of body fat. Children were divided into normal ($n = 17$), overweight ($n = 14$) and obese ($n = 19$). Two qualitative methods of scoring the DAP based on an integrative approach were used to assess self-concept (ESW) and overall level of children's adjustment (EAC). A procedure for judging interpretative skills of clinicians was implemented before they evaluated children's drawings.

RESULTS

As predicted by our hypothesis, BMI was negatively correlated with ESW, $r (50) = -0.29$, $P < 0.05$, but not with EAC, $r (50) = -0.08$, $P = $ ns. To evaluate the effect of gender, Pearson correlations were re-computed

regrouping the sample accordingly: BMI and EAC reached a significant negative correlation in female subjects, r (24) = -0.36, $P < 0.05$, and a positive correlation in male subjects, r (26) = 0.37, $P = < 0.05$; negative correlation between BMI and ESW became stronger in females, r (24) = -0.51, $P < 0.01$ but not in males, whose correlation disappeared resulting not-significant, r (26) = -0.06, P = ns. No effect of age was found. Results indicate that obesity has a negative correlation exclusively on overall adjustment and self-concept in female children.

CONCLUSION
It was concluded that there is a negative bias toward females that reveals how the stigma of obesity is widespread in Western society.

Key words: Obesity; Draw a person; Draw-A-Person test; Projective techniques; Psychopathology

Core tip: This study was executed to investigate psychopathological correlates of child obesity *via* the Draw-A-Person test (DAP). A new procedure for using the DAP was suggested. Results indicate that obesity has a negative correlation exclusively on overall adjustment and self-concept in female children. It is consequently concluded that there is a negative bias toward females that reveals how the stigma of obesity is widespread in Western society. The "intuitive reading" of figure drawings can be considered a valid tool of assessment, even though interpreters' skills should always be assessed before executing each single studies in order to guarantee sound methodological praxis.

INTRODUCTION
Obesity is a serious problem all over the world, both in developing and industrialized countries. It is associated with severe emotional, medical and economical difficulties. According to the World Health Organization (WHO) "in many countries more than half of the adult population is above the overweight threshold, with 20%-30% of adults categorized as clinically obese"[1]. The increasing prevalence of this disorder has led the WHO to declare obesity a global epidemic[2]. The prevalence of childhood obesity has doubled over the last three decades[3]; forecasts for the future are not good: Wang *et al*[4] (2006) have predicted, for the coming years, that almost 50%

of children in North America and 38% of children in the European Union will become overweight. Being obese during adolescence or childhood increases the probability and the severity of long-term health complications[5]; early obesity also enhances the likelihood of being obese as an adult[6] and, furthermore, it is a strong predictor of expected mortality[7].

Though no doubt exists about the medical consequences of obesity, the same can't be said about psychopathological correlates. Reviews addressing this association, indeed, have led to contradictory findings, so that some authors have concluded that there is no link between these two conditions[8,9]. Friedman *et al*[10] (1995) have instead maintained that the absence of overall significant results contradict clinical experience and can be explained by specific theoretical or methodological limitations in reviewed studies (*e.g.*, sampling errors and narrow measurement). They maintain that obesity will determine psychopathological consequences only in individuals carrying definite risk factors and that it prevalently affects specific domains of psychological functions (like self-concept, pessimistic attributions or body image disturbance) rather than overall psychopathology or personality (obesity has thus been defined as a "syndrome of subclinical suffering"). They propose a second generation of studies addressing factors likely to place overweight individuals at psychopathology risk and a third generation of studies to comprehend the relationship between obesity and psychopathological correlates.

Following Friedman *et al*[10]'s (1995) considerations, the present research was conducted to investigate, utilizing the Draw-A-Person test (DAP), two possible psychopathological correlates of childhood obesity: Self-concept, a specific domain of psychological function, and general psychopathology. We also considered the effect of developmental age and gender on these correlations.

There are some reasons why obesity and child psychopathology are expected to be correlated. The stigma of obesity is widespread in Western society: Obese people are easily blamed and condemned for their condition since our culture favours aesthetic models characterized by thinness[11,12]. Staffieri[13] found that obese individuals are easily labelled with negative attributions such as "stupid" or "cheats" compared to normal weight individuals. Negative attributions by others can easily affect self-concept as this schema also evolves by feedbacks from the perceptions of others[14,15]. The habit of being negatively addressed as obese can thus determine the development of a negative perception of oneself as worthless, inadequate, inferior or inept. Some research has in fact found a negative correlation between body image dissatisfaction and self-esteem[16-18]; other studies have found this relation to be mediated by childhood teasing[19,20].

Another hypothesis worth considering is the opposite one: Excessive eating may develop to reduce emotional suffering stemming from an aversive self-concept. This so-called "psychosomatic" hypothesis maintains that

food consumption is an attempt to cope with negative emotions like depression and anxiety[21,22]. The ability of self-esteem to affect eating habits is evident if we consider that the manipulation of self-esteem (*e.g.*, by telling subjects they have failed on a problem solving task) has been shown to provoke disinhibited eating[23]. Furthermore, studies tracking mood and eating by periodic self-monitoring have shown that binge eating is preceded by greater negative moods and followed by emotional relief[24-26]. Heatherton *et al*[27] (1991), in an extensive review, have proposed the so-called "escape model" according to which binge eating arises as a way to narrow a person's attention to immediate food sensations and consequently to avoid broadly and threatening thoughts concerning the self.

In spite of this, research addressing self-concept in obese children has led to contradictory findings, some indicating an association between negative self-worth and excessive weight[28,29] and others which do not evidence this relationship[30-34]. A meta-analysis of Miller *et al*[35] (1999) showed a moderate negative effect size was present as a measure of the relation between self-esteem and weight; they also found this relation to be higher for high school and college students than for children.

We think that obesity and children's self-worth are correlated and that, at least in part, contradictory findings can be explained by two kinds of factors.

The first is question of methodology. Studies of the psychopathological functioning of obese patients have prevalently employed assessment instruments like diagnostic interview schedules, self-made measurements, questionnaires, scales and inventories[10]. The problem here is that children usually have significant difficulties in comprehending and correctly expressing inner feelings and, in general, all subjective states (especially those related to discomforting emotions). Consequently, projective measures have been expressly developed with the aim of capturing unconscious personality dynamics and functioning utilizing samples of behaviours which go beyond self description or clinical observations. It has been demonstrated that they can provide useful and valid information concerning personality and psychopathology (inner conflicts, perception of self and others, fears, relationships with family members or other relevant emotional figures) missed by self-report measures[36,37]. This is one of the reasons why draw a person and other projective techniques are routinely applied for the assessment of children's psychopathology: They are among the first ten most used assessment tools in clinical practice[38,39]. The assumption underlying the DAP is that when a person draws a human figure, he represents the way he views himself[40]. According to this so-called "body image" hypothesis, the representation of one's body becomes a way to express inner emotions and beliefs related to the "self": "The general style and content of a drawing should give an interpreter a sense of how the respondent experiences him- or her-self in the world"[41]. Given the low effect that obesity seems

to have on psychopathology and the methodological difficulties associated with the psychological assessment of children, a projective evaluation of adjustment level and self-concept through the DAP could be particularly effective. Most of all, we think that a projective evaluation of self-worth in obese children could be useful since negative self-attributions are seldom far from an almost tacit level of self-awareness[42].

The 2nd factor which can shed light on the contradictory findings concerning self-worth in obese children is the effect of certain moderator variables like gender and age.

As far as the effect of gender is concerned, though the prevalence of obesity is about the same for both sexes, a number of results and clinical observations indicate that female subjects are at higher risk of developing emotional suffering than male subjects[35,43,44]. Given the importance that body self-esteem plays in the development of female self-concept, women are surely stigmatized more than men[45,46]. It's not a coincidence that treatment for obesity is predominantly requested by women rather than men and this could be explained by corresponding emotional suffering[10]. We consequently hypothesize that obese female children should show higher levels of negative self-attributions than male ones.

As regards the effect of age, Friedman *et al*[10] (1995) maintained that the absence of a clear relationship between self-worth and obesity can be explained if we consider that the link between body esteem and self-esteem during childhood is weak and becomes stronger approaching adolescence. They maintain that this can explain why older obese individuals (adolescents and college-age women) show clearer evidence of negative self-attributions. We agree that this link is more evident during adolescence, but we think it slowly develops during childhood, becoming stronger as adolescence approaches.

These two factors can affect the correlation between obesity and psychopathology whatever the direction of the causal link between them. If we take into consideration the "psychosomatic" model, we can hypothesize that, once it develops in order to reduce emotional suffering, obesity might, in turn, have a worse effect on females and on adolescents for the same reason.

These are, consequently, the hypotheses of this study: (1) obesity has no clear correlation with children's overall level of adjustment; (2) obesity has a specific negative correlation with self-worth; and (3) being female and older in age have an effect on the psychopathological correlates of obesity.

Traditionally, two methods of scoring have been developed for DAP evaluation: A quantitative approach based on the aggregation of multiple variables and a qualitative approach based on holistic clinical evaluation[41]. We used this last method to evaluate drawings since qualitative methods have shown to be sounder than quantitative ones[47,48]. Holistic evaluation scales have been developed on the assumption that the intuitive judgement of clinical psychologists can be a valid and reliable assessment tool[49]. Overall adjustment was assessed through the so-called "Estimated Adjustment of the

Client" (EAC)[50], while we tried a new procedure we called "Estimated Self-Worth" (ESW) to evaluate self-concept of obese children.

Before evaluating children's drawings, it was decided to ascertain the interpretative skills of the two clinicians who were instructed to evaluate adjustment and self-worth through the DAP. Hammer[51] stressed how human figure drawing can be a sensitive tool in the hands of some evaluators while it could be a misleading instrument in the hands of others. Hunt[52] and Fowler[53] maintained that the same clinician should be considered as an assessment instrument and that interpretative performance should be evaluated just like we ordinarily do for tests. Personality characteristics like empathy, intuition, creativity and "affiliative" inter-personal orientations have been underlined as factors predicting good interpretive skills[54]. Above all, we found this evaluation essential since we decided to use a holistic approach which - differently from quantitative scales - relies almost entirely on the clinician's emotional and cognitive reactions to the observation of drawings. We validated interpretative skills using two drawing samples of patients with two different personality disorders and a control group made by subjects with no history of psychopathological problems. The assignment of a patient to a group was decided according to the specific self-concept associated with the correspondent personality disorder (low self-worth *vs* overvalued self-worth). We expected patients belonging to low-self worth personality to have the lower mean score on the ESW measure, patients belonging to the overvalued self-worth personality to reach the higher mean score and subjects with no history of psychopathological problems to have the middle mean score. As regards the evaluation of adjustment, we expected both groups with personality disorders to have lower levels of psychological adjustment than the control group.

MATERIALS AND METHODS

Preliminary analyses regarding interpreters judges
Raters were two experienced clinical psychologists with a formal education and at least five years of clinical practice in the field of assessment through projective techniques and psychotherapy.

DAP scoring
EAC: Following the instructions of Albee *et al*[49,55] judges were first introduced to a definition of adjustment as the capacity for emotional investment in relationships and as the ability to translate reality in a way that is compatible with conventional perceptions of others. No specific DAP indicators were recommended to them: They were instructed to feel free to choose any sign they thought helpful in making a conclusion concerning children's well being. They were also told to rely on clinical experience, empathy and intuition. Adjustment was rated on a 5-point scale ranging from very maladjusted (1) to extremely

well-adjusted (5); the midpoint (3) was labelled as normally adjusted. Intuitive adjustment evaluation has already been successfully applied to evaluate the presence of psychopathology[55,56], to predict psychiatric hospital admission[57] and foster-care placements[50] and to differentiate children with mood and mood/anxiety disorders from control group children[48].

ESW: As regards the measurement of self-esteem, judges were first introduced to a definition of self-esteem as a global judgement of self-worth[58]; it was rated on a 5-point scale ranging from viewing oneself as worthless (1) to viewing oneself as special (5); the midpoint (3) was labelled as balanced view of self-worth. Judges were instructed to choose level 1 when they thought children had a negative view of themselves as worthless; this adjective was further explained using terms referring to personality characteristics like feeling inadequate, fragile, weak, lacking in self-confidence and self-esteem, inept, inferior. Level 5 was defined as feeling special: This term was further explained with other words like superior, admirable, unique, grandiose. Level 3 was labelled as balanced self-worth: Judges were told to select this midpoint level when they thought children were characterised by a balanced self-confidence and self-esteem. As with EAC, no specific DAP indicators were suggested to judges who were left free to choose any indicator they thought helpful in making a conclusion concerning children's self-concept.

Reliability of interpretative skill
Training was carried out to reach at least 80% agreement of all DAP measures on two samples of drawings before working on the experimental ones. A first training sample of twenty-five human figure drawings was randomly selected from the files of a neuropsychiatry service; a second training sample of twenty-five drawings was taken from a local primary school. These drawings were selected to assure that the training could be reliable both with psychopathological and normal subject evaluations; the random selection of training drawings was realized controlling the participants in the main study for age and sex. The choice of experienced clinical psychologists and a period of training were preferred since it has been proved that experience can improve both the interpretative ability and reliability rates of skilled interpreters[59]. Pearson correlations between the two judges were computed for continuous variables: Interrater reliability for EAC was 0.79, for ESW it was 0.81.

Validity of interpretative skill
The overall sample of participants used for the validation of interpreters consisted of 45 individuals, 30 of whom had been seen for psychological testing and psychotherapy at a public psychiatric health service; 15 more participants with no history of psychological counselling or intervention were selected as a control group, controlling for age and sex. A diagnosis of personality disorders was carried out

Table 1 Evaluation of interpretative skill: Mean scores by group membership

Measure	DPD group (n = 15)		NPD group (n = 15)		n (n = 15)	
	M	SD	M	SD	M	SD
EAC	1.86	0.65	1.8	0.96	3.93	0.79
ESW	2.13	1.06	4.27	0.88	3.13	1.12

EAC: Estimated adjustment of the client; ESW: Estimated self-worth; DPD: Dependent personality disorder; NPD: Narcissistic personality disorder; *n*: Subjects with no history of psychopathological problems.

through the SCID-Ⅱ, the Structured Clinical Interview for DSM-IV Personality Disorders[60]. Since all the patients involved in the study entered psychotherapy soon after the assessment procedure, initial SCID diagnoses were confirmed by subsequent clinical observations. Two groups of 15 patients were formed: The first one who received a diagnosis of "dependent personality disorder" and the second one received a diagnosis of "narcissistic personality disorder". It was not possible to assess self-esteem through questionnaires or other measures, since we used randomly selected archival data which were collected before planning and executing this study. However both experimental and clinical research have widely demonstrated the association between low self-esteem and dependency[61-63] while high self-esteem and narcissism are obviously linked. We essentially relied on a clinical diagnosis of the whole personality to assess self-worth, the single domain of psychopathological functioning we were interested in. Drawings were scored on a blind basis: Scorers were unaware of diagnoses of personality disorders (they only knew sex and age).

In accordance with our predictions, mean scores on EAC resulted significantly lower for both groups with personality disorders and higher for the group with no history of psychopathological problems (Table 1); the ANOVA test yielded significant differences between groups on the mean total score, $F(44, 2) = 34.24$, $P < 0.0001$. Planned contrasts indicated that the two groups with personality disorders did not differ on mean EAC score, but they all had significant lower mean scores than the group without psychopathological problems. To examine the validity of ESW, an ANOVA test was computed yielding significant differences between groups on the mean total score, $F(44, 2) = 16.16$, $P < 0.0001$. As predicted, the sample with dependent personality disorder had the lowest ESW mean score while the sample with narcissistic personality disorder had the highest ESW value; balanced self-worth personalities had the middle mean score (Table 1).

Evaluation of obese psychopathology
Subjects and procedure: The participants were 78 Italian children from two Roman schools. To assure generalizability of data, the two schools were randomly selected from the whole sample Roman public schools. Eight children refused to participate in the study, data

coming from 20 children had to be dropped from the sample because of missing information. Thirty-three children were signalled by their teachers due to their excessive weight, thus suggesting the possible risk of obesity. The remaining 17 were taken from the same schools as a control group (controlling for age and sex). Body mass index [BMI; weight (kg)/height (m²)] was used as a measure of body fat. Height was measured to the nearest millimeter with a portable stadiometer while weight was assessed to the nearest 0.1 kg using digital scales. Children did not dress shoes and wore light clothing. Heightbar[10] was fixed on the wall, and children were standing with back and heels pressed to the wall. Measurements were performed by three trained examiners according to standard procedures (Lohman *et al*[64], 1992). Children were subdivided into normal (n = 17), overweight (n = 14) and obese (n = 19) using the standard definition established by Cole *et al*[65] (2000). Participants had a mean (± SD) age of 9.74 ± 1.84 years and a mean BMI of 22.01 ± 2.81; mean BMI were 18.87 (normal), 22.25 (overweight) and 24.65 (obese).

Human figure drawings were obtained by providing children with a pencil and instructing them to simply "draw a person" on a sheet of white typing paper. They were scored on a blind basis: Scorers were unaware of the child's weight, they only knew their sex and age. Pearson correlations between the two judges were computed for continuous variables: Interrater reliability for EAC was 0.81, for ESW it was 0.82. Disagreements were solved by computing the mean for each of the two divergent scores. The research project was described to the parents of obese children as a study of the psychological functioning of obese and overweight children. Parental consensus was requested and obtained for each subject before starting assessment procedures. All of the children's parents gave their assents.

Statistical analysis
A one-sample Kolmogorov-Smirnov test was conducted beforehand to detect excessively skewed data; data which were not normally distributed were subjected to natural log transformations. Analysis of variance (ANOVA) was used to compare the dependent variables across the three groups. Pearson correlation test was used to search for possible association between the different variables under investigation. χ^2 test was used to investigate for possible differences whenever qualitative variables were involved. Statistical analyses were performed in SPSS for Windows 16.0 (SPSS, 2007).

RESULTS
Before testing experimental hypotheses, it was verified that EAC and ESW were not correlated through the Pearson correlation test ($r = -0.034$; $P =$ ns). Nor were differences found regarding distribution of sex, χ^2 (2, n = 50) = 0.31, $p =$ ns and mean age, $F(47, 2) = 2.81$,

Table 2 Evaluation of anthropometrical outcomes and Draw-A-Person test measures by gender				
Measure	**Male (*n* = 24)**		**Female (*n* = 26)**	
	M	**SD**	**M**	**SD**
BMI	21.47	0.24	23.56	0.34
EAC	3.32	0.28	2.91	0.32
ESW	3.12	0.36	2.93	0.37

BMI: Body mass index; EAC: Estimated adjustment of the client; ESW: Estimated self-worth.

Table 3 Evaluation of obesity: Mean scores by group membership						
Measure	**Normal weight group (*n* = 17)**		**Over weight group (*n* = 14)**		**Obese (*n* = 19)**	
	M	**SD**	**M**	**SD**	**M**	**SD**
BMI	18.87	0.37	22.25	0.31	24.66	0.32
EAC	3.18	0.32	3.43	0.27	2.89	0.23
ESW	3.24	0.36	2.79	0.37	2.11	0.32

BMI: Body mass index; EAC: Estimated adjustment of the client; ESW: Estimated self-worth.

P = ns; mean BMI across the groups gave significantly different results, $F_{(47, 2)}$ = 84.43, $P < 0.0001$ (means and standard deviations for BMI, EAC and ESW are shown in Tables 2 and 3).

Two univariated ANOVA tests were computed to examine possible differences on EAC and ESW among children subdivided into groups according to weight; analyses yielded negative results both for EAC, $F_{(47, 2)}$ = 0.92, P = ns, and for ESW, $F_{(47, 2)}$ = 2.88, P = ns. Since ESW means showed the trend we expected on the basis of our hypothesis, diminishing from normal to obese children, we decided to run a Pearson correlation test to verify our hypothesis: It was thought to be a more sensible measure of the association between DAP scores and BMI. As predicted by our hypothesis, BMI was negatively correlated with ESW, $r_{(50)}$ = -0.29, $P < 0.05$, but not with EAC, $r_{(50)}$ = - 0.08, P = ns.

To evaluate the effect of gender, Pearson correlations were re-computed regrouping the sample accordingly: BMI and EAC reached a significant negative correlation in female subjects, $r_{(24)}$ = -0.36, $P < 0.05$, and a positive correlation in male subjects, $r_{(26)}$ = 0.37, $P \leqslant 0.05$; negative correlation between BMI and ESW became stronger in females, $r_{(24)}$ = -0.51, $P < 0.01$ but not in males, whose correlation disappeared resulting not-significant, $r_{(26)}$ = -0.06, P = ns.

These data strongly suggest that gaining weight is associated both with an overall level of psychopathology and negative self-worth but only in female children, not in males; better still, males show a positive association between weight and adjustment. The effect of BMI is stronger on the specific domain of self-worth rather than on the general level of psychopathology. If we consider the most obese children (BMI > 24; *n* = 15) we find further confirmation of our conclusions, since nine of them have the lowest level of ESW (ESW = 1).

To calculate the effect of age on EAC and ESW, a possible correlation between them was first verified. Age resulted associated with ESW, $r_{(50)}$ = 0.36, $P < 0.005$ while EAC had a near-significant correlation with age, $r_{(50)}$ = 0.23, $P = 0.06$. Further analysis was performed to test the possible interaction between age and BMI on EAC and ESW. It was found that controlling for BMI, both the correlation between age and ESW, $r_{(47)}$ = 0.37, $P < 0.004$, and between age and EAC, $r_{(47)}$ = 0.23, $P = 0.053$, showed no substantial change.

Contrary to our predictions, we consequently found no effect of age on the level of self-worth and on the overall level of adjustment of obese children.

DISCUSSION

Obesity
The results of the present study suggest that obesity is associated with low self-worth and lower levels of overall adjustment exclusively in female subjects. Obese female children dislike themselves and show a tendency to feel worthless, inadequate and lacking in self-esteem. Male children do not seem to suffer because they are overweight: Our data even suggest that in male children, weight may be associated with higher levels of adjustment.

Our data are consistent with Friedman *et al*[10]'s idea of obesity as a "syndrome of subclinical suffering". It can be hypothesized that being an obese female child is a risk factor for the development of a negative self-concept (self-worth) and for the development of low levels of adjustment. This negative bias toward females supports the hypothesis that obesity is a cultural problem: It may be that aesthetic models characterised by thinness have negative consequences on self-concept.

The other explanation worthy of consideration is that obesity develops as a consequence of emotional suffering coming from low self-worth: Once developed, overeating could be enhanced by the negative affect caused by social stigma, especially for female children for whom cultural models are particularly demanding. According to this different explanation, it is possible that the negative self-image associated with obesity reinforces pre-existent self-deprecating processes which usually affect self-worth.

In any case, whatever the direction of this link, the management of obesity should focus on self-worth since obese young females are prone to emotional distress and negative mood: Working on self-esteem is of primary importance in facilitating mental health and adjustment to body fat. Some non-dietary approaches to obesity have in fact successfully attempted to improve self-esteem and body image through self-acceptance[66-69]. Nevertheless, it is also important to consider the role that sex hormones, and maturity of the hypothalamic, pituitary gonad axes in the psychological symptoms and

self-esteem of adolescent girls. Some researchers have shown that there is a negative relationship between self-esteem and sexual development of girls and adolescents. Specifically, Huerta *et al*[70] showed that girls who are older and achieve highest sexual development had lower self-esteem, more anxiety and depression than girls younger and with less sexual development, independent of the girls' body weight. Consequently, it may be important to consider sexual development of obese female adolescents in the assessment of their possible emotional problems, as it may take an important role in the development of their psychological troubles.

Clinical judgement of DAP made by experienced clinical psychologists can be successfully used to evaluate self-worth and overall adjustment level in obese children. Indeed, the self-defeating and often unconscious convictions concerning the self found in female subjects, may be observed through some specific DAP signs produced by our sample. For instance, insecurity and low self-esteem may have brought participants to draw human figures with light lines, line discontinuity or erasures. Some DAP indicators may be used to evaluate these psychopathological correlates. According to our judges, the most frequent signs influencing their evaluation might have been small human figures, light lines, line discontinuity, erasures, body simplification, sad or frightened posture/facial expression. These signs are usually associated with anxiety[71] and depression[40,72-74]. This is consistent with research findings that show how obese female adolescents spend significantly fewer months at high school[75] and with other studies which have found an association between obesity and depression[76,77]. Both of these conditions - depression and low scholastic achievement - are indeed associated with low self-esteem and low self-efficacy. Since DAP can be administered in a short period of time and is easily complied by patient, it could be used as a preliminary screening test for the selection of children needing therapeutic intervention.

We also found that age does not mediate the relationship between weight and psychopathology. The absence of the effect of age can be related to the typology of measure used in this study. It may be that an implicit negative self-perception develops during childhood and that it does not change during adolescence, while overt emotional suffering concerning the self, may start during adolescence or later during adulthood. Further research is needed to solve this question: Longitudinal studies with different kinds of assessment instruments should be used to distinguish between low self-esteem and emotional suffering.

Methodological considerations concerning the DAP
The "intuitive reading" of figure drawings can be considered a valid and reliable tool of assessment both from a scientific and clinical point of view. The DAP "feeling approaches" consist of using the cognitive and emotional reactions of a clinician when the drawings are examined to obtain information concerning the drawer's personality: They probably rely on primitive layers of "knowing" far beyond an individual's awareness[54,78] because, while evaluating drawings, judges describe empathising as an involuntary and automatic mental activity. When evaluating drawings characterised by low self-worth our judges said they had the "impressions" of feeling sad or frightened, or imagining a weak child lacking in self-confidence. Sometimes they said they didn't know exactly why they attributed specific scores to drawings: They said they intuitively felt the child had a negative view of himself or a grandiose one even when signs didn't suggest anything clear.

Riethmiller *et al*[41] stressed the importance of multivariable scales when discussing quantitative evaluations of DAP. They maintained that the same construct can find expression through different signs: Evaluating a single sign could consequently be misleading since a single item has a low correlation with the associated construct[79]. Qualitative evaluation of DAP could be useful since clinical intuition may produce a deeper and sounder evaluation of single signs whose meaning may vary in relation to the remaining characteristics of the figure. In other words, holistic evaluation of the figure, through clinical intuition, could give the correct importance and meaning to the varying individual signs of the drawing, thus making possible a more valid overall interpretation.

The problem here is that it is not clear what kind of personality factors or experiences are responsible for the development of interpretative skill: This is the reason why assessment is sometimes considered an art rather than a scientific discipline. From a methodological point of view the problem is the correspondence between subjective involuntary mental activity and objective external reality: Subjective impressions could simply be wrong. The reliability of certain indicators is not enough: Joiner *et al*[80] found that size, detail and line heaviness had high rates of reliability but they were not significantly associated with external measures of depression and anxiety. We used the evaluation of personality disorders as a measure of validity by sorting subjects with different levels of self-worth and adjustment. This choice was made on the assumption that DAP is particularly suitable for the assessment of stable personality tendencies rather than for transient behavioural or mood alterations[81]. The evaluator's personality should consequently be considered as an assessment tool to be validated[53], just like tests, before starting an empirical and holistic evaluation of DAP research data[82]. Even when evaluators have good interpretative skills, countertransference problems could bias interpretations of the drawings. Hammer *et al*[83] found a correlation between the degree of evaluators' hostility and their inclination to perceive aggressive tendencies when evaluating drawings. We consequently think that all research involving holistic DAP measures should require an evaluation of the judges' skills, specifically those relating to the variables to be measured.

The validation procedure which discriminates between the different personality disorders here tested can be thus considered a way to scientifically ascertain the intuitive and empathic skills of evaluators, rather than a way of validating EAC or ESW. It is indeed simply impossible to use traditional tests to evaluate clinical skills like empathy or affiliative tendencies. It would be sounder to validate the accuracy of judges' evaluations by using categories of patients which share the same characteristics of the experimental group. To evaluate disordered thinking for instance, it would be useful to test interpretative skills by comparing drawings by subjects with a diagnosis of schizophrenia with other drawings made by subjects with different psychopathological problems and samples from individuals without any kind of psychiatric diagnosis.

DAP research has been characterised by lack of coherent findings and severe methodological criticism, just like other research involving projective techniques. One of the reasons why DAP research has produced contradictory findings is that the interpreters who evaluate the drawings have different skills. Consequently, interpreters' skills should always be assessed and measured before executing each single studies in order to guarantee sound methodological praxis: "…focus should instead be on good interpreters, who demonstrate outstanding ability to interpret the DAP"[78].

This study has different limitations. First of all, it was not considered the possible effect of socioeconomic strata on the relationship between obesity and the EAC and ESW variables; however, random selection of the two samples probably reduced the possible effect of socioeconomic factors on the results of this study. Also, sexual development that children and the antecedent of age at menarche had at the moment of the study were not considered; this is a limitation as these variables are related with different personality characteristics of children. Future research may address this question.

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COMMENTS

Background
Obesity is a serious problem all over the world. Reviews addressing the association between obesity and psychopathology have led to contradictory findings. It has been proposed that obesity will determine psychopathological consequences only in individuals carrying definite risk factors and that it prevalently affects specific domains of psychological functions. Thus, the present research was conducted to investigate two possible psychopathological correlates of childhood obesity (self-concept and general psychopathology) *via* the Draw-A-Person test (DAP). The authors also considered the effect of developmental age and gender on these correlations.

Research frontiers
Current research suggests that clinical judgement of DAP made by experienced clinical psychologists can be successfully used to evaluate self-worth and overall adjustment level in obese children.

Innovations and breakthroughs
This study is, to the authors' knowledge, the first to investigate psychopathological correlates of child obesity *via* DAP.

Applications
Clinical judgement of DAP made by experienced clinical psychologists can be successfully used to evaluate self-worth and overall adjustment level in obese children. Since DAP can be administered in a short period of time and is easily complied by patient, it could be used as a preliminary screening test for the selection of children needing therapeutic intervention.

Terminology
Obesity: Excessive accumulation of body fat that may impair health; Draw-A-Person test: An implicit measure of personality consisting of drawing a person on a sheet of white typing paper.

Peer-review
This is an interesting and methodologically well developed study.

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ORIGINAL ARTICLE

Case Control Study

Chronic pelvic pain, psychiatric disorders and early emotional traumas: Results of a cross sectional case-control study

Flávia L Osório, Ana Carolina F Carvalho, Mariana F Donadon, André L Moreno, Omero Polli-Neto

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Abstract

AIM

To compare the prevalence of psychiatric disorders and early emotional traumas between women with chronic pelvic pain (CPP) and healthy women.

METHODS

One hundred women in reproductive age, 50 of them had CPP (according to the criteria set by the International Association for Study of Pain), and 50 were considered healthy after the gynecological evaluation. The eligibility criteria were defined as follows: chronic or persistent pain perceived in the pelvis-related structures (digestive, urinary, genital, myofascial or neurological systems). Only women in reproductive age with acyclic pain for 6 mo, or more, were included in the present study. Menopause was the exclusion criterion. The participants were grouped according to age, school level and socio-economic status and were individually assessed through DSM-IV Structured Clinical Interview (SCID-I) and Early Trauma Inventory Self-report - short form (ETISR-SF Brazilian version). Descriptive statistics, group comparison tests and multivariate logistics regression were used in the data analysis.

RESULTS

The early emotional traumas are highly prevalent, but their prevalence did not differ between the two groups. The current Major Depressive Disorder was more prevalent in women with CPP. The CPP was associated

with endometriosis in 48% of the women. There was no difference in the prevalence of disorders when endometriosis was taken into account (endometriosis *vs* other diseases: $P > 0.29$). The current Major Depressive Disorder and the Bipolar Disorder had greater occurrence likelihood in the group of women with CPP (ODDS = 5.25 and 9.0).

CONCLUSION
The data reinforce the link between mood disorders and CPP. The preview evidences about the association between CPP and early traumas tended not to be significant after a stronger methodological control was implemented.

Key words: Pelvic pain; Psychiatric disorder; Early trauma; Emotional; Depression

Core tip: There is also evidence about the association between depressive and anxious symptoms and the presence of chronic pelvic pain (CPP). The weakest points in these data refer to the quality of the studies; as most of them are descriptive and assess symptoms, instead of confirming the disorder symptoms, which may affect the understanding of the link between conditions. The current study used "gold standard" psychiatric diagnostic instruments to assess the presence or absence of Axis I mental disorders. The results showed associations between mood disorders and CPP, but the association between CPP and early trauma tends not to be significant after increased methodological control.

INTRODUCTION

The chronic pelvic pain (CPP) is a prevalent condition in women, mainly in those who are in reproductive age. The CPP occurrence is estimated in approximately 4%[1] but, in Brazil, it is close to 10%[2,3]. According to the International Association for Study of Pain (IASP), CPP is featured as a chronic or persistent pain in pelvis-related structures; it is often associated with negative emotional, sexual, behavioral and cognitive consequences, as well as with symptoms that suggest dysfunctions in such systems. Its symptoms include either cyclic or acyclic pain; however, it is not necessary to show the symptoms for more than six months if the patient presents evident signs of central sensitization. The central sensitization is an important event in patients with chronic pain. There are no pathognomonic clinically signals or symptoms. Nevertheless, primary or secondary hyperalgesia, dynamic tactile allodynia, the temporal summation of pain are some of them. When these conditions were presented, the chronicity can be considered before six months[4].

Factors related to the etiology and maintenance of CPP remain unclear. So far, it is known that CPP is a complex condition influenced by, or resulting from, the interaction between many systems, for instance: the gastrointestinal, urinary and genital ones; it is also associated with neurological and psychological aspects[5,6]. There are many studies pointing towards the role played by the emotional factors, mainly towards the presence of Early Emotional Traumas (EET)[7] and mental disorders[1,8-10] in the psychological aspects associated with CPP.

A meta-analysis conducted by Latthe *et al*[1] pin-pointed that sexual and physical EETs increase in approximately 1.5-2.1 times the chances of developing CPP. However, these authors state that the associations between sexual abuse and CPP are more prevalent in low methodological quality studies; thus, it is worth being careful at the time to interpret the results. There are also evidences about the association between depression and anxiety symptoms, and CPP[11]. Nevertheless, one of the weakest points to these evidences is the quality of the studies, most of them are descriptive and just assess the symptoms rather than confirming the disorder. Thus, it may compromise the understanding about the link between different conditions.

The current study was based on the aforementioned panorama, which evidenced the lack of cross sectional case-control studies that use psychiatric "gold standard" diagnostic instruments to assess the presence or absence of axis I mental disorders, as well as the lack of studies focused on etiological factors associated with CPP.

Thus, the main aims of the current study are: (1) to assess the prevalence of psychiatric disorders and EETs in women with CPP; and (2) to verify the hypothesis that these disorders and traumas had greater occurrence likelihood in the group of women with CPP.

MATERIALS AND METHODS

The present research is a cross sectional study and its convenience sample was composed of 100 women in reproductive age, 50 of them had CPP (according to the criteria set by IASP), and 50 were considered healthy after the gynecological evaluation. The eligibility criteria were defined as follows: Chronic or persistent pain perceived in the pelvis-related structures (digestive, urinary, genital, myofascial or neurological systems). Only women in reproductive age with acyclic pain for six months, or more, were included in the present study. Menopause was the exclusion criterion. Women with CPP were recruited in the Chronic Pelvic Pain Center of a university hospital and the healthy women were

Table 1 Sociodemographic features of the samples according to the chronic pelvic pain and control groups

Variable		CPP (*n* = 50)		C (*n* = 50)		Statistics
		n	%	*n*	%	
Age	Mean (SD)	37.44 (8.12)		37.9 (8.72)		U = 1218.00
						P = 0.82
School level	Up to 8 yr	19	38	19	38	χ^2 = 0.45
	From 9 to 11 yr	26	52	26	52	P = 0.98
	Over 12 yr	5	10	5	10	
Marital status	Single/widow/divorced	15	30	24	48	χ^2 = 3.40
	Married/law marriage	35	70	26	52	P = 0.07
Number of kids	Mean (SD)	1.72 (1.67)		2.08 (1.61)		U = 1055.50
						P = 0.17
Professional status	Non-active	24	48	4	8	χ^2 = 19.84
	Active	26	52	46	92	P < 0.001[1]

[1]Statistically significant difference; CPP: Chronic pelvic pain group; C: Control group; U: Mann-Whitney test.

recruited among the employees and outpatients of the primary care center in the same institution. The participants were grouped according to age, school level and socio-economic status. The recruiting process took place in 2014.

The following instruments were used for data collection: (1) Structured Clinical Interview for DSM-IV - clinical version (SCID-I/CV): Which is used to diagnose different axis I mental disorders, it was translated into Portuguese and validated by Del-Ben *et al*[12], its inter-appraiser reliability score was 0.83; (2) Early Trauma Inventory Self-report - short form (ETISR-SF): Which is a self-report instrument used to investigate traumatic experience history before the age of 18. It is composed of 27 items, divided in four dimensions (general trauma, physical abuse, emotional abuse and sexual abuse) and scored in dichotomous scale (Yes/No). The total score and the score of each sub-scale are given by summing the items. The larger the sum is, the larger the number of experienced traumatic events. The version translated into Portuguese and validated by Osório *et al*[13] was used. It presented internal consistency 0.83 and test-retest reliability 0.78-0.90; (3) Socio-demographic Questionnaire: which is composed of items linked to the socio-demographic features of the sample; and (4) Medical records: Were used to get clinical information associated with CPP.

The SCID-I-CV was applied in person, during individual sections, by an appraiser trained and experienced in using this instrument. Subsequently, the participants filled out the self-report instruments. The study was approved by the Local Ethics Committee (Process No. 11798/2012) and conducted according to the ethical principles of research involving human beings. We got the written consent from the participants.

Data were analyzed in the SSPS statistical software. The descriptive statistics (mean and standard deviation), and the χ^2 and Mann-Whitney tests were used to compare the groups. Cohen statistic was used to estimate the magnitude of the differences between groups. The parameters adopted for the interpretation of this parameter will be: < 0.20 = small, 0.2-0.8 = medium; > 0.80 = large[14].

The prediction analysis was performed through multivariate logistics regression (the backward method). The presence or absence of CPP was the endpoint. The independent variables (psychiatric disorders), whose *P* values were lower than 0.20 in the group comparison analysis, were included in the initial regression model and tested as possible predictors[15,16]. The P < 0.05 was adopted as significance value in all the analyses.

RESULTS

The socio-demographic feature is shown in Table 1, which shows differences in the professional status and higher percentage of inactive women in the CPP group. The list of diagnoses comprised endometriosis (*n* = 24), myofascial and neuralgia (*n* = 6), irritable bowel syndrome (*n* = 5); other diagnosis (adhesions, pelvic inflammatory disease, pelvic congestion syndrome, interstitial cystitis, *n* = 13); undetermined symptom (*n* = 2).

The prevalence of EETs is high (Table 2), but it did not differ between groups.

The specific analysis of each traumatic situation assessed through ETIS-SR also did not show statistic differences between the groups (*P* > 0.11).

There was significantly higher prevalence of current Major Depressive Disorder in women with CPP than in the healthy controls, in cases of Axis I psychiatric disorders (Table 3).

There was the general trend of Mood Disorder prevalence in the CPP group. There was no statistical difference in the prevalence of different disorders when the clinical group and the causes were taken into account (endometriosis *vs* other diseases: *P* > 0.29).

The variables tested in the initial model (*P* > 0.20) of the multivariate regression analysis were: Major Depressive Disorder, Bipolar Disorder, Panic Disorder, Hypochondria and Anorexia. However, the model appeared to be inappropriate. New models were tested, and

Table 2 Scores of the early trauma inventory - short form - and their sub-scales according to the chronic pelvic pain and control groups

Type of early trauma		CPP (*n* = 50)	C (*n* = 50)	Statistics	Effect Size
General traumas	Mean[1]	2.56	2.16	*U* = 1159.00	0.20
	(SD)	(2.26)	(1.81)	*P* = 0.52	
	% Yes[2]	84	82		
Physical punishment	Mean	2.04	1.51	*U* = 1011.00	0.36
	(SD)	(1.66)	(1.28)	*P* = 0.12	
	% Yes	72	70		
Emotional abuse	Mean	2	1.92	*U* = 1204.00	0.04
	(SD)	(1.93)	(1.87)	*P* = 0.88	
	% Yes	64	68		
Sexual events	Mean	1.14	0.98	*U* = 1169.00	0.11
	(SD)	(1.5)	(1.36)	*P* = 0.54	
	% Yes	50	42		
Total	Mean	7.8	6.54	*U* = 1091.50	0.24
	(SD)	(5.84)	(4.5)	*P* = 0.44	
	% Sim	94	98		

[1]Mean of traumatic situation sexperienced in each category; [2]Percentage of subjects with at least one type of trauma within the category. CPP: Chronic pelvic pain group; C: Control group; U: Mann-Whitney test.

Table 3 The prevalence of different Axis I Psychiatric disorders according to the chronic pelvic pain and control groups

Psychiatric disorders[1]		CPP (*n* = 50)		C (*n* = 50)		Statistics
		n	%	*n*	%	
Mood	Current major depressive	14	28	4	8	*P* < 0.011
	Bipolar disorder	6	12	1	2	*P* = 0.11
	Dysthymia	1	2	--	--	*P* = 1.00
	Any mood disorder	24	48	15	30	*P* = 0.06
Use substances	Abuse/dependence Substance	10	20	12	24	*P* = 0.63
anxiety	Panic	8	16	3	6	*P* = 0.11
	Obsessive-compulsive	12	24	9	18	*P* = 0.46
	Post-traumatic stress	3	6	2	4	*P* = 0.65
	Social anxiety	10	20	6	12	*P* = 0.28
	Specific phobies	12	24	11	22	*P* = 0.81
	Any anxiety disorder	27	34	26	52	*P* = 0.84
Somatoforms	Somatization	7	14	5	10	*P* = 0.54
	Hypochondria	4	8	1	2	*P* = 0.17
Eating disorders	Anorexia	4	8	--	--	*P* = 0.12
	Bulimia	5	10	5	10	*P* = 1.00

[1]According to the DSM-IV criteria. CPP: Chronic pelvic pain group; C: Control group.

the variables with lower statistical significance level were individually suppressed, until the final model presented in Table 4 was reached.

This table shows that the current Major Depressive Disorder and the Bipolar Disorder emerged with higher occurrence likelihood in women with CPP. Thus, women with current Major Depressive Disorder and Bipolar Disorder have 5.25 and 9.0 more chances of having CPP than women without the referred disorders, respectively.

DISCUSSION

The main results in the current study pointed out significant differences in the prevalence of current Major Depressive Episodes between women with and without CPP. The recent review conducted by Carvalho *et al*[11] highlighted the link between depressive symptomatology and CPP. However, the present study advanced in the knowledge about this association since it used the gold standard diagnostic interview and a control group paired by age, school level and economic status to assess the presence of depressive disorders. Thus, based on the current results, it is possible stating that the depressive disorder is more prevalent in women with CPP, as well as that the occurrence rate in this group is about five times higher than that in the group of women without CPP (OR = 5.25).

Such finding may be associated with the presence of comorbid conditions often found in depressive states such as pain experience and somatization[17]. On the other hand, it is worth highlighting that most of the studies related to such association point towards a two-way relation between these two conditions. The pain and the limitations linked to these conditions may favor

Table 4 Final logistics regression model showing chronic pelvic pain as endpoint variable

Disorder		OR	95%CI	*P* value
Current depressive episode	No	1[1]	(1.57-17.49)	0.007
	Yes	5.25		
Bipolar disorder	No	1[1]	(1.03-18.57)	0.047
	Yes	9		

[1]The reference variable. OR: Odds ratio; *P* value: Significance level.

the depressive symptoms and disorders[18-20]. Hence, by taking the current findings into consideration, as well as the design of the present study, it is more reasonable to state that the presence of current Major Depressive Episodes is an independent factor associated with CPP.

However, when it comes to the association with Bipolar Disorder, it was observed that such disorder also are more likely to occur in the group of women with CPP, although the analysis between groups did not show statistical significance. Prevalence differences were also not observed in the analyses that have considered the presence or absence of endometriosis. Such finding is interesting because the previously conducted studies disagree on the presence of such association, mainly when the presence of endometriosis, as etiologic risk factor, is taken into account. Kumar *et al*[21] compared 27 women who had endometriosis and other 12 endometriosis-free women with CPP, and found that 45% of the women in the first group presented Bipolar Disorder, whereas no woman in the CPP group presented such condition. Before that, Lewis *et al*[22] had assessed 16 women with endometriosis in an observational study and found that 75% of them presented mood disorder, mainly the affective Bipolar disorder (*n* = 10). On the other hand, just as in the current study, Walker *et al*[23] assessed women with and without endometriosis and found different prevalence of Bipolar disorder.

According to the aforementioned authors, the reason for such association among CPP, endometriosis and Bipolar Disorder remains unknown due to lack of studies on the topic. However, they stand for the hypothesis that the gonadotropin-releasing hormone agonist (GnRH) used to treat endometriosis may also favor emotional instability and other affective disorder conditions in the group of women with CPP and endometriosis; thus it may favor the development of such disorder[22]. Due to such contradictory findings, the methodologically refined studies and those that consider the possible association between medication and affective symptoms, mainly regarding the Bipolar disorder, are timely. These studies may help minimizing the impacts of these disorders and favor the correct approach and treatment applied to different conditions in order to diminish comorbidity risks.

Hence, we may conclude that the current study helped overcoming some of the methodological gaps found in previous studies on this topic and was an attempt to better elucidate the link between CPP and psychosocial conditions. The present study evidenced the association between CPP and mood disorders that need deeper investigation, mainly with regard to their specificities. On the other hand, it reinforced the items highlighted in the meta-analysis conducted by Latthe *et al*[1], who found that the association between CPP and EET tend to be insignificant if strict methodological control is taken.

COMMENTS

Background
The chronic pelvic pain (CPP) is a prevalent condition in women, mainly in those who are in reproductive age. CPP is featured as a chronic or persistent pain in pelvis-related structures; it is often associated with negative emotional, sexual, behavioral and cognitive consequences, as well as with symptoms that suggest dysfunctions in such systems. Factors related to the etiology and maintenance of CPP remain unclear. There are many studies pointing towards the role played by the emotional factors, mainly towards the presence of early emotional traumas and mental disorders in the psychological aspects associated with CPP. There is evidence about the association between depressive and anxious symptoms and the presence of CPP.

Research frontiers
The weakest points in these data refer to the quality of the studies; as most of them are descriptive and assess symptoms, instead of confirming the disorder symptoms, which may affect the understanding of the link between conditions.

Innovations and breakthroughs
The data reinforce the link between mood disorders and CPP. The knowledge about this link is improved by the use of the "gold standard" diagnostic interview and of the group control paired according to the socio-demographic variables.

Applications
Hence, they may conclude that the current study helped overcoming some of the methodological gaps found in previous studies on this topic and was an attempt to better elucidate the link between CPP and psychosocial conditions.

Peer-review
The authors did a very well designed and analyzed study about the presence of chronic pelvic pain and affective disorders. It could be better if the authors take one position or other and explain their reasons clearly in the conclusion section.

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ORIGINAL ARTICLE

Case Control Study

Self-reported and behavioural impulsivity in anorexia nervosa

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Abstract

AIM
To examine how self-reported and behavioural impulsivity are related in anorexia nervosa (AN).

METHODS
Twenty-four females with AN and 25 healthy controls (HC) participant in the study. Self-reported impulsivity was assessed with the Barratt Impulsiveness Scale (BIS-11). The scale yields three second-order factors: Attentional, motor and non-planning. Behavioural impulsivity was investigated with the continuous performance test (CPT), a computer-based task of sustained attention in which numbers are flashed briefly on screen and participants are required to click the mouse when the same number appears consecutively. The rate of commission and omission errors can be used a measure of behavioural impulsivity.

RESULTS
AN participants self-reported increased attentional [AN: 20.67 (3.64), HC: 13.88 (2.91), $P = 0.001$] and reduced motor impulsivity [AN: 11.55 (2.28), HC: 14.08 (2.78), $P = 0.002$]. The rate of omission or commission errors on the CPT did not differ between groups ($P > 0.05$). BIS-11 and CPT measures did not significantly correlate, but attentional impulsivity was related to negative mood states in AN (depression: $r = 0.52$, $P = 0.010$, anxiety: $r = 0.55$, $P = 0.006$, stress: $r = 0.57$, $P = 0.004$).

CONCLUSION
The discrepancy between self-reported and behavioural impulsivity are discussed in terms of perfectionism in AN. Furthermore, it is suggested that improving negative mood states may resolve this inconsistency in AN.

Key words: Eating disorder; Continuous performance; Anorexia nervosa; Attention; Inhibition

Core tip: The findings of the study suggest a discrepancy between self-reported and behavioural impulsivity in anorexia nervosa (AN). Although AN patients did not demonstrate differences from healthy controls in behavioural impulsivity, they self-reported reduced motor impulsivity and greater attentional impulsivity. Attentional impulsivity was associated with negative mood states in AN, suggesting that improving these symptoms may improve patients' perceptions of their attentional impulsivity.

INTRODUCTION

Anorexia nervosa (AN) is a psychiatric illness whose core characteristics include significantly low body weight, a fear of weight gain and disturbed perception of one's own body shape or weight. AN is also frequently associated with obsessive behaviours and perfectionistic tendencies[1,2]. In particular, individuals with AN display elevated concerns over making mistakes[3], and relatedly, often self-report lower rates of impulsivity[4]. However, it is unclear whether self-reported rates of impulsivity are influenced by eating disorder symptomatology or are stable traits exhibited by these individuals. It is also unclear whether these self-reported rates of impulsivity translate to behavioural performance on cognitive tasks of inhibition.

For example, Pieters *et al*[5] reported reduced impulsivity on a speeded choice-reaction task in AN; whereas, Butler, Montgomery[4] found increased errors of commission and shorter response times in AN on a continuous performance task (CPT), but lower rates of self-reported impulsivity.

The CPT is typically utilised as a broad measure of sustained attention and vigilance. However, by examining different components of task performance, researchers have also used it to examine impulsivity. In the visual variant of this task, numbers of letters are typically flashed briefly on screen to participants. The task requires a response (usually a mouse click) when the same number appears twice in a row. Errors of omission describe when the same number appears twice consecutively in sequence, but the participant fails to respond (*i.e.*, inattention); whereas errors of commission involve responding when two consecutive numbers do not match (*i.e.*, impulsivity)[6]. The CPT has been utilised to assess both sustained attention and impulsivity in a variety of conditions associated with these features; predominantly attention deficit hyperactivity disorder (ADHD) which is characterised by both inattention and increased impulsivity[7].

The aim of this study was to investigate the relationship between self-reported impulsivity and behavioural impulsivity in AN, assessed through neuropsychological task performance. It was hypothesised that participants with AN would self-report lower levels of impulsivity than healthy controls, and would similarly demonstrate reduced behavioural impulsivity (*i.e.*, fewer commission errors on the CPT). A further aim was to examine whether differences in impulsivity between AN and healthy control groups were related to eating disorder-related factors, including eating disorder symptomatology, negative mood states, illness duration and body mass index (BMI).

MATERIALS AND METHODS

This study was approved by the human research ethics departments at The University of Melbourne, Swinburne University of Technology, The Melbourne Clinic, The Austin Hospital and St Vincent's Hospital; all in Melbourne,

Australia. Informed written consent was obtained from all participants. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Participants

Participants were 24 right-handed females with AN and 25 healthy controls (HC) matched for age and premorbid intelligence quotient (IQ). HCs were recruited through public advertisements, whereas AN participants were recruited through public advertisements; the Body Image and Eating Disorders Treatment and Recovery Service at the Austin and St Vincent's Hospitals; and The Melbourne Clinic; all in Melbourne, Australia.

All participants were English speaking and had no history of significant brain injury or neurological condition. Controls were required to have no history of an eating disorder or other mental illness. The Mini International Neuropsychiatric Interview, 5.0.0 (MINI)[8] was used to screen all participants for major Axis I psychiatric disorders according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). It was also used to confirm diagnoses of AN, with the exception of the amenorrhea criterion which is no longer included in the current DSM-5. AN was required to be the primary diagnosis of the AN group. AN participants with comorbid psychiatric conditions, other than psychotic conditions, were not excluded as this would not have represented a typical AN sample.

Assessments

Premorbid intelligence was estimated using the Wechsler Test of Adult Reading (WTAR)[9]. Eating disorder symptomatology was investigated with the Eating Disorders Examination Questionnaire (EDE-Q)[10], and negative emotional states with the Depression Anxiety Stress Scale (DASS)[11]. Self-reported impulsivity was assessed with the Barratt Impulsiveness Scale (BIS-11)[12]. The scale yields three second-order factors: attentional (consisting of the first-order factors attention and cognitive instability), motor (consisting of the first-order factors motor and perseverance) and non-planning (comprised of the first-order factors self-control and cognitive complexity).

Behavioural impulsivity was assessed with the Continuous Performance Test - Identical Pairs (CPT-IP), a computer-based task of sustained attention in which numbers are flashed on the screen for 50 ms and participants are required to click the mouse when the same number appears consecutively. The task consists of two-, three- and four-digit conditions, each consisting of 150 trials in which the total number of possible hits is 30 (*i.e.*, the inverse of omission errors), the total number of possible false alarms is also 30 (*i.e.*, commission errors), and total number of possible random responses is 90 (detailed findings of this task which contained the current

sample and additional participants are presented in[13]). Response times (*i.e.*, the time taken to click the mouse from the presentation of the stimulus) are also recorded for omission and commission errors.

Statistical analysis

Following normality checking and the removal of outliers, group differences in BIS-11, EDE-Q, DASS and CPT-IP scores were examined with analyses of variance (ANOVAs). Group differences in BIS-11 subscale scores and CPT-IP scores were further explored with Pearson's correlations between these measures and illness duration, BMI, and EDE-Q and DASS scores. Due to the large number of correlations, alpha was set to 0.01 to account for multiple comparisons.

RESULTS

Table 1 presents the group comparisons in BIS-11, EDE-Q and DASS scores. AN participants had significantly higher EDE-Q and DASS scores, relative to controls. BIS-11 scores significantly differed in the second order factor "attentional", and its two first-order factors "attention" and "cognitive instability", with AN participants reporting higher impulsivity. AN participants also reported significantly lower impulsivity in the first-order factor "motor".

Table 2 describes the CPT-IP findings. Groups were not found to differ in the proportion of hits (inverse of omission errors), false alarms (commission errors) or random responses. AN participants were, however, found to have increased false alarm response times, and greater intra-individual variability in this response.

Pearson's correlation analyses did not reveal any significant correlations between measures for the HC group. A number of significant correlations were revealed in the AN group. The first-order factor, "attention" of the BIS-11 was positively correlated with state depression ($r = 0.53$, $P = 0.007$), anxiety ($r = 0.58$, $P = 0.003$) and stress ($r = 0.65$, $P = 0.001$) as measured by the DASS. The second-order factor, "attentional" was also positively correlated with depression ($r = 0.52$, $P = 0.010$), anxiety ($r = 0.55$, $P = 0.006$) and stress ($r = 0.57$, $P = 0.004$).

DISCUSSION

The findings of the current study suggest that individuals with AN self-report different levels of impulsivity relative to healthy individuals, but do not display behavioural impulsivity (*i.e.,* increased commission errors on the CPT).

AN patients reported lower levels of motor impulsivity, compared to the healthy control group. This subscale relates to acting without thinking[13,14], and suggests that individuals with AN regard themselves as controlled individuals who think before they act. Although the AN group reported lower impulsivity on this subscale, they reported increased impulsivity in terms of attention and cognitive instability - *i.e.,* an

Table 1 Participant information

	AN		HC		
	M	SD	M	SD	*P*
Age	23.07	6.88	22.67	3.19	0.798
Premorbid IQ	104.67	8.19	105.6	7.00	0.670
BMI	16.52	1.14	22.4	3.59	0.001
Illness duration	6.67	7.66	-	-	-
Age of illness onset	16.04	3.50	-	-	-
EDE-Q restraint	3.93	1.42	0.43	0.40	0.001
EDE-Q eating concern	3.78	1.24	0.20	0.20	0.001
EDE-Q shape concern	5.01	0.90	0.99	0.59	0.001
EDE-Q weight concern	4.5	1.41	0.42	0.47	0.001
EDE-Q global score	4.3	1.12	0.60	0.43	0.001
DASS depression	25.08	12.41	1.08	1.29	0.001
DASS anxiety	16.00	9.48	1.88	2.13	0.001
DASS stress	24.92	10.23	3.78	2.78	0.001
BIS-11 attentional	20.67	3.64	13.88	2.91	0.001
BIS-11 attention	13.67	2.99	8.48	2.12	0.001
BIS-11 cognitive instability	7.00	1.44	5.40	1.44	0.001
BIS-11 motor	19.67	3.61	21.28	4.02	0.146
BIS-11 motor	11.55	2.28	14.08	2.78	0.002
BIS-11 perseverance	7.54	1.74	6.92	1.96	0.247
BIS-11 nonplanning	23.21	4.33	22.16	5.71	0.474
BIS-11 self-control	11.75	3.98	11.32	3.87	0.703
BIS-11 cognitive complexity	11.46	2.54	10.84	3.04	0.444
BIS-11 total score	61.88	8.48	57.32	10.98	0.112

AN: Anorexia nervosa; HC: Healthy controls; Premorbid IQ: Standardised Wechsler Test of Adult Reading Score; BMI: Body mass index; EDE-Q: Eating Disorders Examination Questionnaire; DASS: Depression, Anxiety, Stress Scale; BIS-11: Barratt Impulsiveness scale; Age: Age of illness onset and duration illness are reported in years.

inability to focus attention or concentrate[14]. Rosval *et al*[15] similarly reported increased rates of attentional impulsivity in AN. However, attentional impulsivity was not related to eating disorder symptomatology, nor was it related to indicators of potential malnutrition (*i.e.,* BMI and illness duration), or to behavioural impulsivity in the current study. It was, however, significantly correlated with all three measures of negative mood state, *i.e.,* depression, anxiety and stress. This findings suggests that attentional impulsivity in AN may not be related to starvation or to the severity of the eating disorder, but the associated negative mood states. Though, this conclusion remains speculative as the findings are based on statistical association, and also do not take into account longitudinal data. Unlike attentional impulsivity, though, motor impulsivity was not correlated with any measure suggesting that a perceived decrease in motor impulsivity is unrelated to eating disorder symptoms, mood state or behavioural impulsivity.

Groups were also found to not differ in behavioural performance on the majority of measures of the CPT-IP. Groups did not significantly differ in the proportion of correct hits, false alarms or random responses. Groups also did not significantly differ in response times of correct hits, but showed similar response times to a large sample of healthy female participants, who had significantly longer response times than male participants[16]. Groups in the current study did, however, differ in the mean and intraindividual variability (IIV) of response times of false alarms, with the AN group demonstrating increased response times and IIV of false alarms. However, this finding is somewhat limited as only a very small proportion of false alarms were elicited in each group (*i.e.,* 11% and 10% for AN and HC, respectively, of 90 potential responses). Similarly to the current findings, a lack of significant group differences in performance on the CPT-IP has also been reported in a small number of other studies in AN[17,18]. Furthermore, the same group of participants were not found to differ on typical saccadic eye movement measures of impulsivity (*i.e.,* antisaccade or no-go saccade error rates), further supporting the lack of behavioural impulsivity in AN (saccadic eye movement findings are to be reported elsewhere).

The findings of the study, however, are subject to a number of potential limitations. First, given the conservative sample size and the number of statistical comparisons, the statistical power of the study is limited. The DASS and BIS-11 are also restricted in their divergent validity, and further, the conclusions based on these measures are based on statistical association and do not take into account longitudinal validity. The findings of the current study do, however, provide preliminary evidence for divergent self-reported and behavioural impulsivity in AN, which requires replication in a larger sample.

The findings of this study have a number of important implications. Firstly, they suggest that self-reported impulsivity in AN may be unrelated to behavioural

Table 2 Continuous performance test - identical pairs results

	AN		HC		
	M	**SD**	**M**	**SD**	***P***
Proportion of hits	0.83	0.97	0.86	0.9	0.319
Hits RT	547.34	59.41	533.94	53.06	0.409
Hits RT IIV	123.35	28.91	115.38	26.31	0.318
Proportion of false alarms	0.11	0.06	0.09	0.06	0.314
False alarms RT	504.65	151.18	365.41	137.89	0.002
False alarms RT IIV	68.38	32.98	46.48	29.28	0.039
Proportion of random responses	0.01	0.01	0.01	0.01	0.278

AN: Anorexia nervosa; HC: Healthy control; RT: Response time; IIV: Intraindividual variability (a comparison of individual's standard deviations).

performance. This finding may be related to the "control paradox" often reported in AN, in which individuals seek to control their surrounding environment as much as possible but report feeling like they are out of control[19]. Furthermore, this may be related to perfectionistic tendencies reported in AN; thus, further research in this area utilising measures of control and perfectionism would be advantageous to further elucidate this inconsistency in AN. The findings also suggest increased reports of attentional impulsivity in AN is related to negative mood state. Thus, addressing negative mood symptoms may be beneficial in resolving the inconsistency and potential distress in how individuals with AN think they behave and how they actually behave.

In conclusion, overall, the findings of the study suggest that individuals with AN report lower rates of motor impulsivity, and higher rates of attentional impulsivity than HCs; the latter of which is associated with increases in negative mood state symptoms. Reported rates of impulsivity were, however, unrelated to behavioural performance. Therefore, the findings suggest an inconsistency between self-reported impulsivity and behaviour in AN, which may be resolved by improving negative mood states in these individuals.

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COMMENTS

Background
Anorexia nervosa (AN) is associated perfectionistic tendencies, particularly displaying increased concerns over making mistakes. Relatedly, patients often self-report lower rates of impulsivity. However, it is unclear whether self-reported rates of impulsivity are influenced by eating disorder symptoms or are stable traits in AN. It is also unclear whether these self-reported rates of impulsivity translate to behavioural performance on cognitive tasks of inhibition in AN.

Research frontiers
Self-reported and behavioural impulsivity appear to be discrepant in AN.

Innovations and breakthroughs
This study found inconsistencies between self-reported and behavioural impulsivity in AN. The results also indicated that self-reported attentional impulsivity in AN was related to negative mood states.

Applications
Improving negative mood symptoms may improve perceived attentional impulsivity in AN.

Peer-review
In this article, the authors claim that there is a discrepancy between self-reported and behavioural impulsivity in patients with AN. The study appears to have been carefully planned with appropriate tests and adequate controls. The results are interesting and can lead to novel therapeutic approaches in AN.

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Oral but not written test anxiety is related to social anxiety

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Abstract

AIM
To examine the associations of test anxiety (TA) in written *vs* oral exam situations with social anxiety (SA).

METHODS
A convenience sample of 204 students was recruited at the Technische Universität Dresden (TU Dresden, Germany) and contacted *via* e-mail asking to complete a cross-sectional online survey based on established questionnaires. The study protocol was approved by the ethics committee of the TU Dresden. Full data of $n = 96$ students were available for dependent t-tests and correlation analyses on the associations of SA and TA respectively with trigger events, cognitions, safety behaviors, physical symptoms and depersonalization. Analyses were run using SPSS.

RESULTS
Levels of TA were higher for fear in oral exams than for fear in written exams (M = 48.1, SD = 11.5 *vs* M = 43.7, SD = 10.1 $P < 0.001$). Oral TA and SA were positively correlated (Spearman's $r = 0.343$, $P < 0.001$; Pearson's $r = 0.38$, $P < 0.001$) contrasting written TA and SA (Spearman's $r = 0.17$, $P > 0.05$; Pearson's $r = 0.223$, $P > 0.05$). Compared to written TA, trigger

events were more often reported for oral TA (18.2% *vs* 30.3%, *P* = 0.007); which was also accompanied more often by test-anxious cognitions (7.9% *vs* 8.5%, *P* = 0.001), safety behavior (8.9% *vs* 10.3%, *P* < 0.001) and physical symptoms (for all, *P* < 0.001).

CONCLUSION
Written, but not oral TA emerged being unrelated to SA and may rather not be considered as a typical facet of SA disorder.

Key words: Social anxiety; Derealization; Test anxiety; Depersonalization; Safety behavior

Core tip: In a convenience student sample, levels of test anxiety (TA) were higher for fear in oral exams than for fear in written exams. Oral TA and social anxiety (SA) were positively correlated, contrasting written TA and SA. Compared to written TA, trigger events were more often reported for oral TA; which was also accompanied more often by test-anxious cognitions, safety behavior and physical symptoms. Results point to overlaps between oral TA and SA. Since written TA appeared unrelated to SA, it may rather not be considered as a typical facet of SA.

INTRODUCTION

The aim of a deepened understanding of social anxiety (SA) disorder, its putative subtypes and differentiation from other mental disorders has stimulated research in the last decade, *e.g.*, on the relationship of public speaking anxiety with other facets of SA disorder[1,2]. Broadly, the diagnostic category of SA disorder represents a multi-faceted phenomenon, that spans from more or less isolated social fears to severe anxiety in social situations related to interactions with others and performance in public[1,3,4]. Results of Hall[5] and Knappe *et al*[6] pointed to notable differences between fear of public speaking and test anxiety (TA) on the one hand, and other social fears and SA disorder on the other, namely in terms of age of onset, social anxious cognitions, physical symptoms and increase of self-reported anxiety levels over time. For example, in a community sample of adolescents and young adults, isolated social fears in test situations were unrelated to catastrophic social anxious cognitions, but associated with significant avoidance (though not with moderate/severe impairment), lower comorbidity, behavioral inhibition, and

parental psychopathology as compared to respondents with other performance or interaction related social fears[6]. Findings suggest that TA might be meaningfully distinguished from SA, and question whether TA is in fact part of the SA spectrum or if it should be better classified as a specific fear or phobia[3,5,6].

Despite efforts to better understand the relationship of TA and SA disorder, little attention has been paid to the fact that TA can occur in oral and written exams and that these two types of situations imply differing cues for anxiety reactions: A convenience sample of college students preferred written exams over oral exams[7,8], probably because social interaction is limited in written exams, whereas oral exams demand both performance and social interaction skills.

To better understand the relationship of TA and SA, this study aims to explore similarities and differences in written and oral exams, as well as similarities and differences between both forms of TA and SA.

Some facets of TA and SA have been explored so far: Sarason[9] hypothesized, that some type of TA is triggered by "quite specific unfortunate experiences, *e.g.*, a traumatizing teacher in the third grade". Accordingly, trigger events may be particularly relevant for TA, whereas for SA, only few of those affected recall a specific event as origin of their fear[10]. With regard to cognitions, Knappe *et al*[6] found that fear of taking tests was negatively associated with socially phobic cognitions like "something embarrassing or shameful could happen", "to be ashamed" and "to blush", whereas there was a positive association between catastrophic anxiety cognitions and the majority of social fears. Bögels *et al*[3] concluded that blushing is a physical, maybe even unique, sign of SA disorder compared with other anxieties. However, they did not differentiate for specific social fears or social situations. Hoyer *et al*[11] found that symptoms of depersonalization were more frequent in social phobia patients (92%) than in controls (52%).

Overall, based on however limited findings on clinical features, it is suggested that: (1) TA in oral exams is associated with higher levels of SA as compared to written exams; that (2) oral TA is more similar to SA in terms of safety behaviors, cognitions and physical symptoms than written TA; and (3) similar to SA, symptoms of depersonalization/derealization are expected to occur more frequently in oral exams than in written exams.

MATERIALS AND METHODS

Procedure and participants
A convenience sample of 204 students was recruited at the Technische Universität Dresden (TU Dresden), Germany, and contacted *via* email asking to complete an online survey. Students received course credits and/or randomly drawn cinema and bowling vouchers for participation. The ethics committee of the TU Dresden approved the study protocol.

Data from 105 students were not available for

Table 1 Description of full and partial responders

	Complete responders (*n* = 99)[1]		Partial responders (*n* = 22)[2]		χ^2 test
	n	%	*n*	%	
Sex					0.290
Males	25	26	8	36.4	
Females	74	74	14	63.6	
Age (M, SD)	22.9 (3.6)		23.0 (2.6)		0.573
Branch of study					0.297
Mechanical engineering	9	9.4	2	9.1	
Psychology	32	33.3	6	27.3	
Sociology	5	5.2	4	18.2	
Medicine	34	35.4	6	27.3	
Architecture	6	6.3	1	4.5	
Biology	7	7.3	1	4.5	
Other	6	6.3	2	9.1	
Degree of study					0.814
Diploma	31	32.3	7	31.8	
Bachelor	33	34.4	9	40.9	
Master	2	2.1	0	0.0	
State exam	33	34.4	6	27.3	
Number of days since last exam					
Written exam (M, SD)	51.1 (69.0)		51.3 (63.5)		0.920
Oral exam (M, SD)	253.7 (342.8)		406.1 (518.5)		0.468
Probable social anxiety disorder					
SSQ	14	14.1	5	22.7	0.317
LSAS-anxiety (M, SD)	15.6 (10.5)		13.0 (10.0)		0.366
LSAS-avoidance (M, SD)	13.6 (10.2)		11.9 (12.1)		0.574
Probable test-anxiety					
Written exam LSAS-anxiety (M, SD)	1.1 (0.8)		0.9 (0.8)		0.804
Written exam LSAS-avoidance (M, SD)	0.2 (0.6)		0.3 (0.6)		0.550
Oral exam LSAS-anxiety (M, SD)	1.8 (1.0)		1.5 (1.1)		0.566
Oral exam LSAS-avoidance (M, SD)	0.2 (0.6)		0.3 (0.9)		0.619

[1]Data of additional 3 participants were not used since they did not report any fear or avoidance of social situations and thus were not asked about trigger events, test anxiety, cognitions, behavior, depersonalization and physical symptoms due to skip rules; [2]Data of additional 14 participants were not used since they did not give written informed consent. M: Mean; SD: Standard deviation; SSQ: Symptom screening questionnaire; LSAS: Liebowitz Social Anxiety Scale (subscales for severity of social anxiety and avoidance of social situations).

analyses, since e-mail addresses were incorrect (*n* = 14), students did not respond (*n* = 55), or filled in less than 50% of the questionnaire (*n* = 36, 18.9%). There were no systematic differences between participants responding only partly (*n* = 22) and those who responded completely (*n* = 99) (Table 1). *Via* skip rule, only participants who reported anxiety in or avoidance of at least one social situation listed in the Liebowitz Social Anxiety Scale (LSAS) (see below) were asked about trigger events, TA, cognitions, behavior, depersonalization and physical symptoms. Indication of TA based on the PAF [Prüfungsangstfragebogen, (TA Questionnaire), see below] was not required as a necessary condition (*i.e.*, no skip rule when no TA was reported) because Knappe *et al*[6] observed that fear of taking tests occurred independently from SA but not vice versa. Three subjects were excluded because they skipped the survey after indicating no fear or avoidance of social situations. Accordingly, analyses were based on *n* = 96 subjects with complete data sets. The sample consisted of 23 males (24%) and 73 females (76%), aged 19 to 46 years (M = 22.9 years, SD = 3.6). Additional information was collected on the target degree (*n* = 32 state examination, *n* = 32 bachelor, *n*

= 2 master, *n* = 30 diploma) and branch of study (*n* = 33 medicine, *n* = 31 psychology, *n* = 8 mechanical engineering, *n* = 7 biology, *n* = 6 architecture, *n* = 5 sociology, *n* = 6 other). Students were also asked to recall any event as a trigger for their anxiety in social situations, oral or written exams.

Measures

SA: The 24-item German self-report Version of the "LSAS"[12] was administered. Item 17 ("taking a test") was splitted for detailed assessment of anxiety and avoidance during written or oral exam situations (namely: Taking a written test, taking an oral examination). The 27-item German "Fragebogen zu sozialphobischem Verhalten" [SPV (Questionnaire for Social Phobic Behavior)][13] was used to assess social anxious motivated safety behavior, *e.g.*, "I try to behave as normal as possible". Ratings were shown in inversed order for comprehensiveness with other measures.

TA: The German questionnaire "Prüfungsangstfragebogen" [PAF (TA Questionnaire), 20 items][14] was used to assess severity of TA. In addition, generic items asked for trigger events for fear of written and oral exams

		α
Social anxiety	LSAS anxiety subscale	0.914
	LSAS avoidance subscale	0.897
	SPK	0.926
	SPV	0.821
	BSPS-G physical subscale	0.737
	CDS-9 frequency subscale	0.848
Written test anxiety	PAF	0.897
	TAC	0.884
	TAB	0.780
	BSPS-G, physical subscale	0.775
	CDS-9 frequency subscale	0.911
Oral test anxiety	PAF	0.906
	TAC	0.888
	TAB	0.811
	BSPS-G, physical subscale	0.756
	CDS-9 frequency subscale	0.919

α: Cronbach's α; LSAS: Liebowitz Social Anxiety Scale; SPV: FragebogenzusozialphobischemVerhalten (Questionnaire for Social Phobic Behavior); BSPS-G: Brief Social Phobia Scale, German Version; CDS-9: Cambridge Depersonalisation Scale; PAF: Prüfungsangstfragebogen (test anxiety questionnaire); TAC: Test anxious cognitions (generic questionnaire); TAB: Test anxious coping and safety behavior (generic questionnaire).

separately, as well as for test-anxious cognitions (TAC, 10 items) and test-anxious coping and safety behavior (TAB, 17 items). The subscale "physical symptoms" of the German "Brief Social Phobia Scale" (BSPS-G, 4 items)[15] was used to assess bodily symptoms of TA. All instructions were slightly modified to allow for separate self-assessment in written and oral exams.

Symptoms of depersonalization/derealization: The German version of the "Cambridge Depersonalisation Scale 9" (CDS-9, 9 items)[16] was used to assess symptoms of depersonalization/derealization. The instruction was slightly modified for separate assessment with regard to social situations, as well as written and oral exams. The items were presented following the numerical order of the full CDS (in contrast to Michal *et al*[16]). To increase reasonableness of the questionnaire, the duration scale of the CDS-9 was omitted.

The PAF, TAB TAC, BSPS-G und CDS-9 were administered twice for separate assessments in oral and written TA situations. Internal consistencies (Cronbach's α) for scores of all modified scales ranged between 0.737 and 0.919 (Table 2). Convergent and discriminant validity of all modified scales were explored based on their correlation patterns, indicating sufficient validity (Table 3).

Statistical analysis

Dependent *t*-tests for paired samples were used to compare mean values of the number of trigger events, TA, test-anxious cognitions, test-anxious coping and safety behavior, symptoms of depersonalization/derealization and physiological symptoms (blushing, palpitation, tremor and sweating) in written exams to

oral exams. Associations of SA (based on LSAS) with TA during written exams and TA during oral exams (based on PAF) were compared using Steiger's Z[17], which allows to compare overlapping correlations. In absence of normally distributed data, Spearman correlations were computed. Pearson correlations were additionally computed since they are necessary for the computation of Steiger's Z. The correlations were similar and therefore the Pearson correlations were compared within a sample using Steiger's Z. Analyses were run using SPSS[18]. A Bonferroni correction ($\alpha_{corr} = 0.025$) was applied for analyses on safety behaviors to account for multiple testing (*i.e.*, comparison between SA and oral TA; comparison between SA and written TA). It was not applied for explorative analyses, where a higher α error can rather be tolerated than an increase of the β error[19].

RESULTS

Frequencies and associations of SA and written or oral TA

Isolated oral and written TA was reported in 14.6% (*n* = 14) and 2.1% (*n* = 2), respectively, of the sample. More than one third of the sample reported at least one condition out of SA, written TA or oral TA (36.5%, *n* = 35). Symptoms of depersonalization/derealization occurred most often in social situations (*n* = 13; 13.5%), followed by oral exams (*n* = 11; 11.5%) and written exams (*n* = 8; 8.3%) (Table 4).

As expected, SA was unrelated to written TA (Spearman's *r* = 0.17, *P* > 0.05; Pearson's *r* = 0.22, *P* > 0.05), but positively related to oral TA (Spearman's *r* = 0.34, *P* < 0.001; Pearson's *r* = 0.38, *P* < 0.001). Correlations of SA with written *vs* oral TA however did not differ (Steiger's *Z* = -1.18, *P* > 0.05).

Comparisons between written and oral TA

Analyses revealed substantial differences between oral and written TA in terms of trigger events, clinical characteristics and physiological symptoms (Table 5). Specifically, trigger events were more often reported for oral TA (30.3%) than written TA (18.2%) [*t*(95) = 2.78, *P* = 0.007]. As expected, the level of TA was higher among those reporting fear of oral exams than in those with fear of written exams (Table 5) [*t*(95) = -4.86, *P* < 0.001]. Further, TAC and TAB were reported more often for oral TA than for written TA. For symptoms of depersonalization/derealization, no differences between oral and written TA were observed. Physical symptoms were reported more often for oral TA than for written TA.

Comparison between SA, and written and oral TA

SA, based on the LSAS, was positively related to safety behaviors (Spearman's *r* = 0.64, *P* < 0.001; Pearson's *r* = 0.70, *P* < 0.001). Both forms of TA were moderately associated with safety behaviors (written: Spearman's *r*

Table 3 Convergent and discriminant validity of scales and measures

	LSAS anxiety subscale	LSAS avoidance subscale	PAF written	PAF oral	TAC written	TAC oral	TAB written	TAB oral
Oral PAF	0.379[d]	0.304[d]	0.663[d]	1	0.625[d]	0.806[d]	0.332[d]	0.442[d]
Written PAF	0.223[b]	0.167	1	0.663[d]	0.770[d]	0.650[d]	0.467[d]	0.236[b]
SPK	0.673[d]	0.592[d]	0.283[d]	0.453[d]	0.425[d]	0.562[d]	NE	NE
SPV	0.701[d]	0.678[d]	0.425[d]	0.524[d]	NE	NE	0.301[d]	0.412[d]
CDS-9 frequency subscale social	0.338[d]	0.320[d]	0.323[d]	0.311[d]		NE	NE	NE
CDS-9 frequency subscale written	0.274[d]	0.306[d]	0.398[d]	0.383[d]	NE	NE	NE	NE
CDS-9 frequency subscale oral	0.390[d]	0.341[d]	0.337[d]	0.488[d]	NE	NE	NE	NE
Tremor social	0.322[d]	0.352[d]	0.258[b]	0.223[b]	NE	NE	NE	NE
Sweating social	0.351[d]	0.298[d]	0.200	0.311[d]	NE	NE	NE	NE
Blushing social	0.242[b]	0.115	0.218[b]	0.278[d]	NE	NE	NE	NE
Palpitation social	0.379[d]	0.351[d]	0.206[b]	0.313[d]	NE	NE	NE	NE
Tremor written	0.294[d]	0.141	0.640[d]	0.452[d]	NE	NE	NE	NE
Sweating written	0.096	0.028	0.484[d]	0.361[d]	NE	NE	NE	NE
Blushing written	-0.099	-0.094	0.137	0.201[b]	NE	NE	NE	NE
Palpitation written	0.168	0.050	0.657[d]	0.488[d]	NE	NE	NE	NE
Tremor oral	0.289[d]	0.151	0.488[d]	0.608[d]	NE	NE	NE	NE
Sweating oral	0.182	0.048	0.291[d]	0.434[d]	NE	NE	NE	NE
Blushing oral	0.120	-0.060	0.189	0.332[d]	NE	NE	NE	NE
Palpitation oral	0.172	0.054	0.499[d]	0.612[d]	NE	NE	NE	NE

Pearson's correlation coefficient [b]$P < 0.005$, two-tailed [d]$P < 0.001$, two-tailed. NE: Not estimated; CSD-9: Cambridge Depersonalisation Scale-9 items; LSAS: Liebowitz Social Anxiety Scale;PAF: Prüfungsangstfragebogen. SPK Fragebogen zu sozialphobischen Kognitionen; SPV Fragebogen zu sozialphobischem Verhalten.

Table 4 Frequencies of social anxiety, written or oral test anxiety and depersonalization (*n* = 96)

Social anxiety disorder and test anxiety	*n*	%
Overall		
Social anxiety disorder	12	12.5
Written test anxiety	13	13.5
Oral test anxiety	28	29.2
Isolated conditions		
Social anxiety disorder	4	4.2
Written test anxiety	2	2.1
Oral test anxiety	14	14.6
Co-occurrence of conditions		
Written and oral test anxiety	7	7.3
Social anxiety disorder and written test anxiety	1	1
Social anxiety disorder and oral test anxiety	4	4.2
Social anxiety disorder, written and oral test anxiety	3	3.1
Symptoms of depersonalization/derealization		
Social situations	13	13.5
Written exams	8	8.3
Oral exams	11	11.5

Social anxiety disorder was assessed using the anxiety subscale of the LSAS and test anxiety was assessed using the PAF. Symptoms of depersonalization/derealization were measured using the CDS-9. LSAS: Liebowitz social anxiety scale; PAF: Prüfungsangstfragebogen.

= 0.31, $P < 0.001$; Pearson's $r = 0.28$, $P < 0.001$; oral: Spearman's $r = 0.42$, $P < 0.001$; Pearson's $r = 0.45$, $P < 0.001$); these correlations were however lower than the correlation between LSAS and safety behaviors and did not differ (written TA: Steiger's $Z = 3.22$, $P < 0.001$; oral TA: Steiger's $Z = 2.23$, $P < 0.005$; written compared to oral TA: Steiger's $Z = -0.93$, $P > 0.05$).

Symptoms of depersonalization/derealization were similarly frequent across all conditions (scale: 0 = never to 4 = constantly; SA: M = 3.7, SD = 2.7, oral TA: M = 3.2, SD = 5.2, written TA: M = 2.7, SD = 1.9), and only the difference between SA and written TA was statistically significant [$t(95) = 2.95$, $P = 0.004$].

DISCUSSION

Compared to written TA, SA was more closely related to oral TA and oral TA was triggered more often by an event and accompanied more often by test-anxious cognitions, safety behavior and physical symptoms. In terms of safety behaviors and symptoms of depersonalization/derealization, TA conditions and SA were quite similar. Hence, both the differences between TA conditions and associations between oral TA with SA indicate that SA and oral TA are overlapping entities.

Notably, TA in oral exams was associated with SA, unlike with TA in written exams. Hence, TA seems to be a heterogeneous phenomenon that comprises of different types of exam situations which are more or less social in nature. In fact, oral test situations could be presumed as social situations that require social skills, interaction and communication with others and that may elicit fear of negative evaluation. Further, oral exams are difficult to predict and to control, similar to social situations in general. In contrast, written test situations do not necessarily include interacting with others, and often follow familiar structures or schedules. Written exams may thus elicit lower levels or even no SA. Accordingly, Fehm *et al*[20] reported higher levels of prolonged rumination about past social situations (*i.e.*, post-event processing) in interaction-related social situations as compared to performance-related social situations. When written exams are

Table 5 Clinical correlates of test anxiety in written and oral exams (*n* = 96)

| | Test anxiety | | | | | | |
| | Written | Oral | | | | | |
	M (SD)	M (SD)	Δ (SD)	95%CI	df	*t*	*P*
Test anxiety	43.66 (10.12)	48.12 (11.52)	-4.46 (8.98)	(-6.28, -2.64)	95	-4.86	< 0.001
Test-anxious cognitions	20.02 (7.89)	21.72 (8.49)	-1.69 (4.71)	(-2.65, -0.74)	95	-3.53	0.001
Test-anxious behavior	39.00 (8.99)	44.57 (10.34)	-5.57 (6.74)	(-6.94, -4.21)	95	-8.11	< 0.001
Any trigger event (*n*, %)	18 (18.2%)	30 (30.3%)	-12 (-)	(0.036, 0.214)	95	2.775	0.007
Symptoms of DP/DR	2.71 (4.71)	3.167 (5.22)	-.46 (2.85)	(-1.04, 0.12)	95	-1.58	0.118
Blushing	0.27 (0.62)	1.17 (1.19)	-0.90 (1.00)	(-1.10, -0.69)	95	-8.78	< 0.001
Palpitation	1.47 (1.05)	2.11 (1.11)	-0.65 (0.78)	(-0.80, -0.49)	95	-8.1	< 0.001
Tremor	0.70 (0.95)	1.33 (1.26)	-0.64 (0.94)	(-0.83, -0.45)	95	-6.61	< 0.001
Sweating	1.22 (1.05)	1.81 (1.14)	-0.59 (0.87)	(-0.77, -0.42)	95	-6.72	< 0.001

All values represent raw, non-standardized scores. Test anxiety was measured using the PAF. Test-anxious cognitions were assessed using a generic questionnaire. Test-anxious coping and safety behavior was assessed using a generic questionnaire. Symptoms of depersonalization/derealization were measured with the CDS-9. Blushing, palpitation, tremor and sweating were assessed using the respective item of the BSPS-G. *P* two-tailed at $P < 0.05$; CI: Confidence interval; df: Degrees of freedom; *t*: *t*-value; DP/DR: Symptoms of depersonalization/derealization; PAF: Prüfungsangstfragebogen; BSPS-G: German "Brief Social Phobia Scale".

perceived as aversive, this may be related to other factors than to SA, such as inefficient study skills and/or test-taking skills, intolerance of uncertainty[21], avoidance temperament[22], perfectionism[23] or low self-efficacy. Nonetheless, cognitive interference may affect performance in both written and oral exams[24].

Findings need to be considered in light of some limitations: The limited convenience sample (*n* = 96) with overrepresentations of students of psychology and medicine did not allow for analyses on isolated conditions. Though standardized or structured measures are preferred for a more comprehensive evaluation, indications of SA and TA were deduced from established self-report questionnaires (LSAS and PAF) which were modified for separate evaluations of anxiety in oral *vs* written exams. Rates for SA were however in line with population-based data in similar age ranges[6]. Given the limited sample size and the absence of normally distributed data for some variables, multivariate statistics were not applied. Statistical analyses based on correlation analyses and dependent *t*-tests for paired samples were sufficient for testing hypotheses and for the exploration of similarities and differences in written and oral exams, as well as of similarities and differences between both forms of TA and SA. For larger samples with independent assessments for written and oral tests, ANOVA models may be more adequate. All questionnaires were conceptualized as paper-pencil-measures but administered in a web-based format. Internal consistencies for the modified scales were however medium to high. In addition, putative influences on TA like stereotype threat or language difficulties, perceived difficulty or relevance of the exams, or indicators of performance (*i.e.*, grades, results of previous exams) were not assessed.

The similarities and differences of oral and written TA with SA again support the conceptualization of SA as a multi-faceted phenomenon. Because of the notable differences between oral and written TA in terms of trigger events, test-anxious cognitions, safety behavior and physical symptoms, it may be concluded that TA could be rather used to describe anxiety in oral exams than in written exams, similar to the recently introduced DSM-5 "performance only" specifier for SAD where SA is limited to speaking or performing in public[1]. Further differentiation of situation-specific types of TA would allow clarifying the facets of TA and their relationships to SA.

COMMENTS

Background

The diagnostic category of social anxiety (SA) disorder spans from more or less isolated social fears to severe anxiety in social situations related to interactions with others and performance in public. Studies so far pointed to notable differences between fear of public speaking and test anxiety (TA) on the one hand, and other social fears and SA disorder on the other. Findings suggest that TA might be meaningfully distinguished from SA. Some suggest even an alternative diagnostic classification of TA apart from the SA spectrum, for example as a specific fear or phobia.

Research frontiers

Little attention has been paid to the fact that TA can occur in oral and written exams and that these two types of situations imply differing cues for anxiety reactions. More details on the clinical features may help to inform the diagnostic classification of TA.

Innovations and breakthroughs

Compared to written TA, SA was more closely related to oral TA and oral TA was triggered more often by an event and accompanied more often by test-anxious cognitions, safety behavior and physical symptoms. In terms of safety behaviors and symptoms of depersonalization/derealization, TA conditions and SA were quite similar.

Applications

Because of the notable differences between oral and written TA, it may be concluded that TA could be rather used to describe anxiety in oral exams than in written exams, similar to the recently introduced DSM-5 "performance only" specifier for SA disorder.

Terminology

TA refers to excessive stress, marked anxiety or fear and discomfort during

and/or before taking a test or examination. Associated symptoms include physiological over-arousal, tension and somatic symptoms, cognitive symptoms such as worry, dread, fear of failure, and catastrophizing of anticipated consequences of the test situation.

Peer-review

Nice little study with a defined focus.

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ORIGINAL ARTICLE

Prospective Study

Agreement and conversion formula between mini-mental state examination and montreal cognitive assessment in an outpatient sample

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Abstract

AIM
To explore the agreement between the mini-mental state examination (MMSE) and montreal cognitive assessment (MoCA) within community dwelling older patients attending an old age psychiatry service and to derive and test a conversion formula between the two scales.

METHODS
Prospective study of consecutive patients attending outpatient services. Both tests were administered by the same researcher on the same day in random order.

RESULTS
The total sample ($n = 135$) was randomly divided into two groups. One to derive a conversion rule ($n = 70$), and a second ($n = 65$) in which this rule was tested. The agreement (Pearson's r) of MMSE and MoCA was 0.86 ($P < 0.001$), and Lin's concordance correlation coefficient (CCC) was 0.57 (95%CI: 0.45-0.66). In the second sample MoCA scores were converted to MMSE scores according to a conversion rule from the first sample which achieved agreement with the original MMSE scores

of 0.89 (Pearson's r, *P* < 0.001) and CCC of 0.88 (95%CI: 0.82-0.92).

CONCLUSION
Although the two scales overlap considerably, the agreement is modest. The conversion rule derived herein demonstrated promising accuracy and warrants further testing in other populations.

Key words: Mini mental state examination; Montreal cognitive assessment; Cognition; Equation; Assessment; Old age psychiatry

Core tip: In this study we examined the agreement between mini-mental state examination and montreal cognitive assessment in an older population attending mental health service outpatients. Although both scales assess the same construct (cognition) the agreement between them was modest. Further we delivered a conversion rule which can allow conversion of scores between these scales. The converted scores had a high agreement with original ratings. Finally, this new conversion rule was superior to three previously suggested equating rules.

INTRODUCTION
The mini-mental state examination (MMSE)[1] and montreal cognitive assessment (MoCA)[2] are cognitive screening tests that are widely used in both everyday clinical practice and research. However, some evidence suggests that the MMSE is less sensitive for detecting milder cognitive deficits compared to the MoCA, while other studies indicate that it's relative insensitivity to visuospatial and executive deficits impact limit it's suitability in particular populations, *e.g.*, vascular cognitive impairment or Parkinson's disease[3-6]. Comparison of these two tests in specific populations such as patients with Parkinson's disease[7,8] brain metastases[9] or sub-arachnoid haemorrhage[10] indicate that the MoCA is more suitable because it can detect mild forms of cognitive impairment and especially where this includes executive dysfunction. Similarly, population based studies of mild cognitive impairment (MCI) indicate that the MoCA is more sensitive than the MMSE in detecting mild forms of cognitive impairment[11]. However, to our knowledge no specific

comparison of those two tests has been conducted in a general psychogeriatric population where cognitive testing is routine practice.

Furthermore, clinical trials vary in their use of these two scales which makes comparisons between studies and meta-analyses difficult. Equating methodologies can facilitate comparison between studies using different scales to measure the same construct. Previous studies have developed conversion rules for the MMSE and MoCA using either equipercentile equating and/or log-linear smoothing methods. These studies relate to specific populations; Roalf *et al*[12] studied a selected population with Alzheimer's disease (AD), MCI and cognitively intact participants, van Steenoven *et al*[8] studied a population with Parkinson's disease, and Trzepacz *et al*[13] studied a selected population of AD, MCI and participants deemed cognitively intact. However, given that these studies used specific and biased populations there is a lack of data in general elderly psychiatric patients. In addition, only one of these conversion rules (van Steenoven *et al*[8]) has been subject to further examination[7] which although conducted in a similar population only found moderate agreement [Pearson correlation coefficient 0.66 (95%CI: 0.56-0.75)].

Given that both scales are widely used in clinical settings, as well as in clinical trials and cohort studies, a rule to facilitate conversions and comparison of data from different centres and different clinical trials which have used these instruments would have utility.

Therefore, the aims of the present study were threefold (1) to estimate the level of agreement between MMSE and MoCA within an old age psychiatry population; (2) to derive a conversion formula for the two scales and test it in a random population of similar setting; and (3) to compare the new conversion formula with those described in previous studies.

MATERIALS AND METHODS

Subjects and design
This is an observational cross sectional study of performance using two screening cognitive scales in consecutive patients attending an old age psychiatry outpatient clinic and Day Hospital. The "single group design" method was used in this study reflecting that the same population was assessed with the two cognitive tests (MoCA and MMSE).

Procedures
All assessments were conducted by the same psychiatrist who was trained in the use of MoCA and MMSE (McCarthy G). Both tests were administered on the same day with a maximum of 3 h time gap to avoid boredom and/or learning effects. The tests were administered with no particular order (randomly).

Clinical assessments
Demographics: Demographic data (gender, age) were collected from medical records (files and hospital com-

puterised database). In addition, information about years of education was collected from patients and relatives.

Diagnosis: ICD-10 psychiatric diagnoses were collected from the files and collapsed to main ICD-10 F categories. Where multiple diagnoses were evident the most predominant was chosen.

Cognitive assessments: (1) MoCA[2]. The MoCA assesses visuospatial/executive function, naming, memory, attention, concentration, language, abstract thinking, recall memory and orientation. It is scored on a 30-point scale. Higher scores indicate better cognitive performance. Administration typically takes about 12-15 min. Its psychometric properties have been investigated in many studies and it has been found to be superior to the MMSE for the detection of MCI[14]. In addition unlikely to MMSE it takes to account the education level; and (2) MMSE[1]. The MMSE comprises 11 questions assessing orientation to time and place, attention, immediate and short-term recall, language and visuospatial abilities. It is a brief cognitive screening instrument that takes less than ten minutes for administration. Over the past 40 years it has been the most widely used tool in clinical and research settings for brief assessment of cognitive status in elderly individuals. It's psychometric properties have been thoroughly reviewed and indicate moderate-to-high levels of reliability and good evidence of criterion and construct validity[15]. It has a total score of 30, with higher scores indicating better cognitive performance. Disadvantages of the MMSE include a ceiling effect, the influence of education especially for the serials sevens component[15,16], and a documented learning effect[17].

Ethics

The procedures and rationale for the study were explained to all patients but because many patients had cognitive impairment at entry into the study it was presumed that many might not be capable of giving informed written consent. Because of the non-invasive nature of the study, Sligo Regional Ethics Committee approved an approach to establishing consent by virtue of augmenting patient assent with proxy consent from next of kin (where possible) or a responsible caregiver for all participants in accordance with the Helsinki Guidelines for Medical research involving human subjects.

Statistical analysis

Statistical analyses were conducted using the R "equate" package[18]. Z scores were used to compare MMSE and MoCA scores because although they are from the same sample they follow different distributions. The overall agreement between the two scales was assessed using Pearson's product-moment correlation coefficient (r). However this estimation has been criticised by Bland *et al*[19] as misleading and therefore the concordance correlation coefficient (CCC) was also calculated[20]. The CCC measures agreement by assessing how well the
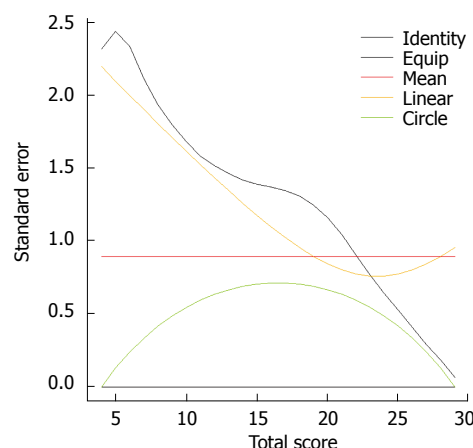

Figure 1 Graphical representation of standard errors after bootstrapping of different equating methods.

relationship between the measurements is represented by a line through the origin at an angle of 45 degrees (as would be generated if the two measurements generated identical results).

To convert MoCA scores to MMSE (and *vice versa*), we generated an equating table to link the two scales. The conversions were extracted from a random population of the studied group and then tested in the remaining sub-population. Given that both scales have the same lower and higher scores but with different difficulty we used the Circle - Arc Method[21]. However, we also applied other methods of equating models (linear, mean and equipercentile) and compared the standard errors of each model after bootstrapping (Figure 1).

By doing this we made the following assumptions: (1) that both scales measure the same latent construct (cognition); (2) that the two scales are not free of errors but the errors are small (both scales must have high reliability); and (3) that the ratings have been conducted by experts and the conversion rule will apply again in measurements that have been performed by experts.

Although both scales are continuous they are discretized continuous meaning that for a person A the score in MoCa (or MMSE) will be for example 11 and never 11.2 and thus the delivered score in MMSE was converted to the nearest integer. Finally, in the second sample we evaluated the conversion methods suggested by (1) Roalf *et al*[12]; (2) van Steenoven *et al*[8]; and (3) Trzepacz *et al*[13] using Pearson's r and CCC to measure the agreement between the original scores and the converted scores.

RESULTS

The total sample (*n* = 135) was randomly divided in two groups; one which was used to derive the equating table (called experimental sample, *n* = 70) and a second evaluation sample (*n* = 65) in which the derived conversion rule was tested.

Table 1 Demographic characteristics and cognitive test scores of the two samples

	Experimental *n* = 70 range (min-max)		Evaluation *n* = 65 range (min-max)	
Males	21 (30%)		21 (32.3%)	
Age	77.36 (SD: 7.06)	62-89	78.83 (SD: 6.6)	66-91
MoCA	19.03 (SD: 6.35)	4-29	18.57 (SD: 6.78)	4-30
MMSE	24.47 (SD: 4.87)	9-30	24.45 (SD: 4.71)	8-30
Years of education	10.71 (SD: 2.47)	6-18	10.20 (SD: 2.15)	7-17

MoCA: Montreal cognitive assessment; MMSE: Mini-mental state examination.

Descriptive statistics

Table 1 shows demographic data as well as the MoCA and MMSE scores in the two samples. The two samples did not significantly differ regarding gender distribution ($\chi^2 = 0.084$, df: 1, $P = 0.772$), age ($t = 1.25$, df: 133, $P = 0.214$), MoCA scores ($t = 0.406$, df: 133, $P = 0.686$), MMSE scores ($t = 0.31$, df: 133, $P = 0.976$) and years of education ($t = 1.29$, df: 133, $P = 0.200$). In addition, Table 2 shows the principal diagnoses in the two samples in percentages. A comparison of the two samples in terms of diagnoses did not identify significant difference ($\chi^2 = 0.644$, df: 3, $P = 0.886$). However, as shown in Table 1 the total MoCA scores were significantly lower than the total MMSE scores in both samples (For experimental sample: $n = 70$, z scores: -4.77, -8.19 respectively for MMSE and MoCA; $P < 0.001$; for the evaluation sample, $n = 65$, z-scores: -4.35, -7.88; $P < 0.001$).

Agreement of the two scales in the experimental sample

The Pearson's product-moment correlation coefficient for MoCA and MMSE was 0.86 ($P < 0.001$) which indicates very good agreement. However, the more conservative CCC was 0.57 (95%CI: 0.45-0.66), indicating a lower agreement between the two scales. Figure 2 depicts a scatterplot including a fitted linear line and a cubic. As evident from the scatterplot, the scores do not fit well to a linear model.

Linking the two scales (MoCA and MMSE)

The "circle-arc" method was used. Table 3 shows the conversion table. Also other equating methods were used but as expected the "circle-arc" had the least standard errors and less biases compared to the others. Figure 1 shows the standard error of the different methods after bootstrapping.

Evaluation of the derived conversion

In the 2nd sample (evaluation sample) we converted MoCA scores to MMSE scores according to the above table and then examined the agreement between the converted MMSE scores and the originals. The Pearson's product-moment correlation coefficient was 0.89 ($n = 65$, $P < 0.001$) and the Lin's CCC was 0.88 (95%CI: 0.82-0.92).
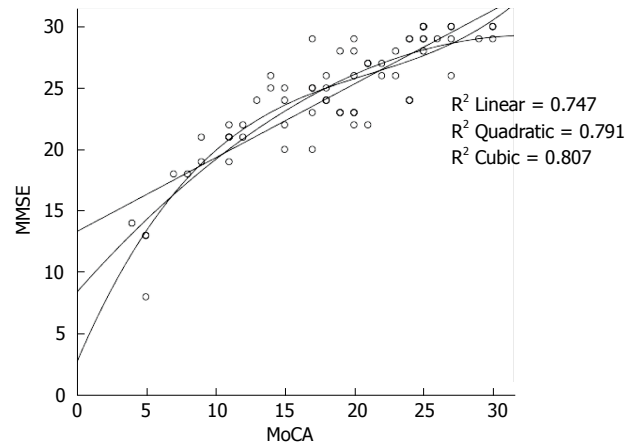

Figure 2 Linear Quadratic and Cubic relationship of montreal cognitive assessment and mini-mental state examination scores.

Thus the converted MMSE scores from MoCA have a high level of agreement with the actual MMSE scores.

Evaluation of the other methods suggested

With the Roalf *et al*[12]'s method, the Pearson's product-moment correlation coefficient was equal to 0.88 ($n = 65$, $P < 0.001$) and the CCC equal to 0.86 (CI: 0.79-0.81). With the van Steenoven *et al*[8]'s method the agreement between the converted and the actual MMSE scores was high with the Pearson's product-moment correlation coefficient of 0.86 ($n = 65$, $P < 0.001$) and the CCC of 0.84 (CI: 0.76-0.90). Finally, using the method suggested by Trzepacz *et al*[13] the Pearson's product-moment correlation coefficient was 0.85 ($n = 65$, $P < 0.001$) and the CCC was 0.82 (CI: 0.72-0.88). All three previously described conversion rules were inferior to that derived herein.

DISCUSSION

It is often assumed that because the MoCA and MMSE measure the same general construct (cognition) that they can be used interchangeably. However, they each emphasise different aspects of cognition and, as our results demonstrate, their agreement is modest. For instance, the MMSE allocates more points for orientation (10 out of 30) compared to only 6 out of 30 in the MoCA, while the MoCA places greater emphasis on visuospatial domains (5 out of 30) compared to only 1 point out of 30 with the MMSE. As a consequence, it is not surprising that these two tests do not have a linear relationship (Figure 2). Furthermore because performance is more difficult in visuospatial and executive domains than orientation, scores in MoCA were significantly lower compared to scores in MMSE. In addition, although both tests are used as continuous scales (ranging from 0 to 30) in fact neither is a true ratio scale such that a score of 10 does not indicate half the cognitive ability of a score of 20. Similarly, both scales include arbitrary anchor points (*e.g.*, a score of 0 does not mean that someone has no

Table 2 Main diagnoses in the two samples

Diagnoses	Experimental sample			Evaluation sample			Total
	n (%)	MMSE mean (SD)	MoCA mean (SD)	*n* (%)	MMSE mean (SD)	MoCA mean (SD)	
Organic, including symptomatic, mental disorders (F00-F09)	32 (45.7)	22.31 (4.95)	15.93 (5.68)	32 (49.2)	21.75 (4.61)	13.91 (5.11)	64
Schizophrenia, schizotypal and delusional disorders (F20-F29)	4 (5.7)	23.5 (3.7)	18.25 (4.43)	2 (3.1)	26 (5.66)	20 (7.07)	6
Mood (affective) disorders (F30-F39)	18 (25.7)	26.06 (4.42)	21.61 (6.29)	17 (26.2)	26.17 (3.46)	22.29 (5.47)	35
Neurotic, stress-related and somatoform disorders (F40-F48)	16 (22.9)	27.25 (3.49)	22.5 (5.33)	14 (21.5)	28.28 (1.85)	24.5 (3.69)	30

MoCA: Montreal cognitive assessment; MMSE: Mini-mental state examination.

Table 3 Conversion table

MoCA scores	MMSE scores
1	3
2	6
3	8
4	10
5	11
6	13
7	14
8	15
9	16
10	17
11	19
12	19
13	20
14	21
15	22
16	23
17	24
18	25
19	25
20	26
21	27
22	27
23	28
24	28
25	29
26	29
27	30
28	30
29	30
30	30

MoCA: Montreal cognitive assessment; MMSE: Mini-mental state examination.

cognitive function at all).

Our second aim was to derive an equating rule to allow for accurate conversion of scores between the two scales. This has important utility for standardising multiple assessments of patients who are assessed using different scales over time. However, most importantly this conversion rule can allow for comparisons between multiple centers in clinical trials which use MoCA or MMSE alternately and can be used for pooling data from different studies to facilitate meta-analyses. Our conversion rule compared very favourably with those described in previous studies in terms of a better

(higher agreement). We examined this issue using both Pearson's correlation coefficients as well as the CCC which provides a more conservative method as, in comparison to Pearson's correlation coefficient, it emphasises level of actual agreement over the general pattern of relationship[19]. Of note, the new method described herein performed better than previous methods by both measures of agreement (Pearson's r or CCC). One explanation for these findings is that our sample is more representative of a general old age population in comparison to the three previous studies in which the samples were more restricted. However, one of the assumptions for equating methods is that the equating relationship is group invariant and as such does not change across the groups[22], if the sample or sampling method influenced the converted scores the conversion rule is not valid.

Although the sample can influence some psychometric values, the most likely explanation for the higher agreement is the equating method that we used as it provides a better fit for our data. The circle-arc which we used does not requires the estimated equating transformation to be linear. It constrains the end points in the two pre-specified end points and a middle point determined from the data and it is thus robust even for small samples[21]. In addition, the circle-arc method produces the most accurate results for different sample sizes compared to other methods like equipercentile with smoothing, linear equating, and mean equating[23]. Therefore, it is likely that the greater accuracy of the conversion rule described herein relates substantially to the methods that were used in its development rather than to the sampling method. These observations are further supported by Armstrong *et al*[7] who found a moderate agreement between the converted and actual scores when they applied the conversion rule suggested by van Steenoven *et al*[8], even though they tested the rule in a similar sample to that in which the rule was originally derived (*i.e.*, patients with Parkinson's disease). However, when the two scales or tests are different in content, reliability, or intended population, it is expected that the scales will be less equivalent to some degree[24], but this is not the case for the MoCA and MMSE as they both have high reliability, assumes that measure the

same construct, (cognition) and are used in populations with possible cognitive deficits.

In conclusion, we found that the MMSE and MoCA have moderate agreement when used to assess general cognitive function reflecting their different emphasis into particular neuropsychological domains. Further, we found that their relationship is non-linear such that non-linear methods of equating should be used to compare performance on these scales. Finally, we derived a conversion rule which performed well in comparison to previously suggested methods and which merits further assessment in other larger and clinically diverse samples.

COMMENTS

Background

Mini-mental state examination (MMSE) and montreal cognitive assessment (MoCA) are widely used assessments of cognition in older people populations.

Research frontiers

Given the different tests of cognition the challenge is how to interpret them to a common "language".

Innovations and breakthroughs

This study applies advance and robust techniques to overcome the above challenges.

Applications

The authors have derived an equation rule to convert the scores from MoCA to MMSE which can be used to pull together data from different studies.

Terminology

Equation models can be used to transform the scores from one scale or instruments to another.

Peer-review

The manuscript is a generally well-written and interesting paper. The topic is important because the rate of dementias increases around the world.

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ORIGINAL ARTICLE

Randomized Controlled Trial

Comparative effectiveness of quetiapine and haloperidol in delirium: A single blind randomized controlled study

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Abstract

AIM
To evaluate the effectiveness of quetiapine and haloperidol in patients of delirium referred to psychiatry consultation liaison services.

METHODS
The study followed a single blind randomised controlled trial design. Thirty-two patients in the haloperidol group and 31 patients in the quetiapine group were assessed at the baseline and 6 consecutive days. Flexible dosing regimen (haloperidol: 0.25-1.25 mg; quetiapine 12.5-75 mg/d) was used. Delirium Rating Scale-Revised-98 (DRS-R-98) and mini mental status examination (MMSE) were the primary and secondary efficacy measures respectively.

RESULTS
Baseline DRS-R-98 severity score and MMSE scores did not differ between the 2 study groups. From baseline to day 6, there was significant reduction in the total DRS-R-98 scores, DRS-R-98 cognitive domain scores, DRS-R-98 non-cognitive domain scores and significant increase in the MMSE scores in both the groups. Both the groups did not differ on any of the assessments in terms of DRS-R98 and MMSE scores. The effectiveness of both the medications was similar in adult and elderly ($\geq$ 60 years) patients. At the end of the trial, 68.75% and 67.74% of subjects in the haloperidol and quetiapine group respectively had mean DRS-R-98 scores below 10. By 6th day, 12 (37.5%) patients in haloperidol group and 9 (29.03%) patients in the quetiapine group had

DRS-R98 score of "0" with no significant difference between the two groups ($P = 0.47$).

CONCLUSION
Quetiapine is as effective as haloperidol in the management of delirium.

Key words: Delirium; Quetiapine; Effectiveness; Atypical antipsychotics

Core tip: This Comparative study showed that quetiapine when used in the doses of 12.5-75 mg/d is as effective as haloperidol in the doses of 0.25-1.25 mg in management of delirium. The effectiveness of both the medications was similar in adult and elderly ($\geqslant$ 60 years) patients. By 6th day, 37.5% patients in haloperidol group and 29.03% patients in the quetiapine group had Delirium Rating Scale-Revised-98 score of "0" with no significant difference between the two groups. Accordingly, this study suggests that quetiapine is as effective as haloperidol in the management of delirium.

INTRODUCTION

Delirium is considered to be a psychiatric emergency seen among medically compromised patients. Management of delirium involves addressing the underlying etiology, providing reorientation cues, ensuring safety of the patients along with improvement in the patient's functioning. Over the years haloperidol has been the main antipsychotic, which has been recommended for management of delirum. However, in view of the extrapyramidal side effects associated with haloperidol, over the last 15 years or so, many researchers have evaluated atypical antipsychotic in the management of delirium[1].

Quetiapine is an atypical antipsychotic considered to have very low extrapyramidal side effect potential and good sedating effect. Due to these, over the years this has been evaluated in the management of delirium in few case reports[2-5], retrospective studies[6], open label trials[7-13] and randomised controlled trials with some following open label design and others followed blinded assessments[14-16]. These studies suggest that quetiapine is better than placebo[15,16] in the management of delirium and as effective as amisulpiride[9] and haloperidol[14]. Data also suggests that compared to placebo, quetiapine is associated with shorter time to first resolution of

delirium, shorted duration of delirium and had lower level of agitation among the intensive care unit patients[15]. In further analysis of the data authors also showed that compared to placebo, quetiapine is associated with faster first resolution of symptoms of fluctuation, inattention and disorientation. However, it took longer time to first resolution of symptoms of agitation and hyperactivity[17].

However, it is important to note that the data with regard to usefulness of quetiapine in management of delirium is limited with total number of patients treated with quetiapine in all the studies less than 200 patients, with none of the studies having more than 25 patients in the quetiapine arm. Hence, there is a need to expand this data. This led to the present single blind randomized, controlled trial, which assessed the effectiveness of quetiapine and haloperidol in patients of delirium, admitted in medical and surgical wards and referred to psychiatry consultation-liaison services.

MATERIALS AND METHODS

This study was conducted in multispeciality tertiary care hospital. Institute Ethics Committee approved the study. The trial was submitted to Clinical trial registry of India. Proxy written informed consent was obtained from the primary caregivers of the patients who were staying with the patient during the hospitalization prior to randomization. The purpose of the study was explained to the caregivers. The caregivers were told about the currently available pharmacotherapy for management of delirium. The caregivers were explained about the commonly used pharmacological agents along with their efficacy and side effect profile. They were informed about the evidence available for quetiapine for management of delirium. The primary caregivers were informed that they could withdraw consent at any stage.

The study was an equivalence trial which followed a single blind randomised controlled trial design. Randomization was done based on the computer generated randomization table, which was done prior to starting of the study. Consecutive patients diagnosed with delirium by the consultation liaison psychiatry team were considered for this research.

Only those patients who fulfilled a diagnosis of delirium (based on the Diagnostic and Statistical Manual, 4th Revision)[18] and were aged more than 18 years were included into the study. Patients with delirium associated with alcohol or benzodiazepine withdrawal, poisoning due to overdose and delirium associated with dementia (based on clinical history) were excluded. Patients who were unresponsive to any verbal or physical stimulus, those with history of aphasia, profound hearing or visual loss, those with prolonged QTc interval (> 500 ms) and past history of hypersensitivity to any of the drugs were also excluded. Patients who had developed neuroleptic malignant syndrome were also not considered for this study. Those with comorbid Parkinson's disease, psychotic or mood disorders and terminal illnesses were also excluded.

For this study, 101 patients were assessed initially. Twenty-seven patients were excluded because they did not fulfil the selection criteria for the study, *i.e.*, 4 patients had comorbid psychiatric illness, drug withdrawal state was present in 5 cases, prolonged QTc interval (> 500 ms) in electrocardiogram (ECG) was seen in 4 patients, 1 patient had Parkinson's disease, 3 patients had terminal illness, in 5 cases the delirium was associated with organophosphorous poisoning and 2 patients were younger than 18 years of age. According only 77 out of 104 patients were eligible for the study and their primary caregivers were approached for the study, out of which written informed consent was given by 70 caregivers. These patients were allocated to receive haloperidol (*n* = 35) or quetiapine (*n* = 35) based on predetermined random number generated prior to recruitment.

Four patients (2 in the quetiapine and 2 in the haloperidol group) were not available for assessment after the initial assessments of 1-2 d as they left the hospital against the medical advice (LAMA). One patient in quetiapine group, the primary treating team used Inj. Haloperidol for management on the second day and as a result the patient was excluded. Two patients (one from each group) could not be started on the assigned medication, due to detoriation in the clinical status [1 subjects went into coma and 1 was transferred to intensive care unit (ICU)] on the same day.

Accordingly out of 70 patients only 63 completed the trials with 32 in the haloperidol group and 31 in the quetiapine group.

The dose of medication was adjusted as per the clinical judgement. Flexible dosing regimen (haloperidol: 0.25-10 mg and quetiapine: 12.5-75 m/d) was used. At our consultation-liaison psychiatry practice setup, haloperidol is usually administered in the dose of 0.25 mg two to three times a day and titrated as per the requirement and majority of the patients are managed with 0.75 to 2.5 mg of haloperidol per day. In case of agitation, a dose of 1.25 to 2.5 mg is given intravenously and same is repeated as per need. For quetiapine a regiment of 12.5 mg/d OD dose was started and depending on the need the dose was increased to 75 mg/d.

Based on the daily clinical assessment, dose titration was done; however, in case the patient was agitated, dose titration was done as per the requirement. In case the delirium improved, the dose used on the previous day was continued till the end of the trial.

One of the investigators (SG) was responsible for the randomization and dose adjustments and another investigator (SM) who was blinded for the medication being administered carried out the clinical assessments.

Besides use of haloperidol or quetiapine, patients were continued on medications for their medico-surgical ailments. However, any medication (like benzodiazepines, steroids, *etc.*) that could possibly contribute to delirium or those medications which were not essential were discontinued. The underlying etiologies for delirum were managed with appropriate measures. The primary caregivers of all the patients were advised to provide optimal level of environmental stimulation, avoid sensory impairments of the patient and make the environment familiar to the patient by ensuring proper environmental cues that could facilitate orientation.

Primary efficacy measure

Delirium Rating Scale-Revised-98 (DRS-R-98)[19] was used as the primary outcome measure. The DRS-R98 is a 16-item scale with 13 severity and 3 diagnostic items that rate the preceding 24-h period. Each item is rated 0 (absent/normal) to 2/3 (severe impairment). Higher severity scores (0-39) indicating more severe delirium. The scale has high interrater reliability, sensitivity and specificity for detecting delirium in mixed neuropsychiatric and other hospital populations[19]. Both, the severity scale (13 items) and the total scale (16 items) have been validated for repeated measures.

Secondary efficacy measure

Additionally the patients were assessed on Mini mental status examination (MMSE)[20] and this was used as a secondary outcome measure for the study. It is a 30 point scale widely used in delirium and dementia research.

Assessments

Patients were initially evaluated at the baseline and 6 subsequent consecutive 6 d, at a particular time of the day (6-8 PM) on DRS-R-98 and MMSE.

Additionally, at the baseline, patients were assessed on Amended Delirium Motor Checklist[21,22], Short IQCODE[23] and etiology checklist.

Amended Delirium Motor Checklist (Amended DMC)[21,22] comprises of 13 items (5 hyperactive features and 8 Hypoactive features) of activity patterns that can be rated by both physicians and nurses. Each item is evaluated in absolute manner, *i.e.*, there is some evidence of the particular behaviour in last 24 h or not. Based on the number of criteria met for hyperactive and hypoactive checklists, the patients are categorized into hyperactive, hypoactive, mixed or no subtypes.

Short IQCODE[23] was used to evaluate the cognitive functions in the last 6 mo. It is a 16-item instrument that allows for the assessment of cognitive status for a defined period prior to the interview point, *e.g.*, six months previously. It is rated based on an interview with a key relative. Each item is rated on a 5 point scale with a score of 3 indicating no change (higher scores denote worsening while lower denote improved cognition). The scale is scored by adding all items and then dividing the total score by 16 to get a mean score per item. The suggested cutoff for suspected dementia is a score > 3.31-3.38.

The etiology checklist was designed for this study and included 47 commonly associated factors which are known to be associated with delirium. Each item was rated as present or absent. For the laboratory parameters, if any of the values was out of the laboratory

range of the hospital it was considered as present.

Statistical analysis

SPSS-14 was used to analyse the data. Mean and standard deviation were calculated for the continuous variables and frequency and percentages were calculated for the ordinal or nominal variable. Comparisons between the groups were done by using students t test/χ^2 test. If the DRS-R-98 data had non-normal distribution, then these were compared by using non-parametric tests. For the same, instead of paired t test, Mann-Whitney U test and Wilcoxon sign rank test were used. Repeat measure ANOVA was used to evaluate the effectiveness of medications on the primary and secondary outcome measure.

RESULTS

The mean age of the participants was 46.42 (SD: 18.26) and slightly less than one-third of the study sample was ≥ 60 years. The mean duration of education in years for the participants was 9.60 (SD: 4.22). Majority of the patients were male, from urban locality and had hospital emergent delirium. The average duration of delirium was 2.61 (SD: 2.08) d prior to enrollment into the trial. The mean IQCODE score was 3.07 (SD: 0.29) with only 3 patients scoring above 3.31, however, clinically these patients were never diagnosed with dementia. In terms of motor subtype, majority of the patients had hyperactive delirium and the mean number of etiologies associated with delirium was 6.82 (SD: 3.60). The mean baseline DRS-R-98 total score for the study sample was 31.52 (SD: 3.34). There was no statistically significant difference between the groups on any of the above variables (Table 1). Two patients with short IQCODE score were in the quetiapine group and 1 patient was in the haloperidol group.

The average dose of haloperidol was 0.67 mg (SD-0.35; range 0.25-1.25) and that of quetiapine was 31.83 mg (SD: 4.10; range 12.5 -75).

For the haloperidol group the average baseline DRS-R98 severity score and MMSE scores were 24.81 (SD: 2.19) and 7.50 (SD: 3.83) respectively and those for quetiapine group were 25.48 (SD: 3.60) and 6.83 (SD: 4.45) respectively with no significant difference between the two groups. As shown in Table 1 there was no significant difference in the DRS-R98 scores and MMSE scores from day 1 to day 6 between the two groups.

Effectiveness of haloperidol and quetiapine

In terms of both DRS-R-98 and MMSE, there was significant improvement in both the study groups from day 1 through day 6 (Table 2). Additionally, repeat measure ANOVA was used to evaluate the effectiveness for both the groups. As there was significant difference in the Mauchly's test of Sphericity, Green-House Geissier within subject effect was considered while interpreting the "F"

and "P" values in the repeat measure ANOVA. Accordingly, ANOVA with repeated measures with a Greenhouse-Geisser correction, showed significant reduction in the mean scores for DRS-R-98 for haloperidol group (F value = 134.25, corrected DF = 82.44; $P < 0.0005$) and also in the quetiapine group (F value = 118.78, corrected DF = 91.23; $P < 0.0005$). Repeat measure ANOVA for MMSE scores with a Greenhouse-Geisser correction for haloperidol group (F value = 73.86, corrected DF = 74.84; $P < 0.0005$) and quetiapine group (F value = 69.62, corrected DF = 77.83; $P < 0.0005$) were also significant.

For both the groups, there was significant difference between the DRS-R-98 scores for each day except for lack of significant difference between day 5 and 6, indicating that with each subsequent day, there was significant improvement from baseline to day 5. As with DRS-R-98, in both the groups, there was significant difference between the MMSE scores for each day except for lack of significant difference between day 5 and 6 in the haloperidol group and 4 and 5, day 4 and 6 and day 5 and 6, indicating that with each subsequent day, there was significant improvement in MMSE from baseline to day 5 in haloperidol group and baseline to day 4 in the quetiapine group.

No significant difference was seen between the two groups, in terms of percentage of patients whose DRS-R-98 score dropped down below 10 (Table 1). Overall by using a cutoff of DRS-R98 severity score of < 10, haloperidol was found to be efficacious in 68.75% and quetiapine was found to be efficacious in 67.74% of cases, with no significant difference between the two groups. As is evident from Table 1, with each passing day there was increase in proportion of patients achieving the DRS-R-98 score of < 10, from baseline to day-5.

At the end of the trial, 12 (37.5%) patients in haloperidol group and 9 (29.03%) patients in the quetiapine group had DRS-R98 score of "0" with no significant difference between the two groups (χ^2 value: 0.508; $P = 0.47$).

Further analysis was done for each day to evaluate the effect of both the medications on the cognitive and non-cognitive domains of DRSR-98 and no significant difference emerged between both the groups for assessment on any given day. In terms of efficacy measure when the repeat measure ANOVA was used, scores on the non-cognitive domain in the haloperidol group showed significant reduction for each day except for lack of significant difference between day 4-5, day 4-6 and day 5 and 6. Similarly in the quetiapine group, there was significant difference between the scores for each day except for lack of significant difference between day 3-4, day 3-5, day 3-6, day 4-5, day 4-6 and day 5 and 6. In terms of cognitive symptoms there was significant difference between the scores for each day in the haloperidol and quetiapine groups except for lack of significant improvement between day 5 and 6 in the quetiapine group.

Data was also analysed separately for young and

Variables	Haloperidol *n* = 32 mean (SD)	Quetiapine *n* = 31 mean (SD)	χ^2/*t*-test
Age (yr)	44.40 ± 16.76 (range 18-76)	48.51 ± 19.75 (range 18-85)	0.89 (P = 0.37)
Age ⩾ 60 yr	7 (32%)	12 (38.7%)	2.11 (0.146)
Education (No. of years)	9.81 ± 4.46 (range 0-15)	9.38 ± 4.03 (range 0-17)	0.396 (P = 0.693)
Male	28 (87.5%)	21 (67.74%)	3.55 (P = 0.06)
Locality- Urban	21 (65.6%)	24 (77.4%)	1.073 (P = 0.300)
Type of onset - hospital emergent	23 (71.87%)	25 (80.64%)	0.668 (P = 0.414)
Duration of delirium prior to assessment (d)	2.38 ± 1.81	2.83 ± 2.32	0.85 (P = 0.398)
Total IQCODE	3.01 ± 0.053	3.13 ± 0.40	1.57 (0.12)
Delirium subtype as per amended DMSS			
Hyperactive	28 (87.5%)	27 (87.09%)	
Hypoactive			5.36 (P = 0.76)
Mixed	3 (9.37%)	2 (6.45%)	
	1 (3.12%)	2 (6.45%)	
Mean dose (mg/d)	0.67 ± 0.35 (range 0.25-1.25)	26.63 ± 15.61 (range 12.5-75)	
Mean number of etiologies	7.06 ± 3.31	6.58 ± 3.92	0.52 (P = 0.60)
DRS-R-98 total score at baseline	31.21 ± 2.40	31.83 ± 4.10	0.73 (P = 0.46)
DRS-R-98 scores (severity items only)			
Day 0	24.81 ± 2.19	25.48 ± 3.60	0.89 (P = 0.37)
Day 1	20.46 ± 3.93	19.54 ± 6.40	0.68 (P = 0.49)
Day 2	15.43 ± 6.19	13.54 ± 7.67	1.07 (P = 0.28)
Day 3	11.46 ± 6.58	9.51 ± 7.29	1.11 (P = 0.26)
Day 4	8.65 ± 6.73	7.83 ± 7.42	0.45 (P = 0.64)
Day 5	6.46 ± 6.06	6.48 ± 6.84	473 (P = 0.749)[1]
Day 6	5.43 ± 5.84	5.58 ± 5.84	466.5 (P = 0.679)[1]
DRS-R-98 < 10 on day 0	0	0	-
DRS-R-98 < 10 on day 1	0	3 (9.67%)	FE = 0.11
DRS-R-98 < 10 on day 2	5 (15.62%)	8 (25.80%)	0.99 (P = 0.31)
DRS-R-98 < 10 on day 3	14 (43.75%)	16 (51.61%)	0.39 (P = 0.53)
DRS-R-98 < 10 on day 4	21 (65.62%)	20 (64.51%)	0.009 (P = 0.92)
DRS-R-98 < 10 on day 5	23 (71.85%)	22 (70.96%)	0.006 (P = 0.93)
DRS-R-98 < 10 on day 6	22 (68.75%)	21 (67.74%)	0.007 (P = 0.93)
MMSE scores			
Day 0	7.50 ± 3.83	6.83 ± 4.45	0.63 (P = 0.53)
Day 1	11.31 ± 5.91	11.80 ± 6.02	0.328 (P = 0.74)
Day 2	15.50 ± 5.16	16.00 ± 6.37	0.343 (P = 0.73)
Day 3	18.28 ± 0.73	18.38 ± 6.26	0.070 (P = 0.94)
Day 4	20.34 ± 5.72	20.67 ± 6.41	0.218 (P = 0.828)
Day 5	21.93 ± 5.01	21.58 ± 5.74	0.263 (P = 0.794)
Day 6	23.00 ± 4.75	22.54 ± 5.34	0.354 (P = 0.724)

Table 1 Sociodemographic, clinical profile, delirum subtype, Delirium Rating Scale-Revised-98 and mini mental status examination ratings for both the study groups

[1]Mann-Whitney *U* value. DRS-R98: Delirium Rating Scale-Revised-98; MMSE: Mini mental status examination; FE: Fisher Exact test.

elderly patients (⩾ 60 years). No significant difference was noted in the DRS-R-98 and MMSE scores on any of the assessments between the haloperidol and quetiapine groups among the elderly (⩾ 60 years) and the young adults.

DISCUSSION

In 2 decades or so some data has emerged for the efficacy of atypical antipsychotic medications in management of delirium. Present study was also a step in the same direction. Most of the earlier studies which have evaluated efficacy of quetiapine have done so in sample sizes less than 25 in quetiapine arm. Most of the previous studies have been open label studies[7-14], with only few studies following randomization and blinding[15,16].

Like our previous study[24], the present study too followed a single blind randomised controlled trial design, included patients with delirium with different etiologies in

a sample which predominantly comprised of young adult subjects (< 60 years) admitted to medico-surgical wards. Outcome was assessed by using DRS-R-98 and MMSE, which are considered to be useful for serial evaluation of delirium. However, unlike the previous study[24], in the present study, besides analysing the data for the whole group, separate analysis was done for adult and elderly groups. Further, the DRS-R-98 data was evaluated separately for cognitive and non-cognitive symptoms. Motor subtypes were assessed by using validated scales, and besides ruling out dementia on the basis of clinical history cognitive functions in the last 6 mo were assessed by using short-IQCODE.

The demographic profile (age and gender distribution) of the participants included in the present study is characteristics of patients with delirium seen in psychiatry consultation liaison services at our centre[25,26] and those included in a previous antipsychotic trial from this centre[24]. The dose of quetiapine in the present

Table 2 Efficacy of haloperidol and quetiapine

	Haloperidol group	Quetiapine group
	Paired *"t"* test/ Wilcoxon sign rank test	Paired *"t"* test/ Wilcoxon sign rank test
DRS-R98 severity scores		
Day 0 and day 1	7.10 (*P* < 0.001)	5.12 (*P* < 0.001)
Day 0 and day 2	9.48 (*P* < 0.001)	8.60 (*P* < 0.001)
Day 0 and day 3	11.69 (*P* < 0.001)	11.68 (*P* < 0.001)
Day 0 and day 4	14.08 (*P* < 0.001)	12.90 (*P* < 0.001)
Day 0 and day 5	17.63 (*P* < 0.001)	4.86 (*P* < 0.001)[1]
Day 0 and day 6	4.94 (*P* < 0.001)[1]	4.86 (*P* < 0.001)[1]
Day 3 and day 6	4.70 (*P* < 0.001)[1]	3.98 (*P* < 0.001)[1]
MMSE		
Day 0 and day 1	3.83 (*P* = 0.001)	4.81 (*P* < 0.001)
Day 0 and day 2	8.53 (*P* < 0.001)	7.40 (*P* < 0.001)
Day 0 and day 3	9.00 (*P* < 0.001)	8.66 (*P* < 0.001)
Day 0 and day 4	10.07 (*P* < 0.001)	9.45 (*P* < 0.001)
Day 0 and day 5	11.68 (*P* < 0.001)	10.55 (*P* < 0.001)
Day 0 and day 6	12.38 (*P* < 0.001)	12.23 (*P* < 0.001)
Day 3 and day 6	6.50 (*P* < 0.001)	5.66 (*P* < 0.001)

[1]Wilcoxon Sign Rank test. DRS-R98: Delirium Rating Scale-Revised-98.

study is lower than the mean dose used in most of the previous studies (42.2 to 93.7 mg/d)[6,8,11,14,16], evaluating quetiapine in delirium. This can be understood from the Pharmacogenomic evidence, which suggests that compared to people from West, patients from countries like India require lower doses of psychotropics[27].

The present study suggests that quetiapine in low dose is as beneficial as haloperidol in management of delirium. This finding supports the available literature which suggests that quetiapine is efficacious in management of delirium[7-16]. Present study also provides credence to the available evidence that quetiapine is as efficacious as haloperidol in management of delirium[14]. This research also suggests that quetiapine is equally efficacious in adults and elderly population. Usefulness in elderly provides support to the previous studies[11]. Accordingly it can be said that quetiapine can be considered as another option in the management of delirium.

There are few limitations of the present study. The sample size for the study was small and due to the same the possibility of a type I error cannot be ruled out. No power calculation was done for estimation of sample size for the study. We did not include a placebo control arm and the side effects of both the study medications were not evaluated. As the rater was aware that all the patients were receiving active treatment and hence this could have affected the ratings. The study was limited to referred patients. Due to very few patients in the hypoactive group and those with short IQCODE score above the cut-offs, efficacy could not be compared in different motoric subtypes and those with possible dementia and without dementia. The treating psychiatrist was not blinded to the medication and this would have some bearing on the dose used. Hence, these limitations

must be considered while interpreting the results of this study. This study suggests that quetiapine is as effective as haloperidol in the management of delirium in adult and elderly patients.

COMMENTS

Background
There is limited data on use of quetiapine in management of delirium.

Research frontiers
Very few studies have evaluated the effectiveness of atypical antipsychotics in delirium.

Innovations and breakthroughs
Few studies have evaluated the usefulness of quetiapine in management of delirium.

Applications
Quetiapine can be considered as an alternative to haloperidol in management of delirium.

Peer-review
This is an interesting randomized controlled trial comparing haloperidol and quetiapine in delirium not related to substance withdrawal. The study has been adequately performed and is well presented.

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SYSTEMATIC REVIEWS

Cognitive behavioural therapy for auditory hallucinations in schizophrenia: A review

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Abstract

AIM
To provide an updated of recent findings about efficacy of cognitive-behavior therapy (CBT) in reduction of command hallucinations.

METHODS
PubMed/MEDLINE, Cochrane Library, the Cumulative Index to Nursing and Allied Health Literature, PsycINFO, ClinicalTrial.gov searches were performed using the keywords "hallucinations", "behavioural therapy" and " cognitive therapy" in order to identify relevant articles published during the years of 2011 to 2016. No language limits were used. Studies conducted within control group, reviews, editorials, were excluded. Data on efficacy, acceptability and tolerability were extracted by three authors independently. Disagreements were resolved in a consensus meeting or by another reviewer.

RESULTS
A total of eight articles were eligible for inclusion. Two are randomized clinical trials (RCTs) and six are observational studies. The two RCTs included showed a greater efficacy of CBT compared to standard care on auditory hallucinations (AHs). Nevertheless, they considered different CBT models, particularly Treatment of Resistant Command Hallucinations and Cognitive Therapy for Command Hallucinations. As regards non RCT-studies, all papers included showed reduction on frequency and severity of AHs and distress related to them. However, the lack of content details within non-RCTs studies decreased their comparability. In terms of predictive variables,

our findings show that negative symptoms at baseline appeared to be the strongest predictor of the treatment efficacy. Indeed, negative symptoms showed a significant negative correlation on outcome.

CONCLUSION
Although more conclusive studies are still needed, we found some preliminary evidence for the efficacy of CBT in the treatment of command hallucinations.

Key words: Auditory hallucinations; Cognitive-behavior therapy; Schizophrenia; Psychotic disorder; Treatment; Distress; Functional impairment

Core tip: Auditory hallucinations (AHs), especially command hallucinations, represent a special problem for the clinical management of schizophrenia and contribute significantly to distress and disability related to this disorder. The aim of this article is to review the current knowledge and evidence on the efficacy of cognitive-behavior therapy interventions in AHs.

INTRODUCTION

Hallucinations can be defined as sensory experiences in any sensory modality, occurring in the absence of a corresponding external stimulation whilst in a fully conscious state, and resembling veridical perceptions[1-3]. In schizophrenia, hallucinations occur with a high frequency of up to 50%-80%[4]. Among hallucinations, auditory hallucinations (AHs) are considered the highest, with prevalence estimates in schizophrenia ranging between 40% and 80%[5-7].

AHs, especially command hallucinations, are also associated with an increased risk of harmful or dangerous actions[8-13]. Shawyer *et al*[8] reported a median 53% prevalence of command hallucinations in adult participants with psychiatric disorders, 48% of these participants said the commands stipulated harmful or dangerous actions, rising to 69% for participants in medium secure unit. However, the link between the presence of command hallucinations and harm to self or others is not straightforward. In the MaCarthur study[14], no association was reported between the presence of delusions or command hallucinations and violence. Thoughts about violence, however, were a strong predictor of violence 6 mo later[14].

Besides the high prevalence, AHs experienced in psychotic illness contribute significantly to distress and disability[8]. Indeed, several clinical studies show that AHs appraised as malevolent are significantly and positively associated with distress[9-11]. These findings were confirmed in a recent systematic review by Mawson *et al*[12].

Antipsychotic agents are considered to be the first choice for the treatment of psychotic symptoms[15], but at least one third of patients exhibit persistent psychotic symptoms, despite drug treatment[16]. Treatment of drug-resistant patients can be complicated by adverse effects, due to the use of second-line drugs such as Clozapine[17] or combination therapy with multiple antipsychotic agents[18]. Moreover, there are many concerns regarding patients' refusal to adhere with drug regimes[19] and long-term compliance to therapy[20]. Consequently, there is a growing interest on psychological interventions, which are now recognized as important components of a comprehensive therapeutic approach in the treatment of schizophrenia. AHs are some of the most prominent and distressing of the treatment-resistant symptoms, and command hallucinations are the most high risk of these[21]. Command hallucinations represent a special problem for the clinical management of psychosis. Previous research suggests cognitive-behavior therapy (CBT) to be a useful treatment for reducing compliance with harmful command hallucinations[8,22].

Specifically, CBT applied to the treatment of command hallucinations does not focus on reducing the experience of voices, but on reducing the perceived power of voices to harm the individual and to motivate compliance[8,22]. Indeed, the main rationale is that by challenging key beliefs about the power of commanding voices, the patients would show a lower level of compliance and appeasement behavior and an increase in resistance to the same voices. In a recent meta-analysis, van der Gaag *et al*[23] showed that CBT is effective in the treatment of AHs and delusions. Specifically, individually tailored case formulation CBT showed larger effect-size than broad CBT including standard training programs. However, in this study va der Gaag *et al*[23] have considered both the AHs that delusions.

The aim of our review is to provide an updated overview on the efficacy of CBT interventions in AHs. Specifically, we focus on the efficacy of CBT in reducing command hallucinations.

MATERIALS AND METHODS

This is a review of the literature published between 2011 and 2016 on trials using CBT targeted on AHs in schizophrenia and related psychotic disorders.

A comprehensive literature search of the PubMed/MEDLINE, Cochrane Library, the Cumulative Index to Nursing and Allied Health Literature (CINHAL), PsycINFO, ClinicalTrial.gov databases were conducted. A search algorithm based on a combination of the terms: (hallucinations) AND (behavioral therapy OR cognitive
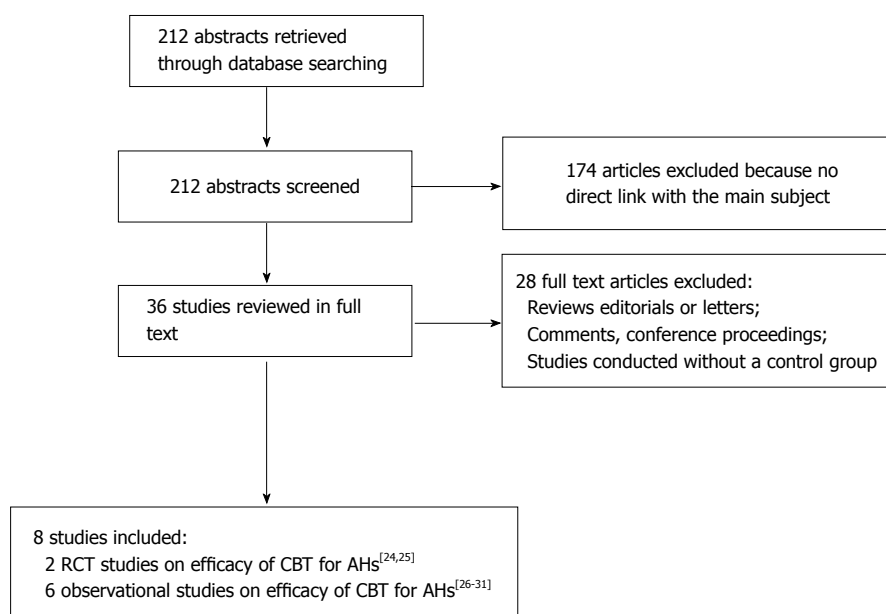
Figure 1 Flow chart of literature review. AHs: Auditory hallucinations; CBT: Cognitive-behavior therapy; RCT: Randomized clinical trial.

therapy) was used. Moreover the bibliographies of the most relevant published articles in the field were screened. The last update of the search was on March 2016. Data on efficacy, acceptability and tolerability were extracted by three authors independently (Franco De Crescenzo, Maria Laura Pucciarini, Maria Pontillo). Disagreements were resolved in a consensus meeting or by another reviewer (Marco Armando). No language limits were used.

The search algorithm resulted in 212 articles, of which 38 referred to potentially eligible studies. Of these, 30 articles were non-empirical studies, reviews and commentaries. We found a total pool of eight studies on CBT for the treatment of AHs.

Two randomized clinical trials (RCTs)[21,24] and six observational studies[25-30] fulfilled the inclusion criteria. In terms of evidence-based medicine, the quality of these studies was moderate.

We decided to focus on the past five years (2011-2016) because this is the period in which CBT models specifically targeted on AHs were developed. In fact, for the previous period, van der Gaag *et al*[23] (2014) had already published a meta-analysis. However, this meta-analysis does not focus specifically on AHs.

In Figure 1 are represented the search strategy with inclusion/exclusion criteria for the papers.

RESULTS

RCTs

During the last four years two RCTs have been conducted, proving the efficacy of CBT for the treatment of AHs, both were specifically on Command Hallucinations. Details on the methodologies and results of the studies are shown in Table 1.

Shawyer *et al*[24] evaluated the efficacy of a cognitive behavioural intervention model called Treatment of Resistant Command Hallucinations (TORCH) compared

with befriending, which is a fully manualised control intervention[31] that provides the patients with the same amount of therapist engagement and expectancy as CBT. The treatment program was conducted for 15 weekly sessions lasting approximately 50 min and with a follow up at six months. Despite TORCH participants subjectively reporting greater improvement in command hallucinations compared to Befriending participants, the study found no significant group differences in the primary outcome (*e.g.*, degree of compliance with harmful command hallucinations), nor in the secondary outcomes (*e.g.*, severity illness, global functioning, level of distress related to command hallucinations, quality of life), based on blinded assessment data.

Birchwood *et al*[21] performed a multi-centre RCT of Cognitive Therapy for Command Hallucinations (CTCH)[32] which is a subtype of CBT specifically targeted on AHs, compared to usual treatment, on 197 participants with command hallucinations, to prevent harmful compliance. This RCT was programmed to follow the patients for 19 sessions, delivered steadily over the 9 mo post-randomisation. The results showed a better efficacy of CTCH respect to treatment as usual.

Both the studies[21,24] were powered on the compliance behavior to voices. In Shawyer *et al*[24] the Voices Acceptance and Action Scale (VAAS)[33] was used, while in Birchwood *et al*[21] the Voice Compliance Scale (VCS)[34] was preferred.

The two RCTs differed in many ways. First of all, Shawyer *et al*[24] did not find any statistical difference between the treatments considered (TORCH *vs* befriending). Birchwood *et al*[21] is a much larger trial which found a significant difference between the treatments considered (CBT *vs* treatment as usual) and which was based and powered on a previous pilot study[22]. The comparisons used for the two RCTs were different as well. In Shawyer *et al*[24] the intervention "befriending" was used. It has a similar amount of therapist engagement and expectancy as CBT,

Table 1 Randomized clinical trials of cognitive behavioral therapy for auditory hallucinations

Ref.	Sample	Methods	Criteria for diagnosis	Criteria for outcome	Focused treatment	Results	Follow-up
Shawyer *et al*[14]	n = 44 Mean age: 39	RCT	(1) diagnosis of schizophrenia or related condition based on DSM-IV criteria (2) command hallucinations within the previous 6 mo that caused distress or dysfunction despite treatment with antipsychotic medication at therapeutic doses	Assessor-rated degree of compliance with harmful command hallucinations on a scale of 0-7 Self-rated confidence to resist obeying harmful commands and confidence in coping with general commands on a scale of 0-100 PANSS Modified GAF PSYRATS Quality of Life Enjoyment and Satisfaction Questionnaire Client Satisfaction Questionnaire VAAS BAVQ-R 8-item self-report Insight Scale RSQ	Randomized to 15 sessions of the intervention "TORCH" or the control, Befriending. A sub-sample of 17 participants was randomized to a waitlist control before being allocated to TORCH or Befriending Pharmacological treatment: Chlorpromazine equivalent dose (mg) Mean = 742.9 SD = 388.7	Confidence to resist harmful CHs (P = n.s.) Confidence in coping with CHs ($P < 0.01$) PANSS total ($P < 0.05$) Modified GAF (P = n.s.) Distress PSYRATS ($P < 0.01$) Disruption PSYRATS ($P < 0.01$) Quality of Life ($P < 0.05$) VAAS (P = n.s.) BAVQ-R (P = n.s.)	6-mo
Birchwood *et al*[15]	n = 197 Mean age: 37.4	RCT	(1) ICD-10 schizophrenia, schizoaffective, or mood disorders, under care of a clinical team (2) history of harmful command hallucinations of at least 6 mo duration with recent (< 9 mo) history of harm to self, others or major social transgressions as a result of the commands (full or partial compliance); or harmful command hallucinations where the individual is distressed and appeasing the powerful voice	VCS VPD Personal knowledge questionnaire/omniscience scale BAVQ-R PSYRATS Calgary Depression Rating Scale for Schizophrenia Beck Hopeleness Scale Beck Scale for Suicidal Ideation PANSS	Randomized to cognitive Therapy for command Hallucinations + treatment as usual or treatment as usual alone Adherence to cognitive therapy was excellent: only 12 (12%) of 98 participants not attending any sessions, and 79 (81%) completing the therapy (all manualised elements) Pharmacological treatment: Olanzapine equivalents dose (mg) 25.79 (SD: 21.73).	RSQ (P = n.s.) VCS experimental group: 41; control group: 49 VPD total, experimental group: 21.31; control group: 23.98 Personal knowledge questionnaire (P = 0.09) BAVQ-R (P = n.s.) PSYRATS total (P = n.s.) Calgary Depression Rating Scale for Schizophrenia (P = n.s.) Beck Scale for Suicidal Ideation (P = n.s.) PANSS total (P = n.s.)	18-mo

AHs: Auditory hallucinations; BAVQ-R: Beliefs about the voices questionnaire-revised; CHs: Command hallucinations; GAF: Global assessment of functioning scale; n.s.: Not significant; PANSS: Positive and negative syndrome scale; RCT: Randomized clinical trial; PSYRATS: Psychotic symptom rating scale; RSQ: Recovery style questionnaire; VAAS: Voices acceptance and action scale; VCS: Voice compliance scale; VPD: Voice power differential scale.

with similar drop-out rates[35]. Befriending involves a series of conversations that resemble conversations with a friendly social acquaintance. In Birchwood *et al*[21] the treatment as usual[15] was used as comparison.

On the one hand, in Shawyer *et al*[24] no differences were found between or within the TORCH and befriending

groups on confidence to resist harmful commands at endpoint or follow up. On the other hand in Birchwood *et al*[21] the CTCH intervention showed to be significantly superior to the usual treatment and the efficacy interpreted as the effect that is common to the 9 and 18 mo follow-up points, was calculated as an odds ratio of

0.574 (95%CI: 0.33-0.98, *P* = 0.042). Both the trials had high quality and a low risk of bias (Table 1).

Non-RCT clinical studies

We found six non-RCT studies examining the efficacy of CBT for AHs in patients with psychosis. Details on the methodologies and results of the studies are shown in Table 2.

In the latest study, Zanello *et al*[30] investigated the effectiveness of a group cognitive behavioural therapy for AHs, the Voices Group, in 41 patients with schizophrenia or schizoaffective disorders. The program Voices Group was conducted for seven specific sessions. The results showed a significant reduction in the severity of AHs (*P* < 0.005) and in total symptoms severity score of BPRS 4.0 [Brief Psychiatric Rating Scale[36]; without hallucination (*P* < 0.01)]. This result remained stable after the 6-mo follow-up.

Thomas *et al*[25] conducted an open label trial on the efficacy of CBT in reducing AHs in 33 subjects with schizophrenia. They also investigated the role of insight, beliefs about the origin of hallucinations, negative symptoms and cognitive disorganization as predictors of the outcome. The study observed post-treatment improvements in hallucination severity when AHs were considered a specific target of psychological treatment. Only overall negative symptoms showed a significant negative correlation on (rpb = -0.60, *P* = 0.001) with outcome. This effect appeared to be independent of length of illness, drop-out and number of sessions.

Mortan *et al*[26] evaluated the effectiveness of a group-based CBT program for AHs on 7 inpatients with schizophrenia and other psychotic disorders compared to 5 in patients treated with treatment as usual. The CBT treatment program was conducted for 9-10 sessions twice/wk. The results showed a significant reduction (*P* < 0.005) in the severity and frequency of hallucinations, delusions, negative symptoms, distress and anxiety after group-based CBT.

A case study by Hutton and Morrison[27] described the effectiveness of brief CBT (12 wk) in an 18-year-old male with psychotic disorder and AHs who refused antipsychotic medication. By week 12, the frequency and duration of AHs had reduced to zero.

Dannahy *et al*[28] examined the impact of group person-based cognitive therapy (PBCT) for distressing voices in a sample of 62 participants with treatment-resistance and subjectively distressing voices. Participants were divided in nine groups and PBCT was conducted over 8-12 sessions. Results demonstrated significant improvements in the outcomes measure of general well-being (*P* < 0.001), voice-distress (*P* < 0.001), control (*P* < 0.01) and dependence upon voice (*P* < 0.05).

Gottlieb *et al*[29] tested the feasibility and effects of a 10 session web-based CBT for AHs in a sample of 17 individuals with schizophrenia spectrum disorder. Results showed a significant reduction of AHs, including the perception of voices as an outside entity and intensity of negative commentary. Interestingly, participants improved in depression and delusion severity, although these symptoms were not directly targeted in the program.

DISCUSSION

The present review describes the efficacy of CBT in patients with AHs. In summary, the two RCTs included showed a greater efficacy of CBT compared to standard care on AHs. However, in Shawyer *et al*[24], TORCH participants subjectively reporting greater improvement in command hallucinations compared to Befriending but no significant group differences on primary outcome measure that was level of compliance with harmful command hallucination. In Birchwood *et al*[21] instead, CTCH participants showed an improvement in this measure.

One possible explanation of the discrepancy between the two RCTs in term of efficacy on reducing level of compliance with harmful command hallucinations is that, within the general framework of CBT, different theoretical approach can play a different role on the efficacy of the intervention. Indeed, the two RCTs were built on different theoretical frameworks. The TORCH framework is based on the "acceptance" of voices by "cultivating the capacity to just notice voices and associated thoughts rather than believing and acting on them". The CTCH focuses on targeting individuals' appraisals, behavior and affect, and not necessarily symptoms. It is based on the nature of the relationship with the personified voice. Therefore, if the voice hearer believes the voice to have malevolent intent, and crucially to have the power to deliver the threat, this can motivate compliance or appeasement behavior. In addition, in Shawyer *et al*[24] the intervention "befriending" was used as the control condition and it has a similar amount of therapist engagement and expectancy as CBT. This is likely to have resulted in smaller between-group effect sizes respect to Birchwood *et al*[21].

As regards non RCT-studies, all papers included showed reduction on frequency and severity of AHs and distress related to them. However, the lack of content details and on rationales within non-RCTs studies decreases their comparability and therefore the chance to draw final conclusions.

In terms of predictive variables, negative symptoms appeared to be the strongest predictor of the treatment efficacy. It may be that negative symptoms are a barrier to treatment specific to hallucinations, although it would be important to verify this association in other studies. However, based on this finding, it is possible to propose that negative symptoms interfere with engagement in therapy, in rapport with the therapist, and completion to homework. This might lead to modifications of CBT to treatment for the presence of negative symptoms, such as the use of more behavioral methods.

Some limitations and strenghts should be con-

Table 2 Non randomized clinical trials of cognitive behavioral therapy for auditory hallucinations

Ref.	Sample	Methods	Criteria for diagnosis	Criteria for outcome	Focused treatment	Results	Follow-up
Zanello et al[21]	n = 41 age-range: 18-65	Naturalistic Study	(1) Diagnosis of a schizophrenia or schizoaffective disorder (2) Current AHs in the form of voices, occurring at least once per week	Reduction of AHs: BPRS Total symptom severity without AHs: BPRS	7 sessions of CBT based upon the program "Voice Group" of Wikes et al 1999 Pharmacological treatment: New antipsychotic Combined antipsychotic Anxiolytic, mood stabilizer, hypnotic or antidepressant medication Dosage: Changed when clinically required	Decrease in the hallucinations item score of Bprs ($P < 0.05$) Decrease in the total symptoms severity score of BPRS ($P < 0.01$)	6-mo
Thomas et al[16]	n = 33 Mean age: 36.4	Non-RCT Open trial	1) Diagnosis of a schizophrenia or schizoaffective disorder (2) Current AHs in the form of voices, occurring at least once per week (3) Voices associated with significant subjective distress (4) History of voices for at least one year; and (5) currently prescribed antipsychotic medication	Correlation between PSYRATS, PANSS, SAI and Outcome Main Outcome measure: Improvement of five points of more on the PSYSTRATS	24 sessions of CBT based upon the manual of Fowler et al (1995) Pharmacological treatment: Chlorpromazine-equivalent pre-treatment: M = 793.1 mg, SD = 468.6 mg; post–treatment: M = 768.1 mg, SD = 473.8 mg	Only negative symptoms showed a statistically correlation with outcome (rpb = -0.60; $P \leqslant 0.001$)	None
Mortan et al[17]	n = 12 age range: 18-55	Pilot study	(1) Criteria for schizophrenia or schizoaffective based on DSM-IV-R (SCID I) (2) At least 1 psychotic attack with hospitalization (3) Ongoing AHs (4) Use of oral and injectable antipsychotic	Presence of Positive Symptoms: SAPS Presence of Negative Symptoms: SANS Comorbid symptoms: BDI HDI	9-10 sessions of CBT upon the manual of Morrison, 2002, Goldberg, 2007) Pharmacological treatment: Oral and injectable antipsychotic medication	Difference between pre-treatment and post-treatment Treatment goup: SAPS hallucination subscale score ($P = 0.027$) SAPS delusion sub-scale score ($P = 0.028$) SANS total scored ($P = 0.046$) KSQ (P = n.s.) BDI (P = n.s.) Control group: SAPS hallucination subscale score (P = n.s.) SAPS delusion sub-scale score (P = n.s.) SANS total scored (P = n.s.) BDI ($P = 0.043$) HDI (P = n.s.)	1-yr post-treatment follow-up
Hutton et al[18]	Single case, An 18-year-old man	Case report	Criteria for schizophrenia spectrum disorder based on DSM-IV Symptoms and psychosocial functioning: GAF; BPRS; Clinical questionnaire	Positive Symptoms: PSYRATS/CAARMS Beliefs about control of AHs: IVI	Brief CBT upon the mindfulness approach Pharmacological treatment: None	Pre-treatment: IVI score: 62 Post treatment: IVI score 2 The frequency and duration of AHs had reduced to zero	1, 3, 4, 9 mo post therapy
Dannahy et al[19]	n = 62 divided in nine groups	Pilot study	The individual had been experiencing treatment-resistant and subjectively distressing voices for at least the preceding	Primary outcome measure: Improve general psychosocial well-being (CORE-OM); Secondary measures:	Group person-based cognitive Therapy (PBCT) conducted over 8-12 sessions based upon the manual of Chadwick et al (2006) Pharmacological treatment: Standard psychiatric care	CORE-OM Total score: Post-group: 1.90[b] (0.70) VOICE-DISTRESS Total score:	1 mo

	Mean age: 41.1 SD: 9.2		2 yr, with the voice-distress rated at 3 or greater on at least one of the two PSYRATS voice-distress items	Reduce distress and perceived voice-control; Evaluate the relationship with voice (VAY)	Group person-based cognitive Therapy (PBCT) conducted over 8-12 sessions based upon the manual of Chadwick *et al* (2006) Pharmacological treatment: Standard psychiatric care	Post-group: 3.57[b] (0.83) VOICE-CONTROL total score: Post-group: 53.47[b] (23.59) VAY Voice Dependence total score: Post group: 6.76 (5.69) VAY Voice Intrusiveness total score: Post group: 9.03 (4.32) VAY Voice Dominance total score: - Post group: 14.46 (6.37) VAY Hearer distance total score: Post group: 12.93 (5.93)	
Gottlieb *et al*[20]	*n* = 17 Mean age: 40.10 SD: 13.63	Pilot study	(1) Criteria for schizophrenia, schizoaffective disorder, or psychosis, NOS based on DSM-IV (2) At least "moderate" level of AHs severity over the past week (BPRS Hallucinations item 4 or higher); (3) Between the ages of 18-65; (4) No exposure to CBTp within the past 3 yr (5) No current suicidal ideation or hospitalization within the past month (6) Taking a stable dose of an antipsychotic medication for at least one month; (7) No active substance abuse/dependence (8) MMSE score ≥ 24)	Primary outcomes: Reduce the frequency, intensity, loudness, associated distress, perceived degree of controllability of, and interference from AHs (PSYRATS) Secondary outcomes: Evaluate beliefs about AHs (BAVQ-R); Evaluate overall psychopathology (BPRS), and depression (BDI-II)	Web-based cognitive-behavioral therapy for AHs: - 10 session: - psychoeducational video tutorials - games - interactive exercises - social network to examine the coping strategies of other users. Pharmacological treatment: stable dose of antipsychotic medication for at least one month	Significant reductions from baseline to post-treatment in several measures of AHs and in overall psychopathology on the BPRS: PSYRATS AHs subscale total: *P* = 0.007 PSYRATS AHs Subscale: Voices location: *P* = 0.029 Voices intensity of negative statements: *P* = 0.049 PSYRATS delusions subscale total: *P* = 0.101 BPRS total score: *P* = 0.001 BPRS Subscale: BPRS Psychosis: *P* = 0.002 - BPRS Depression: *P* = 0.004 BPRS Activation: *P* = 0.001 BAVQ-R total score: *P* = 0.902 (n.s.) BDI-II-total score: *P* = 0.085 (n.s.)	None

[b]*P* < 0.001. BPRS: Brief psychiatric rating scale; PSYRATS: Psychotic symptom rating scale; PANSS: The positive and negative syndrome scale for schizophrenia; SAI: The Schedule for the Assessment of Insight; SCID-I: Structured Clinical Interview for DSM; SAPS: Scale for the assessment of positive symptoms; SANS: Scale for the assessment of negative symptoms; BDI-II: The Back Depression Inventory II; HDI: Hamilton depression inventory; CAARMS: Comprehensive Assessment of At-Risk Mental States; GAF: Global Assessment of functioning; IVI: Interpretation Voices of Inventory; CORE-OM: Clinical outcomes in routine evaluation-outcome measure; VAY: Voice and You; BAVQ-R: The Belief about Voices Questionnaire-Revised; MMSE: Mini Mental State Examination; AHs: Auditory hallucinations.

sidered in our review. Firstly, the role and the possible interference of antipsychotic medications with psycho-therapy should be further assessed in the primary studies. Secondly, there is a discrepancy of study design and outcome measures between studies, which did not allow a quantitative analysis of the results. Thirdly, most studies are only preliminary and underpowered. Among strenghts, we have two RCTs with 241 individuals randomized in total and both of them conclude that CBT may be an alternative for individuals with schizophrenia who experience AHs despite antipsychotic treatment.

Overall, several CBT models were tested in the studies included. Apart TORCH and CTCH, Mindfulness approach, PBCT or web-based CBT were used.

We propose that further RCTs are needed. In particular, based on our findings, future studies should be drawn with reference to validated theoretical framework that predicts individuals' compliance with voices and the associated distress, rather than the presence of psychotic symptoms *per se*. This validated theoretical framework should also consider the role of negative symptoms in predicting the effectiveness of the intervention on Ahs.

Finally, due to the efficacy and high tolerability and acceptability of RCT-studies, we believe that the treatment with CBT should be integrated into standard care for AHs, taking into account that individuals with AHs and command hallucinations especially, and more in general with psychotic disorders, show often a poor compliance to pharmacological treatments.

COMMENTS

Backgrounds

In schizophrenia, auditory hallucinations (AHs) occur with a high frequency ranging between 40% and 80%. AHs, especially command hallucinations, are also associated with an increased risk of harmful or dangerous actions and are some of the most prominent of the pharmacological treatment-resistant symptoms. Consequently, there is a growing interest on psychological interventions. The aim of this review is to provide an updated of recent findings about efficacy of cognitive-behavior therapy (CBT) in reduction of command hallucinations.

Research frontiers

Previous research suggests CBT to be a useful treatment for reducing compliance with harmful command hallucinations. Specifically, CBT applied to the treatment of command hallucinations does not focus on reducing the experience of voices, but on reducing the perceived power of voices to harm the individual and to motivate compliance. Indeed, the main rationale is that by challenging key beliefs about the power of commanding voices, the patients would show a lower level of compliance and appeasement behavior and an increase in resistance to the same voices.

Innovations and breakthroughs

In literature evidence on efficacy of CBT in reduction of command hallucinations are still few. Only in recent years CBT models specifically targeted on AHs were developed. Studies published in the last five years were critically reviewed by the authors who make a comparison between the current existing CBT models for AHs and data to support each.

Applications

This review suggests that the treatment with CBT should be integrated into standard care for AHs, taking into account that individuals with AHs and command hallucinations especially show often a poor compliance to pharmacological treatments.

Terminology

AHs can be defined as sensory experiences in auditory modality, occurring in the absence of a corresponding external stimulation whilst in a fully conscious state, and resembling veridical perceptions. CBT is a psychosocial intervention that is the most widely used evidence-based practice for treating mental disorders. CBT focuses on the development of personal coping strategies that target solving current symptoms and changing unhelpful patterns in cognitions (*e.g.*, thoughts, beliefs, and attitudes), behaviors, and emotional regulation.

Peer-review

The topic is interesting, informative and useful for a clinician. The paper is clearly written.

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CASE REPORT

Understanding the paranoid psychosis of James: Use of the repertory grid technique for case conceptualization

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Abstract

In this paper we illustrate the potential of the repertory grid technique as an instrument for case formulation and understanding of the personal perception and meanings of people with a diagnosis of psychotic disorders. For this purpose, the case of James is presented: A young man diagnosed with schizophrenia and personality disorder, with severe persecutory delusions and other positive symptoms that have not responded to antipsychotic medication, as well with depressive symptomatology. His case was selected because of the way his symptoms are reflected in his personal perception of self and others, including his main persecutory figure, in the different measures that result from the analysis of his repertory grid. Some key clinical hypotheses and possible targets for therapy are discussed.

Key words: Persecutory delusions; Personal constructs; Schizophrenia; Repertory grid technique; Case formulation

Core tip: The repertory grid measures indicated that the patient's meaning system was strongly articulated around a very negative view of self, and by symptomatic constructs related to fear, anxiety, sense of loneliness, and perceived aggressiveness in others. Furthermore, constructs related

to hostility dominated his perception of his persecutory figure and also of his parental figures. Based on this appraisal, the case formulation was suggested as a focus for psychotherapy to enhance his self-esteem and deal with family conflicts.

INTRODUCTION

Psychotic disorders are complex conditions with a wide range of clinical symptoms. One of the central psychotic experiences is persecutory delusions, which are present in over 70% of early psychotic patients[1], and which are accompanied by many clinically important symptoms such as anxiety and depression[2]. Despite the common administration of antipsychotic medication, more than half of patients still have persistent positive symptoms that interfere with their daily functioning[3,4]. In this context of drug-resistant cases, the use of psychotherapy, with cognitive-behavioral therapy (CBT) the most widely studied, is of increasing interest and importance, and is recommended either as an adjuvant or even an alternative treatment. To this effect, a recent meta-analysis demonstrated the efficacy of CBT in reducing symptom severity in these cases[5]. In addition, one randomized controlled trial demonstrated similar results for cases not taking antipsychotic drugs[6].

In cognitive models of psychosis and other mental disorders, an essential element for planning psychological therapy is the use of case conceptualization. From a person-based approach to psychosis[7], paranoid psychotic symptoms cannot be studied in isolation because the distress experienced by these patients is not a direct consequence of psychotic symptoms. Rather, it is mediated by the meaning patients ascribe to them. With this approach more attention is paid to personal meanings than to symptoms when developing each patient's case conceptualization.

From a more general perspective, this focus on the subjective construction of the symptoms and problems experienced by the client was previously highlighted by personal construct theory (PCT)[8]. This theory sees psychological activity as a subjective meaning-making process for the events people encounter in life[9]. Thus, the person's cognitive system is formed by a complex network of bipolar dimensions of meaning, interdependent and hierarchically organized, denominated personal constructs (as opposed to theoretical constructs), such as "friendly-hostile". This system of constructs is used to interpret the person's

experience and to organize his or her actions.

In PCT, case conceptualization is understood as a way to see the world through the patient's eyes[10]. To study personal views of the world, the most widely used method is the Repertory grid technique (RGT). It allows us to access the idiosyncratic meanings of the person about self and others, in his or her interpersonal world. Also, it yields some measures regarding the cognitive structure of the subject. Both sources of information have interesting clinical implications: They allow the therapist to formulate clinical hypotheses and identify possible targets for therapy[11]. In addition, in a review by Freeman *et al*[2], perceptions of self and others have been linked to the development and maintenance of persecutory delusions, as well as being proposed as important targets for therapy.

Despite its possibilities as an instrument for the detailed exploration of the individual's belief system to be employed for case conceptualization, little use is made of the RGT in common psychiatric practice and psychotherapy, and no recent studies have been found involving cases with paranoid psychotic symptoms. This is even more surprising given the line of research led by Bannister in the 1960s and 1970s[12] (although they were using another type of grid with provided rather than elicited personal constructs).

In this article, we present the possibilities for the application of the RGT toward the understanding of paranoid psychosis, showing the sense that symptoms can have for the person with schizophrenia. To this end, we have selected the case of James (fictitious name), a man diagnosed with schizophrenia and personality disorder, who presents severe persecutory delusions that have not improved with medication. He was one of the first patients to participate in a Spanish clinical trial based in Metacognitive Training (MCT+) for psychosis by Moritz *et al*[13]; in the initial evaluation, a battery of instruments was administered, including instruments that assess psychophatology and the RGT. The main indices obtained from the RGT are described and used to understand his personal meanings about himself and others at the delicate moment when his persecutory delusions are very intrusive. Possible clinical hypotheses derived from this analysis, and targets for the therapeutic process of James, are discussed.

CASE REPORT

James is a Spanish man 25 years of age who lives with his parents and is unable to study or work although he completed high school studies. He reports a relationship with a girl, Ana, at the current moment, who lives in a distant part of Spain. He refers to her as his girlfriend but their only contact has been by internet and telephone.

He has a diagnosis of schizophrenia and personality disorder not otherwise specified. The disturbances started 2 years ago, and the last psychotic episode occurred 10 mo before the present assessment, for which he had

Table 1 Assesment of psychopathology	
	Raw scores
PANSS positive	23
P1: Delusions	6
P6: Suspiciousness/persecution	6
PANSS negative	17
PANSS general psychopathology	37
PSYRATS delusions	21
PSYRATS hallucinations	33
BDI-II	32

to be hospitalized. Since this last hospitalization, he has been experiencing persecutory delusions and auditory hallucinations that have not been reduced, despite taking antipsychotic medication, olanzapine 20 mg/d and aripiprazole 15 mg/d, last one administrated monthly as depo.

As relevant background to the psychotic symptoms, James had a relationship two years ago with another girl, Mary, which lasted eighteen months. During the assessment, he told us that it was a good relationship most of the time. He also stressed that both of them felt the lack of support from other people ("We were both alone, we had in common that we had no one else, and we relied on each other"). At the end of the relationship, James uploaded a song on line in which he talked about Mary and their relationship, and he also made comments on social networks about the girl, resulting in conflict with Mary and her family and friends, and turning all of them against him. In this context, James developed intense fears of the girl and her relatives, which culminated in a psychotic crisis experienced 10 mo before this assessment.

Since then, James says that he lives in fear every day, convinced that his ex-girlfriend and her relatives seek to harm or even kill him, even when he recognizes not having had any contact with them for months. He has a general feeling of being threatened and persecuted, being very alert to signs in the environment, which makes him afraid to go outside. His psychotic experiences seem to increase at night when he hears noises at his window. He is afraid that they could be caused by his persecutors, so most nights he has problems falling asleep. From time to time, he also presents auditory hallucinations of which he is unaware, hearing voices on the street, which always have threatening content, referring to hurting or killing him.

Regarding the relationship with his parents, he says that the family atmosphere is not good, there having been severe conflicts since adolescence, when James had episodes of aggression toward his parents. James maintains a discourse greatly focused on his paranoid ideas, and when he talks about his fears at home he says he feels unheard and slighted, and that he has received aggressive and dismissive responses. He experiences family life as hostile, and describes having suffered episodes of aggression from his father not only in the past but also recently. He also notes that since

the psychotic crisis, he has lost the few friends he had, feeling very alone and with little support.

Assessment of psychopathology

In the Metacognitive Training study in which James is enrolled, a battery of instruments was administered in two sessions before the beginning of the therapy.

The instruments assessing psychopathology, shown in Table 1, were administered in the first session. James's scores on the Positive and Negative Syndrome Scale (PANSS), Spanish adaptation of Peralta *et al*[14], and the Psychotic Symptom Rating Scales (PSYRATS), Spanish adaptation by González *et al*[15], showed a high severity for the positive symptoms. In addition, on PANSS items related to passive social withdrawal and active social avoidance the scores indicate moderate-severe social isolation, which is associated with his suspiciousness and persecutory fears. He also presented severe depressive symptomatology, as measured with the Beck Depression Inventory (BDI-II), Spanish adaptation by Sanz, Perdigón *et al*[16].

The severity of his psychopathology could also be observed during the second interview, when the repertory grid was administered. He was very cooperative and talkative, but he was also invaded by his delusions and made repeated verbalizations about them and the suffering that they brought him ("I'm scared. Before coming here I heard a man in a bar talking about killing me").

The administration of the repertory grid technique

The RGT is a structured interview exploring the patient's personal meanings. The first phase is the selection of elements, which represent a sample of the most significant people for the patient. In the case of James, 17 elements were chosen. Four of them were provided by the clinician according to their possible clinical implications: "self now", "ideal self" (which represents how he would like to be), "self before the psychotic crisis", and the "non-grata person", which represents a person he does not like (for James, this was his ex-girlfriend, his main persecutory figure). The remaining elements, elicited by James, were his parents, six members of the extended family who live far away but for whom he felt much appreciation (maternal grandparents, two uncles, and two cousins), two good friends from the time before the psychotic crisis, Ana, identified by him as his current partner, and a friend of his partner's with whom he often talks. The selected items are recorded in the upper part of the protocol of the grid (Figure 1) defining the columns, the first column for the "self now" and the last for the "ideal self".

In the second phase, constructs are not provided by the clinician (like items in nomothetic research); rather, they are elicited directly from the person evaluated as a way to express his or her personal meanings. Personal constructs are elicited using the dyadic method, which consists of asking the subject about similarities and then differences in each pair of elements in terms of their

Date :

Name: James
Grid number: PRE-THERAPY

				1 Self now	2 Father	3 Mother	4 Grand-father	5 Grand-mother	6 Aunt	7 Uncle	8 Cousin-1	9 Cousin-2	10 Friend-1	11 Friend-2	12 Current partner	13 Friend-3	14 Self-before	15 Mary	16 Ideal self
1.	Harsh	1.	Tolerant	3	2	3	7	7	6	7	5	6	6	6	7	7	7	2	7
2.	Worrier	2.	Careless	2	5	1	1	1	1	1	1	2	7	2	1	1	1	6	5
3.	Attentive	3.	Non-attentive	1	3	3	1	1	1	3	2	5	3	3	1	1	7	2	1
4.	Calm	4.	Nervous	7	5	7	1	1	2	5	5	5	2	1	1	1	6	6	1
5.	Argumentative	5.	Assertive	6	1	1	7	7	7	3	3	3	5	7	7	7	2	2	7
6.	Empathetic	6.	Egocentric	4	6	2	1	1	1	1	1	5	4	2	1	1	1	2	1
7.	Friendly	7.	Unfriendly	4	5	5	1	1	1	1	1	2	2	1	1	1	6	2	1
8.	Hysterical	8.	Relaxed	4	3	2	7	7	6	6	3	3	5	7	7	7	1	6	6
9.	Tidy	9.	Untidy	6	1	3	1	1	1	1	1	5	2	2	1	2	7	1	1
10.	Happy	10.	Sad	5	3	3	1	1	1	1	2	1	2	1	1	1	5	5	1
11.	Funny	11.	Boring	5	3	2	2	1	1	6	6	2	2	2	2	5	2	2	1
12.	Capable of loving	12.	Incapable of loving	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1
13.	Fighter	13.	Coward	7	1	1	1	1	1	1	1	1	3	1	1	1	7	3	1
14.	Generous	14.	Mean	1	3	3	1	1	1	1	1	1	3	3	1	1	5	5	1
15.	Respects-family	15.	Detached	6	1	1	1	1	1	1	1	1	1	1	1	1	6	1	1
16.	Tires easily	16.	Even-tempered	3	1	1	3	7	6	5	3	3	3	7	7	7	1	3	7
17.	Altruist	17.	Selfish	4	2	1	1	1	1	1	1	1	5	1	1	1	6	5	2
18.	Goodperson	18.	Badperson	2	2	1	1	1	1	1	1	2	1	1	1	1	6	2	1
19.	Aggressive	19.	Non-aggressive	6	2	2	6	7	7	7	7	7	3	7	7	7	2	3	7

1 Very   4 Middle point 5 Slightly
2 Quite   6 Quite
3 Slightly   7 Very

Figure 1 The repertory grid of James.

perceived personality or character traits. For example, the first question for James was: "In terms of their personality, in which way would you say your mother and father are alike?" James answered, "Both of them are harsh". This answer constitutes one pole of the first construct, so to obtain the other pole we asked "What would be the opposite of harsh for you?" and the answer was "tolerant". Thereby, the first construct ("harsh *vs* tolerant") was obtained and more similarity and difference questions were made for this and other pairs of elements. Elicited constructs were written down by the interviewer in the horizontal entries of the grid (Figure 1). After 19 constructs were obtained from James he started to repeat many constructs and showed fatigue. This is usually the time to end the elicitation process, which is known as the "saturation point".

The last phase is the rating of the elements. Each element is assigned a value in a seven-point Likert-type scale for each construct. Taking as an example the cited construct, 1 means "very harsh", 2 "quite harsh", 3 "slightly harsh", 4 "middle point", 5 "slightly tolerant", 6 "quite tolerant", and 7 "very tolerant".

Once the grid data matrix was filled (see James's grid in Figure 1), the administration process ended, and the data were ready to be analyzed qualitatively or quantitatively, for which there is software available, the GRIDCOR, with a manual to guide interpretation of the data[17]. This software allows synthesizing and analyzing the great amount of information reflected in the grid using different statistical methods.

Qualitative analysis of the repertory grid: How does James define himself in his own words?
From the data of the grid we may grasp the personal views of James about himself. To do so, we should focus on the constructs in which James rates himself with extreme values (scores of 1-2 and 6-7). Once obtained, we can narratively formulate his self-definition: "I am a very attentive, generous person, with a great capacity to love. But I'm also very nervous and very cowardly. I also consider myself quite a good person, concerned and assertive, but I am also quite messy and detached from my family, for whom I feel I have little respect".

We identify what James values about himself by finding congruent constructs in his grid. These are the constructs where the elements "self now" and "ideal self" are rated in the same pole, dimensions for which James does not wish any change. We found six such constructs: Attentive, assertive, capable of loving, generous, good person, and non-aggressive.

In contrast, discrepant constructs reveal aspects that James does not like about himself and which he would like to change. These are constructs in which the "ideal self" is rated at the opposite pole to the "self now". These discrepant constructs can be also expressed narratively: "Contrary to what I am (harsh, nervous, messy, sad, boring, cowardly, detached, and someone who tires easily), I would like to be tolerant, quiet, happy, funny, a

Table 2 Main significant measures of the repertory grid of James

Self-construing		Cognitive structure	
Self-ideal distance	0.5	PVAFF	54.11%
Self-others distance	0.4	Polarization	60.53%
Ideal-others distance	0.25		

fighter, and even-tempered".

James's congruent constructs are his "strong points", those which might be central to his identity. Additionally, they may be seen as resources to validate and protect during the therapy process. However, James presents more discrepant than congruent constructs (eight to six), which could be a reflection of his current life moment in which he lives dominated by fears that stem from his persecutory delusions ("I would like to be a fighter, but I am a coward"), in a state of suffering and dissatisfaction with himself ("I would like to be happy and quiet, but instead I am sad and nervous").

Main cognitive measures derived from the repertory grid technique

The GRIDCOR program outputs many quantitative measures that explain different aspects of how the patient construes himself and others, and also about the structure of his cognitive system. In the case of James, the most significant indices are shown in Table 2.

Construing self and others: Various aspects of the self can be evaluated taking the Euclidean distances between the elements "self now", "ideal self" and "others" (an artificially generated element taking into account the average of the scores of all elements but "self now" and "ideal self").

Self-ideal differentiation: The discrepancy in the ratings of the elements "self now" and "ideal self" can be considered as a measure of self-esteem, since by comparing these two elements the patient is evaluating himself on his own terms. High differentiation (*e.g.,* d > 0.32) is usually taken as indicative of low self-esteem. This is the case with James: He feels very far away from the way he would like to be and he therefore feels great dissatisfaction with himself and serious distress. This finding matches with the self-definition of James, with many discrepant constructs, and with the clinical observations made during the assessment process.

Self-others differentiation: The discrepancy between self and others becomes an index of how people see themselves as different (or similar) with respect to the other elements in the grid. This differentiation is considered as a measure of perceived social isolation. High differentiation (*e.g.,* d > 0.35) is an indication that a person experiences himself or herself as different from others, feeling that he or she shares few features with other people.

James presented high perceived social isolation,

viewing himself as very different, which is compatible with feeling like "the weird guy", accompanied by notable feelings of loneliness.

Ideal-others differentiation: The discrepancy between the ideal self and others is considered as an index of the degree of perceived adequacy of others. High dissimilarity (*e.g.,* d > 0.28) means that the person has great dissatisfaction with others, while a lower score suggests a positive perception of them, as was the case with James. For a wider perspective, we can take into account his self-esteem, which is very low and negative, with others being closer to his ideal self than his current self; they are the "good ones".

Self-construction profile: Five different self-construction profiles can be identified taking into account the joint interpretation of the three differentiation indices explored: Positivity, superiority, negativity, depressive isolation, and resentment profiles.

The conjoint interpretation of the three indices of James suggests a depressive isolation profile. This profile represents the combination of having a negative view of oneself, high perceived social isolation, and a positive perceived adequacy of others. This combination suggests that James views himself in negative terms and different from others, as if he was saying: "The others are great, but not me. I am the only one who is weird". This profile usually applies to depressive patients and people with other psychiatric categories who manifest hopelessness, which is congruent with James's depressive symptoms.

The structure of the cognitive system

Interpersonal cognitive differentiation: Interpersonal cognitive differentiation refers to the extent to which a person can construe his or her social experiences from different points of view. The more differentiated a cognitive structure is, the more meaningful dimensions are available to the person to perceive and understand the behavior of others.

Several measures have been proposed to assess cognitive differentiation, but the percentage of variance accounted for by the first factor (PVAFF) resulting from the factor analysis is the one with the strongest reputation. This percentage indicates the importance or weight of the main dimension of meaning. It is estimated that a low PVAFF indicates a differentiated cognitive structure, favoring multidimensional thinking and allowing other dimensions to play relevant roles in the way the subject construes, while a high PVAFF indicates low cognitive differentiation, with a tendency to one-dimensional thinking. James's score indicates a cognitive structure with low differentiation, with one dimension which plays the main role for the construction of himself and the others.

Polarized thinking: Polarization refers to the extent to which a person construes reality in an extreme
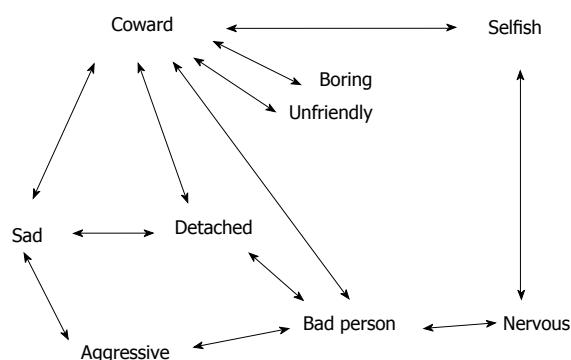
Figure 2 Main construct correlations for "coward".

way, and it is considered as a measure of cognitive rigidity. It is computed as the percentage of extreme scores in the grid. High percentages are indicative of a polarized structure. This score is very high in the case of James, suggesting a very rigid cognitive structure, with a tendency to construe himself and others in a dichotomous way.

Centrality of symptomatic constructs: The variance accounted for by each construct in the grid data matrix is calculated with Bannister's[18] Intensity score, which is based on the strength of the correlations with the other constructs. Thus, those constructs with the highest intensity scores tend to be the ones with greater weight or importance in the cognitive system. When these constructs express aspects which can be considered as symptomatic, then potential difficulties for change in the therapeutic process may appear. For James, five constructs are the most intense or central to his cognitive system: "coward *vs* fighter", "aggressive *vs* non-aggressive", "unfriendly *vs* friendly", "tires easily *vs* even-tempered" and "nervous *vs* calm". Analyzing their content, most of these core constructs could be considered as symptomatic, reflecting his emotional experience of fear and anxiety, and his perception of threat in others, both in the context of the persecutory delusions. The construct "coward *vs* fighter" is central to the sense of identity of James. Additionally, it does have a very high percentage of polarization (87.50%). Checking his grid raw data matrix (Figure 1), we observe that he considers himself as the only element who is a "coward", while all the others are perceived as "fighters" (as he would like to be). Clinically, this construct might be related to the suffering and permanent sense of fear and alertness that invades his personal life.

To analyze the identity implications of this construct, the correlation matrix among constructs can be used to explore its personal meaning in the context of his cognitive system. In Figure 2, the network of constructs associated with the pole "coward" is represented. All the constructs that are associated with it have negative connotations. The construct pole "coward" is strongly associated, by this order, with the poles "detached" (r =

0.94), "boring" (r = 0.90), "selfish" (r = 0.83), "sad" (r = 0.81), "bad person" (r = 0.79) and "unfriendly" (r = 0.61). Therefore, this highly interrelated meaning configuration articulated around the core construct "coward *vs* fighter" helps us to understand how invalidating it must have been for James to experience intense fears (such as those caused by the perceived threat of others). We may infer here a massive invalidation of his most central aspirations (becoming "fighter", "funny", "altruist", "happy", a "good person", "friendly", and someone who "respects his family"). In PCT, this invalidation of core constructs is linked to intense negative emotions.

A graphic display of the main axes of construction: The GRIDCOR program employs Correspondence Analysis, a multivariate statistical technique similar to principal component analysis, in order to simultaneously compute both constructs and elements expressed in the grid data matrix. It aims to represent the main dimensions of meaning employed by the subject in order to understand his interpersonal world. Each axis or dimension is composed of both elements and constructs with their corresponding loads (which varies across axes).

In the case of James, as mentioned before, the first factor explained 54.11% of variance while the second one accounted for 16.54%. Taken together, these two axes are responsible for 70.66% of the variance in the grid data. The GRIDCOR software yields a graph placing both axes orthogonally, creating a two-dimensional space, which allows us to get an approximate picture of how James perceives himself and others from his main dimensions of meaning (Figure 3). As explained before, each axis represents a dimension of meaning, comprising a particular combination of specific constructs and elements, which are arranged along the axis, being allocated the ones that account for major weight in each axis at the extremes, and around the central area the ones with less weight in that dimension of meaning. In this graph, the final allocation of both constructs and elements results from the combination of the dimensions of meaning of both axes, the first axis represented in the horizontal (abscissa) plane and the second axis in the vertical (ordinate). In this case, for instance, the extremes of the first axe are delimited by the elements "self before" and the "ideal self" or "current partner", whereas the extremes of the second axis are represented by "father" and "self now". The selected constructs and elements that appear in the graph account for the major weight in both axes, and, therefore, it represents the meanings that James gives the most importance to in his view of his interpersonal world, according to grid data. From the distribution of people and meanings in this graph we may derive three different groupings with which James categorizes and interprets his interpersonal world.

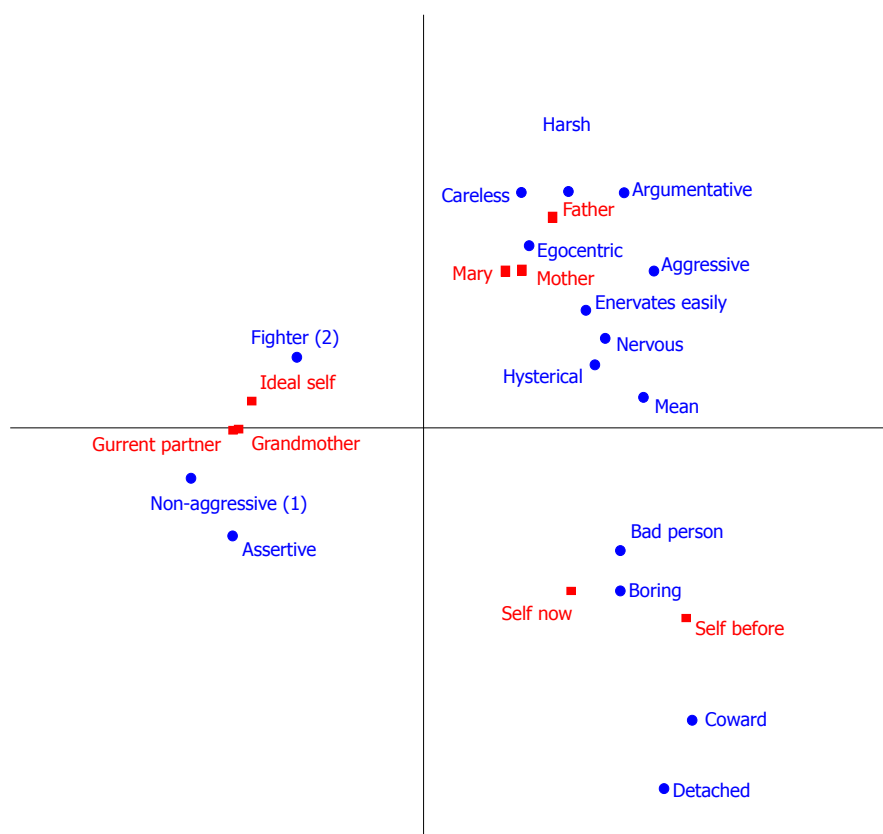James's group, the lonely guy: Me and my "self before the crisis". According to his grid, James perceived

Figure 3 Graph output for the main axes of James. Note: (1) The construct poles "even-tempered", "quiet" and "calm" are also allocated here; (2) The construct poles "happy", "respects family" and "good person" a real so allocated here.

his current and past selves in negative terms ("coward", "detached", and "boring"), different and far from other people, reflecting his experience of self-isolation. Looking at the direct ratings in the grid, the only notable difference is that his current self is now seen as quite a "good person" while the self before the crisis is rated as quite a "bad person". This could be an important aspect to explore in the therapy process to understand the meanings of this change for James.

The group of the good ones: Where James wants to belong. The ideal self of James is situated in an opposite quadrant, with his current partner and his grandmother. Although they do not appear in the graph due to lack of space and lesser variance loading, most of the other elements are located there as well. All the constructs with positive connotations appear there; James would like to be a "fighter", which would imply also being "happy" and "respectful of family", as others are perceived. Another constellation of constructs in this area is related to a desired change in the anxiety of James: He would like to become "even-tempered", "quiet" and "calm", like the others.

The threatening group: The parents and the persecutory figure. In this group we find Mary, his ex-girlfriend, whom he identifies as one of his main persecutors. His parents, with whom he has had many severe conflicts and from whom he feels little support, are there as well. Unexpectedly, these three people are located very close to each other. It may be seen that James gives meaning to them mainly in terms of a constellation of constructs with hostile content ("harsh",

"argumentative", "aggressive", and as people who "tire easily").

DISCUSSION

In this article, the main objective was to illustrate how the RGT can provide clinicians with a systematic portrayal of the personal views of a patient with paranoid psychotic symptoms. This approximation might help in uncovering a patient's personal meanings, and their relationship with symptoms, in order to enhance case formulation and identify therapy targets. We can also focus on the repertory grid indices found for James and contrast them with the current literature about psychosis. His self-definition and self-construction profile denote low self-esteem, with many negative evaluations about the self, which correspond to a set of discrepant constructs ("I am harsh, nervous, messy, sad, boring, a coward..."). Both low self-esteem and negative self-evaluations have been associated with the development and maintenance of positive symptoms[19,20]. More specifically, paranoid delusions have been linked to reflections of specific negative evaluations about the self[21]. Similarly, the high perceived social isolation reflected in James's grid seems to be common in people with early psychosis, along with depressive symptoms[22]. Effectively, depression is common in psychotic patients, and following acute psychosis it may be a psychological response to the apparently uncontrollable life event that psychosis episodes represent for patients[23]. Furthermore, depression can be a contributing factor in

the maintenance of persecutory delusions[24].

A first clinical hypotheses derived from these findings is that the negative self-concept and depressive isolation profile of James play a significant role in his paranoid symptoms. He would benefit from therapy having as a target the enhancement of his self-esteem, reconstructing his discrepant constructs and protecting his congruent constructs from further invalidation, which could be expected to have a positive effect on his paranoid delusions as well as on his depressive symptoms. Another important target would be ways to help James feel integrated with others, which might also have a positive effect on his depressive symptomatology.

At this point some of the results derived from the analysis of the relationships among James's personal constructs must be taken into consideration. First, we have to remember that some of these discrepant and symptomatic constructs for James constitute part of his identity, and being a "coward" and "detached" has many negative implications in his cognitive system. From the perspective of PCT, to the extent that symptomatic constructs define the patient's self-identity we may foresee difficulties for change in therapy. On the other hand, it may be observed that all the positive construct poles are located together, close to the ideal self and far from the self now (which could be expressed like this: "If only I was fighter and respectful with family... everything would change"). For James, the change in one construct implies a change in many others, which renders the objective of change too large and difficult to achieve, as it becomes overly idealized and magnified.

Another feature of James's grid is the low differentiation and high polarization of his construct system. Actually, cognitive rigidity has been associated with delusions[25,26] and with severity of the course of depression[27-29]. Polarization could be considered as a measure of cognitive rigidity as it reflects dichotomous thinking, the "all-or-nothing" style[17]. Also, the high PVAFF of James's grid indicates a tendency to one-dimensional thinking. Therefore, reducing the tendency to making extreme judgments and increasing his cognitive differentiation would be reasonable targets for his therapeutic process, which is one of the lines of the MCT + work, introducing doubt into reasoning[30].

Another issue of the case conceptualization of James is the perceived relationship of his parents and his main persecutory figure. Constructs related to malevolence and hostile content have been associated with the perceived main persecutors in paranoid psychotic patients in a repertory grid study[31]. Within the constructs employed by James, those with a hostile intent reflect both his paranoid thoughts (about his ex-girlfriend) and the bad atmosphere experienced at home. These constructs employed by James might be related with the tradition of Expressed Emotion, conceptualized for the first time by Brown *et al*[32]. We do not have any direct assessment of James's parents but his perception of them is based on hostility and criticism, which has been related with higher severity of positive symptoms[33] and risk for relapse in early psychosis populations[34]. Many studies have demonstrated the efficacy and importance of working with families with high expressed emotion in psychosis[35], so this would be another therapy focus.

We may also focus on the perceived similarity within the hostility constructs for the parents and the persecutory figure. Is there any possible explanation for this phenomenon? From a constructivist perspective, Gara[36] developed a set-theory model of person perception. Following this model, there are some main people in an individual's life, called "supersets", who provide the perceptual categories for the construing of other people, so the personality characteristics attributed to them would probably be first observed in these supersets. Usually supersets are found to be significant people in the subject's life, very often his or her parental figures. Following this line of thought, it would be possible to consider as a clinical hypotheses that James's supersets would be his mother and his father, and that he might be construing his persecutory figure in line with them in terms of hostility constructs, probably developed within the context of many years of conflict at home. This hypothesis reinforces the previous suggestion of including a family intervention in James's therapeutic process. The intervention would focus on increasing the understanding of James disorder for the parents and on easing their supposedly conflictive family interactions. According to this clinical hypothesis, these improvements should facilitate changes in the tendency of James to perceive others in terms of threat and hostility, thereby also changing the structure of these core constructs for his identity and becoming less central. Thus, the intervention would also be expected to have a positive effect on his positive symptoms.

In conclusion, the use of the RGT in exploring the case of James has made it possible to understand how he construes his personal world at such a delicate moment, when his persecutory delusions are so severe. Furthermore, some possible key clinical hypotheses have been constructed with this information, signaling important areas such as self-concept and family relationships, as possible targets for therapy. However, the measures and clinical hypotheses derived from the repertory grid analysis must not be the only ones to consider in the implementation of therapy. RGT furnishes detailed information about the self and personal identity of patients, which is only one factor to consider in case formulation and therapy planning. The RGT is an assessment technique that provides the clinician with relevant systematic information about the personal meanings, self-concept, and cognitive structure of patients, which can also be applied to psychotic patients. This instrument has already demonstrated its utility in case formulation and research in psychology and psychotherapy[37,38], but its clinical and research potential for psychotic disorders has not been sufficiently exploited to date.

COMMENTS

Case characteristics

A 25-year-old man with severe persecutory delusions and hallucinations with threatening content without improvement following antipsychotic medication.

Clinical diagnosis

Psychiatric diagnosis of schizophrenia and personality disorder not otherwise specified. The onset of the disorder was 2 years ago.

Treatment

Olanzapine 20 mg/d and Aripiprazole 400 mg/mo as depo. The case is going to start metacognitive individual training for psychosis.

Experiences and lessons

The authors highlight the possibilities of the repertory grid technique to understand the personal meanings behind the symptoms and to identify targets for psychotherapy.

Peer-review

The article is very good as of its scientific.

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Catatonia: Our current understanding of its diagnosis, treatment and pathophysiology

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Abstract

Catatonia is a psychomotor syndrome that has been reported to occur in more than 10% of patients with acute psychiatric illnesses. Two subtypes of the syndrome have been identified. Catatonia of the retarded type is characterized by immobility, mutism, staring, rigidity, and a host of other clinical signs. Excited catatonia is a less common presentation in which patients develop prolonged periods of psychomotor agitation. Once thought to be a subtype of schizophrenia, catatonia is now recognized to occur with a broad spectrum of medical and psychiatric illnesses, particularly affective disorders. In many cases, the catatonia must be treated before any underlying conditions can be accurately diagnosed. Most patients with the syndrome respond rapidly to low-dose benzodiazepines, but electroconvulsive therapy is occasionally required. Patients with longstanding catatonia or a diagnosis of schizophrenia may be less likely to respond. The pathobiology of catatonia is poorly understood, although abnormalities in gamma-aminobutyric acid and glutamate signaling have been suggested as causative factors. Because catatonia is common, highly treatable, and associated with significant morbidity and mortality if left untreated, physicians should maintain a high level of suspicion for this complex clinical syndrome. Since 1989, we have systematically assessed patients presenting to our psychiatry service with signs of retarded catatonia. In this paper, we present a review of the current literature on catatonia along with findings from the 220 cases we have assessed and treated.

Key words: Catatonia; Schizophrenia; Benzodiazepines; Electroconvulsive therapy; Extrapyramidal disorders

Core tip: Catatonia is a complex clinical syndrome occurring in more than 10% of patients with acute psychiatric illnesses, and it is associated with multiple life-threatening complications. In the last several decades, renewed interest in this syndrome has led to a great deal of research and debate regarding its

diagnosis and treatment. In this paper, we present a review of the current literature on catatonia along with findings from the 220 cases we have assessed and treated since 1989. Catatonia itself is readily treated using low-dose lorazepam, and it also has important implications for how other underlying psychiatric conditions should be treated.

INTRODUCTION

Catatonia is a clinical syndrome characterized by a distinct constellation of psychomotor disturbances. Two subtypes have been described: Retarded and excited. Catatonia of the retarded type is associated with signs reflecting a paucity of movement, including immobility, staring, mutism, rigidity, withdrawal and refusal to eat, along with more bizarre features such as posturing, grimacing, negativism, waxy flexibility, echolalia or echopraxia, stereotypy, verbigeration, and automatic obedience[1-3]. Excited catatonia, on the other hand, is characterized by severe psychomotor agitation[4], potentially leading to life-threatening complications such as hyperthermia, altered consciousness, and autonomic dysfunction. This so-called "malignant" or "lethal" catatonia can be rapidly fatal if not appropriately treated[5,6]. The relative prevalence and diagnostic significance of catatonic signs differ among studies and patient populations, but there is general agreement that catatonia occurs in 9%-17% of patients with acute psychiatric illnesses[1,2,7] and that retarded catatonia is the more frequently observed subtype[4,8-10].

The catatonic syndrome is associated with other disorders, underscoring the necessity of rapid diagnosis and treatment. In particular, catatonia appears to be a risk factor for the development of neuroleptic malignant syndrome[11-13], which has a mortality rate of approximately 10%[14] and may be clinically indistinguishable from malignant catatonia[15,16]. This has important implications for the treatment of catatonia in the context of psychosis, which will be discussed later in this review. Additionally, the immobility and refusal to eat or drink associated with catatonia can give rise to potentially serious medical complications, including dehydration[17], malnutrition[18,19], deep vein thrombosis and pulmonary embolism[20,21], pneumonia and other infections[17], pressure ulcers[19], and muscle contractures[18,19]. The very nature of catatonia can make it challenging, if not impossible, to carry out patient interviews and examinations, thereby interfering with the recognition of underlying diagnoses. These complications of catatonia highlight the importance of recognizing the syndrome

and quickly initiating treatment.

Overall, it is clear that catatonia is a common and serious problem that often remains unrecognized. Despite a renewed interest in the disorder over the last several decades[22], a number of questions remain regarding its causes and treatment. In this paper, we review the current understanding of the diagnosis, treatment, and pathophysiology of catatonia, and we identify several areas of uncertainty where further research is required.

DIAGNOSIS

Clinical features
While catatonia has long been considered a subtype of schizophrenia or a clinical feature of other medical and psychiatric conditions, the earliest descriptions by Kahlbaum *et al*[23] in fact suggested a unique entity with a distinct clinical course. This proposal was not universally accepted, however, and a great deal of debate has ensued regarding the most appropriate classification of catatonia. Largely due to the influence of Emil Kraepelin, catatonia eventually came to be "officially" seen as a type of schizophrenia[24]. Early descriptions of catatonia in both the diagnostic and statistical manual of mental disorders (DSM) and international classification of diseases included it only under the category of schizophrenia, and this view persisted for many years. Things began to change in the 1970's, when multiple reports indicated that catatonia is more closely associated with affective disorders than schizophrenia[4,25]. More recently, it has been proposed that catatonia is also relatively common in patients diagnosed with autism[26]. In 1994, catatonia was recognized in the DSM-IV as a disorder that could either complicate general medical conditions or be a specifier in mood disorders. At the same time, there were continued arguments in support of catatonia being its own distinct diagnostic category[7,27].

A number of different criteria have been proposed for the diagnosis of catatonia. In our own ongoing assessment and treatment of consecutively referred patients with catatonia who present to either our acute-care inpatient psychiatric unit or to the consultation-liaison service, we diagnose patients based on the presence of at least four of the catatonic signs originally described by Karl Kahlbaum in 1874[1]. These signs, along with their frequency in our patient series, are presented in Table 1. As we originally reported in 1990[1], immobility and mutism are the most common signs, each present in over 90% of patients. In keeping with this finding, the diagnostic criteria proposed by Taylor *et al*[27] include immobility and mutism (along with stupor) as core criteria for catatonia. A systematic effort to identify the catatonic signs with the best diagnostic performance was conducted by Peralta *et al*[2]. Immobility and mutism were again identified as the most common signs, observed in 90.6% and 84.4% respectively of catatonic patients. Rigidity was also common in their

Table 1 Frequency of various catatonic signs in our series of 220 consecutive catatonic cases

Sign	% of patients
Immobility	97
Mutism	97
Withdrawal and refusal to eat	91
Staring	87
Negativism	67
Posturing	58
Rigidity	54
Waxy flexibility/catalepsy	27
Stereotypy	25
Echolalia or echopraxia	14
Verbigeration	14

sample, observed in 75.0% of catatonic patients. The use of 4 or more catatonic signs as a diagnostic criterion resulted in 100% specificity, but also led to a small number of catatonic patients failing to be identified. As a result, Peralta *et al*[3] suggest the use of three or more catatonic signs as a diagnostic criterion for catatonia, and this recommendation has been supported by more recent work from the same group[3]. The DSM-V defines catatonia as the presence of three or more of the following: Catalepsy, waxy flexibility, stupor, agitation, mutism, negativism, posturing, mannerisms, stereotypies, grimacing, echolalia, and echopraxia[28]. A number of scales have been developed to quantify catatonic signs[29]. While these scales may prove useful for research, we have not found them to be necessary for clinical purposes.

The most important step in the diagnosis of catatonia is recognition of the syndrome's characteristic clinical signs. Immobility and mutism are particularly common, and the appearance of either of these signs in the absence of another explanatory condition should raise the clinical suspicion of catatonia, at which point the presence of other catatonic signs can be determined. In our experience, patients are often incontinent, disheveled, and cachectic depending on the duration of illness. The lack of meaningful responses to external stimuli in these patients should not be interpreted as a lack of awareness of their surroundings. Indeed, many of the patients we have treated reported being completely aware and were able to recall their catatonic state in detail after they recovered.

Differential diagnosis
A number of neurological conditions may appear similar to catatonia, and may even have substantial overlap with respect to pathophysiological mechanisms. The following is a partial list of conditions that, in our experience, have considerable clinical overlap with catatonia and should be carefully considered.

Extrapyramidal side-effects: Extrapyramidal side-effects are commonly associated with both typical and atypical antipsychotic drugs[30,31], so they are of special concern in patients with psychiatric illness. Like

patients with catatonia, patients with drug-induced parkinsonism may present with immobility, staring, and rigidity. On numerous occasions we have been asked to see a patient with a tentative diagnosis of catatonia who in fact had antipsychotic-induced parkinsonism. This distinction is an important one to make, since the benzodiazepine medication used to treat catatonia may exacerbate the postural instability that is often associated with parkinsonism. One notable difference between the syndromes is that parkinsonian patients are typically cooperative and interactive, in contrast to catatonic patients who are often withdrawn and negativistic. Also, tremor, which is often present in patients with parkinsonism, is not a feature of catatonia. Unusual features like echophenomena and posturing are typically absent in parkinsonism. We have, however, seen parkinsonian patients whose freezing was mistaken for posturing. Additionally, some patients treated with antipsychotic drugs may develop signs consistent with both catatonia and parkinsonism[32]. Other extrapyramidal side-effects may also resemble some aspects of catatonia. For example, the posturing and immobility of catatonic patients can be mistaken for dystonia, while the psychomotor agitation of excited catatonia can appear similar to akathisia. In patients being treated with antipsychotic medication, care must be taken in assessing these clinical features to ensure diagnostic accuracy.

Neuroleptic malignant syndrome: Neuroleptic malignant syndrome is a life-threatening reaction to antipsychotic treatment (including treatment with atypical antipsychotics[33]) in which patients develop rigidity, mutism, and delirium accompanied by diaphoresis, hypertension, tachycardia, and fever[34,35]. Autonomic instability helps to distinguish this syndrome from uncomplicated catatonia, but it may sometimes be indistinguishable from malignant catatonia except for the precipitating factor of antipsychotic treatment. Cessation of antipsychotic medication, along with supportive therapy, is often sufficient to treat these patients, but additional pharmacological treatment or electroconvulsive therapy (ECT) may be indicated.

Nonconvulsive status epilepticus: Nonconvulsive status epilepticus can be clinically indistinguishable from catatonia. In both cases, patients can be immobile, mute, rigid, and unable to eat, drink, or cooperate with an examination. Although electroencephalogram (EEG) findings in nonconvulsive status epilepticus can be highly variable, these investigations are nonetheless crucial to making the correct diagnosis[36,37].

Abulia or akinetic mutism: Disorders of diminished motivation exist on a spectrum including abulia (moderate) and akinetic mutism (severe)[38]. In the extreme case, neurological dysfunction results in a complete lack of spontaneous speech or movement due to a lack of motivation or drive. Patients are fully aware and visual

tracking is preserved. Overt signs of catatonia such as negativism and echophenomena may differentiate the two disorders, but more subtle presentations can make the two conditions difficult to distinguish[39]. In such cases, a trial of lorazepam may be helpful in identifying catatonia.

Locked-in syndrome: Locked-in syndrome is usually associated with ventral pontine lesions, and results in near complete paralysis, while blinking and vertical eye movements are spared[40]. Patients are aware and, unlike catatonic patients, generally eager to communicate through blinking. However, it should be noted that some patients with locked-in syndrome are unable to blink or move their eyes. As with catatonic patients, EEG investigations are often normal. Abnormalities identified using magnetic resonance imaging (MRI) or brainstem evoked potentials help to identify patients with the locked-in syndrome.

Vegetative state: The vegetative state is characterized by a complete lack of awareness of the self or surroundings, often secondary to a severe cerebral injury[41]. The patient makes no voluntary responses to stimuli, and does not visually track objects, but sleep-wake cycles are preserved. Although this definition of the persistent vegetative state is reasonably clear, confidently assessing a lack of awareness can be problematic. EEG and MRI techniques have been used to demonstrate awareness in a disturbing number of patients who otherwise met criteria for a vegetative state[42,43]. Unlike the normal EEG of catatonia, the EEG in vegetative states is almost always abnormal[44].

Stiff person syndrome: Stiff person syndrome is an autoimmune disorder frequently presenting with low back and lower extremity stiffness and spasms, as well as exaggerated lumbar lordosis[45], which can be mistaken for posturing. Like catatonia, the condition can render patients immobile. Episodes are typically triggered when patients are startled or experience emotional stress. In contrast with what is observed in patients with catatonia, patients with stiff person syndrome are not mute and will often indicate that they are in great pain as a result of the muscle spasms. Since most patients are GAD65 antibody seropositive[45], antibody testing may be helpful if there is diagnostic uncertainty. The syndrome generally improves in response to benzodiazepine treatment, perhaps supplemented by adjunctive immunotherapy where appropriate.

INVESTIGATIONS

All patients suspected of having catatonia should have EEG testing as a screen for other neurological conditions. This will typically show epileptiform activity in nonconvulsive status epilepticus and slowing in cases of encephalopathy. The EEG in catatonia is typically normal

unless there is a concurrent condition that may be causing the abnormality[1,9,46]. Given that catatonia can develop in the context of a wide array of neurological conditions, brain imaging, preferably by MRI, is recommended[1,47]. In cases of retarded catatonia, immobility generally allows these investigations to be conducted easily. Laboratory investigations should include a complete blood count, blood urea nitrogen, creatinine, muscle and hepatic enzymes, thyroid function tests, electrolytes, blood glucose, and urinalysis to assess for comorbid conditions, causes, or complications of catatonia. Marked dehydration is not uncommon in catatonic patients, and must be attended to. Vital signs should be assessed frequently, as hypertension and fever (often accompanied by elevated creatine phosphokinase, decreased serum iron, and leukocytosis) may herald the onset of malignant catatonia or neuroleptic malignant syndrome if the patient has received antipsychotic agents[35,48-50]. When possible, a careful review of the patient's recent medications and any changes should be conducted. It is important to determine whether or not a patient has been receiving antipsychotic agents or benzodiazepines, as we have reported, and continue to see, the development of catatonia following abrupt discontinuation of benzodiazepines[51,52].

Unfortunately, the nature of catatonia makes some aspects of a physical and neurological exam impossible. Components of the neurological exam that can usually be assessed include the pupillary reaction, ocular movements, corneal reflex, reaction to pain, the presence of drooling, blink response to threat, reaction to light or sound, frontal release signs, assessment of tone, deep tendon reflexes, and the plantar response.

TREATMENT

A characteristic feature of catatonia is its striking responsiveness to benzodiazepine treatment. We recommend an initial dose of 1-2 mg lorazepam, administered sublingually or intramuscularly. The ability to administer lorazepam intramuscularly is a major advantage, since many catatonic patients refuse to eat or take medication by mouth. A lower lorazepam dose is preferable in patients who are young, elderly, or medically compromised, especially when there is a diagnosis or high likelihood of sleep apnea. If the initial dose is ineffective, it should be repeated in 3 h and again after another 3 h. We have analyzed treatment response in 153 patients treated with lorazepam. In this group, we have observed a response in 132 (85.7%), 90 of whom experienced complete recovery within 3 h. This robust response to low-dose lorazepam has also been reported by others[46,53], but higher doses may be necessary in some cases[54,55]. If a patient responds adequately to benzodiazepine treatment, they should continue on the same dose (provided that this dose is not overly sedating or causing any other problematic side-effects) until treatment of any underlying disorder is underway. Relapse into a catatonic state can occur

Table 2 Rates of response to lorazepam treatment in catatonic patients with various underlying diagnoses

Diagnosis	Patients responding (%)
Bipolar disorder (*n* = 31)	97
Unipolar depression (*n* = 30)	93
Other psychoses (*n* = 24)	92
Medical/neurological condition (*n* = 11)	82
Schizophrenia (*n* = 22)	59

if benzodiazepines are discontinued before this. In our experience, a subset of patients may develop catatonia whenever attempts are made to discontinue lorazepam, and these patients may require long-term maintenance treatment[56]. This phenomenon has also been reported by others[57].

It should be noted that patients with long-standing catatonia may not respond as robustly or as rapidly to benzodiazepine treatment as those with acute catatonia[54,58]. We have reported the cases of two brothers, one of whom had been catatonic for 2 wk prior to treatment, while the other had been hospitalized with catatonia for 5 years[59]. The first brother recovered completely in 2 wk on a lorazepam dose of 3 mg/d. The second brother, on the other hand, showed only gradual improvement on lorazepam 4 mg/d before being discharged from hospital a year after treatment initiation.

An underlying diagnosis of schizophrenia may be associated with a less robust response to benzodiazepine treatment[53]. We have observed a response rate of only 59.1% in patients with schizophrenia, compared with a response rate of over 90% in patients with other psychiatric diagnoses (Table 2). The poorer treatment response in patients with schizophrenia may be related to the chronicity of symptomatology, or it may suggest a distinct underlying pathophysiology, perhaps reflecting the prominence of psychosis affecting their motor behaviour. Nevertheless, benzodiazepines can be effective for treating catatonia in many patients with schizophrenia, and a therapeutic trial is warranted. This is especially the case given the overall safety of benzodiazepine medication.

ECT is another highly effective option for the treatment of catatonia[9,60], and even patients who do not respond to benzodiazepines are likely to respond to ECT[61,62]. Despite its effectiveness, ECT has an important drawback: It requires clear consent. Catatonic patients are unable to discuss ECT or consent to its administration, and consent from a substitute decision maker is often difficult to obtain. Because of these problems, and because benzodiazepines are easily administered and have a high margin of safety, we recommend that benzodiazepines be used as the first line of treatment. ECT should be considered in patients who fail to respond to benzodiazepines after several days and surrogate consent should be sought. The exception to this strategy is the patient with malignant catatonia, for whom ECT should be administered early, since the condition has a high rate of mortality if it is not rapidly and effectively treated[6,49].

Of the catatonic patients we have assessed, 77.7% later reported having experienced psychotic symptoms during the catatonic episode. This raises a difficult problem in treatment, since antipsychotic medications may be associated with an increased risk of neuroleptic malignant syndrome in patients with catatonia. White *et al*[12] identified 17 consecutive patients with neuroleptic malignant syndrome, all of whom exhibited catatonic signs prior to antipsychotic exposure. In our own patients, we have observed that 3.6% of catatonic patients treated with antipsychotic medications developed neuroleptic malignant syndrome[63]. This is in contrast to an incidence of 0.07%-1.8% in all patients treated with antipsychotic drugs[64,65]. Raja *et al*[11] identified 3 cases of neuroleptic malignant syndrome in a series of consecutive patients presenting to the psychiatric emergency service, all 3 of whom demonstrated catatonic signs and low serum iron prior to the onset of neuroleptic malignant syndrome. The relationship between catatonia and neuroleptic malignant syndrome is not limited to patients treated with typical antipsychotics, as clozapine has also been reported to be a precipitating factor[13]. Although more research is required in order to identify which patients are most susceptible to neuroleptic malignant syndrome, we feel that the existing evidence is sufficient to recommend the avoidance of antipsychotic drugs in acutely catatonic patients. In our experience, once catatonic symptoms have been treated by benzodiazepines or ECT and patients are eating, drinking, and walking, antipsychotic treatment can be initiated safely.

Although lorazepam and ECT have long been recognized as effective treatments for patients with catatonia, other options have been suggested. Several case reports have described patients effectively treated with zolpidem[66,67], which, like typical benzodiazepines, may treat catatonia through interactions with GABA-A receptors[68]. As well, amantadine and memantine, which act as NMDA antagonists but also interact with a number of other neurotransmitter systems, have shown efficacy in a small number of patients[69,70]. It is not yet clear whether these options are likely to be helpful in the small fraction of patients who do not respond to either lorazepam or ECT.

PATHOPHYSIOLOGY

While the pathophysiology of catatonia is still unclear, several theories have been proposed based on the available data. One possible interpretation of catatonia is that the syndrome is an outward manifestation of intense anxiety[22,71]. The majority of catatonic patients we have treated reported feeling extremely anxious before and during their catatonic episode, to the extent that some believed they were about to die, had already died, or that they needed to remain immobile

in order to avoid threats from others. Benzodiazepines reduce anxiety by enhancing chloride conductance through GABA-A receptor ion channels, and may treat catatonia through this mechanism. However, a number of our patients - particularly those with schizophrenia - reported little anxiety during their catatonic episode. This observation does not exclude the possibility that anxiety is an important component of catatonia, but suggests that it is not an essential component for all patients with the syndrome.

A second interpretation of catatonia is that it is essentially a movement disorder similar to parkinsonism. As noted previously, the clinical features of catatonia overlap with those of parkinsonism, which is understood to be caused by dysfunction of the basal ganglia. Since most projection neurons in the basal ganglia are GABAergic, it is plausible that benzodiazepines could treat catatonia by influencing GABA signaling in the basal ganglia. Functional imaging studies have shown that catatonia is associated with altered activity in orbitofrontal, prefrontal, parietal, and motor cortical regions[72], suggesting that these cortical structures may also play a role in the pathophysiology of catatonia. This interpretation is reinforced by observations that GABA-A binding is reduced in cortical regions of catatonic patients, motor and affective symptoms are correlated with these abnormalities in GABA-A binding, and cortical abnormalities in catatonic patients are normalized following exposure to lorazepam[72].

Whatever the pathophysiology of catatonia may be, it is clear that a wide variety of underlying disorders can be associated with the emergence of catatonic signs. These include mood disorders, nonaffective psychotic disorders, a number of medical and neurological conditions, and genetic disorders[73]. How - or if - these diverse etiologies converge upon a final common pathway causing catatonia is unknown, and it is possible that variations in the clinical presentation of catatonia represent distinct underlying mechanisms that would respond preferentially to different treatments. For instance, future research may allow physicians to identify patients who are unlikely to respond to lorazepam treatment and should receive ECT or another pharmacological treatment as a first line option.

CONCLUSION

The syndrome of catatonia encompasses a wide range of psychomotor abnormalities, none of which are present in all patients. Immobility and mutism are especially common, and the presence of these signs should prompt physicians to actively assess other markers of catatonia. The differential diagnosis of catatonia is complex, and catatonia itself can arise from a diverse array of psychiatric and medical etiologies, complicating the investigation of these patients. Affective disorders are the most common underlying psychiatric diagnoses. Fortunately, most catatonic patients rapidly respond to low-dose lorazepam. Some patients, particularly those

with long-standing catatonia or schizophrenia, may respond more gradually or not at all to lorazepam, and may require ECT or other pharmacological treatments. We feel that the use of antipsychotics should generally be avoided until the acute catatonic episode has resolved in order to avoid precipitating neuroleptic malignant syndrome. The pathophysiology of catatonia is still poorly understood, and it is unclear whether different constellations of clinical signs might represent distinct underlying mechanisms. Recognizing and treating catatonia usually results in rapid resolution of the syndrome, whereas failing to recognize it may lead to potentially fatal complications including infection, neuroleptic malignant syndrome, and pulmonary embolism. Because of this, physicians should maintain a high level of suspicion for the catatonic syndrome, especially in patients experiencing an acute psychiatric illness.

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REVIEW

Treatment-adherence in bipolar disorder: A patient-centred approach

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Abstract

About half of the patients diagnosed with bipolar disorder (BD) become non-adherent during long-term treatment, a rate largely similar to other chronic illnesses and one that has remained unchanged over the years. Non-adherence in BD is a complex phenomenon determined by a multitude of influences. However, there is considerable uncertainty about the key determinants of non-adherence in BD. Initial research on non-adherence in BD mostly limited itself to examining demographic, clinical and medication-related factors impacting adherence. However, because of inconsistent results and failure of these studies to address the complexities of adherence behaviour, demographic and illness-related factors were alone unable to explain or predict non-adherence in BD. This prompted a shift to a more patient-centred approach of viewing non-adherence. The central element of this approach includes an emphasis on patients' decisions regarding their own treatment based on their personal beliefs, life circumstances and their perceptions of benefits and disadvantages of treatment. Patients' decision-making processes are influenced by the nature of their relationship with clinicians and the health-care system and by people in their immediate environment. The primacy of the patient's perspective on non-adherence is in keeping with the current theoretical models and concordance-based approaches to adherence behaviour in BD. Research over the past two decades has further endorsed the critical role of patients' attitudes and beliefs regarding medications, the importance of a collaborative treatment-alliance, the influence of the family, and the significance of other patient-related factors such as knowledge, stigma, patient satisfaction and access to treatment in determining non-adherence in BD. Though simply moving from an illness-centred to a patient-centred approach is unlikely to solve the problem of non-adherence in BD, such an approach is more likely to lead to a better understanding of non-adherence and more likely to yield effective solutions to tackle this common and distressing problem afflicting patients with BD.

Key words: Non-adherence; Bipolar disorder; Attitudes; Health-beliefs; Treatment-alliance; Familial influences; Knowledge; Stigma

Core tip: Treatment non-adherence in bipolar disorder (BD) is a complex phenomenon determined by a multitude of influences, but its critical determinants are yet to be identified with certainty. Demographic and illness-related factors have not been able to explain or predict non-adherence in BD. On the other hand, patient-centred variables such as attitudes and beliefs regarding medications, treatment-alliance, family attitudes, knowledge, stigma and access to treatment may be the more seminal influences on medication-taking in BD. A move from an illness-centred to a patient-centred approach is more likely to lead to a better understanding and more effective solutions for non-adherence in BD.

INTRODUCTION

To write prescriptions is easy, but to come to an understanding with people is hard. A country doctor: Franz Kafka, 1917.

Bipolar disorder (BD) is a commonly prevalent and enduring condition characterized by recurrent episodes and often followed by residual symptoms. The high rates of comorbidity, suicide and functional impairment in BD also ensure that it is a common cause of disability as well as economic and social burden[1,2]. Pharmacological treatments are efficacious in both acute and long-term treatment of BD in clinical trials of these medications. Nevertheless, the effectiveness of medication treatments, particularly long-term treatment with medications is less impressive in day-to-day practice. Inadequate treatment-adherence is the single most important hurdle in translating efficacy in research settings into effectiveness in clinical practice[3]. In common with other chronic medical conditions with intermittent symptoms and delayed effects of discontinuing treatment, non-adherence is widespread in BD and is associated with several adverse consequences. Apart from undermining the usefulness of treatment and leading to poor outcomes, non-adherence also increases the risk of relapse, re-hospitalization and suicide several folds[2,4]. Non-adherence in individuals with BD leads to greater utilization of health-care services and increased mental health expenditures[5-7]. Finally, the poorer quality of life, stigmatization and functional impairment which accompany non-adherence lead to added burden on the family and society as a whole[8].

HOW COMMON IS NON-ADHERENCE IN BD?

Adherence has been defined as "the extent to which a person's behaviour, taking medication, following a diet, and/or executing lifestyle changes, corresponds with agreed recommendations from a health care provider"[9]. Studies of BD have largely focused on medication non-adherence rather than other aspects of treatment-adherence. Not surprisingly, there is a considerable variation between the rates of such non-adherence ranging from universal adherence in some studies to more or less universal non-adherence in others[10-12]. Much of this variability in rates can be attributed to methodological differences across studies. Adherence has been defined and assessed differently in different studies. Studies also differ in the settings in which they have been conducted (*e.g.,* clinics or community), in their designs (*e.g.,* cross-sectional or longitudinal), in the patient samples included, and in the phase of illness or the duration during which non-adherence has been estimated[13-17]. Extremely high or low rates have usually been obtained from studies with small patient samples and from specialized settings, or from randomized controlled trials of medication efficacy[10-12,18-20]. If these extremes are ignored most studies report non-adherence rates from 20% to 50%, with a few reporting rates from 60% to 70% for all patients with BD[21-23]. This is very similar to the estimates of several reviews on the subject, which conclude that on the average about 40% to 50% of patients with BD (range 9%-66%) do not take their medications regularly[2,4,24-28]. These rates are essentially the same regardless of whether patients are on mood stabilizers or antipsychotics. Moreover, rates of non-adherence seem to have remained unchanged or even increased over the years despite the introduction of several new medications[1,4,13,26,29]. The fact that about half of patients with BD become non-adherent during long-term treatment puts it on par with several other chronic psychiatric and medical disorders[4,9,30-32]. Moreover, a large part of this commonly prevalent non-adherence remains undetected in real-world treatment of BD as well; clinicians appear to be particularly likely to underestimate non-adherence among their patients[17,33-36]. However, the blame for lack of detection in day-to-day practice cannot be entirely laid at the clinician's door, because it is notoriously difficult to predict who is likely become non-adherent. Non-adherence is obviously a complex phenomenon determined by a multitude of influences. However, despite best efforts it is still unclear as to which of these factors is most critical in determining non-adherence in BD[4,13,26].

DEMOGRAPHIC AND ILLNESS-RELATED DETERMINANTS OF NON-ADHERENCE IN BD

In a seminal article about 40 years ago Jamison *et al*[37] proposed four mutually interacting domains to explain non-adherence to prescribed lithium among patients with BD. These included factors related to

the patient (*e.g.*, demographic characteristics), the illness (*e.g.*, severity), the effect of medications (*e.g.*, side effects) and characteristics of the clinicians (*e.g.*, relationship with patients). These determinants have been subsequently adopted by others working in the field, but some significant additions have been made in each category. For example, patient-related factors have come to include personal attitudes and beliefs about medication-taking in addition to demographic factors. Clinician-related factors has been broadened to include an environmental domain, which encompasses the influence of clinician-patient interactions and health-system related factors on adherence, as well as the influence of the family and significant others in the patient's life[13,25,28,38,39].

In the 1980s and 1990s research on treatment non-adherence amongst those diagnosed with psychiatric disorders including BD mostly limited itself to examining demographic, clinical and medication-related factors impacting adherence[2,17,38,40]. The exclusive focus on these factors appeared to be driven by biologically and medically orientated conceptualizations of the illness, although the primacy of the biological approach had been the object of criticism for long. Unfortunately, these efforts to predict non-adherence on the basis of demographic and illness-related factors were not very successful[40-44]. This failed quest could be due to several reasons, the principal one being the equivocal and inconsistent results obtained from different studies[40,45,46]. For example, although certain demographic correlates have emerged as likely determinants in some studies[4,26,27,42], several other studies have found no association between demographic variables and non-adherence in BD[17,47-51]. Among individual demographic attributes there has been some evidence for an association of non-adherence in BD with younger age[13,27,39,52,53], minority ethnicity[7,54-58], and social disadvantage[27,34,52,56,57,59], but the evidence for such links is either limited or often contradictory[2,4,34,38,48,49,60]. The role of clinical factors in determining non-adherence in BD seems to have been similarly inconsistent and ambiguous[42,61]. Overall severity of the illness in terms of symptom-burden, greater number of episodes and prior hospitalizations appeared to influence non-adherence in some[25,27,28,35,62], but not all studies[2,16,47,51,59]. A majority of studies among patients with BD found that poor insight and denial of the illness was associated with non-adherence[4,24,26,38,63-65]. However, though it might be difficult for a patient to be adherent without a basic level of insight, simply having insight may not be sufficient to ensure adherence[1,16,36,65,66]. The presence of comorbid disorders, particularly substance use disorders has also emerged as a consistent correlate of non-adherence in BD[26,28,39,42,46], but the evidence for associations with most other clinical variables has been either scarce or inconsistently replicated[35,62,67-70]. Among medication-related factors the role of the efficacy-tolerability balance in determining adherence has been the focus of most

studies in BD. A large number of studies have found that treatment side effects negatively influenced adherence in BD[7,35,46,59,70], though many of these have exclusively investigated the side effects of lithium[34,67,71-74]. On the other hand, an almost equally large number of studies and patient surveys have revealed that side effects are not associated with non-adherence in BD[2,4,26,27,39,75]. It appears that fear or concerns regarding side effects rather than their actual prevalence may be more important in determining non-adherence in BD[17,26,41,45,76,77]. The influence of treatment-efficacy on adherence has been examined less often, though some studies suggest that medications alleviating depressive symptoms are more likely to promote adherence[7,51,78-80].

The uncertain influence of clinical and demographic factors on adherence in BD could also be due to the fact that many of the studies examining this association have failed to take into account complex interactions between several such variables, which could eventually determine the risk of non-adherence in BD[28]. For example, the higher prevalence of non-adherence during manic episodes could well be due to a lack of insight or the presence of cognitive impairment during such episodes[1,4,41]. Similarly, the greater risk of non-adherence in men could be related to the more frequent use of substances among them[81,82]. Additionally, the simple and dichotomous categorizations of demographic, clinical and treatment-related factors adopted by these studies ignored the subjective experience of medication-taking and the importance of factors such as attitudes and health-beliefs, which might underlie the link between demographic and clinical factors and non-adherence[28,81,83]. Accordingly, there is ample evidence to suggest that variables such as age, gender, severity of illness, effects of substance use, side effects and other treatment-related factors may impact adherence through their effects on subjective patient experiences such as their attitudes to medications, their knowledge of the illness and the availability of social support[2,19,51,84-88]. Moreover, while certain demographic and clinical variables such as young age, symptom-severity, substance use comorbidity and lack of insight may be useful in delineating groups at high risk for non-adherence, they do not accurately predict non-adherence at the level of the individual patient[11,40,41,45]. Finally, because many of these factors may not be amenable to change they do not provide opportunities for adherence enhancement through targeted psychosocial interventions[48,49]. These limitations of attempting to predict and target non-adherence based on demographic and illness-related factors indicates the need for an alternative perspective on treatment-adherence. The newer perspective lays greater emphasis on the patient's point of view of medication-taking, while acknowledging that problems with adherence are likely to be determined by complex interactions between the patient, the illness, its treatment and the wider socio-cultural environment in which such treatment takes

place[11,27,41,61].

A PARADIGM SHIFT TO A PATIENT-CENTRED APPROACH TO ADHERENCE IN BD

As with other chronic medical conditions, research on predictors of non-adherence in BD over the last two decades has undergone a gradual shift in thinking from an illness-centred to a patient-centred approach[40,89]. In this patient-centred paradigm, adherence is viewed as a dynamic rather than a static process which is influenced by many factors within and outside the patient[2,13,26,42,43]. At the core of this process lie the patients' abilities to make decisions about their own treatment[11,27,90]. Patients are the "final decision-makers" who have a right to choose whether or not continue treatment based on their own beliefs, personal circumstances and their perceptions of benefits and disadvantages of treatment. This right of patients to have a say in their treatment is acknowledged and prioritized in the patient-centred approach to medication-taking[2,7,27,32,50,90]. The emphasis on the patient's decision-making prompts a shift in the patient-clinician relationship to one in which both are equal and active collaborators. This approach is in keeping with the move away from earlier compliance-based models to those that place emphasis on concordant relationships between patients and clinicians[1,4,32,50,89,90]. While compliance-based paradigms treated patients as passive recipients of treatments and ignored the centrality of their viewpoints, the currently prevalent adherence- and concordance-based approaches place greater stress on the need for an agreement on decisions regarding treatment between patients and clinicians. The cornerstone of the concordance approach rests on open discussions of mutual views about taking medications, and a shared decision-making alliance between patients and clinicians while retaining the primacy of patients' choices. This shift in paradigms has been further propelled by the formulation of a number of health-behaviour models, which have been used to explain non-adherence in BD with a certain degree of success[11,41,45,76,90,91]. Though consisting of divergent social, cognitive and behavioural perspectives on adherence, they give central importance to the very same elements such as patients' attitude and health-beliefs, the treatment-alliance and factors in patients' immediate environments influencing adherence. Finally, the move from illness-related determinants to patients' perspectives on adherence has aided the development of several adjunctive psychosocial interventions to enhance medication-adherence in BD. These treatments, which use the framework of a collaborative alliance with patients and families to address non-adherence through educative, cognitive and behavioural means have had some success in optimizing adherence in BD[2,4,26,28,38,61].

PATIENT-CENTRED DETERMINANTS OF NON-ADHERENCE IN BD

Attitudes and beliefs regarding medications

In the research on adherence a distinction is often made between unintentional and intentional non-adherence. Unintentional non-adherence arises from personal or environmental restrictions which hamper medication-taking, while intentional non-adherence arises from patients' views on medications which affect their willingness to take them[92,93]. In an influential study, Horne and Weinman[94] categorized patients' beliefs about medications into general beliefs related to the intrinsic nature of medicines and ways in which medicines are used by doctors, and specific beliefs comprised of the perceived necessity of taking medications coupled with concerns about their adverse consequences. Both general health-beliefs and specific beliefs (attitudes) regarding medications have been examined among patients with BD. Barring a few exceptions[10,27,74,80,95], the majority of such studies of patients with BD have found that both health-beliefs and attitudes to medications are associated with non-adherence in BD[2,4,13,26,28]. Some of the studies have actually concluded that adverse attitudes and health-beliefs among patients have a much greater influence on non-adherence than demographic, illness or treatment-related factors such as side effects[17,26,45,51,76]. In other studies attitudinal factors such as doubts about the need for medications, as well as concerns about their adverse effects have been found to account for a substantial proportion of variance in intentional non-adherence[11,19,37,41,87,96]. All kinds of negative attitudes have been found in these studies though the commonest ones appear to be fear of side effects and harm caused by medications[17,19,37,51,75,97], denial of severity of illness and the need for treatment[11,41,46,86,98,99], the negative impact of long-term medications on daily routines and competing priorities of life[37,85,86,98,100,101], perceived ineffectiveness of medications[19,51,69,46,98,102], fears regarding dependence, being controlled or stigmatized by taking medications[35,37,60,75,103], and missing the pleasure experienced during manic episodes because of the mood-controlling effects of medications[37,44,101,104,105]. On the other hand, some patients perceive medications to be helpful and seem to realize the necessity of taking medications to prevent relapses, hospitalizations and other negative consequences[11,69,79,98,106,107]. Attitudes towards medications among patients might be relatively independent of their demographic and clinical characteristics[50,51,108,109], or they might differ according to age and illness-related factors such as the severity of the illness and its course, comorbid substance use and side effects of medications[85,100,110-113]. Additionally, patients' attitudes are more likely to be influenced by their knowledge of the illness, attitudes among their family members and ethno-cultural groups, the clinician-patient relationship and the overall quality of life among patients[35,60,84,101,103,114]. However, regardless of the

substantial evidence in favour of attitudes and health-beliefs influencing adherence behaviour in BD, the number of studies is relatively small. Moreover, because of the cross-sectional designs and the small numbers of patients in most studies it is not possible to make any inferences about causality[41,86].

Treatment alliance

Apart from attitudes and health-beliefs the other principal influence on non-adherence in BD is the treatment-alliance between the doctors or clinicians and patients. The concept of the treatment-alliance as a collaborative and affective bond between clinicians and patients has its origin in psychoanalytic and psychotherapeutic literature[115,116]. In keeping with the research-evidence on treatment-alliance in psychotherapy and other psychiatric disorders such as schizophrenia[115-117], an effective alliance appears to have a significant influence on treatment-adherence in BD as well. Though research on the influence of treatment-alliance on adherence is relatively scarce, the more or less unequivocal finding from several studies is that a strong therapeutic alliance is associated with improved adherence among patients with BD[18,58,91,114,118-120]. A strong alliance appears to enhance treatment-adherence in BD in several ways such as fostering more positive attitudes to treatment and enhancing the acceptance of treatment among patients[1,2,13,14,26,78]. The importance of a genuinely collaborative alliance in determining adherence is also in accord with the current emphasis on the active involvement of patients in decision-making and concordance-based approaches to understanding adherence in BD[1,13,52,78,121]. Moreover, a patient-centred approach and a collaborative clinician-patient alliance appear to be essential ingredients of all psychosocial interventions designed to enhance adherence in BD[1,7]. Definitions of therapeutic alliance in psychotherapy have three common elements including the collaborative nature of the relationship, the affective bond between patients and therapists, and the patient's and therapist's ability to agree on treatment goals and tasks[115]. Research among patients with BD indicates that the very same components of treatment-alliance are intimately related to adherence behaviour. Forging a successful treatment-alliance in BD begins with a two-way, reciprocal communication between the patient and the clinician[18]. There are a number of studies among patients with BD showing that not only do clinicians tend to overestimate the degree of adherence among patients, but there is also considerable discrepancy between clinicians and their patients regarding the reasons for non-adherence as well as the meaning of non-adherence[3,24,43,76,96]. Clinicians might also fail to acknowledge the patient's concern or distress about long-term treatment. Therefore, clinicians first need to create an atmosphere in which patients are able to freely discuss their problems about taking medications. Clinicians also need to play an active role in attempting to understand the patient's views on illness and medi-cation-taking. To further this open and active stance clinicians must not only listen more effectively, but also learn to value this communication with patients in order to forge effective links with them[18,26,107,122]. Empathy, compassion and skilful counselling are much valued by patients and positively associated with adherence in BD[120,123]. This bi-directional communication also forms the vehicle for imparting information about the illness and its treatment since patients frequently express the need for such information[75,106,122]. Moreover, information can be used to effectively dispel incorrect beliefs about medications, reduce feelings of stigma and foster positive attitudes to treatment among patients[50,114]. The other necessary component of an effective treatment-alliance is a genuinely collaborative relationship between the patients and clinicians. This collaborative relationship is built on respect for patients' rights to decide about their own treatment and a shared decision-making process with patients and clinicians as active and equal partners[8,18,121]. Evidence suggests that patients place substantial emphasis on this interactional component of the treatment-alliance and that the degree of agreement between clinicians and patients on decisions regarding treatment is a high priority for patients[18,122]. Adherence is also enhanced when both patients and clinicians agree on their roles and responsibilities within the alliance. Patients also want this interactional relationship to be flexible and responsive to changes in clinical status and their treatment needs[18]. Feelings of trust also help in building a strong treatment-alliance as studies have found that trust in medication, trust in the clinician and absence of negative treatment expectations are all associated with adherence in BD[85]. Other elements of importance are regular contacts and reviews by clinicians, ongoing support and the long-term stability of the clinician-patient relationship[49,58,106,114,124]. Finally, factors such as attitudes of patients about the illness and its treatment, their perception of the clinician, ethnic and cultural backgrounds of patients, expectations of patients and the extent to which these have been met, and personality attributes such as locus of control have emerged as some of the more significant influences on the treatment-alliance[60,86,114,118,125,126].

Knowledge about the illness and its treatment

Another potential determinant of adherence in BD is the knowledge about the illness and its treatment among patients. Lack of such knowledge is widespread and a prevailing source of dissatisfaction among patients with BD[75,101,106,113,127,128]. Patients appear particularly unhappy with the lack of information provided on side effects and other aspects related to medication-treatment[75,86,127-129]. Although enhancing knowledge should improve adherence among patients, results of studies in BD have been somewhat equivocal in this regard. While several studies have found that inadequate knowledge of the illness among patients appears to affect treatment-adherence[19,75,80,95,102,128], quite a few others have concluded that patients' level of knowledge is not

associated with adherence in BD[10,34,51,84,118,130]. Similarly, psychosocial interventions imparting information in an effort to reduce non-adherence have met with mixed success[38,90,109,126,131,132]. There could be several reasons for these unexpected results including factors such as old age and longer duration of treatment, which influence the levels of knowledge and may act as potential confounders[10,19,84,86,130,133]. Insufficient knowledge could be due to insufficient efforts and ineffective means of imparting information by clinicians, as well as cognitive impairments, lack of insight and motivation among patients[127,128,134]. Patient's perceptions about their need for information and the extent to which these are met also have some bearing on adherence. Provision of information is more likely to be effective only if it is tailored to the specific needs of patients[128].

The role of the family and significant others

Families influence patients' medication-adherence in several ways. A disorganized or dysfunctional family environment has been associated with higher prevalence of non-adherence in BD[101,135,136]. A disturbed family atmosphere often leads to non-adherence by reducing the social support available to the patient[108,137]. Such an outcome would be more likely among patients who are more dependent on family members. This probably explains why a number of studies have found that patients with a greater external locus of control are more likely to become non-adherent[50,83,95,126]. Perceived criticism, negative affective responses and stigmatization or rejection within the family are also associated with non-adherence among patients with BD[75,138]. Finally, attitudes and health-beliefs of family members and their knowledge of the illness and treatment have been shown to have a significant influence of the patient's own beliefs and attitudes. Accordingly, negative attitudes and improper understanding of the illness among family members may affect the patient's decision whether or not to continue treatment[2,4,75,91,101,138].

Stigma, patient satisfaction and system-related factors

For many people with BD the stigma of having a chronic mental illness and needing to take long-term treatment for it may deter adherence. Consequently, studies among patients with BD have found their perceived sense of stigma to be associated with non-adherence[75,100,135,139]. Feelings of stigma regarding BD and its treatment also appear to promote negative attitudes towards treatment and adversely affect the treatment-alliance[114]. Patient satisfaction with various aspects of treatment has been found to be a determinant of non-adherence in BD. Dissatisfaction with the efficacy of treatment, with lack of information, with clinicians and with the treatment-alliance have all been found to adversely affect adherence in BD[59,80,106]. On the other hand, patients who are contended with their own lives are more likely to adhere with treatment[28,103,140]. Access to treatment and affordability of treatment also constitute significant barriers to continued adherence in BD. Bhugra et al[141] have suggested that only about half of the patients receive appropriate treatment BD because of systemic barriers to gaining access to appropriate care. This has been endorsed by the results of a number of other studies[38,49,80,101,119]. Finally, further elaboration of the concepts of shared decision-making, personal recovery and integrated or collaborative care are being increasingly applied to understand treatment-adherence in chronic medical disorders[142]. However, as of now these concepts have been only sparingly used to explain treatment-adherence in psychiatric disorders such as schizophrenia and depression. They have not yet been widely applied to BD. A discussion of the importance of these factors is thus beyond the scope of this brief review.

CONCLUSION

This brief examination of the literature on treatment-adherence in BD clearly suggests that research in this area is in the process of making a decisive shift towards the patient's perspective on non-adherence. Given the failure of demographic, illness and treatment-related factors to explain and predict non-adherence in BD, greater emphasis on factors such patients' attitudes and health-beliefs, the clinician-patient relationship and the impact of the immediate environment on treatment-adherence, certainly appears to be more appropriate. Moreover, such a stance is congruent with the current theoretical perspectives of adherence and concordance-based models of adherence. However, non-adherence continues to be rife in BD and simply adopting a patient-centred approach is unlikely to be a panacea for the problem. Nevertheless, it seems to be the only way forward for both research and clinical practice. Research focusing on non-adherence from the viewpoint of patients is more likely to lead to a greater understanding of this complex phenomenon in BD. A patient-centred approach also encourages clinicians to improve their understanding of the critical elements of adherence behaviour, to enhance their sensitivity to their patients' needs and to develop a collaborative and trusting relationship with them while attempting to tackle the problem of non-adherence. Thus, while there is no looking back to an earlier era of considering non-adherence to be only a patient's problem, we can only hope that adopting a patient-centred approach will lead us to find effective solutions to this common and distressing problem of non-adherence in BD.

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Basic Study

Reasoning and Rehabilitation cognitive skills programme for mentally disordered offenders: Predictors of outcome

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Abstract

AIM

To investigate factors predicting treatment completion and treatment outcome of the Reasoning and Rehabilitation Mental Health Programme (R&R2MHP) cognitive skills programme for mentally disordered offenders.

METHODS

Secondary analysis of data previously obtained from 97 male patients who were sectioned and detained under the United Kingdom Mental Health Act in low, medium and high security hospitals and who had completed R&R2MHP. Predictors of treatment completion included background variables and five outcome measures: Four self-reported measures of violent attitudes, social problem-solving skills, reactive anger and locus of control and an objective measure of behaviour on the

ward that was completed by staff. Completion of the 16 session programme, which was delivered on a weekly basis, was classified as ≥ 12 sessions.

RESULTS

It was found that the R&R2MHP is appropriate for delivery to participants of different ages, ethnic background, and at different levels of security without the completion rate or treatment effectiveness being compromised. Participants taking oral typical psychotropic medication were over seven times more likely to complete the programme than other participants. Behavioural disturbance on the ward prior to commencing the programme predicted non-completion (medium effect size). As far as treatment completion was concerned, none of the background factors predicted treatment effectiveness (age, ethnic background, level of security, number of previous convictions and number of previous hospital admissions). The best predictor of treatment effectiveness was attitude towards violence suggesting that this should be the primary outcome measure in future research evaluating outcomes of the R&R2MHP cognitive skills program.

CONCLUSION

The findings suggest that a stable mental state is a key factor that predicts treatment completion.

Key words: Treatment; Completion; Outcomes; Mentally disordered offenders; Reasoning and Rehabilitation Mental Health Programme; Cognitive skills program

Core tip: This study adds important new information to understanding factors that predict treatment completion of the Reasoning and Rehabilitation Mental Health Programme cognitive skills programme for mentally disordered offenders. Out of 97 male patients, 76 (78.4%) completed the programme. There were two factors that predicted treatment completion, low level of behavioural disturbance on the ward prior to treatment commencing, and most importantly patients currently being on oral typical psychotropic medication, which increased over seven times the likelihood that they would compete the programme. The findings suggest that a stable mental state is a key factor that predicts treatment completion.

INTRODUCTION

An increasing number of mentally disordered offenders (MDOs), who have severe mental illness, are at far greater risk of committing violent offences and within these populations of MDOs recidivism is high. In the United Kingdom, within 5 years of release 15% of MDOs re-offend, 3% of whom commit serious violent offences[1]. In a large longitudinal cohort study of 47326 Swedish prisoners, Chang *et al*[2] reported that up to 20% of violent reoffending in men and 40% in women was attributable to the diagnosed psychiatric disorders.

There are well-recognised predicators of recidivism in MDOs, with examples including beliefs and attitudes supporting a criminal lifestyle and poor social problem-solving skills[3-4]. Consequently, there is a rising demand for evidence-based treatments designed to minimise antisocial behaviour in MDOs and manualised programmes have been developed in an attempt to reduce the rates of offending through cognitive skills training[5]. The most widely applied programme is the 36 session "Reasoning and Rehabilitation" (R&R) programme, which employs a cognitive-behavioural paradigm and is accredited for use by the correctional services[5]. R&R aims to encourage self-control, meta-cognition, social skills, interpersonal cognitive problem-solving skills, creative thinking, critical reasoning, social perspective-taking, values enhancement, emotional management and helper therapy[6]. While R&R has previously been shown to be effective in reducing recidivism rates in young offenders and juveniles, completion rates may be poor[7,8].

In an attempt to be more responsive to the needs of offenders, Young *et al*[9] developed a revised version of the original R&R, referred to as "Reasoning and Rehabilitation Mental Health Programme" (R&R2MHP), which specifically focuses on helping offenders with serious mental health problems (*e.g.,* psychosis) and has substantially fewer sessions (*i.e.,* 16 *vs* 36). While the original R&R had been shown to be effective in reducing offending in both institutional and community settings with moderate effect sizes[10], it was not considered sufficient to meet all the needs of special offender groups, including those with mental disorders[9].

In their multi-site controlled trial using the R&R2MHP, Rees-Jones *et al*[11] found that 52 out of 67 (78%) of participants completed the programme (*i.e.,* completing 80% or more of the sessions). C-Y Yip *et al*[12] found a completion rate of 80% in a maximum secure unit setting. A completion rate of 92% was found among patients with intellectual disability[13]. These studies have reported a number of positive outcomes relating to attitudes towards violence, social-problem solving skills, reactive anger, locus of control and behaviour on the ward.

In spite of the high completion rate of the R&R2MHP, it is nevertheless important to identify factors that may either facilitate or hinder successful completion of the programme. Young *et al*[14] found that most non-completers were from maximum security, suggesting that the institution's level of security is a possible factor. No other predictors of non-compliance were examined

in this study. Rees-Jones *et al*[11] found that there were no significant differences between the completers and non-completers in age, previous convictions, previous admissions, and motivation to engage in treatment. The only difference was that non-completers had significantly better problem-solving skills at baseline than the completers (Cohen's d = -0.65, medium effect size), which seems counter-intuitive. C-Y Yip *et al*[12] found no significant difference between completers and non-completers in age, number of previous convictions or admissions, but the non-completers were rated by informants to be significantly more behaviourally disordered (Cohen's d = -0.88, large effect size) as measured by the Disruptive Behaviour and Social Problem Scale (DBSP)[15]. This provides strong evidence that participants who are rated by nursing staff as behaviourally disturbed prior to the commencement of the R&R2MHP are more likely than other participants to not complete the programme.

The purpose of the current study is to combine data from the Rees-Jones *et al*[11] and C-Y Yip *et al*[12] studies, which include all three levels of security (low, medium, maximum), in order to answer the following research questions: (1) what factors predict treatment completion/non-completion; and (2) what factors predict treatment outcome among those completing the programme.

The variables we investigated in the current study include the age of the participant, ethnic background ("white" *vs* "other"), level of security (*i.e.*, low, medium and high), number of previous convictions, number of previous hospital admissions, medication status, and the scores on typical psychometric outcome measures at baseline (*i.e.*, prior to the commencement of the programme) relating to attitudes towards violence, social-problem solving skills, reactive anger, locus of control and behaviour on the ward. Of particular interest is type and form of administration of the psychotropic medication the patient is prescribed at the time of the programme, because deteriorating mental state is an important factor that leads to non-completion[16]. The distinction drawn in this study is between the "First" and "Second" generation antipsychotic drugs and whether they are prescribed orally or by a depot injection. We also analysed differences between completers and non-completers in the outcome measures at baseline.

MATERIALS AND METHODS

Participants
Participants were a mixed sample of 97 males who were sectioned and detained under the United Kingdom Mental Health Act (1983) in either a low, medium or high secure hospital setting [*n* = 25 (25.8%), 42 (43.3%), 30 (30.9%) respectively] in 13 secure forensic facilities across the United Kingdom (three low secure, nine medium secure and one high secure). These settings differ in their staffing arrangements

and physical security measures. Patients are stratified based on whether they present a serious danger to themselves and others and have potential to abscond, hence reside within a graded care system relative to their individual needs.

All patients participated in the treatment condition (R&R2MHP) and inclusion criteria included an age range of 19-63, history of severe mental illness (*e.g.*, schizophrenia, schizoaffective disorder, bipolar disorder), no previous experience of participating with R&R2, and proficiency in the English language. Exclusion criteria included intellectual disability, patients who were mentally unstable (*e.g.*, experiencing serious current psychotic symptoms), and/or who posed a risk of violence to the researcher.

Intervention
R&R2MHP[9] is a structured, manualised CBT programme comprised of sixteen 90-min sessions, delivered on weekly basis, and developed for antisocial youths and adults with mental health problems. The programme is a revised version of the original 36 session Reasoning and Rehabilitation programme, initially developed for use in correctional facilities[5]. The aim of the programme is to reduce anti-social behaviour and attitudes and improve pro-social thinking, emotional and behavioural control and problem-solving skills. R&R2MHP consists of five treatment modules: (1) a neurocognitive model which introduces techniques to increase attention control, impulse control, memory, and constructive planning; (2) a problem-solving module which encourages problem identification, generation of multiple alternative solutions, and consequential thinking; (3) an emotional control module which involves management of anxiety, anger, and conflict; (4) a social skills module which aims to increase awareness of the thoughts and feelings of others; and (5) a critical reasoning module which aims to develop skills in the assessment and evaluation of information, *e.g.,* evaluating options and effective behavioural skills. The programme offers a novel approach by allowing participants to engage in both individual and group therapy, with the latter being achieved by the inclusion of a mentoring paradigm whereby a member of staff meets with the patient between group sessions to assist the participant to transfer skills learned in the group into their daily lives. Mentors receive written guidance about how to structure each mentoring session and received training and on-site supervision from programme facilitators. As a structured, manualised programme, R&R2MHP fosters consistency in delivery and programme integrity. A steering committee, attended by site principal investigators and clinical staff, met regularly to maintain a consistent approach to research and treatment.

Treatment completion
A cut-off of ≥ 12 sessions was used to classify patients as completers, in line with the methodology

and recommendation provided by Cullen *et al*[7] thus representing at least 80% attendance of the programme. Hence, non-completers were classified as those attending < 12 sessions.

Baseline assessments

Demographic data (*e.g.,* age, and ethnic background), psychiatric diagnosis, medication status, and index offence information were obtained from clinical file review at the beginning of the study. Medication status at the time of study was categorised into the following groups according to the type of medication and method of delivery (*i.e.,* oral *vs* depot injection): (1) currently on oral typical psychotropic medication; (2) currently on oral atypical psychotropic medication; (3) currently on depot typical psychotropic medication; (4) currently on depot atypical psychotropic medication; (5) currently on antidepressant psychotropic medication; and (6) currently on mood stabilisers psychotropic medication.

The "typical" psychotropic medication' category included: Haloperidol, Thioridazine, Thiothixene, Fluphenazine, Trifluoperazine, Perphenazine, Molindone, Loxapine and Prochlorperazine.

The "atypical" psychotropic medication category included: Risperidone, Olanzapine, Quetiapine, Clozapine, Ziprasidone, and Aripiprazole.

Outcome measures

The following outcome measures were administered at baseline (Time 1) and repeated at post group (Time 2) to assess the violent attitudes and social problem-solving skills, reaction to provocation (anger), and disruptive behaviour and social functioning. All measures are self-reported with the exception of the DBSP which is rated by an informant.

Maudsley Violence Questionnaire[17,18]: Is a 56-item true/false questionnaire with a score range of 0-56. The Maudsley Violence Questionnaire (MVQ) measures cognitive style in relation to violence attitudes and is designed for use across a spectrum of violent offenders and non-violent individuals. Following factor analysis the 56 items can be stratified into two factors: *Machismo* - endorsing stereotypical expectations of men as strong and tough (42 items based on this factor) and *Acceptance* - accepting and enjoying violent behaviour (14 items based on this factor). The MVQ has high internal consistency (Cronbach's α ranges from 0.76 to 0.91) and validity[17].

Social Problem-Solving Inventory-Revised Short[19]: Is a 25-item questionnaire with a 5-point Likert-type response format. The Inventory is comprised of five subscales: Two of which measure problem-solving orientation (positive and negative problem orientation) whilst the remaining three assess problem-solving style (rational problem-solving, impulsivity/carelessness, and avoidance) (scores range between 0 and 20 for each

domain). An adjusted total score was obtained (score range = 0-20) with higher scores reflecting better problem-solving ability. The measure is reported to have high test-retest reliability (0.68-0.91) and internal consistency (Cronbach's α ranged from 0.69 to 0.95).

The Novaco Anger Scale and Provocation Inventory: Reaction to Provocation/Personal Affect Questionnaire[20]: Was used to assess cognitive, arousal, and behavioural domains of anger experience. Forty-eight items, each rated on a 3-point Likert-type format scale, provide these domains with higher scores indicating higher anger levels (score range between 16 and 48 for each domain); a total score can also be obtained by summing the domain scores (score range from 48-144). The Reaction to Provocation/Personal Affect Questionnaire (NAS-PI) has been shown to have good reliability (test-retest coefficients ranged from 0.78 to 0.91) and internal consistency of 0.92[21,22].

The Locus of Control Scale[23]: Was used to assess the extent to which participants believe events to be internally or externally controlled. The Locus of Control Scale (LoC) is a 40-item yes/no questionnaire with a high score indicating that the person perceives events as externally controlled, whereas a low score indicates that a person believes they control events internally (score range from 0-40). The scale has been found to have varied level of internal consistency, ranging from 0.37 to 0.86[24].

The Disruptive Behaviour and Social Problem Scale[15]: Is an informant-rated questionnaire consisting of 14 statements rated on a 7-point Likert-type format scale relating to a person's behaviour and social interactions over the past month (score range of 14-98) in their current environment (*i.e.*, in this study, this was completed by a member of the healthcare staff who knew the patient well and rated their behaviour on the ward). The scale consists of two factors: (1) disruptive behaviour, for example, whether the participant is difficult to manage; if they are verbally aggressive or attention seeking (score range 8-56); and (2) social and psychological functioning, for example, insight into behaviour, feelings of guilt, and positive social interactions with others (score range of 6-42). Higher scores indicate a greater degree of problems. Both factors have good internal consistency in male offenders (Cronbach's α 0.92 and 0.84, respectively).

Procedure

We combined the existing data bases from the Rees-Jones *et al*[11] and C-Y Yip *et al*[12] studies. The two studies included 67 and 30 male participants in the treatment group, respectively. Both studies involved non-randomised controlled trials. For treatment effectiveness we relied on differences in the outcome measures between baseline and end of treatment for

those participants who completed the programme. In controlled trials the failure to complete the programme reduces the real differences between the treatment and control groups[25].

Statistical analysis

Descriptive statistics summarised demographics, clinical and forensic baseline characteristics. To assess differences between groups t-tests were performed on continuous data and χ^2-tests on categorical data. Change scores in the outcome measures between baseline (Time 1) and end of treatment (Time 2) were measured in two ways: (1) change in mean scores over time and use of a paired *t* test (Cohen's d was calculated by the mean difference score over the standard deviation of the difference); and (2) by categorising an improvement of one or more points on each test as an "improvement" and no change or a worse score as "no improvement". A binary logistic regression was used to investigate which of the outcome measures best predicted completion *vs* non completion.

We ran a binary logistic regression for each of the outcome measures with improvement between Time 1 and Time 2 being the independent variable and predictors being participants' age, ethnic background ("black" *vs* "other"), oral typical psychotropic medication (yes *vs* no), and level of security (low/medium *vs* high).

RESULTS

Patient demographics and baseline characteristics

The sample were of mixed ethnicity; White (*n* = 52, 53.6%), Black Caribbean (*n* = 13, 13.4%), Black African (*n* = 11, 11.3%), Black Other (*n* = 12, 12.4%), Asian (*n* = 2, 2.1%), Mixed Race (*n* = 4, 4.1%) or Other (*n* = 2, 2.1%). These were reclassified as "White" (*n* = 52, 53.6%) and "Other" (*n* = 44, 45.4%). The age range of participants was 19-63 with an average age of $\overline{X}$ = 35.31, SD = 9.16. All participants had a history of severe mental illness, most commonly psychotic disorders (*n* = 87, 89.7%), as well as mood disorders (*n* = 9, 9.3%) and developmental disorders (*n* = 1, 1%).

The majority of index offences were violence related (*n* = 85, 73.9%), for example homicide and assault; other index offences for current admission included financial (*n* = 6, 5.2%), drug (*n* = 4, 3.5%), sexual (*n* = 12, 10.4%), arson (*n* = 7, 6.1%) and other (*n* = 1, 0.9%).

Treatment completion rate

The average number of sessions attended was 13.22, SD = 3.84; 78.4% (*n* = 76) participants completed R&R2MHP and 21.6% (*n* = 21) did not (*i.e.,* they did not complete the minimum of 12 sessions). Information on the reason for drop out was only available for 10.3% (*n* = 10) of cases: These were due to non-compliance (*n* = 6), poor mental state (*n* = 1) and "other unknown reason" (*n* = 3).

Table 1 Differences in medication status of completers and non-completers

	Completers n (%)	Non-completers n (%)	χ^2 df = 1	OR (95%CI)
Currently on oral typical psychotropic medication	22 (29.7)	1 (5.3)	4.86[1]	7.62 (0.97-60.62)
Currently on oral atypical psychotropic medication	39 (52.7)	13 (68.4)	1.51	0.51 (0.18-1.50)
Currently on depot typical psychotropic medication	10 (13.5)	3 (15.8)	0.65	0.83 (0.21-2.38)
Currently on depot atypical psychotropic medication	6 (8.1)	0 (0)	1.65	0.91 (0.86-0.98)
Currently on antidepressant psychotropic medication	16 (21.6)	2 (10.5)	1.2	2.34 (0.49-11.2)
Currently on mood stabilisers psychotropic medication	20 (27.0%)	5 (26.3)	0.01	1.04 (0.33-3.25)

[1]$P < 0.05$.

Factors predicting treatment completion

Background measures: There was no significant age difference (*t* = 1.0) between the completers (mean = 35.8, SD = 9.4) and non-completers (mean = 33.5, SD = 8.0).

The completion rates for the three levels of security (low, medium, high) were 76.0% (*n* = 19), 78.6% (*n* = 33) and 89.0% (*n* = 24), respectively. The difference was not significant (χ^2 = 0.131, df = 2).

Similarly there was no significant difference between the number of "White" (*n* = 43, 82.7%) and "Other" (*n* = 32, 72.7%) ethnic participants who completed the programme. This difference was not significant (χ^2 = 1.39, df = 1).

There was no significant difference (*t* = -0.32, df = 86, ns) in the number of previous convictions between the completers (mean = 8.34, SD = 14.88) and non-completers (mean = 8.45, SD = 9.62).

No significant difference (*t* = -0.85, df = 82, ns) was found in the number of previous hospital admissions between the completers (mean = 3.89, SD = 3.86) and non-completers (mean = 4.79, SD = 4.60).

Table 1 shows the differences in the medication status between completers and non-completers. Out of the six medication categories only "Currently on oral typical psychotropic medication" showed a significant difference between the two groups (χ^2 = 4.86, df = 1, OR = 7.62, 95%CI: 0.97-60.62).

Baseline measures: Out of the five baseline psycho-

	Completers Mean (SD) (*n*)	Non-completers Mean (SD) (*n*)	*t* value (df)	Cohen's d
MVQ (total)	15.8 (12.2) (76)	17.4 (13.3) (21)	-0.52 (95)	0.05
SPSI-RS	11.8 (3.0) (76)	12.6 (3.3) (21)	-1.15 (95)	0.22
NAS-PI	81.0 (19.9) (76)	80.3 (18.4) (21)	0.14 (95)	0.01
LoC	16.77 (5.4) (52)	13.93 (4.4) (15)	1.85 (65)	0.57
DBSP	35.2 (11.4) (63)	43.1 (14.6) (15)	-2.27 (76)[1]	0.60

[1]$P < 0.05$. MVQ: Maudsley Violence Questionnaire; DBSP: Disruptive behaviour and social problem scale; NAS-PI: Novaco Anger Scale and Provocation Inventory; LoC: Locus of Control Scale; SPSI-RS: Social problem-solving inventory-revised short.

metric measures, only the DBSP discriminated significantly between completers and non-completers (see Table 2). Completers had a significantly lower score (*t* = -2.27, df = 76, Cohen's d = 0.60). A further analysis of the DBSP showed that the Disruptive Behaviour subscale (*t* = -2.19, df = 76, *P* < 0.05, Cohen's d = 0.59) differentiated better between the completers and non-completers than the Social Problem subscale (*t* = -1.36, df = 76, ns, Cohen's d = 0.38).

Factors predicting treatment outcome among completers
Background predictors of therapeutic outcome: The binary logistic regression for each of the outcome measures showed that none of the predictors (age, ethnic background, oral typical psychotropic medication, and level of security) predicted therapeutic outcome (categorical measure).

Outcome predictors of therapeutic outcome: Table 3 shows the difference in the outcome measured between Time 1 and Time 2. There was a significant improvement over time on four of the outcome measures: MVQ, SPSI-RS, NAS-PI and DBSP with the effect sizes (Cohen's d for a paired sample) being 0.43, 0.27, 0.23, and 0.27, respectively. No significant improvement was found for LoC.

DISCUSSION
The findings suggest that R&R2MHP can be used with participants of different ages, ethnic background, and at different levels of security without the completion rate or treatment effectiveness being compromised.

Two specific findings are relevant to the completion rate, namely psychotropic medication and ward behaviour. The medication status of the participants appears to influence the completion rate. Those participants who were on oral typical psychotropic medication at the time of the study were over seven times more likely to complete the programme. Yet being on oral

typical psychotropic medication did not predict treatment effectiveness on any of the five outcome measures. The implication is that this type of medication helped participants complete their required sessions, but it did not have any additional benefit relevant to treatment effectiveness. Participation in cognitive skills group programmes of this type require a reasonably stable mental state, however none of the other types of medication predicted completion. There is evidence that atypical antipsychotics do not offer clinical superiority over typical antipsychotics (with the exception of clozapine)[26,27], and we have found that those patients on oral route of typical antipsychotics are more likely to complete the programme. Oral medication may provide greater flexibility to cope with changes in mental state and prevent deterioration. Furthermore patients who are on an oral route of antipsychotic administration rather than depot are likely to be more clinically stable in terms of insight and attitude towards treatment, and this is likely to translate into better compliance with psychological treatment[28]. This is a novel finding and merits further research.

At baseline the completers had a significantly lower total score on a measure of ward behaviour rated by staff (the DBSP) than the non-completers with a medium effect size. The disruptive behaviour subscale was a much better predictor of non-completion than the social and psychological functioning subscale (Cohen's d 0.59 *vs* 0.38). This suggests that patients whose behaviour is often disruptive on the ward are at much greater risk of non-completion than other patients. The implication is that their behavioural disturbance on the ward needs to be addressed before they are able to participate fully in a cognitive skills intervention. Future research should investigate the causal and contributory factors to behavioural disturbance in the ward setting and this may relate to a range of problems, including poor mental state[29] and symptoms of attention deficit hyperactivity disorder[30].

As far as treatment effectiveness is concerned, the MVQ performed much better in terms of effect size than the other outcome measures. The two main violent attitudes measured by the MVQ, which have implications for treatment targets, are the use of violence to defend or enhance vulnerable self-esteem and the general acceptance that violence is justified as a way of life. Typically, controlled treatment trials compare the treatment group with a control group with the former including outcome measures of those who did not complete the programme ("Intention to Treat"; "ITT"), which in fact reduces the effect size where there is a poor completion rate[25]. This may bias the apparent effectiveness of specific outcome measures. The answer is either to delete the non-completers from the group differences comparison (*i.e.,* conduct a per-protocol analysis) or control for factors that may cause drop-out. The latter is methodologically sounder than the former[31].

Table 3 Differences between pre and post measures on the psychometric tests

Measure	*n*	(pre)	SD (pre)	Mean (post)	SD (post)	*t*(df)	Cohen's d
MVQ (total)	76	15.78	12.19	12.23	9.61	3.75 (75)[2]	0.43
SPSI	76	11.73	3.00	12.54	3.04	-2.33 (75)[1]	0.27
NAS-PI	76	80.99	19.89	77.09	15.86	2.09 (75)[1]	0.23
LoC	52	16.77	5.42	16.32	5.39	1.91 (51)	0.08
DBSP	63	35.21	11.40	32.57	11.32	2.16 (62)[1]	0.27

[1]$P < 0.05$; [2]$P < 0.01$. MVQ: Maudsley violence questionnaire; DBSP: Disruptive behaviour and social problem scale; NAS-PI: Novaco anger scale and provocation inventory; LoC: Locus of control scale; SPSI-RS: Social problem-solving inventory-revised short.

Everitt and Pickles (2004) outline six factors that influence treatment adherence, including completing all the sessions: (1) the amount of time and inconvenience; (2) the perceived importance of the procedure; (3) the potential health benefits *vs* potential risks; (4) the amount of discomfort caused by the treatment; (5) the amount of effort required; and (6) the number and type of side effects caused by the treatment[31]. They point to a number of factors that may improve treatment adherence, including short treatment trials, close supervision (*e.g.*, inpatient settings), and staff maintaining a positive attitude during the trial. Future research should investigate the effects of these six factors. Reducing the sessions of the original R&R has clearly improved treatment completion; completion in institutional settings may be better than programmes delivered in the community[32,25].

LoC showed no significant treatment effects in the current study. It failed to distinguish between completers and non-completers, using a categorical measure of improvement. In addition, it showed no significant difference between the Time 1 (baseline) and Time 2 (end of treatment) measures, unlike the four other outcome measures. Rees-Jones *et al*[11] (2012) found no difference in LoC between Time 1 and Time 2 for males in low and medium security, but there was a significant improvement at Time 3 (at three month follow-up). In contrast, Jotangia *et al*[16] (2013), investigating females in low and medium security, found an improvement on the LoC scale both at Time 2 and Time 3. This suggests two possibilities. Firstly, LoC is more effecting in measuring treatment improvement in females than males. This possibility merits further research. Secondly, LoC may take longer than the other measures to show treatment effects; this has been found for other outcome measures[25].

The main limitations of the study are the lack of documented reasons for the non-completion, the relatively low number of participants in the non-completion group, which resulted in limited power, the lack of information about institutional factors that may have influenced non-completion, and the fact that the participants were a convenience sample from previously published studies. In addition, the effects of gender could not be ascertained and this should be investigated in future studies.

This is a cross-sectional study that investigates associations rather than causation, nevertheless, this study has added important new information to understanding factors predicting treatment completion/non-completion among MDOs. For patients who were on oral typical psychotropic medication, this very significantly improved completion. In contrast, disturbed ward behaviour prior to commencing treatment was significantly associated with non-completion. No background factors were found to predict treatment outcome among those who completed the programme but among outcome measures attitudes towards violence was the best predictor of treatment effectiveness suggesting that this should be the primary outcome measure in future research.

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COMMENTS

Background
Cognitive skills programmes have been found to be effective in reducing offending through reduced violent attitudes and improved social problems skills. It is important to understand the factors that best predict completion of programmes, as well as those predicting a successful treatment outcome among those who completed the programme. The factors that best predict completion may not be the same factors as those that predict treatment outcome.

Research frontiers
Identification of the variables that predict treatment completion and treatment outcome will lead to more personalised treatment and better use of resources.

Innovations and breakthroughs
No previous research has investigated the effects of typical *vs* atypical psychotropic drugs as predictors of treatment completion and treatment outcome among mentally disordered offenders. The findings show that typical psychotropic drugs, administered orally, increased seven-fold the likelihood of the patients completing the programme, whereas it had no effect on treatment effectiveness. This is a novel finding.

Applications
The mental state of patients engaging in cognitive skills programmes needs to

be carefully assessed and continually reviewed during the programme as well as their medication status. In addition to mental state, this includes the behaviour of the patient on the ward. The fact that the age of the patient, ethnic background, number of previous convictions, number of hospital admissions, and level of security did not predict treatment completion or treatment outcome shows that the Reasoning and Rehabilitation Mental Health Programme (R&R2MHP) can be applied to most patients at different levels of security provided their mental state is stable.

Terminology
A typical medication comprised the first generation of psychotropic drugs, followed by the atypical (second generation) drugs.

Peer-review
This is, in summary, an interesting research paper aimed to investigate factors predicting treatment completion and treatment outcome of the R&R2MHP cognitive skills programme in a sample of 96 mentally disordered offenders.

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ORIGINAL ARTICLE

Retrospective Study

Infectious, atopic and inflammatory diseases, childhood adversities and familial aggregation are independently associated with the risk for mental disorders: Results from a large Swiss epidemiological study

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Abstract

AIM
To examine the associations between mental disorders and infectious, atopic, inflammatory diseases while adjusting for other risk factors.

METHODS
We used data from PsyCoLaus, a large Swiss Population Cohort Study (*n* = 3720; age range 35-66). Lifetime diagnoses of mental disorders were grouped into the following categories: Neurodevelopmental, anxiety (early and late onset), mood and substance disorders. They were regressed on infectious, atopic and other inflammatory diseases adjusting for sex, educational level, familial aggregation, childhood adversities and traumatic experiences in childhood. A multivariate logistic regression was applied to each group of disorders. In a complementary analysis interactions with sex were introduced *via* nested effects.

RESULTS
Associations with infectious, atopic and other chronic inflammatory diseases were observable together with consistent effects of childhood adversities and familial aggregation, and less consistent effects of trauma in each group of mental disorders. Streptococcal infections were associated with neurodevelopmental disorders (men), and measles/mumps/rubella-infections with early and late anxiety disorders (women). Gastric inflammatory diseases took effect in mood disorders (both sexes) and in early disorders (men). Similarly, irritable bowel syndrome was prominent in a sex-specific way in mood disorders in women, and, moreover, was associated with early and late anxiety disorders. Atopic diseases were associated with late anxiety disorders. Acne (associations with mood disorders in men) and psoriasis (associations with early anxiety disorders in men and mood disorders in women) contributed sex-specific results. Urinary tract infections were associated with mood disorders and, in addition, in a sex-specific way with late anxiety disorders (men), and neurodevelopmental and early anxiety disorders (women).

CONCLUSION
Infectious, atopic and inflammatory diseases are

important risk factors for all groups of mental disorders. The sexual dimorphism of the associations is pronounced.

Core tip: This study adds to the evidence that infectious, atopic and inflammatory diseases make up an important group of risk factors for neurodevelopmental and common mental disorders. They contribute independently of further major risk factors such as childhood adversities, traumatic experiences and familial aggregation. Each group of mental disorders (neurodevelopmental, early and late anxiety, mood, substance) attracts different combinations of risk factors. The sexual dimorphism of the associations is pronounced. The hypothesized biological mechanism that acts as a common denominator in this group of risk factors involves imbalances, *e.g.*, within the development of the immune system interfering with critical stages of brain development.

Ajdacic-Gross V, Aleksandrowicz A, Rodgers S, Mutsch M, Tesic A, Müller M, Kawohl W, Rössler W, Seifritz E, Castelao E, Strippoli MPF, Vandeleur C, von Känel R, Paolicelli R, Landolt MA, Witthauer C, Lieb R, Preisig M. Infectious, atopic and inflammatory diseases, childhood adversities and familial aggregation are independently associated with the risk for mental disorders: Results from a large Swiss epidemiological study. *World J Psychiatr* 2016; 6(4): 419-430 Available from: URL: http://www.wjgnet.com/2220-3206/full/v6/i4/419.htm DOI: http://dx.doi.org/10.5498/wjp.v6.i4.419

INTRODUCTION

There is an increasing awareness that infectious diseases, atopies and inflammatory conditions contribute to the risk for neurodevelopmental disorders (ND) and common mental disorders (CMD). A great number of the empirical results documented below underline the eminent role of the immune system. Nevertheless considerable scepticism abounds. Among other things, it is not clear how immunological risk factors are balanced against other risk factors in ND and CMD. The main aim of this study was, therefore, to assess the associations of infectious, atopic and inflammatory diseases with ND and CMD while adjusting for socio-demographic characteristic, familial aggregation, traumatic experiences and childhood adversities. A simple vulnerability-trigger model will serve to introduce the state of empirical research, thus reducing the potential variability of single and multiple hit models to a minimal general form.

Associations related to triggering mechanisms

The most intuitive example of a triggering factor in CMD is a postinfectious condition such as fatigue[1]. Infectious mononucleosis, *i.e.*, typically an Epstein Barr virus (EBV) infection in adolescence or adulthood, is a well known cause of postinfectious fatigue. However, also several other pathogens are also able to upregulate psychiatric symptoms, such as persistent pathogens: Borna disease virus, herpes simplex virus (HSV)-1, varicella zoster virus, and *Chlamydophila trachomatis*[2]. Apart from the first attack, a reactivation of an endogenous infection can increase the risk of depression[3].

It is noteworthy that the reciprocal causal direction also exists[4,5]. Generally speaking, it is not only the case that pathogens can trigger psychiatric illness, but, conversely, that psychiatric disorders can lead to an increased risk of infection. The two should not be confounded, keeping in mind that the causal direction is not always clear[6]. The examples above illustrate a trigger mechanism of ND and CMD, *i.e.*, the second part of conventional vulnerability-trigger (or, by analogy, diathesis-stress) models.

Associations related to vulnerability mechanisms

The first part of the vulnerability-trigger model are vulnerability factors occurring very early in life: Infections, atopic and inflammatory processes that establish, apart from their immediate effects, a lasting, possibly life-long vulnerability for CMD. A well known example of an early vulnerability is comprised in the pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS) model. This model has been applied in attention deficit hyperactivity disorder (ADHD), obsessive-compulsive disorder (OCD), and tic disorders such as the Gilles de la Tourette Syndrome[7,8]. It suggests that some persons with ND or CMD might actually suffer from an autoimmune disorder due to autoantibodies directed against basal ganglia tissue and appearing after infections with group A streptococci.

Evidence for associations between early infections and ND and CMD goes far beyond PANDAS and other autoimmune processes such as NMDA receptor encephalitis[9]. A compelling example is the link between EBV infections in childhood and risk of psychotic experiences in adolescence demonstrated in the ALSPAC cohort[10]. In a similar vein, studies from the Goodwin group which suggested that respiratory diseases in childhood and severe infections requiring the use of antibiotics in the first year of life increase the risk for several mental disorders such as depression, anxiety disorders and oppositional defiant disorder (ODD) later on in life[11,12].

The temporal sequence between pathogens and CMD may apply later in life as well. For instance, Danish record linkage studies have shown that individuals hospitalized because of an infection, particularly a bacterial infection, were more likely to develop schizophrenia later in life[13]. Apart from studies demonstrating a temporal sequence, many cross-sectional antibody based studies have pointed at associations between ND and CMD and selected pathogens. Serological studies have been particularly proliferative in psychosis research by implicating a broad spectrum of viral, bacterial and protozoan pathogens. For illustrative purposes, these are: (1) herpes viridae (cytomegalovirus[14], human herpesvirus-6[15], HSV-1[16,17], EBV[18]); (2) Toxoplasma gondii[14,19-23]; (3) Chlamydia infections: trachomatis[23,24], psittaci and pneumoniae[25,26]; (4) Mycoplasma pneumoniae (case study)[27]; (5) *Helicobacter pylori*[28]; and (6) gastrointestinal pathogens[29,30].

Associations related to parallel mechanisms

Not only were pathogens shown to precede psychotic experiences but also atopic diseases such as asthma and atopic dermatitis[31]. Similarly, the first occurrence of atopic dermatitis was reported to precede major depressive disorder and anxiety disorders[32] or ADHD[33]. Also other atopic diseases preceded ADHD[34]. However, evidence for the converse temporal sequence between atopic diseases and ND and CMD was also found with ND and CMD occuring first[35,36].

Again, the number of cross-sectional comorbidity studies providing evidence for a simple link between ND and CMD on the one hand and atopic diseases on the other is much greater than those focusing on temporal succession. They involve in particular asthma[37-44], hay fever[45], and eczema[46]. The association between atopic dermatitis and ADHD has gained particular attention since it emerges typically in the first years of life[33,47,48].

Beside atopies, chronic or relapsing inflammatory diseases have been shown to be linked to a great variety of CMD, and both theoretically qualify as triggers and as vulnerability markers. Skin diseases such as acne[49,50], psoriasis[51] and rosaceae[52] also contribute to the list of associations. Moreover, this list includes gastric inflammatory diseases[53-56], and gastrointestinal diseases/syndromes: Irritable bowel syndrome[57,58], Crohn's disease[59], interstitial cystistis[60,61] as well as recurrent cystitis[62], autoimmune diseases[63-65] and others[51]. This is only a small selection of associations, and the list could be extended with ease.

Aims of the analysis

To summarize, the complex picture of associations entails any variant of temporal sequences and almost any combination between groups of somatic diseases and groups of ND and CMD. Thus, in so far as infectious, atopic and chronic inflammatory diseases precede ND and CMD or share a mutual vulnerability with them, the relevant mechanisms cannot be determined on the level of single pathogens. Taken together, the literature provides important pieces of a larger puzzle with, however, still blurred contours. Comprehensive analyses enabling a broader understanding of these links are still missing. The present study takes advantage of a

large epidemiological data base from the PsyCoLaus study[66] to further investigate whether major groups of infectious, atopic and inflammatory diseases are associated with major groups of mental disorders.

MATERIALS AND METHODS

The Colaus/PsyCoLaus study
The data used in this analysis stem from CoLaus/PsyCoLaus[66,67], a cohort study designed to study mental disorders and cardio-vascular risk factors in the community and to determine their associations. The sample was randomly selected from the residents of the city of Lausanne (Switzerland) from 2003 to 2006 according to the civil register. Sixty-seven percent of the 35 to 66 years old participants of the physical baseline exam (n = 5535) also accepted the psychiatric evaluation, which resulted in a sample of 3720 individuals who underwent both the somatic and psychiatric exams.

Measures
A French version of the semi-structured Diagnostic Interview for Genetic Studies (DIGS)[68] was used in the PsyCoLaus study to assess a broad spectrum of lifetime DSM-IV Axis I criteria. The French version has shown excellent inter-rater and adequate test-retest reliability for major mood and psychotic disorders[69] as well as for substance use disorders[70]. Moreover, the DIGS allowed for gathering additional information on the course and chronology of comorbid features[66]. However, the brief phobia section of the DIGS was replaced by the corresponding sections from the Schedule for Affective Disorders and Schizophrenia - Lifetime Version (SADS-L)[71] in the current study. The anxiety sections of the French version of the SADS-L also revealed satisfactory reliability[72]. All diagnoses were lifetime diagnoses.

Grouping of mental disorders
We considered the following major groups of mental disorders based on the typical age of onset and common classifications: (1) neurodevelopmental diseases [typically starting during childhood: Tic disorders, ADHD, conduct disorder (CD), ODD]; (2) early-onset anxiety disorders (typically starting during childhood: separation anxiety disorder, overanxious disorder, animal phobias, social phobia); (3) late-onset anxiety disorders [typically starting after adolescence: Generalized anxiety disorder (GAD), panic, agoraphobia, specific phobias (excl. animal phobias[73])]; (4) mood disorders (typically starting after adolescence: major depressive disorder, dysthymia, bipolar disorders); and (5) substance use disorders (typically starting after adolescence: alcohol, cannabis, other illicit drug abuse/dependence).

Disorders with low frequencies (schizophrenia, schizoaffective disorders) or inadequately fitting in with the major groups (OCD, personality disorders, eating

disorders) were not included in the analyses.

Assessment of infectious, atopic and inflammatory diseases
The information on infectious diseases and related conditions was derived using an extended version of the medical history parts of the DIGS and the SADS-L and was based on self-reporting. In the interview participants were asked questions about ever having been diagnosed with various infectious diseases, diseases of the nervous system, cardiovascular, respiratory, gastrointestinal, metabolic and dermatological conditions as well as allergies and hormonal problems. For each disease, a screening question was asked and followed up in the case of an affirmative response.

In the current analyses the infectious diseases and related conditions were selected: (1) diseases typically related to streptococcal infections of the respiratory tract (scarlet fever, tonsillitis, rheumatic fever); (2) measles/mumps/rubella (MMR); the age range of the sample implies that most participants had not received an MMR vaccine in childhood, as routine measles and later MMR vaccinations schedules were only introduced by the Swiss government only in the 1960s; (3) urinary tract infections (UTIs) (cystitis, pyelitis, pyelonephritis, other nephritis, urethritis, prostatitis); (4) irritable bowel syndrome; (5) peptic ulcer/gastritis; (6) asthma and atopic diseases; (7) acne; and (8) psoriasis.

Covariates
We adjusted the analysis for the following variables which might account for the relationship between infectious diseases and mental disorders: (1) sex; (2) education level (low: Basic school and apprenticeship level; medium: Pre-university and high-level technical schools; high: University); (3) familial aggregation assessed by the semi-structured Family History - Research Diagnostic Criteria[74,75] which includes information on first and second degree relatives; subtypes parallelized to the groups of mental disorders mentioned above; dichotomized into any *vs* none; (4) childhood adversities dichotomized into any *vs* none if one of the following questions was confirmed: Did your parents fight frequently amongst themselves (interparental violence)?; Did your parents ever do anything that frightened you (like lock you in a closet)? (fear of maltreatment by parents); Did any of the following occur before your 16th birthday: .... put in foster care? (foster care); Overall, how would you characterize your childhood (N/A, happy, either happy not unhappy, unhappy, very unhappy)? categorized as yes, if unhappy or very unhappy (unhappy children); and (5) traumatic experiences in childhood below the age of 10 (serious accident or disaster, victim of violent attacks (self or loved ones), witnessed homicide or other forms of violent deaths; the age limit was chosen in order to focus on experiences mostly generating a vulnerability for mental disorders instead of acting as a trigger

themselves); the questions were taken from the French version of the SADS-LA (see above) and dichotomized into any *vs* none.

Statistical analysis

The data were analyzed using binary logistic regression and displaying odds ratios (OR) and 95%CI. The regression analysis was redone for men and women separately before including interaction effects. In order to better figure out the source of sex-specific divergences - either men or women - the interaction effects were modeled *via* nested effects, *i.e.*, by nesting each infectious, atopic and inflammatory variable in men and in women. All analyses were carried out using SAS version 9.3. The statistical analysis was reviewed by Viktor von Wyl from the Epidemiology, Biostatistics and Prevention Institute of the University of Zürich.

RESULTS

Table 1 shows the overall and sex-specific prevalence estimates for five major groups of mental disorders (neurodevelopmental, early-onset anxiety, late-onset anxiety, mood and substance disorders) together with education level, familial aggregation, trauma below the age of 10, childhood adversities and various infectious and atopic/inflammatory diseases. In bivariate analyses, mental disorders were consistently associated with familial aggregation, trauma and childhood adversities. Trauma showed distinct sex-specific associations in early disorders and in substance abuse. The associations of ND and CMD with infectious, atopic and inflammatory diseases spread across the whole table in a less consistent way. Moreover, they displayed more sex-specific divergencies. Therefore, and since some variables, *e.g.*, UTI, are skewed by sex, an additional look at the sex-specific associations was necessary in multivariate analyses.

In multivariate analysis (Table 2), the associations with familial aggregation and childhood adversities remained relatively stable across all five models for each group of mental disorders (ORs up to 3). The effect of trauma clearly diminished. Each group of ND /CMD displayed associations with any of the infectious, atopic and inflammatory diseases included in the analysis. Many associations occurred at trend level, thus suggesting more in-depth analyses either related to sex-specific associations or to the level of specific disorders.

Analyses involving interaction effects by nesting infectious, atopic and inflammatory diseases within sex (Table 3) uncovered further hetereogenity. In detail, ND disorders were associated with streptococcal infections specifically in men (OR = 1.98, 95%CI: 1.08-3.66) but not in women. Peptic ulcer/gastritis was significant only in the men model (OR = 1.95, 95%CI: 1.08-3.53), and showed a similar tendency in women. The opposite applies for UTI, where only women (OR = 1.68, 95%CI: 1.11-2.54) reached the conventional significance level.

Early-onset anxiety disorders showed associations with MMR, which were similar in both groups; again only women (OR = 1.46, 95%CI: 1.01-2.10) reached the conventional significance level. Another shared issue is irritable bowel syndrome with a strong impact in men (OR = 3.15, 95%CI: 1.58-6.28) and a trend level impact in women. Associations found specifically in men comprise peptic ulcer/gastritis (OR = 1.85, 95%CI: 1.13-3.05), psoriasis (OR = 2.02, 95%CI: 1.20-3.39) and, at trend level, acne. Moreover, associations with UTI emerged specifically in women (OR = 1.44, 95%CI: 1.16-1.79), at trend level also with atopic disease, but not in men.

In late-onset anxiety disorders, UTI (OR = 2.13, 95%CI: 1.19-3.82) were predictive not in women but in men. The significant predictors in women comprise MMR (OR = 1.81, 95%CI: 1.12-2.90) and peptic ulcer/ gastritis (OR = 1.60, 95%CI: 1.02-2.51), whereas irritable bowel syndrome and atopic disease remain significant at the trend level.

Mood disorders were associated with UTI in women (OR = 1.47, 95%CI: 1.19-1.81) and in men (OR = 1.63, 95%CI: 1.00-2.65). Also the impact of peptic ulcer/gastritis is apparent in both groups (in women: OR = 1.58, 95%CI: 1.02-2.46, and in men: OR = 1.98, 95%CI: 1.26-3.09). Acne (1.96, 95%CI: 1.35-2.85) predicts mood disorders in men, whereas irritable bowel syndrome (OR = 2.25, 95%CI: 1.35-3.76) and psoriasis (OR = 2.02, 95%CI: 1.14-3.58) contribute in women.

Finally, substance abuse/dependence did not yield any relevant associations in women. In men, it was linked with peptic ulcer/gastritis (OR = 1.88, 95%CI: 1.18-2.99) and with acne (OR = 1.74, 95%CI: 1.17-2.59).

As a side effect of the analysis involving interaction effects, the sex main effect in early and late anxiety disorders disappeared and greatly diminished in mood disorders. The models proved to be stable even when the strongest predictors in each model were omitted. Preliminary analyses on a more detailed level focusing on specific ND and CMD revealed a heterogeneity of results that clearly surpassed the findings presented in this study (results not shown).

DISCUSSION

This is the first study to apply a comprehensive epidemiological perspective on the associations of major groups of ND and CMD with infectious, atopic and inflammatory diseases. It adds to the evidence that infectious, atopic and inflammatory diseases make up an important group of risk factors. The main outcome was the great range of associations although the statistical models had been adjusted for trauma, childhood adversities, familial aggregation and education. Provided that the analyses were carried out on grouped CMD and somatic diseases, the results reported in this study represent only the tip of an iceberg. In addition,

Table 1 Groups of mental disorders and risk factors in the PsyCoLaus study: Frequencies and crude odds ratios (with 95%CI), overall and by sex

	n (%)	Education level low	Education level medium	Education level high	Familial aggregation[6]	Trauma below age of 10	Childhood adversities[7]	Streptococcal diseases[8]	MMR	Peptic ulcer/ gastritis	Irritable bowel syndrome	Atopic diseases	Acne	Psoriasis	Urinary tract infections[9]
Total n (%)															
All	3720 (100.0)	1965 (53.4)	916 (24.9)	798 (21.7)	2071 (55.7)	160 (4.3)	1013 (27.2)	230 (6.2)	3033 (86.7)	317 (6.5)	187 (3.8)	2129 (43.8)	474 (9.7)	220 (4.5)	844 (22.7)
Males	1750 (47.0)	888 (51.3)	366 (21.1)	477 (27.6)	876 (50.1)	45 (2.6)	417 (23.8)	87 (5.0)	1353 (83.2)	159 (7.0)	52 (2.3)	895 (39.6)	191 (8.4)	108 (4.8)	86 (4.9)
Females	1970 (53.0)	1077 (55.3)	550 (28.2)	321 (16.5)	1195 (60.7)	115 (5.8)	596 (30.3)	143 (7.3)	1226 (89.6)	158 (6.1)	135 (5.2)	1234 (47.4)	283 (10.9)	112 (4.3)	758 (38.5)
Odds ratios															
Neurodevelopmental mental disorders[1]															
All	308 (8.3)	1 (ref)	1.17 (0.89-1.55)	0.94 (0.69-1.29)	1.75 (1.26-2.42)	1.93 (1.22-3.05)	2.93 (2.31-3.71)	1.67 (1.11-2.50)	1.18 (0.83-1.67)	1.68 (1.14-2.49)	1.74 (1.07-2.83)	0.98 (0.78-1.24)	0.87 (0.59-1.29)	1.07 (0.63-1.80)	1.13 (0.86-1.48)
Males	189 (10.8)	1 (ref)	1.39 (0.96-2.00)	0.85 (0.58-1.24)	1.98 (1.27-3.10)	1.28 (0.53-3.06)	3.10 (2.27-4.23)	2.65 (1.57-4.48)	1.35 (0.87-2.08)	1.52 (0.89-2.57)	2.18 (1.03-4.61)	1.15 (0.85-1.56)	0.88 (0.50-1.53)	1.09 (0.57-2.08)	1.36 (0.73-2.56)
Females	119 (6.0)	1 (ref)	1.03 (0.67-1.57)	0.93 (0.54-1.58)	1.75 (1.08-2.85)	2.98 (1.72-5.12)	3.22 (2.21-4.69)	1.05 (0.52-2.11)	1.13 (0.63-2.04)	1.94 (1.08-3.49)	1.86 (0.97-3.58)	0.89 (0.62-1.30)	0.97 (0.55-1.72)	0.93 (0.37-2.35)	1.94 (1.34-2.82)
Early anxiety disorders[2]															
All	951 (25.6)	1 (ref)	0.98 (0.82-1.17)	0.93 (0.77-1.12)	2.75 (2.36-3.21)	1.96 (1.41-2.71)	1.85 (1.58-2.17)	1.51 (1.13-2.00)	1.43 (1.12-1.81)	1.33 (1.01-1.76)	2.42 (1.74-3.37)	1.23 (1.06-1.43)	1.32 (1.05-1.66)	1.41 (1.03-1.94)	1.90 (1.61-2.24)
Males	327 (18.7)	1 (ref)	1.03 (0.76-1.41)	1.06 (0.80-1.41)	2.48 (1.93-3.20)	2.01 (1.06-3.82)	2.01 (1.55-2.61)	1.71 (1.05-2.78)	1.27 (0.89-1.80)	1.75 (1.15-2.66)	2.84 (1.53-5.27)	1.03 (0.81-1.31)	1.59 (1.08-2.32)	2.22 (1.42-3.48)	1.25 (0.74-2.11)
Females	624 (31.7)	1 (ref)	0.90 (0.72-1.13)	1.01 (0.77-1.32)	2.76 (2.26-3.36)	1.65 (1.13-2.42)	1.65 (1.35-2.02)	1.29 (0.91-1.84)	1.35 (0.96-1.88)	1.11 (0.77-1.61)	1.97 (1.33-2.92)	1.25 (1.03-1.51)	1.11 (0.83-1.47)	1.01 (0.64-1.59)	1.51 (1.24-1.83)
Late anxiety disorders[3]															
All	554 (14.9)	1 (ref)	0.96 (0.77-1.20)	0.82 (0.64-1.04)	2.15 (1.67-2.76)	1.64 (1.12-2.42)	1.92 (1.59-2.32)	1.38 (0.98-1.94)	1.44 (1.07-1.95)	1.66 (1.21-2.27)	2.30 (1.59-3.32)	1.34 (1.12-1.61)	1.13 (0.85-1.49)	1.13 (0.75-1.69)	1.49 (1.22-1.82)
Males	200 (11.4)	1 (ref)	1.14 (0.78-1.67)	1.22 (0.87-1.72)	1.96 (1.24-3.08)	0.97 (0.38-2.48)	1.60 (1.16-2.20)	1.01 (0.51-1.99)	1.03 (0.68-1.56)	1.32 (0.77-2.25)	2.04 (0.97-4.31)	1.19 (0.89-1.61)	1.56 (0.99-2.46)	0.70 (0.33-1.47)	2.68 (1.60-4.49)
Females	354 (18.0)	1 (ref)	0.84 (0.65-1.11)	0.64 (0.45-0.92)	2.06 (1.53-2.79)	1.67 (1.08-2.57)	2.02 (1.59-2.55)	1.47 (0.98-2.20)	1.75 (1.11-2.75)	1.93 (1.29-2.86)	2.14 (1.39-3.29)	1.35 (1.07-1.69)	0.88 (0.61-1.27)	1.56 (0.95-2.56)	1.06 (0.83-1.34)
Mood disorders[4]															
All	1765 (47.4)	1 (ref)	1.21 (1.03-1.41)	0.99 (0.84-1.16)	2.14 (1.87-2.45)	1.90 (1.37-2.63)	2.07 (1.79-2.40)	1.05 (0.80-1.38)	1.18 (0.97-1.44)	1.71 (1.32-2.22)	2.31 (1.63-3.26)	1.27 (1.11-1.44)	1.29 (1.05-1.59)	1.45 (1.07-1.96)	2.19 (1.87-2.56)
Males	628 (35.9)	1 (ref)	1.43 (1.11-1.84)	1.26 (1.00-1.59)	1.93 (1.57-2.37)	1.91 (1.05-3.44)	1.85 (1.48-2.31)	1.01 (0.51-1.69)	1.15 (0.88-1.52)	1.70 (1.17-2.47)	1.65 (0.91-3.00)	1.29 (1.06-1.57)	1.76 (1.27-2.45)	1.38 (0.91-2.09)	2.04 (1.32-3.14)
Females	1137 (57.7)	1 (ref)	1.00 (0.82-1.24)	1.02 (0.79-1.31)	2.08 (1.73-2.50)	1.52 (1.02-2.27)	2.12 (1.72-2.60)	1.01 (0.72-1.43)	0.93 (0.70-1.26)	1.83 (1.25-2.69)	2.25 (1.44-3.52)	1.09 (0.91-1.30)	0.93 (0.71-1.23)	1.79 (1.12-2.85)	1.47 (1.22-1.77)
Substance abuse/ dependence[5]															
All	576 (15.5)	1 (ref)	0.77 (0.61-0.96)	0.89 (0.71-1.12)	2.10 (1.62-2.72)	1.22 (0.81-1.84)	1.82 (1.51-2.19)	0.89 (0.56-1.40)	0.90 (0.69-1.17)	1.55 (1.13-2.12)	1.26 (0.83-1.92)	0.97 (0.81-1.16)	1.17 (0.89-1.54)	1.42 (0.98-2.06)	0.78 (0.61-0.98)
Males	429 (24.5)	1 (ref)	0.82 (0.62-1.09)	0.66 (0.51-0.87)	2.65 (1.87-3.75)	1.00 (0.50-1.98)	1.94 (1.52-2.47)	0.98 (0.59-1.62)	0.98 (0.73-1.33)	1.62 (1.09-2.41)	1.79 (0.96-3.35)	1.12 (0.90-1.40)	1.64 (1.15-2.33)	1.24 (0.78-1.97)	1.36 (0.84-2.18)
Females	147 (7.5)	1 (ref)	0.80 (0.53-1.21)	1.00 (0.63-1.59)	2.11 (1.35-3.29)	2.48 (1.46-4.24)	2.60 (1.85-3.65)	0.82 (0.41-1.65)	1.58 (0.82-3.06)	1.53 (0.86-2.74)	1.64 (0.88-3.07)	1.06 (0.75-1.48)	0.89 (0.52-1.53)	1.66 (0.84-3.28)	1.41 (1.01-1.98)

[1]Tics, attention deficit hyperactivity disorder, conduct disorder, oppositional defiant disorder; [2]Separation anxiety disorder, overanxious disorder, specific phobias, panic, agoraphobia, specific phobias (excl. animals); [3]Generalized anxiety disorder, social phobia; [4]Major depression disorder, dysthymia, bipolar disorders; [5]Alcohol, cannabis, other illicit drugs abuse/dependence; [6]Overall figures; Ns relating to each subgroup of mental disorders: 382 (neurodevelopmental), 1110 (early anxiety), 386 (late anxiety), 1446 (mood), 349 (substances); OR's were based on subgroup specific information; [7]Interparental violence, fear of maltreatment by parents, growing up in a children's home, unhappy childhood; [8]Tonsillitis, scarlet fever, rheumatic fever. MMR: Measles/mumps/rubella; [9]cystitis, pyelitis, pyelonephritis, other nephritis, urethritis, prostatitis.

Table 2 Mental disorders regressed on infectious, atopic and inflammatory diseases, odds-ratios and 95%CI derived from logistic regression models

	Model 1 Neurodevelopmental disorders	Model 2 Early anxiety disorders	Model 3 Late anxiety disorders	Model 4 Mood disorders	Model 5 Substance abuse/ dependence
Sex	0.38 (0.27-0.52)	1.60 (1.33-1.94)	1.50 (1.19-1.87)	2.05 (1.74-2.41)	0.19 (0.14-0.24)
Education level					
Low	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Medium	0.91 (0.66-1.24)	1.24 (1.01-1.52)	1.11 (0.87-1.41)	0.86 (0.72-1.04)	1.19 (0.92-1.54)
High	1.10 (0.78-1.56)	1.08 (0.87-1.34)	1.07 (0.83-1.39)	0.88 (0.72-1.06)	1.27 (0.98-1.66)
Familial aggregation of CMD	1.55 (1.07-2.23)	2.54 (2.14-3.01)	1.75 (1.34-2.29)	1.77 (1.52-2.06)	2.12 (1.59-2.82)
Trauma below age of 10	1.43 (0.84-2.44)	1.07 (0.73-1.57)	1.12 (0.73-1.71)	1.11 (0.76-1.62)	1.36 (0.84-2.20)
Childhood adversities	2.74 (2.09-3.60)	1.51 (1.25-1.81)	1.89 (1.53-2.33)	1.87 (1.57-2.23)	1.81 (1.45-2.27)
Streptococcal infections	1.29 (0.79-2.10)	1.11 (0.80-1.55)	1.22 (0.84-1.78)	0.80 (0.59-1.10)	0.80 (0.51-1.25)
Mumps, measles, rubella	1.37 (0.91-2.06)	1.36 (1.04-1.77)	1.33 (0.97-1.83)	1.07 (0.86-1.34)	1.15 (0.85-1.54)
Peptic ulcer/gastritis	1.72 (1.11-2.68)	1.23 (0.89-1.71)	1.47 (1.03-2.11)	1.74 (1.27-2.39)	1.58 (1.09-2.29)
Irritable bowel syndrome	1.30 (0.71-2.36)	1.81 (1.24-2.64)	1.74 (1.15-2.62)	1.87 (1.26-2.79)	1.70 (1.06-2.73)
Atopic diseases	0.95 (0.73-1.25)	1.11 (0.94-1.31)	1.24 (1.01-1.51)	1.06 (0.91-1.24)	1.02 (0.83-1.26)
Acne	0.83 (0.53-1.30)	1.10 (0.85-1.43)	1.02 (0.74-1.39)	1.23 (0.97-1.57)	1.27 (0.92-1.76)
Psoriasis	1.22 (0.69-2.16)	1.45 (0.99-2.11)	1.05 (0.66-1.69)	1.59 (1.11-2.28)	1.41 (0.91-2.19)
Urinary tract infections	1.51 (1.06-2.14)	1.37 (1.12-1.67)	1.06 (0.83-1.35)	1.49 (1.22-1.80)	1.20 (0.88-1.64)

CMD: Common mental disorders.

Table 3 Mental disorders regressed on infectious, atopic and inflammatory diseases, odds-ratios and 95%CIs derived from logistic regression models with nested effects

		Model 1 Neurodevelopmental disorders	Model 2 Early anxiety disorders	Model 3 Late anxiety disorders	Model 4 Mood disorders	Model 5 Substance abuse/ dependence
Sex		0.41 (0.23-0.72)	0.79 (0.54-1.15)	1.08 (0.68-1.69)	1.56 (1.08-2.26)	0.34 (0.21-0.54)
Education level						
Low		1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Medium		1.09 (0.79-1.49)	0.79 (0.65-0.98)	0.89 (0.70-1.13)	1.15 (0.96-1.38)	0.83 (0.64-1.08)
High		0.88 (0.62-1.25)	0.91 (0.73-1.14)	0.92 (0.71-1.20)	1.12 (0.92-1.36)	0.77 (0.59-1.01)
Familial aggregation of CMD		1.53 (1.06-2.21)	2.53 (2.14-3.00)	1.78 (1.36-2.33)	1.77 (1.52-2.06)	2.14 (1.60-2.86)
Trauma below age of 10		1.47 (0.86-2.50)	1.08 (0.73-1.58)	1.11 (0.73-1.71)	1.12 (0.76-1.64)	1.40 (0.86-2.27)
Childhood adversities		2.78 (2.12-3.65)	1.52 (1.26-1.83)	1.91 (1.55-2.36)	1.89 (1.59-2.25)	1.83 (1.46-2.29)
Streptococcal infections	Women nested	0.69 (0.29-1.67)	1.15 (0.76-1.73)	1.32 (0.84-2.07)	0.91 (0.60-1.36)	0.87 (0.42-1.82)
	Men nested	1.98 (1.08-3.66)	1.08 (0.60-1.92)	1.04 (0.51-2.09)	0.67 (0.40-1.12)	0.78 (0.44-1.39)
Mumps, measles, rubella	Women nested	1.16 (0.58-2.32)	1.46 (1.01-2.10)	1.81 (1.12-2.90)	0.97 (0.70-1.35)	1.62 (0.79-3.29)
	Men nested	1.47 (0.88-2.45)	1.29 (0.88-1.91)	0.96 (0.62-1.49)	1.12 (0.83-1.52)	1.04 (0.74-1.46)
Peptic ulcer/gastritis	Women nested	1.72 (0.88-3.34)	0.97 (0.63-1.49)	1.60 (1.02-2.51)	1.58 (1.02-2.46)	1.19 (0.61-2.35)
	Men nested	1.95 (1.08-3.53)	1.85 (1.13-3.05)	1.25 (0.67-2.35)	1.98 (1.26-3.09)	1.88 (1.18-2.99)
Irritable bowel syndrome	Women nested	1.23 (0.56-2.70)	1.48 (0.95-2.30)	1.60 (0.99-2.57)	2.25 (1.35-3.76)	1.63 (0.84-3.16)
	Men nested	1.74 (0.70-4.36)	3.15 (1.58-6.28)	1.97 (0.86-2.48)	1.33 (0.67-2.62)	1.80 (0.90-3.61)
Atopic diseases	Women nested	0.74 (0.48-1.12)	1.19 (0.96-1.47)	1.28 (0.99-1.66)	0.97 (0.79-1.18)	1.02 (0.69-1.49)
	Men nested	1.14 (0.80-1.61)	1.02 (0.77-1.34)	1.19 (0.86-1.66)	1.22 (0.97-1.53)	1.05 (0.81-1.35)
Acne	Women nested	0.75 (0.39-1.47)	0.94 (0.68-1.31)	0.82 (0.55-1.23)	0.90 (0.66-1.24)	0.74 (0.40-1.37)
	Men nested	0.92 (0.50-1.67)	1.47 (0.96-2.25)	1.52 (0.92-2.51)	1.96 (1.35-2.85)	1.74 (1.17-2.59)
Psoriasis	Women nested	0.84 (0.29-2.43)	1.05 (0.60-1.82)	1.48 (0.83-2.66)	2.02 (1.14-3.58)	1.70 (0.77-3.73)
	Men nested	1.44 (0.73-2.86)	2.02 (1.20-3.39)	0.60 (0.25-1.42)	1.33 (0.82-2.17)	1.30 (0.76-2.21)
Urinary tract infections	Women nested	1.68 (1.11-2.54)	1.44 (1.16-1.79)	0.94 (0.72-1.22)	1.47 (1.19-1.81)	1.30 (0.89-1.89)
	Men nested	1.26 (0.63-2.51)	0.99 (0.55-1.76)	2.13 (1.19-3.82)	1.63 (1.00-2.65)	1.04 (0.61-1.79)

CMD: Common mental disorders.

many associations were sex-specific. Intriguingly, accounting for interaction effects of infectious, atopic and inflammatory diseases with sex had different consequences for ND and CMD. In early and late anxiety disorders the sex main effect came down to one, meaning that the sex ratio in these disorders was fully determined by sex-specific associations with these risk factors.

Challenges
In view of the broad spectrum of results, the discussion will not focus on particular pathogens or findings as was done in the introduction, but will attempt to systematize them. Their interpretation encounters several basic challenges. First, the general heterogeneity of the associations between ND/CMD and infectious/atopic/ chronic inflammatory diseses is enormous. The extent

and heterogeneity of associations require appropriate, *i.e.*, neither universal nor parsimonious explanatory approaches. This methodological argument also applies also for the surprising sexual dimorphism of associations between ND/CMD and infectious, atopic and inflammatory diseases: There must be several mechanisms inducing sex-specific differences in rates of ND/CMD. Not least, this also applies to the different ages when CMD risk factors may emerge. While much attention has been paid to prenatal and perinatal events[76-78], the impact of MMR or scarlet fever in the current results shows that the age range can vary broadly. In brief: The same infectious disease or immune system imbalance could yield different vulnerability outcomes, depending on the age when it occurs.

Interpretation approaches
On a formal level the interpretation of the findings can follow three basic pathways (see, for example)[47]: (1) infectious, atopic and inflammatory diseases induce a risk for ND and CMD; (2) ND and CMD increase the risk for infectious, atopic and inflammatory diseases; and (3) both ND/CMD and infectious, atopic and inflammatory diseases share the same intermediate mechanisms or etiopathogenetic processes. These pathways will be used in the following to categorize and interpret the results.

Most of the current results point to the pathways one and three. In instances such as childhood infectious diseases the interpretation seems to be relatively unambiguous. Childhood infections lend themselves to the first pathway since they mostly precede other disorders or diseases. The range of potentially relevant pathogens, that figure as risk factors for mental disorders extends beyond well investigated prenatal infections (in the first place those summarized under the label TORCH - toxoplasmosis, rubella, cytomegalovirus, herpes)[79,80] and the PANDAS model (related to group A streptococcal infections in early childhood)[81]. In the current analysis it includes viral pathogens (MMR) in addition to streptococcal diseases. Moreover, the brief list of infectious diseases involved is to be understood as a preliminary compilation. More specific analyses, for example on anxiety disorders[82], would contribute additional links. In addition, several frequently occurring infectious agents in childhood cannot be adequately assessed by self-report data (*e.g.*, Haemophilus influenzae, respiratory syncytial virus, influenza).

Similar reasoning about the sequence of events also applies to atopic diseases. They often start in childhood and adolescence, *i.e.*, mostly before mood disorders (men) and late anxiety disorders (women). Thus, atopic diseases also seem to contribute to CMD rather than the other way round. However, atopic diseases represent a different type of immune system imbalance than infectious childhood diseases. It is a puzzling finding that the same disorder can be associated with risk factors which represent different, partly even antagonistic or competing immune system responses, such as Th1 *vs* Th2 or Th17 *vs* Treg[83].

This phenomenon can be perceived in associations related to chronic inflammatory diseases which represent pathway 3 above. For example, acne[84] and psoriasis[85] are assumed to be Th1/17 related skin diseases, whereas atopic eczema or the irritable bowel syndrome[57] are considered to have mainly[86] a Th2 related background.

Pathways 1 and 3 suggest that immunological processes are the common denominator of the related risk factors of ND/CMD. The immunological hypothesis in ND and CMD has many direct contributors, such as the TORCH (Toxoplasma gondii, rubella virus, cytomegalovirus, and herpes simplex virus)[80] and PANDAS models in ND disorders, serological studies, for example in schizophrenia (see above), leucocyte counts in depression[87], gastrointestinal inflammation in psychosis[29], the autoantibodies link[88], the inflammation topic in mood disorders[89], and, finally, evidence for upregulated proinflammatory mediators such as IL-1β, IL-6 and TNF-α[90]. However, in some instances such as UTI or ulcer the categorization of immune processes is less clear and may involve different basic mechanisms.

Hypotheses regarding the neurophysiological background mechanisms
The basic assumption of the immunological hypothesis within a two or three hit model (*i.e.*, a vulnerability-trigger model) of CMD is that immune system imbalances impact brain development during critical stages. Animal models referring to neonates have shown that bacterial infections may have an impact both on brain development and on the programming of the immune system[91-93]. While this research is based on *E. coli* models, the implications might generalize to other microbes, including streptococci, as well. It has been suggested that this pathway relies on the impact of cytokines on microglia, which in turn crucially influence brain development at different stages of life by influencing cell proliferation, synaptogenesis and immune processes in exchange with astrocytes, neurons and oligodendroglia[94,95]. An interesting perspective that has emerged recently is that mast cells are able to activate microglia[96].

In agreement with epidemiological research, the microglia pathway offers new perspectives for the understanding of the sex-ratios in mental disorders. Microglia numbers in males and females are differently skewed at different age stages. In early childhood, more microglia can be discerned in various brain regions of males, whereas in adolescence and adulthood, there are more microglia in the brains of females[97]. If more frequent, microglia are at the same time more "active"[94].

Limitations
While the promise of this study relies on a comprehensive epidemiological approach not feasible in most other subdisciplines in psychiatry, the study also has several limitations. First, all information is based on

the self-reporting of study subjects, which implies a substantial recall bias, both regarding mental problems and infectious diseases. Provided that infectious diseases remain asymptomatic in many instances and that underreporting is the most probable biasing effect regarding adverse experiences and stigmatized issues, our results represent rather conservative approximations of the "real" associations. Second, herpes as well as measles, mumps and rubella infections were presumably reported more frequently by subjects with a more severe or an exanthematic appearance of the infection. Thus, while these infections were underreported in this study, their frequencies implicitly provide a measure of disease severity. A similar limitation also applies to UTI and streptococcal infections. Third, the age of onset in streptococcal infections, herpes infections and in UTI could not be reliably assessed, the first two because of the inclusion of related diseases and late sequels, the latter because of the large proportion of undiagnosed or asymptomatic UTI in childhood. Finally, several further infectious agents of interest could not be identified by self report (see above) and thus could not be considered for the analysis.

In conclusion, atopic and inflammatory diseases make up an important group of potential risk factors for ND and CMD. They contribute independently of further major risk factors such as childhood adversities, traumatic experiences and familial aggregation. While the amount of evidence is enormous and continuously growing, the interpretational framework is compromised by the fact, that - similarly to research on smoking and cancer - direct experimental proofs are not feasible. Meanwhile, prevention in this field might already be going on unnoticed due to classical tools such as vaccinations and appropriate treatment of infectious diseases in childhood[98].

COMMENTS

Background
There are numerous results in the literature documenting the associations between inflammatory diseases of any kind (including infectious and atopic diseases) and neurodevelopmental disorders (ND)/common mental disorders (CMD). They complement other groups of risk factors (psychosocial stressors, traumatic experiences, pre-/perinatal risk factors, hormonal processes, substances, cerebral injury).

Research frontiers
In contrast to detailed knowledge about bivariate associations and particular issues, a systemic understanding is missing: How do these associations aggregate to more general mechanisms, how do they interact and compete? A better systemic understanding of the links between inflammatory diseases and ND/CMD might help us to understand even unresolved issues such as the sex ratios or the heterogeneous age at onset in these disorders.

Innovations and breakthroughs
This is the first study to apply a comprehensive epidemiological perspective on the associations of inflammatory diseases with major groups of ND and CMD while adjusting for other groups of risk factors. It confirms that inflammatory diseases make up an important group of risk factors of ND and CMD. However, the pathways are heterogeneous and sex-specific.

Applications
Inflammatory diseases are indicators of upregulations and imbalances in immune system activity. Since the immune system activity can be modulated and infectious diseases can be prevented, new potentials for intervention and prevention become apparent.

Terminology
Th1, Th2, Th17 are T helper cells, Treg are regulatory T cells. They represent different modes of immune system activity.

Peer-review
This is a very interesting body of work as a part on renewed interest in inflammatory processes and major psychiatric diseases.

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ORIGINAL ARTICLE

Observational Study

Health-care needs of remitted patients with bipolar disorder: A comparison with schizophrenia

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Abstract

AIM

To investigate health-care needs and their correlates among patients with remitted bipolar disorder (BD) compared to patients with remitted schizophrenia.

METHODS

Outpatients with BD (*n* = 150) and schizophrenia (*n* = 75) meeting clearly defined remission criteria were included in the study along with their relatives. Diagnostic ascertainment was carried out using the Mini International Neuropsychiatric Interview. Demographic and clinical details were recorded using structured formats. Residual symptoms were assessed using standardized scales. Health-care needs were assessed on two separate scales. The principal instrument employed to assess health-care needs was the Camberwell Assessment of Need-Research version (CAN-R). To further evaluate health-care needs we felt that an additional instrument, which was more relevant for Indian patients and treatment-settings and designed to cover those areas of needs not specifically covered by the CAN-R was required. This instrument with a structure and scoring pattern similar to the CAN-R was used for additional evaluation of needs. Patients' level of

functioning was assessed using the Global Assessment of Functioning Scale and their quality of life (QOL) using the World Health Organization Quality Of Life-BREF version in Hindi.

RESULTS
An average of 6-7 needs was reported by patients with BD as well as their relatives. Commonly reported needs were in the areas of economic and welfare needs, informational needs, social needs and the need for treatment. According to the CAN-R, both patients and relatives reported that more than 60% of the total needs were being met. However, over 90% of the needs covered by the additional evaluation were unmet according to patients and relatives. Needs in the areas of economic and welfare-benefits, information, company, daytime activities and physical health-care were largely unmet according to patients and relatives. Total, met and unmet needs were significantly higher for schizophrenia, but the most common types of needs were quite similar to BD. Relatives reported more needs than patients with certain differences in the types of needs reported. Level of patients' functioning was the principal correlate of greater total and unmet needs in both groups. Significant associations were also obtained with residual symptoms and QOL.

CONCLUSION
The presence of unmet needs in remitted patients with BD was an additional marker of the enduring psychosocial impairment characteristic of the remitted phase of BD.

Key words: Health-care needs; Bipolar disorder; Schizophrenia; Remission; Patients; Relatives

Core tip: Health-care needs, functioning and quality of life (QOL) were assessed in 150 outpatients with remitted bipolar disorder (BD) and 75 with remitted schizophrenia. A high number of needs were found in BD; economic, welfare and information needs were mostly unmet. Total, met and unmet needs were significantly higher for schizophrenia, but the pattern of needs was similar to BD. Relatives reported more needs than patients with differences in the types of needs. Patient-functioning, residual symptoms and QOL were associated with higher needs. Unmet needs in remitted patients with BD were indicative of the enduring psychosocial impairment during remission.

INTRODUCTION
The traditional view of bipolar disorder (BD) is that of a condition characterized by good outcome and complete recovery from acute episodes of the illness. However, research over the past few decades has clearly shown that a substantial proportion of remitted patients with BD continue to display subsyndromal symptoms, neurocognitive deficits and impairments in occupational and social functioning[1-3]. The diverse nature of these disabilities in BD suggests that measures beyond mere clinical symptoms are required to truly estimate the psychosocial impact of the condition during remission. Measures such as quality of life (QOL) or level of functioning are useful, but they do not generally provide much information about how the illness-related dysfunction or lack of satisfaction with treatment can be alleviated. The concept of "health-care needs" appears to overcome this shortcoming being a measure of outcome, in which subjective perceptions of patients and caregivers are evaluated in order to determine ways of improving the outcome of the illness[4].

The National Health Service and Community Care Act[5] defines "need" as the "requirement of the individual to achieve, maintain and restore an acceptable level of social independence and QOL". A health-care need is considered to be present when because of symptoms, distress or disability, the patient's level of functioning is not optimal due to some potentially remediable or preventable cause[4]. Accumulating evidence indicates that the extent to which needs of patients is met predicts their levels of disability, QOL, and satisfaction with treatment[6,7]. Accordingly, examination of needs and their correlates not only serves as the basis for improved treatment and judicious resource allocation, but also as a comprehensive indicator of the psychosocial status of patients and their psychosocial outcome following treatment[8]. Finally, in order for patients to become partners in their own treatment, it is important to understand and prioritize their personal wants and needs[4].

Despite the obvious implications of examining health-care needs of patients, very few studies have chosen to focus exclusively on examining needs among patients with BD. This contrasts with the large amount of literature available on needs of patients with other mental illnesses, particularly schizophrenia. Accordingly, the current study aimed to assess health-care needs and their correlates among patients with BD in remission, compared to those with remitted schizophrenia. Given the paucity of literature in this area, the first objective was to document the number and types of needs found among patients with BD in remission. To provide a context for the findings in BD, comparisons were carried out with health-care needs among patients with remitted schizophrenia and the correlates of health-care needs were also examined. Schizophrenia was chosen as a comparison group because of the substantial

amount of research data on health-care needs available for this condition. Based on previous research it was hypothesized that the number and types of health-care needs in BD would be similar to schizophrenia and would be associated with patient-functioning, symptom-severity and QOL. Since some differences between patients' and caregivers' evaluation of needs has been reported earlier[9,10], health-care needs were assessed both from the perspective of patients and their relatives. The eventual findings were expected to yield a better understanding of health-care needs among patients with BD in remission.

MATERIALS AND METHODS

Approval/consent
The protocol was approved by the ethics and research committees of the institute where it was conducted. Written informed consent was sought prior to induction and other ethical safeguards were maintained during the study.

Participants
Patients along with their relatives were recruited from those attending the outpatient psychiatric services of a tertiary-care hospital in north-India. Patients aged 18-60 years, with a diagnosis of BD or schizophrenia as per DSM-IV criteria[11], determined using the Mini International Neuropsychiatric Interview (MINI)[12] were included. Patients with BD had to be in remission, which was defined cross-sectionally as a score of < 8 on the 17-item Hamilton Depression Rating Scale[13] and a score of < 6 on the Young Mania Rating Scale[14]. Further, only those patients with BD without acute episodes in the 3-mo period prior to intake were included based on information from patients, relatives and case notes. Finally, patients had to be on a stable dose of psychotropics, *i.e.*, not more than 50% hikes or reductions in dosages in these 3 mo. Patients with schizophrenia were included if they met remission criteria of Andreasen *et al*[15] on the Positive and Negative Syndrome Scale for Schizophrenia (PANSS)[16]. Similar to patients with BD, only patients with no exacerbations of positive or negative symptoms and on stable doses of psychotropics in the 3-mo period prior to intake were included. Additionally, both patient groups were matched on age, gender, residence (urban/rural) and duration of illness. Patients with comorbid psychiatric or physical illnesses, substance use disorders (except nicotine use) and organic brain syndromes were excluded. Over a period of about a year, 150 outpatients with BD and a matched group of 75 outpatients with schizophrenia who fulfilled the selection criteria were inducted along with their relatives.

Assessments
Apart from confirming diagnoses with the MINI and rating symptoms, demographic and clinical details were recorded using structured formats. The principal instrument employed to assess health-care needs was the Camberwell Assessment of Need-Research version (CAN-R)[17]. The CAN-R consists of clinical and social needs divided into 22 areas. In each of these areas there are four sections, which assess the severity of need, current help received from friends or relatives, help from social services and outpatient clinics, the adequacy of help and satisfaction with the help received on a four-point scale. Though the CAN-R is a valid and reliable instrument for assessing needs of people with severe mental illnesses, it appeared to leave out some of the needs commonly reported by Indian patients. To evaluate health-care needs felt to be more relevant for Indian patients and treatment-settings, an additional instrument which was designed to cover those areas of needs not specifically covered by the CAN-R was used. The structure and scoring pattern of this instrument was similar to the CAN-R, but it had 21 items/areas not covered by the CAN-R. This scale has been used in a multi-centric Indian study on needs of patients with severe mental illnesses[18]. Finally, the level of functioning of patients was assessed using the Global Assessment of Functioning Scale (GAF)[11] and their QOL using the World Health Organization Quality Of Life-BREF version in Hindi (WHOQOL-BREF)[19].

Statistical analysis
Data were analyzed using the Statistical Package for Social Scientists, version 15.0. Continuous variables in the two groups were compared using "*t*" tests or Mann-Whitney tests, and ordinal and nominal variables using χ^2 tests. To examine the association between health-care needs and clinical and demographic correlates, Pearson's Product Moment Correlation coefficients (for normally distributed continuous data) and Spearman's Rank Correlation coefficients (for ordinal data with non-normal distributions) were estimated. Significance was set at 5%; *P* values were also adjusted for the multiple correlations carried out by using the Bonferroni correction. Separate stepwise multiple regression analyses with total and unmet needs on the CAN-R as dependent variables were carried out using patients' and relatives' reports to determine the correlates of health-care needs.

RESULTS

Profile of participants (Table 1)
Patients with BD were more likely to be married and in paid employment compared to those with schizophrenia. Relatives of patients with BD were more likely to be women and more likely to be their spouses, whereas parents outnumbered spouses in the schizophrenia group. All patients were on treatment. Clinical profiles of both groups were comparable.

Needs assessment on the CAN-R: Patients' reports (Table 2)
Though the total number of needs was relatively high

Table 1 Profile of the patients and their relatives

	Patients		Relatives		
	Bipolar disorder (n = 150)	Schizophrenia (n = 75)	Bipolar disorder (n = 150)	Schizophrenia (n = 75)	
Age (yr) mean (SD)	36.1 (10.1)	33.4 (9.9)	42.3 (12.8)	43.7 (11.7)	
Gender n (%)					Relatives' gender χ^2 = 6.74; P < 0.01
Male	101 (67)	42 (56)	64	46 (61)[a]	
Female	49	33	86 (57)	29	
Marital status n (%)					Marital status of patients χ^2 = 7.15; P < 0.01
Not married	43	35	12	5	
Married	107 (71)	40 (53)[a]	138 (92)	70 (93)	
Years of schooling mean (SD)	11.4 (4.9)	11.5 (4.4)	11.1 (5.9)	11.6 (6.24)	
Occupation n (%)					Occupation of patients χ^2 = 21.87; P < 0.001
Paid employment	103 (69)	27	72	44 (59)	
Others	47	48 (64)[b]	78 (52)	31	
Family type n (%)					
Nuclear	69	35			
Non-nuclear	81 (54)	40 (53)			
Residence n (%)					
Urban	86 (57)	39 (52)			
Rural	64	36			
Relationship with the patient n (%)					χ^2 = 11.69; P < 0.01
Spouse			68 (45%)	17 (23)[a]	
Parents			48 (32%)	30 (40)	
Sibs			16 (11%)	14 (19)	
Others			18 (12%)	14 (19)	
Age of onset (in years) mean (SD)	26.7 (9.5)	26.1 (12.7)			
Duration of illness (mo) mean (SD)	110.3 (78.9)	93.7 (65.9)			
Duration of treatment (mo) mean (SD)	99.1 (69.5)	100 (94.3)			
Number of hospitalizations in the past mean (SD)	0.7 (1.0)	0.5 (0.9)			
PANSS positive score mean (SD)	-	10.6 (5.6)			
PANSS negative score mean (SD)	-	12.3 (7.2)			
PANSS general psychopathology score mean (SD)	-	27.4 (12.6)			
YMRS score mean (SD)	2.0 (3.1)	-			
HDRS score mean (SD)	1.0 (2.1)	-			
GAF score mean (SD)	70.07 (17.79)	66.63 (15.63)			
WHOQOL-BREF scores mean (SD)	93.87 (15.17)	88.77 (17.85)			

[a]P < 0.01; [b]P < 0.001: Comparisons between BD and schizophrenia on marital status and occupation of patients, relatives' gender and relationship with patient. PANSS: Positive and Negative Syndrome Scale for Schizophrenia; YMRS: Young Mania Rating Scale; HDRS: Hamilton Depression Rating Scale; GAF: Global Assessment of Functioning; WHOQOL-BREF: World Health Organization Quality of Life Bref version; BD: Bipolar disorder.

among both patient groups, more than 60% of the total needs were perceived as being met. The mean number of total, met and unmet needs reported by patients was significantly higher for schizophrenia than BD. However, the pattern of individual needs was largely similar with the eight most common domains of needs in both groups being: Help with providing welfare-benefits, information about the condition and its treatment, help with household skills, help for allaying psychological distress, the need for company and social life, help regarding physical-health problems, help with daytime activities, help with self-care, and help for coping with psychotic symptoms. Among these domains, needs were perceived to be unmet in the areas of welfare-benefits, company, and information (mainly for schizophrenia) by a greater proportion of the patients. Patients with schizophrenia reported significantly greater needs in some additional domains including help with psychotic symptoms, the need for company, telephones and transport, and financial needs.

Needs assessment on the CAN-R: Relatives' reports (Table 3)

The overall pattern of needs and the eight most common needs reported by relatives was similar to that of patients. Like patients about 60% of the total needs were perceived to be met by relatives. Additionally, the mean number of total needs and met and unmet needs were significantly higher for those with schizophrenia than those with BD. Certain significant differences were, however, noted between patients and relatives. For the BD group, the mean number of total needs (t = 1.97; P < 0.05) and unmet needs (t = 2.01; P < 0.05) was significantly higher according to the relatives. Similar to patients' reports, the need for help with psychotic symptoms and for transport were greater among those with schizophrenia, but unlike patients, relatives reported significantly greater total needs in the domains of accommodation and help with the household skills. Finally, while in the BD group, the need for welfare benefits and company (among the eight most common needs) were perceived as being largely unmet, in the schizophrenia group unmet needs were greater in two additional areas of help with daytime activities and information about the condition and its treatment, where the proportion of relatives reporting unmet needs was significantly greater than those reporting information needs to be met (χ^2 = 13.79; P < 0.01).

Help received and satisfaction with help: Patients' and relatives' reports

Patients' and relatives' reports about the help received from formal (health-care services) and informal sources (family), and their satisfaction with this help revealed certain common trends across both patient groups. Firstly, both patients and relatives reported that they had hardly received any help from either formal or informal sources and were largely dissatisfied with the help received in three of the eight areas where needs

Table 2 Health-care needs on the Camberwell Assessment of Need-Research version - as reported by patients						
	Bipolar disorder *n* = 150			Schizophrenia *n* = 75			*t* values
Total needs - mean (SD)	6.03 (2.87)			7.45 (2.80)			3.54[d]
Met needs - mean (SD)	3.92 (2.21)			4.58 (2.32)			2.07[a]
Unmet needs - mean (SD)	2.1 (1.70)			2.87 (1.88)			3.01[b]
Domains	Total needs *n* (%)	Met needs *n* (%)	Unmet needs *n* (%)	Total needs *n* (%)	Met needs *n* (%)	Unmet needs *n* (%)	χ^2 values
Accommodation	11 (7)	11 (7)	0 (0)	9 (12)	7 (9)	2 (3)	1.34
Food	16 (11)	16 (11)	0 (0)	12 (16)	10 (13)	2 (3)	1.3
Household skills	92 (61)	86 (57)	6 (4)	55 (73)	52 (69)	3 (4)	3.17
Self care	28 (19)	25 (17)	3 (2)	18 (24)	15 (20)	3 (4)	0.87
Daytime activities	65 (43)	44 (29)	21 (14)	41 (55)	21 (28)	20 (27)	2.57
Physical health	68 (45)	47 (31)	21 (14)	32 (43)	27 (36)	5 (7)	0.14
Psychotic symptoms	44 (29)	38 (25)	6 (4)	67 (89)	60 (80)	7 (9)	72.01[d]
Information about condition and treatment	106 (71)	61 (41)	45 (30)	60 (80)	24 (32)	36 (48)	2.25
Psychological distress	87 (58)	73 (49)	14 (9)	40 (53)	32 (43)	8 (11)	0.44
Safety to self	14 (9)	14 (9)	0 (0)	5 (7)	4 (5)	1 (1)	0.46
Safety to others	21 (14)	16 (11)	5 (3)	8 (11)	5 (7)	3 (4)	0.49
Alcohol	8 (5)	8 (5)	0 (0)	0 (0)	0 (0)	0 (0)	2.73
Drugs	13 (9)	8 (5)	5 (3)	4 (5)	1 (1)	3 (4)	0.39
Company	74 (47)	36 (24)	38 (25)	50 (67)	24 (32)	26 (35)	6.07[a]
Intimate relationships	17 (11)	7 (5)	10 (7)	10 (13)	3 (4)	7 (9.3)	0.04
Sexual expression	14 (9)	9 (6)	5 (3)	3 (4)	0 (0)	3 (4)	1.34
Child care	21 (14)	18 (12)	3 (2)	10 (13)	6 (8)	4 (5)	0.019
Basic education	9 (6)	7 (5)	2 (1)	7 (9)	6 (8)	1 (1)	0.84
Telephone	33 (22)	28 (19)	5 (3)	27 (36)	23 (31)	4 (5)	5.01[a]
Transport	19 (13)	14 (9)	5 (3)	18 (24)	12 (16)	6 (8)	4.67[a]
Money	30 (20)	22 (15)	8 (5)	30 (40)	12 (16)	8 (11)	10.22[a]
Welfare benefits	114 (76)	1 (1)	113 (75)	63 (84)	0 (0)	63 (84)	1.91

[a]$P < 0.05$; [b]$P < 0.01$; [d]$P < 0.001$: Comparisons between BD and schizophrenia on total, met and unmet needs and different types of needs. BD: Bipolar disorder.

were commonly expressed including welfare-benefits (93%-98%), information about the condition and its treatment (59%-73%), and the need for company and social life (45%-56%). In the areas of help regarding physical-health problems and with daytime activities, some help was received from friends and family; still, about a-third to half of the respondents were dissatisfied with help received. In the areas of help with the household skills and for allaying psychological distress, majority of the respondents (73%-100%) reported receiving help from informal sources, and were satisfied with the help received. In the area of psychotic symptoms, a majority of the patients with schizophrenia and their relatives (85%-90%) acknowledged receiving help from health-care services and were satisfied with the help received; though respondents in the bipolar group did not receive much help from formal sources, the majority were still satisfied by the help received in this area (83%-90%).

Additional evaluation of health-care needs of patients (Table 4)
Results of the additional evaluation of needs showed that a larger proportion of the needs (over 90%) reported by patients or their relatives were unmet in contrast to the CAN-R evaluation. Similar to the CAN-R evaluation, total, met and unmet needs were significantly greater among those with schizophrenia. Common areas of

needs included those for free treatment, reimbursement of medical expenses, financial help, help with work or job reservations, travel concessions, and the need for psychoeducation. Patients expressed the need for travel concessions, disability certificates which would enable them to avail welfare-benefits, and the need for self-help groups, while relatives reported needs in the areas of rehabilitation and help with the stress of caregiving. Not unsurprisingly, the majority of the respondents (79%-100%) reported that they had received little help in these areas. Unlike the CAN-R evaluation, there were no differences between the patients' and relatives' reports.

Correlates of health-care needs (Table 5)
Univariate associations between health-care needs and demographic, clinical and psychosocial variables revealed that the GAF and the WHOQOL-BREF scores demonstrated significant inverse associations with total needs based on relatives' reports, and unmet needs based on reports of both patients and their relatives. Table 5 also includes the results of separate stepwise multiple regression analyses with total needs and unmet needs being the dependent variables in each analysis. The GAF scores, PANSS positive scores, and scores on the psychological-health domain of the WHOQOL-BREF explained about 25% variance in the total needs scores (GAF scores - 18%; PANSS positive scores - 5%;

Table 3 Health-care needs of patients on the Camberwell Assessment of Need-Research version - as reported by their relatives

	Bipolar disorder *n* = 150			Schizophrenia *n* = 75			*t* values
Total needs	6.72 (3.19)			8.36 (2.91)			3.37[f]
Met needs	4.15 (2.34)			4.99 (2.18)			2.57[a]
Unmet needs	2.57 (2.31)			3.37 (2.58)			2.37[a]
Domains	Total needs	Met needs	Unmet needs	Total needs	Met needs	Unmet needs	χ^2 values
Accommodation	12 (8)	11 (7)	1 (1)	15 (20)	13 (17)	3 (4)	6.82[b]
Food	32 (21)	30 (20)	2 (1)	23 (31)	20 (27)	3 (4)	2.35
Household skills	103 (69)	90 (60)	13 (9)	63 (84)	56 (75)	7 (9)	6.07[a]
Self-care	36 (24)	31 (21)	5 (3)	25 (33)	19 (25)	6 (8)	2.20
Daytime activities	77 (51)	51 (34)	26 (17)	43 (57)	21 (28)	22 (29)	0.72
Physical health	79 (53)	62 (41)	17 (11)	39 (52)	31 (41)	8 (11)	0.01
Psychotic symptoms	47 (31)	37 (25)	10 (7)	69 (92)	59 (79)	10 (13)	73.68[f]
Information about condition and treatment	106 (71)	62 (41)	44 (29)	60 (80)	17 (23)	43 (57)	2.25[1]
Psychological distress	87 (58)	70 (47)	17 (11)	49 (65)	39 (52)	10 (13)	1.12
Safety to self	8 (5)	8 (5)	0 (0)	6 (8)	5 (7)	1 (1)	0.61
Safety to others	31 (21)	19 (13)	12 (8)	14 (19)	9 (12)	5 (7)	0.12
Alcohol	11 (7)	8 (5)	3 (2)	2 (3)	2 (3)	0 (0)	1.23
Drugs	18 (12)	5 (3)	13 (9)	4 (5)	2 (3)	2 (3)	1.82
Company	83 (55)	37 (25)	46 (31)	45 (60)	18 (24)	27 (36)	0.44
Intimate relationships	25 (17)	10 (7)	15 (10)	12 (16)	3 (4)	9 (6)	0.02
Sexual expression	13 (9)	5 (3)	8 (5)	2 (3)	0 (0)	2 (3)	2.01
Child care	25 (17)	20 (13)	5 (3)	12 (16)	8 (11)	4 (5)	0.02
Basic education	8 (5)	5 (3)	3 (2)	7 (9)	6 (8)	1 (1)	1.28
Telephone	34 (23)	28 (19)	6 (4)	25 (33)	21 (28)	4 (5)	2.94
Transport	26 (17)	13 (9)	13 (9)	24 (32)	14 (19)	10 (13)	6.22[a]
Money	31 (21)	20 (13)	11 (7)	24 (32)	10 (13)	14 (19)	3.47
Welfare benefits	117 (78)	1 (1)	116 (77)	66 (88)	1 (1)	65 (87)	3.29

[a]$P < 0.05$; [b]$P < 0.01$; [f]$P < 0.001$: Comparisons between BD and schizophrenia on total, met and unmet needs and different types of needs. [1]Between A significantly greater proportion of relatives than patients with schizophrenia reported that needs in the area of information about the condition and its treatment were unmet ($\chi^2 = 13.79$; $P < 0.01$). BD: Bipolar disorder.

WHOQOL-BREF psychological-health domain scores - 2%) according to relatives' reports. The same variables explained about 28% to 35% of the variance in unmet needs scores according to patients' or relatives' reports, with 22% to 29% of the variance being explained by the GAF scores alone.

DISCUSSION

There could be two possible reasons for carrying out assessments of health-care needs in any group of patients[4,7,8]. Firstly, the needs elicited serve as a comprehensive index of the psychosocial outcome of the disorder. Secondly, such assessments provide a picture of needs from the perspective of patients and their relatives, indicating areas that could be targeted to improve the outcome of the disorder. The results of this study provide information particularly for remitted patients with BD on these two aspects.

Health-care needs among patients with BD according to patients

The average number of total needs reported by the patients themselves on the CAN-R was about six, which fell within the range of 4 to 10 needs reported by patients with severe mental illnesses on the CAN-R[18,20-22]. Though comparison with other studies was difficult because of differences in patient-samples, methodology and assessment instruments, the mean

number of total needs among patients with BD of the present study was quite similar to previous reports of patients with either BD[23-25], or severe mental illnesses including BD[9,18,22,26-28]. Combining the findings of the CAN-R and the scale for additional evaluation of needs suggested that needs were most frequently expressed in three or four broad clusters. The commonest of these were economic and welfare needs including needs for welfare-benefits, free treatment, financial help, travel concessions or disability benefits and help with obtaining jobs. The second group consisted of the need for information about the condition and its treatment, and for psychoeducational programmes for meeting this need. The third group consisted of social needs such as the need for help with household skills and help with psychological distress, the need for company and help with daytime activities, and the need for self-help facilities to cater to these social needs. Finally, physical health needs and the need for treatment of psychotic symptoms were also commonly expressed. The pattern of needs reported by patients of the current study was broadly similar to the ones reported by other studies of BD, which have found that needs are most frequently expressed in social, treatment, informational, and economic or welfare domains[9,23-25].

On the CAN-R a majority of the patients and relatives reported their needs to have been met. Nevertheless, needs in the areas of economic and welfare-benefits, information, company, daytime activities and physical

Table 4 Additional areas of needs: Patients' and relatives' reports[1]

	Bipolar disorder-mean (SD) (*n* = 150)	Schizophrenia-mean (SD) (*n* = 75)	*t* values
Needs of patients reported by patients			
Total needs	2.39 (1.87)	3.08 (2.08)	2.52[a]
Met needs	0.23 (0.61)	0.27 (0.64)	0.38
Unmet needs	2.15 (1.73)	2.81 (1.9)	2.6[a]
Needs of patients reported by relatives			
Total needs	2.67 (2.26)	3.33 (2.13)	2.13[a]
Met needs	0.19 (0.49)	0.48 (1.37)	2.29[a]
Unmet needs	2.47 (2.09)	2.85 (1.82)	1.34

	Patients			Relatives		
Seven common areas of additional needs-bipolar disorder (*n* = 150)	Total needs	Met needs	Unmet needs	Total needs	Met needs	Unmet needs
Free treatment	69	4	65	4	65	70
Medical reimbursement	56	3	53	3	53	67
Job reservations/occupational help	42	1	41	1	41	44
Financial help	35	4	31	4	31	44
Psychoeducation	30	10	20	10	20	29
Travel concessions	20	2	18	2	18	24
Patient groups, clubs, societies	17	0	17	0	17	22
Seven common areas of additional needs - schizophrenia (*n* = 75)						
Medical reimbursement	53	0	53	50	1	49
Free treatment	44	0	44	48	1	47
Job reservations/occupational help	35	1	34	32	4	28
Financial help	28	1	27	30	1	29
Psychoeducation	19	6	13	15	7	8
Travel concessions	12	0	12	15	2	13
Certification needs	11	1	10	11	4	7

[a]$P < 0.05$: Comparisons between BD and schizophrenia on total, met and unmet needs and different types of needs. [1]This additional evaluation was carried out using an instrument designed to cover those areas of needs not specifically covered by the CAN-R; it had 21 areas with a format similar to the CAN-R; only results pertaining to the seven most common needs are depicted. CAN-R: Camberwell Assessment of Need-Research version; BD: Bipolar disorder.

health-care needs were largely perceived as being unmet, and participants were mostly dissatisfied with the help received from the health-care services. In other areas patients received help from friends and family; therefore, these needs were reported as being met, and patients were satisfied with the help received. The additional evaluation also confirmed that economic, welfare and information needs were the ones most likely to remain unmet. The proportion of met *vs* unmet needs and the types of unmet needs in this study were very similar to several Indian studies, which have assessed health-care needs among patients with severe mental illnesses including schizophrenia and BD[18,22,25,29-31]. The pattern of primacy of economic and welfare needs in Indian studies is also quite unlike the pattern of needs reported in Western studies, where a greater amount of help and benefits are usually received from health-care services; therefore, social needs are more often unmet than economic, welfare or treatment needs[7,24,32-35]. These differences clearly reflect the inadequate support that patients receive from formal health-care services in India, which forces them to turn to their family and friends to fulfil their needs[18,22,26]. They also emphasize the fact that socio-cultural factors such as the pre-eminence of the family in providing care, and the limited reach of the local health-care services probably have a greater bearing on the pattern of needs, particularly

unmet ones, than other factors such as the type of psychiatric disorder[20,36,37].

Comparison of health-care needs between patients with BD and schizophrenia
That the type of psychiatric disorder has minimal influence on expressed needs was endorsed by other results of this study, which indicated that there were very few differences between patients with BD or schizophrenia in most aspects of health-care needs assessed. Nevertheless, the total number of needs, the number of met and unmet needs, and needs in the domains of company, financial help, transport and telephones were all significantly higher for schizophrenia. This was a consistent finding on the CAN-R as well as the additional evaluation of needs and across reports of both patients and their relatives. This was probably because patients with schizophrenia had greater levels of residual psychopathology even in their remitted stage than patients with BD. The fact that patients with schizophrenia reported greater needs in the area of psychotic symptoms, and that the severity of positive psychotic symptoms was associated with the extent of total and unmet needs provided further support for the notion that residual positive symptoms contributed to the greater number of needs in schizophrenia[30]. However, apart from these differences, the pattern of

Table 5 Correlates of health-care needs[1,2]

Univariate associations	CAN-R scores as per patients' reports (n = 225)			CAN-R scores as per caregivers' reports (n = 225)		
	Met needs	Unmet needs	Total needs	Met needs	Unmet needs	Total needs
Duration of illness	-0.237					
GAF scores		-0.422			-0.553	-0.443
WHOQOL total scores		-0.294			-0.367	-0.306
WHOQOL general		-0.288			-0.276	-0.306
WHOQOL physical health					-0.300	
WHOQOL psychological		-0.267			-0.287	-0.295
WHOQOL social relationship		-0.287			-0.304	-0.249
WHOQOL environment					-0.337	-0.242
Multiple regression analyses						
	Unmet needs - patients' reports			Unmet needs - relatives' reports		
	R square		Adjusted R square	R square		Adjusted R square
GAF	0.203		0.199	0.291		0.288
GAF, PANSS positive	0.270		0.264	0.340		0.334
GAF, PANSS positive, Psychological Health domain of WHOQOL- BREF	0.293		0.283	0.361		0.353
				Total needs - relatives' reports		
GAF				0.182		0.178
GAF, PANSS positive				0.236		0.229
GAF, PANSS positive, Psychological Health domain of WHOQOL- BREF				0.258		0.248

[1]Pearson's Product Moment Correlation coefficients or Spearman's Rank Correlation coefficients; [2]Only those significant associations that persisted after adjusting for multiple correlations using Bonferroni correction are depicted. Bonferroni value = 0.05/60 = 0.00083. CAN-R: Camberwell Assessment of Need-Research version; GAF: Global Assessment of Functioning; WHOQOL-BREF: World Health Organization Quality of Life Bref version; PANSS: Positive and Negative Syndrome Scale for Schizophrenia; BD: Bipolar disorder.

needs including the seven or eight areas where needs were commonly expressed, either on the CAN-R or on the additional evaluation were largely similar between the two groups. Other comparisons of health-care needs between schizophrenia and BD have generally reported a similar profile in both disorders[18,25,27,28], though one study found that patients with affective disorders had higher levels of unmet needs in certain areas[9].

Health-care needs: Patients vs relatives

Relatives' reports of needs on the CAN-R, including the most common needs, the overall pattern of needs, the proportion of needs met, and the differences between schizophrenia and BD were mostly similar to that of patients. However, for the BD group the number of total needs and unmet needs was significantly higher according to the relatives. Finally, there some differences between patients' and relatives' reports in individual domains of the CAN-R and the type of unmet needs, with relatives usually placing more emphasis on social and informational needs than the patients themselves. This was in line with most of the previous research on the subject, which has indicated that relatives generally report greater number of needs, and/or their perceptions regarding areas of need differ from those of patients[9,36,38,39]. Differing views of needs among patients and relatives could be a consequence of the additional component of caregiver-burden that relatives have to face, since certain studies have found that a higher level of caregiver-burden is usually associated

with higher levels of expressed needs and differences in the types of needs reported by relatives[9,40].

Correlates of health-care needs

The level of patient-functioning emerged as the single most important correlate of health-care needs, particularly unmet needs among both patient groups. This was similar to earlier reports of a positive association between greater number of needs and higher levels of dysfunction[7,18,21,29,33]. Moreover, the associations between needs and functioning, between needs and residual symptoms and between needs and QOL also underlined the fact the extent and pattern of needs was a useful index of the overall psychosocial status of remitted patients with BD or schizophrenia[6,7,33,34,41,42].

Limitations

The findings of this study need to be viewed in the context of its methodological limitations. Principal among these was that it was a hospital-based study of remitted patients from a single centre; this hinders the generalization of its results to other patient populations with differing clinical profiles. Moreover, though the CAN-R has been used among Indian patients it is yet to be properly validated in Indian settings, particularly among patients with BD. The fact that the additional evaluation carried out using a self-designed instrument yielded somewhat different findings suggests that the CAN-R might need some modifications before being used among Indian patients.

Conclusions

These methodological lacunae notwithstanding, several findings of this study may be of relatively novel significance. Firstly, patients with BD even when they were in remission had wide ranging health-care needs, many of which were unmet. Impaired functioning, residual symptoms and QOL emerged as the principal mediators of total and unmet needs. Thus, the presence of unmet health-care needs is an additional marker of the enduring psychosocial impairment characteristic of remitted BD. Accordingly, treatment of BD should place greater emphasis on addressing the unmet needs of patients with BD even after patients achieve remission. Secondly, relatives expressed a somewhat different pattern of needs than patients, which indicates that their input is vital for comprehensive assessment and management of needs in BD. Lastly, despite some differences the overall pattern in which economic and welfare needs superseded treatment and social needs was very similar across BD and schizophrenia. This suggests that socio-cultural and health-service related factors have a relatively greater impact on the pattern of needs than diagnostic categories. Though examination of health-care needs in BD remains a priority area for further research, it is equally important for future studies to incorporate the socio-cultural context while examining health-care needs of remitted BD, since this appears to be the appropriate way to improve the treatment and outcome of BD.

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COMMENTS

Background

Elicitation of health-care needs in any group of patients serves as a comprehensive index of the psychosocial outcome of the disorder. Such assessments also provide a picture of needs from the perspective of patients and their relatives indicating areas that could be targeted to improve the outcome of the disorder. Despite the obvious implications of examining health-care needs of patients, very few studies have chosen to focus exclusively on examining needs among patients with bipolar disorder (BD), particularly during phases of remission. This contrasts with the large amount of literature available on needs of patients with schizophrenia.

Research frontiers

The type of health-care needs is heavily dependent on the socio-cultural setting and the nature and quality of the local health-care services. However, very few studies from developing countries have undertaken comprehensive assessments of health-care needs among patients with mental illnesses. This information is necessary for judicious allocation of resources for treatment of mental illnesses in these countries.

Innovations and breakthroughs

Given the paucity of research in the area, this study attempted to assess health-care needs and their correlates in BD during remission, compared to remitted schizophrenia. The relatively novel findings of this study were: (1) patients with BD even when they were in remission had wide ranging health-care needs,

many of which were unmet; (2) impaired functioning, residual symptoms and quality of life (QOL) emerged as the principal mediators of total and unmet needs; (3) relatives reported more needs than patients and a somewhat different pattern of needs than patients; and (4) despite some differences the overall pattern in which economic and welfare needs superseded treatment and social needs was very similar across BD and schizophrenia indicating that socio-cultural and health-service related factors have a relatively greater impact on the pattern of needs than diagnostic categories.

Applications

The implications of these findings for the treatment of BD are that: (1) the presence of unmet health-care needs is an additional marker of the enduring psychosocial impairment characteristic of remitted BD; accordingly, treatment of BD should place greater emphasis on addressing the unmet needs of patients with BD even after patients achieve remission; (2) the input provided by relatives is vital for comprehensive assessment and management of needs in BD; and (3) it is important for future studies to incorporate the socio-cultural context while examining health-care needs in order to improve the treatment and outcome of BD.

Terminology

Health-care needs: The National Health Service and Community Care Act, 1990 defines "need" as the "requirement of the individual to achieve, maintain and restore an acceptable level of social independence and QOL". A health-care need is considered to be present when because of symptoms, distress or disability the subject's level of functioning falls below the optimum, and this is due to some potentially remediable or preventable cause.

Peer-review

The manuscript is relatively well-written and easy to follow.

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ORIGINAL ARTICLE

Randomized Clinical Trial

Influence of different second generation antipsychotics on the QTc interval: A pragmatic study

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Abstract

AIM
To investigate whether differential influence on the QTc interval exists among four second generation antipsychotics (SGAs) in psychosis.

METHODS
Data were drawn from a pragmatic, randomized head-to-head trial of the SGAs risperidone, olanzapine, quetiapine, and ziprasidone in acute admissions patients with psychosis, and with follow-up visits at discharge or maximally 6-9 wk, 3, 6, 12 and 24 mo. Electrocardiograms were recorded on all visits. To mimic clinical shared decision-making, the patients were randomized not to a single drug, but to a sequence

of the SGAs under investigation. The first drug in the sequence defined the randomization group, but the patient and/or clinician could choose an SGA later in the sequence if prior negative experiences with the first one(s) in the sequence had occurred. The study focuses on the time of, and actual use of the SGAs under investigation, that is until treatment discontinuation or change, in order to capture the direct medication effects on the QTc interval. Secondary intention-to-treat (ITT) analyses were also performed.

RESULTS
A total of 173 patients, with even distribution among the treatment groups, underwent ECG assessments. About 70% were males and 43% had never used antipsychotic drugs before the study. The mean antipsychotic doses in milligrams per day with standard deviations (SD) were 3.4 (1.2) for risperidone, 13.9 (4.6) for olanzapine, 325.9 (185.8) for quetiapine, and 97.2 (42.8) for ziprasidone treated groups. The time until discontinuation of the antipsychotic drug used did not differ in a statistically significant way among the groups (Log-Rank test: P = 0.171). The maximum QTc interval recorded during follow-up was 462 ms. Based on linear mixed effects analyses, the QTc interval change per day with standard error was -0.0030 (0.0280) for risperidone; -0.0099 (0.0108) for olanzapine; -0.0027 (0.0170) for quetiapine, and -0.0081 (0.0229) for ziprasidone. There were no statistically significant differences among the groups in this regard. LME analyses based on ITT groups (the randomization groups), revealed almost identical slopes with -0.0063 (0.0160) for risperidone, -0.0130 (0.0126) for olanzapine, -0.0034 (0.0168) for quetiapine, and -0.0045 (0.0225) for ziprasidone.

CONCLUSION
None of the SGAs under investigation led to statistically significant QTc prolongation. No statistically significant differences among the SGAs were found.

Key words: Psychosis; QTc prolongation; Antipsychotics; Clinical trial; Pragmatic design

Core tip: Antipsychotic drugs have a bad reputation of prolonging the QTc interval, and thereby possibly leading to fatal incidents of Torsade de pointes arrhythmias and sudden cardiac death. Differential propensities for QTc prolongation among second generation antipsychotics (SGAs) have been claimed, but lack substantial support from pragmatic studies. None of the SGAs was statistically significantly prolonging the QTc interval in the present pragmatic study, and no statistically significant differences among the drug groups were found for this outcome. Even in a situation with a substantial proportion with QTc prolongation at admittance any of the SGAs under investigation seemed to be safe choices in the present study.

INTRODUCTION

Sudden cardiac death (SCD) is a rare, but dramatic event in patients with schizophrenia, with a standardized mortality rate of 4.5 compared to in the general population[1]. Antipsychotic drugs have been implicated as conveying a risk for SCD because of their potential for prolonging the heart rate corrected QT (QTc) interval of the electrocardiogram (ECG) which may lead to polymorphic ventricular tachycardia [Torsade de pointes (TdP)], ventricular fibrillation and heart arrest[2,3]. Some agents have even been temporarily or permanently withdrawn from the market[4,5]. Indeed different propensities for inducing QT interval prolongation have been reported for various antipsychotic drugs, with ziprasidone among the worst offenders[2,6]. Several methodological issues have been raised however, with regards to how well differences, derived mainly from phase III randomized controlled trials (RCTs), might translate into usual clinical practice[2,7]. Potential limitations include the numerous exclusion criteria, with a risk of selection bias, and the short durations of most RCTs of antipsychotic efficacy. To combat some of the limitations, the pragmatic trial of effectiveness design has been launched in an attempt to deliver more relevant data for clinical decision makers. Effectiveness studies are characterized by heterogeneous samples and study settings more representative of usual clinical practice[8].

Pragmatic studies investigating the different propensities of QTc prolongation of second generation antipsychotics (SGAs) in real-life settings are rare, but we have previously reported QTc interval findings from a pragmatic RCT of SGAs in acutely admitted patients with psychosis and followed for 24 mo[9]. Only the intention-to-treat (ITT)/overall change of the QTc intervals during the full 24-mo follow-up were analysed in this study, regardless of the many drug changes that occurred during the study. Furthermore, we have recently published cross-sectional data on the proportion with prolonged QTc intervals at admittance and at the end of the acute treatment phase approximately 4 wk later[10]. About a quarter of the sample had borderline prolonged or prolonged QTc intervals at admittance, with a reduction of this proportion at follow-up. The substantial proportion with prolonged QTc intervals could theoretically be at particular risk if the "wrong" antipsychotic drug were initiated, and we want to compare risperidone, olanzapine, quetiapine, and ziprasidone head-to-head in a consecutive sample in the

period from initiation in the acute phase of psychosis, up until discontinuation, in order to determine which drug, if any, could be considered the safest in this regard.

Accordingly, the primary aim was to investigate whether differential influence on the QTc interval existed among the SGAs under investigation. We hypothesized that ziprasidone would increase the QTc interval during follow-up, and that the QTc interval change in those receiving ziprasidone would be different from that in the other drug groups.

MATERIALS AND METHODS

Study design
The Bergen Psychosis Project (BPP) compared the effectiveness of olanzapine, quetiapine, risperidone, and ziprasidone with a 2-year follow-up[9]. The study was approved by the Regional Committee for Medical Research Ethics (RCMRE) and the Norwegian Social Science Data Services, and was sponsored independently of the pharmaceutical industry. The study investigates the first allocated antipsychotic drugs until treatment discontinuation or change to another antipsychotic drug, to isolate the effect of the drugs on the QTc interval.

Patients
The RCMRE allowed eligible patients to be included before informed consent was provided. This enabled a clinically representative sample. Adults acutely admitted for psychosis were consecutively recruited when antipsychotic drugs in the oral formulation were indicated. A symptom threshold for inclusion was set at ≥ 4 on at least one of the following items of the Positive and Negative Syndrome Scale (PANSS)[11]: Delusions, Hallucinatory behavior, Grandiosity, Suspiciousness/persecution, or Unusual thought content. Diagnostic valuations were conducted by the clinical staff (psychiatrists or specialists in clinical psychology) according to the ICD-10 (http://apps.who.int/classifications/icd10/browse/2010/en). Exclusion criteria were: Antipsychotic drugs in the oral formulation not indicated, manic psychosis because of concerns of reduced cooperativeness with assessments, other behavioral or mental reasons causing inability to cooperate, language barrier towards spoken Norwegian, electroconvulsive therapy indicated, or established clozapine treatment. Drug-induced psychosis was not an exclusion criterium when antipsychotic drug therapy was deemed indicated by the attending clinician.

Treatments
Eligible patients were offered the first SGA in a random order of the investigational agents. The result of the randomization was known both to the clinical staff and the patient. The SGA that was first on the list defined the randomization group. The treating physician and/or the patient could select the next drug in the sequence if the first could not be used. Reasons for unselecting the first drug included contraindications, or negative

experiences with previous use of the drug. In theory, contraindications or previous negative experiences could also include QTc-interval pathologies but this was not the case for any of the patients screened for eligibility. The same procedure was repeated if the next drug could not be used. Doses, concomitant use of other medicines, or antipsychotic drug changes were determined by the attending physician or psychiatrist. Combinations of antipsychotic drugs were not allowed except in some sporadic instances.

Importantly, the present study does not focus on the randomization groups but on the actual chosen SGA from the sequence. We have previously reported that there were no statistically significant differences among the randomization groups regarding the percentage choosing a different SGA from the first one on the list[9].

Clinical assessments
Assessments were conducted at baseline, at discharge or at 6-9 wk from baseline if still admitted, and at 3, 6, 12, and 24 mo from baseline. Other than the PANSS interview, the assessments included the Calgary Depression Scale for Schizophrenia[12], the Clinical Drug and Alcohol Use Scales[13], the Clinical Global Impression-Severity of Illness scale[14], and the Global Assessment of Functioning-Split Version, Functions scale[15]. Blood was collected between 8 am and 10 am for analysis of serum levels of the antipsychotic drugs.

Until discharge, or at 6-9 wk at the latest the study procedures were part of the hospital's routine quality project for patients with psychosis, and the procedures were part of the patients' medical record. At this point, the patients were asked for informed consent to be contacted and included in the follow-up project.

At follow-up visits 3, 6, 12, and 24 mo after baseline, measures of psychopathology, blood sampling, and ECG recordings were repeated, and all medications were recorded.

QTc assessments
The QTc interval estimation was done automatically by a Philips Pagewriter Trim Ⅱ cardiograph at admission and discharge/at 6-9 wk when the patient was still in hospital. At later visits after discharge, a Schiller AT-101 cardiograph was used. Bazett's formula was used for correction. The ECG recording at baseline was done before the first administration of the study SGAs.

Statistical analysis
The baseline data of were analyzed using IBM SPSS software, version 23.0, and by means of exact χ^2 tests (categorical data) and one-way ANOVAs (continuous data). These tests were also applied for baseline comparisons between those lost to follow-up before retesting and those with repeated tests.

Power analyses were run in R (http://www.r-project.org) by means of linear mixed effects (LME) models. The baseline QTc interval and standard deviations were based on the results of the model used in the present
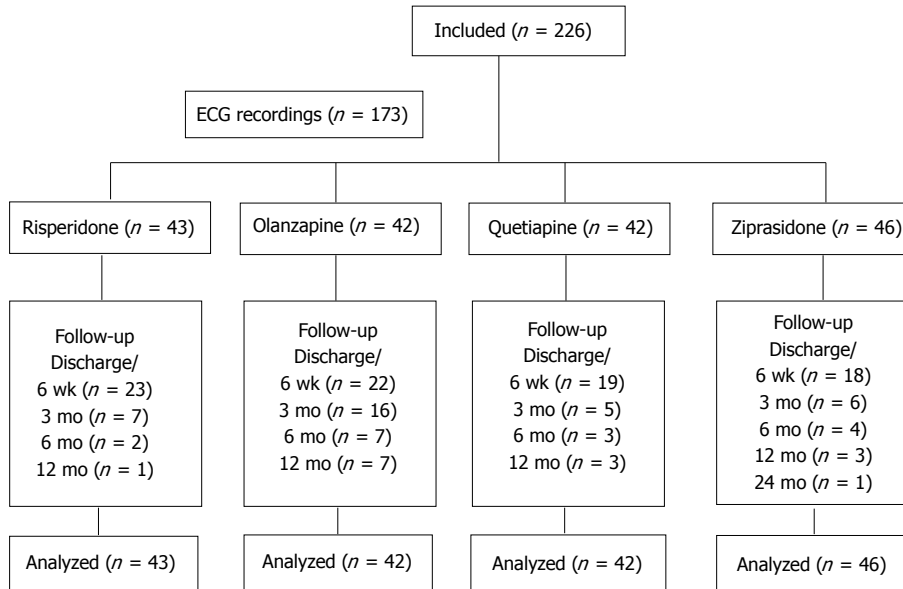
Figure 1 Flow of participants (*n*) through the study. ECG: Electrocardiogram.

study, and slope differences between the groups deemed to be of clinical significance were used in the model. The drop-out rate was set to 3% per month, and 10000 simulations were run. Based on the power analysis the study should have 96% power to detect 2.5% QTc interval differences between the drug groups with 30 subjects in each group.

Changes in the QTc intervals were analysed in R by means of LME models (http://www.r-project.org)[16]. Fixed effects, *i.e.*, systematic differences between the drugs, gave different linear slopes in the four treatment groups, technically a group-by-time interaction with a potential for baseline group differences. The sensitivity analyses based on the ITT groups had no baseline group differences as this was based on the randomization groups. The model calculated overall QTc interval change per day during follow-up that could be visually represented by the slope of a linear curve with time plotted against the QTc interval. The model utilized all available data and handled different numbers of visits by individual patients, as well as differences in time between visits. The LME has demonstrated superior statistical power in studies where missing data cannot be ignored[17], as is the case in the present study. Benjamini-Hochberg adjustments were applied for multiple comparisons. An α-level = 0.05, two-sided, was used as a threshold for statistical significance. The statistical review of the study was performed by a biomedical statistician.

RESULTS

A total of 226 patients were included in the study, and 173 patients underwent ECG assessments. The study enrolment and follow-up is presented in Figure 1.

Information about baseline demographics and clinical descriptives are given in Table 1. There were

no statistically significant differences between those with and without ECG assessments, respectively, for any baseline characteristic except for the distribution of alcohol use (exact χ^2 test: $P = 0.002$), with a larger proportion with alcohol dependency among those without ECG recordings compared to those with ECG recordings (21.2% *vs* 7.0%). There were no statistically significant differences among the SGAs for any of the descriptives except for a higher PANSS positive subscale score in the olanzapine group compared to the risperidone group (one-way ANOVA: $P = 0.025$; mean difference 2.7 points; 95%CI: 0.2-5.1), and compared to the ziprasidone group (one-way ANOVA: $P = 0.045$; mean difference 2.5 points; 95%CI: 0.4-4.9). There were no statistically significant differences for any baseline characteristic between those with only a baseline test and those with repeated tests.

The mean antipsychotic doses in milligrams per day with standard deviations (SD) were 3.4 (1.2) for risperidone, 13.9 (4.6) for olanzapine, 325.9 (185.8) for quetiapine, and 97.2 (42.8) for ziprasidone treated groups. The mean serum levels in nanomoles per liter with SD and dose reference ranges were 73.3 (54.6) (30.0-120.0) for risperidone, 108.2 (70.9) (30.0-200.0) for olanzapine, 414.2 (548.2) (100.0-800.0) for quetiapine, and 131.0 (101.0) (30.0-200.0) for ziprasidone. The time until discontinuation of the SGAs did not differ in a statistically significant way among the groups (Log-Rank test: $P = 0.171$).

There were no statistically significant differences among the groups for the concomitant use of another antipsychotic drug, antidepressant, mood stabilizer, benzodiazepine, or anticholinergic drug at any visit except for a higher proportion of concomitant benzodiazepine use in the quetiapine group at the 12-mo visit (exact χ^2 test: $P = 0.026$)

The maximum QTc interval recorded at any follow-

Characteristics	Risperidone (*n* = 43)	Olanzapine (*n* = 42)	Quetiapine (*n* = 42)	Ziprasidone (*n* = 46)	All patients (*n* = 173)
Gender					
Male	34 (79.1)	28 (66.7)	29 (69.0)	29 (63.0)	120 (69.4)
Antipsychotic naïve	17 (40.5)	15 (35.7)	21 (50.0)	20 (44.4)	73 (42.7)
Alcohol last 6 mo					
None/no misuse	10 (23.3)	7 (16.7)	7 (16.7)	10 (21.7)	34 19.7)
Dependency	2 (4.7)	4 (9.5)	6 (14.3)	0 (0.0)	12 (6.9)
Drugs last 6 mo					
None	26 (60.5)	31 (73.8)	30 (71.4)	32 (69.6)	119 (68.8)
Misuse	10 (23.3)	6 (14.3)	7 (16.7)	7 (15.2)	30 (17.3)
Diagnosis[1]	*n* (39)	*n* (42)	*n* (41)	*n* (44)	*n* (166)
Schz and rel.	22 (56.4)	25 (59.5)	25 (61.0)	22 (50.0)	94 (56.6)
Acute	4 (10.3)	4 (9.5)	2 (4.9)	4 (9.1)	14 (8.4)
Drug-induced	6 (15.4)	6 (14.3)	5 (12.2)	5 (11.4)	22 (13.3)
Affective	4 10.3)	4 (9.5)	6 (14.6)	5 (11.4)	19 (11.4)
Rest	3 (7.7)	3 (7.1)	3 (7.3)	8 (18.2)	17 (10.2)
Age mean (SD)	34.5 (15.6)	32.3 (11.2)	37.2 (14.8)	32.4 (13.5)	34.1 (13.9)
QTc admittance mean (SD)	422.1 (39.7)	420.9 (33.5)	421.1 (25.1)	420.4 (22.0)	421.1 (30.4)
PANSS total mean (SD)	73.4 (14.0)	76.0 (14.3)	73.6 (14.0)	70.8 (12.4)	73.4 (13.7)
PANSS positive mean (SD)	18.6 (4.9)	21.3 (4.6)	20.0 (3.6)	18.8 (3.9)	19.7 (7.5)
PANSS negative mean (SD)	20.8 (8.1)	18.3 (7.3)	19.2 (7.1)	18.4 (7.4)	19.2 (7.5)
PANSS general mean (SD)	34.0 (6.5)	36.4 (6.6)	34.4 (7.6)	33.6 (6.3)	34.6 (6.8)
CDSS mean (SD)	6.8 (4.9)	6.3 (4.9)	6.4 (4.9)	7.8 (6.4)	6.9 (5.3)
GAF-F mean (SD)	30.8 (5.9)	30.1 (6.0)	30.6 (7.2)	32.2 (5.0)	30.9 (6.0)
CGI mean (SD)	5.2 (0.6)	5.3 (0.7)	5.1 (0.7)	5.0 (0.6)	5.2 (0.6)

[1]Patients with missing diagnoses are not included in the list. *n*: Number of patients with ECG at baseline and or ECG at discharge; SD: Standard deviation; Antipsychotic naïve: No life-time exposure to antipsychotic drugs before index admission; First admission: Index admission was the first admission to a mental hospital; Misuse: Misuse or Dependence according to Drake *et al*[13]; Schz and rel.: Schizophrenia and related disorders: Schizophrenia, schizo-affective disorder, acute polymorphic psychotic disorder with symptoms of schizophrenia, acute schizophrenia-like psychotic disorder, delusional disorder; Acute: Acute psychosis other than those categorized under Schz and rel.; Affective: Affective psychosis; Rest: Miscellaneous psychotic disorders; All diagnoses are according to ICD-10; PANSS: The Positive and Negative Syndrome Scale; CDSS: The Calgary Depression Scale for Schizophrenia; GAF-F: The Global Assessment of Functioning, split version, Functions scale; CGI: The Clinical Global Impression, severity of illness scale.

up visit was 462 milliseconds (ms). None of the drug groups had statistically significant changes of the QTc interval (LME: $P \geqslant 0.36$ for all). The QTc interval change per day with standard error was -0.0030 (0.0280) for risperidone; -0.0099 (0.0108) for olanzapine; -0.0027 (0.0170) for quetiapine, and -0.0081 (0.0229) for ziprasidone (Figure 2).
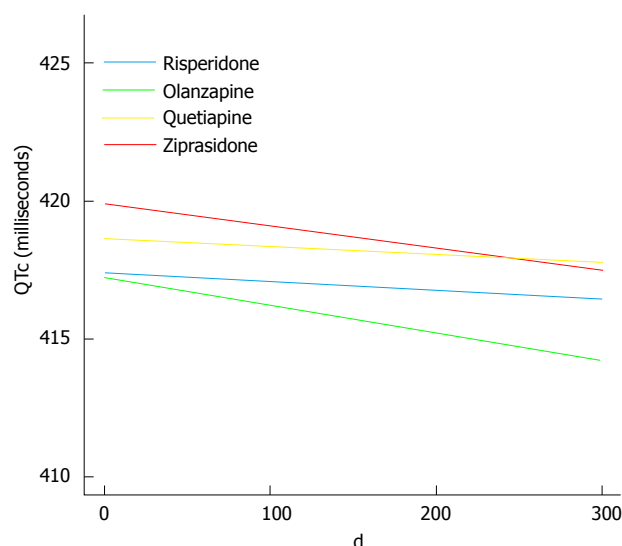

Figure 2 Change of QTc intervals. The curves were generated based on the drug-specific linear mixed effects slopes for risperidone, olanzapine, quetiapine, and ziprasidone, respectively. The curves are confined to the first 300 d because the bulk of data was obtained before this point in time.

There were no statistically significant differences among the groups for change of the QTc interval (LME: $P \geqslant 0.72$ for all). As a sensitivity analysis, we also performed LME analyses based on the ITT groups (the randomization groups), revealing almost identical slopes with -0.0063 (0.0160) for risperidone, -0.0130 (0.0126) for olanzapine, -0.0034 (0.0168) for quetiapine, and -0.0045 (0.0225) for ziprasidone.

Serum potassium, sodium, and calcium were measured at baseline and at first follow-up. There were no statistically significant differences among the drug groups for any of these electrolytes. Serum prolactin was measured at all points of follow-up. The prolactin level was higher in the risperidone group compared to the quetiapine group at baseline (one way ANOVA: $P = 0.015$; mean difference 350.3; 95%CI of the mean 46.7-654.0), and significantly higher in the risperidone group compared to all the other groups at first follow-up (one way ANOVA: $P = 0.004$). These differences did not persist at later visits.

DISCUSSION

SGAs are among the groups of drugs that have a bad reputation for prolonging the QTc interval, and thereby possibly leading to fatal incidents of TdP and SCD. This issue has received a lot of attention in previous studies, and differential propensities for QTc interval prolongation have been found among antipsychotic drugs[2]. The implications for usual clinical practice are unresolved, however, as the experimental designs of the majority of the studies may limit the generalizability of their findings[8]. The present study was conducted as close to clinical practice as possible by virtue of its pragmatic design. It aimed to investigate whether or not four first-line SGAs used in the acute treatment of psychosis

increased the QTc interval - and if so - is there a basis for a ranking between these drugs, regarding the risk of such a prolongation?

The main results of the study were that none of the SGAs prolonged the QTc intervals in a statistically significant way, and that no statistically significant QTC interval differences among the SGAs were found. These findings do not support some of the previous literature, including the comprehensive meta-analysis of 15 different antipsychotics by Leucht and colla-borators[6], where ziprasidone was among the top three antipsychotics with regards to QTc prolongation. A recent review also finds that ziprasidone prolongs the QTc interval, but with heterogeneous results in different studies[18]. Theoretically, short term treatment with antipsychotics may not give sufficient plasma levels to influence the heart cells but we find this unlikely as both mean doses and serum levels for the SGAs under investigation were in the therapeutic range. However, pharmacokinetic estimations are beyond the scope of the present study. Indeed, we have previously demonstrated that the distinct side-effect profiles derived from phase III RCTs are dampened in pragmatic studies[19].

Moving beyond the mean scores one might suspect the existence of a subgroup of patients with patholo-gically prolonged QTc intervals, but not numerous enough to alter the mean scores substantially. All the more there is reason to believe this group could be clinically very important. During follow-up, however, none of the participants had critically prolonged QTc intervals as the maximum QTc interval recorded was 462 ms. In a previous study we reported that approximately 25% of the patients had prolonged QTc intervals, or borderline prolongations, at the time of admittance to hospital[10]. Most likely, this prolongation was due to other causes than the use of SGAs including agitation, because many of the patients had never used antipsychotic drugs before, or those who had used SGAs had, in many instances, discontinued their antipsychotics some time before hospital admission. Taken together, the present study seems to indicate that, even in a situation with a substantial proportion of patients with QTc prolongation at admittance, any of the SGAs under investigation are safe choices.

Some limitations to the study need consideration. For one, attrition was substantial during follow-up. Two hundred and twenty-six patients participated in the study, but only 173 patients underwent ECG assess-ments because of feasibility issues in the acute phase. There were no statistically significant differences in baseline characteristics between those with and without ECG assessments except a small difference in alcohol consumption patterns. Therefore, selection bias seems unlikely. Furthermore, no baseline characteristics showed statistically significant differences between those with only baseline tests and those with repeated tests. Finally we chose LME statistics for the longitudinal data analyses to handle drop-outs and missing data.

Even though the BPP was a randomized study, the randomization was not to a single SGA but to a sequence of all of the four SGAs under investigation to mimic the clinical process of choosing a drug for a patient as closely as possible. The first drug in the sequence defined the randomization group, but about 20% chose a different SGA than the one first on the list in the sequence, although this proportion did not differ in a statistically significant way among the groups, as accounted for in a previous publication[9]. In the present study, we focused not on the randomization groups, but on the drugs actually used as we wanted to investigate the direct drug effects on the ECG. However, this also violates the effects of randomization and could introduce bias. However, the 173 patients divided themselves by chance into fairly even large groups for the four SGAs, and the only statistically significant baseline difference among the drug groups was a higher PANSS positive subscale score in the olanzapine group. Also no statistically significant differences in times to discontinuation were found. Finally, the sensitivity analyses based on ITT groups gave almost identical results. Significant bias therefore seems unlikely.

ECG assessments were carried out using two auto-matic measuring devices which both used Bazett's formula for heart rate correction of the QT intervals. As this formula has a tendency to overcorrect QT inter-vals at higher heart rates, Frederica's formula is now the preferred one. Both the stress associated with acute admissions as well as psychotropic medications themselves could increase the heart rate in patients with mental illness. Nielsen and collaborators[2] des-cribed incident differences in measurements by 12 ms when the heartrate was > 70/min (Bazett's 475 ms *vs* Frederica's 463 ms), and increases to 24 ms (508 *vs* 484) when the heartrate was > 80/min. Accordingly, the use of Bazett's formula in our study may have led to higher QTc measures compared to if Frederica's formula had been used. Ideally, all the recordings should have been carried out with the same equipment, but for practical reasons this was not possible. We have no reason to suspect that this should introduce bias, and believe the use of the same correction formula is the most important factor for comparable recordings.

The use of concomitant medication was equally distributed among the groups. However, it cannot be ruled out with certainty that the effects of other drugs may have influenced the slopes of the QTc recordings. The direction of any such influence would be hard to predict. There were more men than women in the study, which may have led to somewhat lower mean QTc total intervals as women have longer QTc intervals than men. This should not have introduced bias to the group comparisons as gender was evenly distributed among the drug groups. The risperidone group had higher serum prolactin levels at baseline and the first follow-up, but not thereafter. Any influence on the QTc interval from the prolactin level could only be speculative.

Despite the above mentioned limitations we con-clude that our findings do not support that any of the

SGAs under investigation leads to QTc prolongation. No statistically significant differences among the SGAs were found, and all the drugs on which the study is based may be considered to be safe alternatives in this regard.

ACKNOWLEDGMENTS

COMMENTS

Background
The ability of antipsychotic drugs to prolong the QT interval of the electro-cardiogram (ECG) has been associated with increased risk of sudden cardiac death in patients using these drugs.

Research frontiers
Although differential propensities among various antipsychotics for QT prolongation have been found, the generalizability to, and hence the clinical relevance of these findings in, the population have been questioned.

Innovations and breakthroughs
The majority of clinical antipsychotic drug trials typically include highly selected patient samples where concomitant illicit drug use, other mental or physical comorbidities, or the need for other psychotropics are exclusion criteria. Furthermore, the follow-up is often of short duration. The main advantages of the present study include the consecutive recruitment of acutely admitted patients with psychosis; the diagnostically and symptomatically heterogeneous sample, and the long follow-up. These factors should make the results more applicable for pharmacological decision making in clinical departments.

Applications
Based on the present study, all the second-generation antipsychotics under investigation seem to be safe choices in acutely admitted patients with psychosis.

Terminology
The QT interval: The ECG measure of the duration of ventricular de- and repolarization of the heart.

Peer-review
In this paper, the authors investigated investigate whether differential influence on the QTc interval exists among four second generation antipsychotics (SGAs) in 173 patients. They concluded that none of the SGAs under investigation led to QTc prolongation and no differences among the SGAs were found. This study is straightforward and generally well described.

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SYSTEMATIC REVIEWS

Cognitive-behavioural therapy for obsessive-compulsive disorder co-occurring with psychosis: Systematic review of evidence

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Abstract

AIM
To review available evidence on the use of cognitive behavioural therapy (CBT) for treating obsessive compulsive disorder co-occurring with psychosis.

METHODS
In this paper we present a detailed and comprehensive review of the current literature focusing on CBT treatment of obsessive compulsive disorder (OCD) co-occurring with schizophrenia or schizoaffective disorder. We identified relevant literature published between 2001 and May 2016 through MEDLINE/PubMed search using as search string ("obsessive compulsive disorders" or "obsessive compulsive symptoms") and ("schizophrenia" or "schizoaffective disorder" or "psychosis") and ("cognitive behavioural therapy"). Other citations of interest were further identified from references reported in the accessed articles. The search was limited to studies written in English and carried out in adult patients. A total of 9 studies, 8 case reports and 1 case series, were found.

RESULTS
The reviewed evidence indicates that CBT is: (1) safe, *i.e.*, does not worsen psychotic symptoms; (2) well accepted, with a discontinuation rate quite similar to that reported for patients with OCD without psychosis comorbidity; (3) effective, with a symptom reduction quite similar to that reported for patients with OCD without psychosis and for SRIs treatment of OCD co-occurring with psychosis; and (4) effective in patients with OCD induced by second-generation antipsychotic as well as in patients with OCD not induced by second-generation antipsychotic. Alcohol/substance use disorder comorbidity and OCD onset preceding that of SCH/SA was predictors of poor outcome. These results are derived only by additional studies with adequate sample size.

CONCLUSION
Our results support the use of CBT for OCD in patients with psychosis.

Key words: Obsessive compulsive disorder; Obsessive compulsive symptoms; Schizophrenia; Schizoaffective disorder; Cognitive behavioural therapy; Second-generation antipsychotic; Clozapine

Core tip: Ten percent of patients with schizophrenia fulfill criteria for obsessive compulsive disorder (OCD) and in 1/3 of cases OCD onset is related to second-generation antipsychotic (SGA) treatment. Reviewed evidence indicates that cognitive-behavioral therapy for OCD in patients with psychosis is: (1) safe (does not worsen psychotic symptoms); (2) well accepted (discontinuation rate similar to that reported for patients with OCD without psychosis); (3) effective (symptom reduction similar to that reported for patients with OCD without psychosis); and (4) effective in patients with OCD induced by SGA as well as in patients with OCD not induced by SGA. These conclusions are preliminary.

INTRODUCTION
The association of schizophrenia (SCH) or schizoaffective disorder (SA) with obsessive compulsive disorder (OCD) or symptoms (OCS) is quite common. In a meta-analysis of 36 studies, including a total of 3.308 patients with SCH, the pooled prevalence rate reported for OCD was 12% and for OCS of 30%[1]. This prevalence rate is higher than that of OCD in general population (2%-3%)[2] and of SCH in patients with primary OCD (1.7%)[3]. Up to 20% of patients with OCS/OCD co-occurring with psychosis report the onset or aggravation of obsessive compulsive symptoms after beginning treatment with a second-generation antipsychotic (SGA), mainly with serotonergic antagonist antipsychotics as clozapine and, at less extend, with olanzapine[4-9]. Some authors suggested that in these cases OCS might be considered an adverse event of SGA and introduced the term "antipsychotic-induced OCS" or "secondary OCS" (s-OCS)[5,10]. Nevertheless, because sometimes OCS occur or worsen also under no treatment or treatment with first-generation antipsychotics which are not primarily 5HT2-R-antagonistic[11], an interaction between genetic/biological predispositions, psychosocial factors and treatments could better explain the phenomenon[12].

The presence of OCS in patients with schizophrenia is associated with depressive symptoms, high suicide risk, cognitive impairment, poor social functioning, poor perceived quality of life, and poor prognosis[13-18]. The relationship between OCS and positive and negative symptoms is unclear[19]. Although etiological hypotheses have been put forward to explain the high OCS/OCD co-occurrence in patients with schizophrenia, the causes of this comorbidity remain unclear. As reported by Schirmbeck *et al*[20]: (1) epidemiological data do not confirm the hypothesis of a random association between the two syndromes; (2) clinical data do not confirm the hypothesis that OCS/OCD protects against psychotic disintegration[21,22]; and (3) to date, results of neurobiological studies attempting to validate the hypothesis of a separate subtype of psychosis, a so-called "schizo-obsessive disorder"[23,24] comprising typical positive, negative and cognitive symptoms of SCH and OCS are inconsistent.

Despite the increasing awareness that OCS/OCD co-occurring with SCH-SA are common and disabling, research on treatment strategies for these complex and treatment-resistant patients is scanty. The American Psychiatric Association practice guidelines[25] suggest to stabilize first psychotics symptoms using an antipsychotic drug and subsequently to treat OCS by the augmentation with a serotonin reuptake inhibitor (SRI), *e.g.,* citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine, sertraline, venlafaxine, and clomipramine. Evidence on the efficacy and safety of this augmentation strategy is limited and controversial, and is based to our knowledge on 16 studies (132 patients), most of which are single or multiple case reports. Several studies demonstrated the beneficial effect of antipsychotic-SRI combination, while some studies showed poor response, a risk of psychosis worsening and sometimes aggressiveness (for a review see[26]). Furthermore, the antipsychotic-SRI combination produces some clinically significant pharmacokinetic drug interactions: (1) some SRIs (such as fluvoxamine, fluoxetine, paroxetine and venlafaxine) may increase the plasma concentration of particular antipsychotics (such as clozapine, olanzapine, risperidone) by inhibition of hepatic cytochrome P450 isoenzymes (*e.g.,* 1A2, 2D6) and consequently may increase the risk of adverse events; and (2) the anticholinergic properties of clomipramine limit its use in elderly patients and in those treated with low-potency typical antipsychotics or anticholinergic agents. Some authors suggested to treat OCS co-occurring with psychosis by augmenting antipsychotics with a mood stabilizer, but evidence supporting this strategy is limited to 11 patients treated with lamotrigine[27]. As regards s-OCS, several options were proposed: Waiting for spontaneous resolution, gradually reducing the antipsychotic dosage, switching to another antipsychotic, combining an antiserotoninergic SGA with either a dopaminergic SGA (amisulpiride or aripiprazole) or a mood stabilizer, and augmenting SGA with a SRI[25,26]. So far, very limited evidence supports each of these options, that are generally grounded on theoretical

considerations and/or on the findings of single case reports or case series. Furthermore, the use of SRIs in patients with psychosis is not always safe, as previously discussed, and the dose reduction of clozapine or the switch from clozapine to another SGA could induce an exacerbation of psychotic symptoms.

Hence, an alternative to pharmacological approaches to primary and secondary OCS/OCD co-occurring with SCH/SA is needed.

Of the other existing treatment options for non-comorbid OCD, cognitive-behavioral therapy incorporating exposure and ritual prevention (CBT) is the psychological therapy most supported by research evidence[25].

The aim of this study is to review available evidence on the use of CBT with or without ritual prevention for treating OCD co-occurring with SCH/SA.

MATERIALS AND METHODS

In this paper we present a detailed and comprehensive review of the current literature focusing on CBT treatment of OCD co-occurring with SCH/SA. We identified relevant literature published between 2001 and May 2016 through MEDLINE/PubMed search using as search string ("obsessive compulsive disorders" or "obsessive compulsive symptoms") and ("schizophrenia" or "schizoaffective disorder" or "psychosis") and ("cognitive behavioural therapy"). The title and the abstract of the retrieved articles were reviewed by the two authors independently and non-pertinent papers were excluded. Of 182 papers screened, only papers including original articles which directly addressed CBT treatment for OCD co-occurring with psychosis were retained for review and inclusion in this study. Other citations of interest were further identified from references reported in the accessed articles. The search was limited to studies written in English and carried out in adult patients. A total of 9 studies, 8 case reports and 1 case series, including overall 31 patients, were found.

RESULTS

Effectiveness of CBT for OCD comorbid with psychosis

No randomized, controlled trials investigated the efficacy of CBT for OCD in patients with psychosis. However, several important suggestions can be derived from the identified case reports and case series. Table 1 shows the demographic and clinical characteristics and the response to CBT of the 10 patients included in the 8 case reports; characteristics and treatment response of the 21 patients included in the case series are reported separately[28-35]. Briefly, 8 patients were male, the mean age was 28 years (range 19-50), and the mean duration of OCD before starting CBT was 7 years (range 1-15). In 1 patient CBT did not include ERP strategies, and in 6 patients psychological treatment was supplemented with pharmacological treatment (SSRIs). One patient, despite an initial reduction of OCS after starting CBT, dropped out. Of the other 9 patients, 5 showed a full

remission and 4 a clinical relevant decrease of OCS severity. Some studies reported follow-up assessments, lasting from 6 mo to 3 years, suggesting a long-term stabilization of the improvement. Although case reports suggest a potential benefit of CBT for OCD co-occurring in psychosis, caution is needed in interpreting these results because of the small number of cases and the heterogeneity of the treatment as regards CBT duration (from "few" to 45 h) and concomitant use of SSRIs.

More homogeneous and clinically useful information can be derived from the case series reported by Tundo *et al*[36] in a naturalistic study including 21 consecutive patients (age 18-65 years) meeting DSM-IV[37] criteria for OCD of at least moderate severity [Yale-Brown Obsessive Compulsive Scale[38,39] (Y-BOCS) total score $\geq$ 16] and either for SCH or SA of up to moderate psychotic severity (Positive and Negative Symptoms[40] total score < 95). Treatment included antipsychotics, in association with mood stabilizers in SA patients (50% of cases), for SCH or SA and CBT for OCD. Patients were treated in a tertiary care setting, in which treatment guidelines were personalized taking into account each patient's insight into illness, treatment adherence, Axis I comorbidity and alcohol/substance use disorder. ERP strategies were supplemented with cognitive techniques and other ad hoc interventions, when necessary. Psychotherapy was scheduled flexibly: The mean number of CBT sessions was 34 (range 23-41) in patients with SA and 31 (range 8-40) in patients with SCH. During the study, 5 patients with SCH discontinued the therapy: One refused it after the first session, 1 was hospitalized because of the worsening of psychosis and 3 said that CBT was ineffective. Patients who dropped out from the study had their last observation carried forward for statistical analysis, thus 21 patients were analyzed.

The results showed a significant OCS reduction over 12 mo (Y-BOCS total score 30.8 ± 6.7 at baseline, 22.3 ± 8.3 after 12 mo of treatment), as well as improvements in severity of illness, as measured by Clinical Global Impression-Severity[41] (CGI-S) (5.5 ± 1.6 at baseline, 4.5 ± 1.0 after 12 mo of treatment), and functional improvement, as measured by the Global Assessment of Functioning[42] (GAF) (49.2 ± 10.1 at baseline, 55.9 ± 12.3 after 12 mo of treatment). At the end of the trial, 52% patients were rated as much or very much improved, 33% as responders and 19% as remitters. The 1-year change from baseline in the YBOCS score was 8.1 (95%CI: 5.4-10.8), only slightly lower than that observed in pre-to-post treatment comparisons of ERP (mean 11.4; range 10.5-12.2), and CBT studies (mean 10.6; range 8.5-12.8) in primary OCD[43]. Furthermore, insight into illness significantly increased.

Effectiveness of CBT for OCD induced by SGA

MacCabe *et al*[28] first described the case of a men with OCS emerging one year after starting clozapine and responding to 4 mo of CBT (Y-BOCS total score decreased from 12 to 4). The result was maintained at

Ref.	Demographic characteristics	OCD duration	Treatments	CBT duration	OCD response	Follow up
Ganesan *et al*[30]	Male, 33 yr	12 yr	CBT/ERP + SSRI	NA	Remitted	8 wk
	Male, 31 yr	11 yr	CBT/ERP + SSRI	NA	Improved	8 wk
	Female, 25 yr	1 yr	CBT/ERP + SSRI	NA	Improved	8 wk
MacCabe *et al*[28]	Male, 50 yr	5 yr	CBT/ERP	4 mo	Remitted	11 mo
Ekers *et al*[31]	Male, 31 yr	15 yr	CBT/ERP	20 h	Remitted	6 mo
Peasley-Miklus *et al*[29]	Male, 22 yr	12 yr	CBT/ERP	6 mo	Responded	36 mo
Rufer *et al*[32]	Female	NA	CBT/ ERP + SSRI	45 h	Improved	15 mo
Kobori *et al*[33]	Male, 26 yr	6 yr	CBT + SSRI	19 h	Remitted	24 mo
Rodriguez *et al*[34]	Male, 19 yr	< 2 yr	CBT/ERP + SSRI	Few hours	Dropped out	
Hagen *et al*[35]	Male, > 20 yr	several years	CBT/ERP	9 h	Remitted	6 mo

Table 1 Case reports of cognitive-behavioural therapy for obsessive-compulsive disorder co-occurring with psychosis

OCD: Obsessive compulsive disorder; CBT: Cognitive behavioural therapy; ERP: Exposure and responser prevention; SSRI: Selective serotoninergic reuptake inhibitor; NA: Not available.

follow-up, 11 mo later.

Recently, Tundo *et al*[44] reanalyzed their case series to compare the adherence to and the effectiveness of CBT in patients with SCH/SA and comorbid primary OCD (p-OCD) to those with secondary OCD (s-OCD). As suggested by Schirmbeck *et al*[45], they used the order of three events (first psychotic manifestation, start of SGA treatment and subsequent onset of OCD) to define s-OCD. The authors reported an OCD induction in 7 out 21 patients, related to olanzapine in 4 patients and to clozapine in 3 patients. Neither of these drugs nor their dosages were changed during the study.

During the trial the improvement of OCS did not differ significantly between s-OCD and p-OCD (Y-BOCS total score at baseline 28.0 ± 2.3 and 32.1 ± 1.6, respectively; after 12 mo of treatment 24.0 ± 2.1 and 24.5 ± 1.5, respectively), while global functioning, as measured by GAF, improved more rapidly in patients with p-OCD. At 12 mo drop-out rates (s-OCD 14.3% *vs* p-OCD 28.6%) were lower and improvement (s-OCD 57.1% *vs* p-OCD 50%), response (s-OCD 42.9% *vs* p-OCD 28.6%) and remission (s-OCD 42.9% *vs* p-OCD 7.1%) rates proved to be higher in patients with s-OCD, although not significantly. The findings indicate that the adherence to CBT in patients with psychosis and s-OCD did not differ from that of patients with psychosis and p-OCD and the drop-out rate is similar to that reported in the literature for CBT in patients with OCD without psychosis comorbidity[44]. Improvement, response and remission rates in s-OCD group did not differ from those of p-OCD group and are quite similar to those reported in the literature for pharmacological treatment of OCD comorbid with schizophrenia[44].

Predictors of response

Tundo *et al*[46] identified two outcome predictors of CBT effectiveness on co-occurring OCS: The alcohol/substance use disorder comorbidity and the temporal onset of OCD compared to that of SCH/SA.

Patients with alcohol/substance use disorder were significantly less likely to improve than those without this comorbidity (0% *vs* 68%, respectively; P = 0.012).

The rate of improvement was lower in patients in which OCD onset preceded that of SCH/SA than in patients in which OCD onset occurred after that of SCH/SA or in patients in which the onset of the two disorders was simultaneous (0%, 50%, and 83.3%, respectively; P = 0.067).

CBT tolerability

One reason why CBT as treatment for OCS co-occurring with schizophrenia has been scarcely investigated can be related to safety and tolerability concerns[47]. In this regard, a focus group evaluating clinician's perceptions on CBT use among patients with severe mental illness reported the fear that intervention-related arousal would result in severe exacerbation of psychotic symptoms[48].

However, the results of the studies included in this review do not support these concerns and, on the contrary, suggest that CBT not only significantly decreases OCS severity, but also ensures a stable remission of psychosis or even the improvement of psychotic symptoms.

In fact, psychotic exacerbation was reported in 2 of 31 patients reviewed. In one case the patient showed reluctance to commit to ERP so the therapist focused on different cognitive techniques[29]. In the other case CBT was discontinued because of psychotic exacerbation and subsequent hospitalization after more than 6 mo of psychotherapy[36]. The authors argued that the worsening of psychotic symptoms was related to the natural course of schizophrenia and not to the symptom intensification triggered by the involvement in ERP.

Therefore, available results, although limited by the small sample size and the lack of controlled clinical trials, provide encouraging evidence about safety and tolerability of CBT in patients with OCS/OCD co-occurring with psychosis.

DISCUSSION

In patients with schizophrenia or schizoaffective disorder the co-occurrence of OCD or OCS is quite common (12% and 30%, respectively) and it is associated with

high impairment (great burden of disease, anxiety and depressive symptoms, suicide risk, cognitive impairment, poor social and vocational functioning, and poor prognosis). In about 1 in 4 cases the onset or aggravation of OCS took place after the beginning of SGA treatment. In clinical practice the more frequent treatment of p-OCD and s-OCD is pharmacotherapy, mainly the association of antipsychotic and SRI, while data on the efficacy and the safety of pharmacotherapy are limited and controversial.

The results of the present review support the use of CBT for treating OCS/OCD in patients with SCH/SA. The available data show that this psychological treatment is: (1) safe, *i.e.,* it does not worsen psychotic symptoms; (2) well accepted, with a drop-out rate quite similar to that reported for patients with OCD without psychosis comorbidity; (3) effective, with a symptoms reduction quite similar to that reported for patients with OCD without psychosis and for SRIs treatment of OCD co-occurring with psychosis; and (4) effective in patients with s-OCD as well as in patients with p-OCD. Only subjects with lifetime alcohol/substance use disorder pose a challenge.

Our findings should be interpreted taking into account the limitations of studies included in this review: (1) they are all case-reports; (2) they include a small number of patients; (3) their methodological quality is low; and (4) they do not include a control group or control treatments and, as a consequence, it is not possible to attribute the observed effects to CBT, to the natural course of illness or to non-specific therapeutic factors. So, randomized clinical trials and observational studies with larger samples are required to confirm the safety, the tolerability and the efficacy/effectiveness of CBT for OCS/OCD in patients with psychosis. Despite these limitations, however, the available evidence provides useful information for clinicians planning OCS treatment in patients with SCH/SA and suggests that in these patients CBT might be a viable alternative to pharmacological treatment with SRI. So, in our opinion psychiatrists should not only rely on pharmacotherapy, the most common treatment in clinical practice (for a review see[49]), but also on CBT, to select the appropriate treatment for each patient according to their clinical judgment. According to our experience, pharmachotherapy should be used in patients who either refused or did not respond to CBT and, *vice versa,* CBT should be tried in patients who did not respond to medication or are at higher risk of psychotic exacerbation. In high resistant patients further potential options could be some somatic treatments (*e.g.,* electroconvulsive therapy, repetitive transcranial magnetic stimulation, deep brain stimulation)[50-52] and psychological treatments alternative to CBT (*e.g.,* psychodynamic therapy)[53]. Further clinical trials are warranted to accrue evidence on the efficacy of CBT as well as pharmacological treatment and their combination and to provide useful information to define specific guidelines for the treatment of OCS/OCD in schizophrenia.

COMMENTS

Background
More than 10% of patients with schizophrenia (SCH) have comorbid obsessive compulsive disorder (OCD) and in about 20% of cases the onset or worsening of obsessive compulsive symptoms (OCS) is related to treatment with second-generation antipsychotic (SGA), typically serotonergic antagonist antipsychotics as clozapine and olanzapine. The OCS-psychosis comorbidity is associated with high suicide risk, cognitive impairment, poor social functioning and quality of life, and poor prognosis. Management of this condition is a hard challenge for physicians and the evidence on the efficacy and safety of antipsychotic-SRI combination, the most frequent treatment in clinical practice, is limited and controversial.

Research frontiers
An alternative to pharmacological approach to OCS/OCD co-occurring with psychosis could be cognitive-behavioral therapy incorporating exposure and ritual prevention (CBT), the currently available psychological treatment for non-comorbid OCD most supported by research evidence.

Innovation and breakthroughs
The present article aims to review available evidence on the use of CBT for treating OCD co-occurring with SCH.

Applications
The review suggests that CBT for OCD co-occurring with psychosis is: (1) safe, *i.e.,* does not worsen psychotic symptoms; (2) well accepted, with a discontinuation rate quite similar to that reported for patients with OCD without psychosis comorbidity; (3) effective, with a symptom reduction quite similar to that reported for patients with OCD without psychosis and for SRIs treatment of OCD co-occurring with psychosis; and (4) effective in patients with OCD induced by SGA as well as in patients with OCD not induced by SGA.

Terminology
The study does not include terms that may not be familiar to the majority of the readers.

Peer-review
This is a comprehensive review of the literature.

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