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EDITORIAL

A common genetic mechanism underlying susceptibility to posttraumatic stress disorder

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Abstract

We hypothesize that susceptibility to post-traumatic stress disorder (PTSD) may be determined in part by aberrant microtubule-associated protein tau expression in neurons of critical brain structures. The following lines of evidence support this hypothesis. First, epidemiologic data suggest the involvement of genetic factors in the susceptibility to PTSD. Second, the common features of both abnormal tau expression and PTSD include amygdalar and hippocampal atrophy, upregulation of norepinephrine biosynthetic capacity in the surviving locus coeruleus neurons and dysfunction of *N*-methyl-*D*-aspartate-receptors. Finally, our experiments using rTg4510 mice, a model that over-expresses human mutant tau and develops age-dependent tauopathy, demonstrate that these animals display circling behavior thought to be related to states of anxiety. To detect the potential molecular mechanisms underlying PTSD episodes, laser-assisted/capture microdissection can be used with microarray analysis as an alternative approach to identify changes in gene expression in excitatory and/or inhibitory neurons in critical brain structures (*i.e.*, hippocampus and amygdala) in response to the onset of PTSD.

Key words: Amygdalar damage; Anxiety behavior; Microarrays; Microdissection; Microtubule-associated protein tau; Post-traumatic stress disorder; RNA quality

Core tip: We propose that susceptibility to post-traumatic stress disorder (PTSD) may be determined, in part, by aberrant microtubule-associated protein tau expression in neurons of critical brain structures. We review several lines of evidence to support this novel hypothesis. In addition, we review types of PTSD, namely non-classical PTSD, induced by various medical conditions and address this issue of why non-classical PTSD can be reliably elicited. To verify our hypothesis, we propose to use animal models of PTSD combined with laser-assisted/capture microdissection and microarray analysis to examine gene expression changes in selected cellular elements in response to the occurrence of PTSD.

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INTRODUCTION

Defining posttraumatic stress disorder

Posttraumatic stress disorder (PTSD) is an anxiety disorder which can develop following exposure to a traumatic event such as combat, natural disasters, domestic violence, or other catastrophes. Epidemiological studies demonstrate that 4%-23% of those experiencing a traumatic event develop PTSD[1-4]. The lifetime prevalence of PTSD among United States citizens is approximately 8%[5,6]. Functionally, abnormalities in amygdala, prefrontal cortex, and hippocampus as well as abnormalities in neuroendocrinologicol characteristics may be associated with PTSD[7].

The development of PTSD requires exposure to a traumatic event which is then followed by the altered regulation of the neural circuits that govern what is often termed the "fight-or-flight" response. As reviewed by Sherin and Nemeroff[8], this dysregulation likely involves norepinephrine, γ-aminobutyric acid (GABA), serotonin, and neuropeptide Y and includes the hippocampus, amygdala and the prefrontal cortex. Still, the majority of those exposed to a traumatic event do not develop PTSD and thus, risk factors have been identified which indicate increased vulnerability. These risk factors include smaller hippocampal volume, below normal executive function abilities, poorer attention, older age at time of traumatic event, female gender, and co-morbid disease[3,4,9]. Further, gene-environment interactions as well as epigenetic influences are likely to be important factors in the consideration of PTSD risk[10,11]. Still, the molecular and pathogenic bases underlying vulnerability to PTSD are largely unknown.

Non-classical PTSD

PTSD-like symptoms can arise after serious health events that may be associated with brain damage/neural loss. We call this medically induced condition "non-classical PTSD" to discriminate it from the classical PTSD qualified for standards of the fifth edition of the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (DSM-5). Such medical events include stroke[12,13], brain trauma[14], acute coronary syndromes (ACS)[7], a brief treatment in an intensive care unit[15] and even hematopoietic stem-cell transplantation therapy for cancer[16,17]. The diagnostic criteria for non-classical PTSD are similar to those for classical PTSD and include symptoms such as re-experiencing, cognitive or behavioral avoidance of reminders of the event, and physiological hyperarousal following occurrence of the above mentioned critical medical events. Per the DSM-5, non-classical PTSD should not be included in the PTSD category since one of its exclusive criteria is that the medical condition(s) and not a traumatic/catastrophic event(s) is the eliciting cause. Interestingly, the prevalence of non-classical PTSD is similar to that of classical PTSD. For example, the overall prevalence of ACS-induced PTSD is 12% and individual study prevalence estimates range 0%-32%[7]; and incidence of stroke-elicited non-classical PTSD is 10%-31%[13,18]. Further, non-classical PTSD appears to be unrelated to neurologic impairment[18]. In addition, the biological basis underlying the occurrence of non-classical PTSD remains unclear, even when substantial brain damage is involved. One approach is to examine whether non-classical PTSD shares, at least partially, a common biological basis/pathogenic pathway with that described for classical PTSD. Modeling non-classical PTSD should be feasible and/or reproducible in experimental animals because the key PTSD brain structures, such as the amygdala and/or the hippocampus, could be directly targeted.

HYPOTHESIS

Here, we hypothesize that susceptibility to PTSD may be determined in part, by aberrant tau expression in the amygdala and hippocampus. This abnormal expression is thought to then interfere with the normal cognitive processes in response to traumatic events, thus conferring vulnerability to PTSD development.

GENETIC FACTORS AND PTSD

As defined by the DSM-5, PTSD symptoms include four main types: re-experiencing the traumatic event, avoiding reminders of the trauma, negative cognitions and mood, and increased anxiety/emotional arousal. Clinical reports clearly support a role for genetic factors in the development of PTSD[19]. Quantitative genetic analyses of monozygotic and dizygotic male twin pairs reveal that genetic factors account for 13%-30% of the variance in liability for symptoms in the "re-experiencing" cluster, 30%-34% for symptoms in the "avoidance cluster" and 28%-32% for symptoms in the "arousal cluster" [20]. Hyperresponsivity in the dorsal anterior cingulate is proposed as a familial risk factor for the development of PTSD following psychological trauma[21]. A report on 200 members of 12 multigenerational families that experienced an earthquake demonstrated the likelihood of inherited vulnerability to symptoms of PTSD[22]. The specific genes that may cause increased PTSD susceptibility have not been identified. However, in the first genome-wide association study of PTSD, several single-nucleotide polymorphisms (SNPs) were associated with PTSD[23]. It has been hypothesized that strong memory of a traumatic event could contribute to PTSD development and symptoms, and a genetic inclination for strong memories might confer an increased risk. In support of this, a specific SNP within the gene that encodes protein kinase C alpha, a memory-relevant gene, may be linked to increased PTSD risk[24]. Nevertheless, clinical association studies have not established a causative relationship between any specific gene and PTSD.

PTSD AND ALZHEIMER'S DISEASE

At least four million Americans suffer from Alzheimer's disease (AD) and associated disorders in which tau pathology is one of the hallmarks. While there are no

reports directly linking PTSD and AD (or mild cognitive impairment), common features of the disorders include amygdalar and hippocampal atrophy[25-29], upregulation of norepinephrine biosynthetic capacity in surviving locus coeruleus neurons[30], and *N*-methyl-*D*-aspartate (NMDA)-receptor activation dysfunction[31]. A review of imaging studies (single photon emission tomography; positron emission tomography; magnetic resonance imaging; and functional magnetic resonance imaging) described morphological similarities between AD and PTSD in the medial temporal lobe, hippocampus, and cingulate cortex[32]. In addition, there is increasing evidence to suggest that amygdalar degeneration is associated with emotional disorders, including AD and PTSD, and that unilateral amygdalar atrophy can manifest in tauopathies[33].

Anatomical connections may provide an explanation of the aforementioned similarities between PTSD and AD: noradrenergic projections to the amygdalar complex and hippocampus originate in the locus coeruleus[34]. In response to stressful stimuli, the hypothalamic-pituitary-adrenocortical (HPA) axis acts with a surge in adrenocorticotropic hormone and glucocorticoid release which initiates a response in central nervous system circuitry[35]. Locus coeruleus norepinephrine projections are some of the pivotal structures bridging the central stress response pathways to HPA activity[36,37]. The locus ceruleus, *via* release of norepinephrine, can modulate cellular excitability and synaptic efficacy and, thus, influence behavioral performance[38]. Nevertheless, there is little information concerning how this anatomical link may contribute to vulnerability to PTSD in AD or AD-susceptible populations.

ANIMAL MODELS OF PTSD

Several paradigms for inducing PTSD in animal models have been accepted. Generally, they include the use of brief stressors which result in biological and behavioral outcomes that simulate PTSD symptoms. As reviewed by Pitman *et al*[39], models with both face and construct validity include predator exposure, serial exposure to multiple stressors, and footshock with additional stressors. Nevertheless, the complexity and variability of human PTSD symptoms make it difficult to establish animal models that precisely mimic human PTSD.

rTG4510 TRANSGENIC MOUSE MODEL AND PTSD

The rTg4510 transgenic mouse was created as a model of inducible tauopathy[40]. With age, rTg4510 mice develop neurofibrillary tangles (NFTs) and neuronal and memory loss. The tau transgene is driven by a tetracycline-operon-responsive element. Tet transactivator binds the tetracycline operator sequences within the cytomegalovirus promoter and drives the expression of the human tau transgene (human 4-repeat tau containing the P301L mutation). A 15-fold over-expression of tau in the forebrain (hippocampus and cortex) can occur and can be repressed with doxycycline in this model. This model has been widely investigated with reports of decreased amygdala and hippocampal activity[41], loss of synapses[42], and poor spatial learning and memory, particularly in females[43]. Importantly, the cognitive dysfunction in older rTg4510 mice can be reversed by repressing tau expression, despite the pre-existence of brain atrophy, neuronal loss, and the continued accumulation of the 64 kDa insoluble tau species and NFTs[40].

Our hypothesis that tau expression may be linked to PTSD risk is based on our recent report describing injection of 2 μL of 1% fluorogold, a "harmless" fluorescent tracer, into the right amygdala elicited circling behavior thought to be related to an anxiety-like state[44]. This circling behavior was transient in control mice, but persisted for 14 d in rTg4510 mice. The post-injection clinical signs observed in the rTg4510 mice were of the type thought to be relevant to those appropriate for an animal model of PTSD[45]. Specifically, the fluorogold injections elicited: seizure-like attacks which were characterized by high-amplitude motor spasms of the extremities and trunk while the animal was lying on its back; rolling along the longitudinal body axis and/or turning over spontaneously; persistent circling behavior, in the presence or absence of stimuli, that occurred primarily during the light period when mice would normally be sleeping; and hyperexcitablity (the circling behavior often occurred following minimal stimulation, such as a gentle push)[44].

MECHANISMS UNDERLYING THE VULNERABILITY OF THE rTG4510 MOUSE MODEL TO PTSD

Reduced volume in key brain structures

Reduced hippocampal and anterior cingulate volumes appear to be a characteristic of PTSD[46-50] as well as dysfunction in the medial prefrontal cortex, amygdala, and hippocampus[51]. rTg4510 mice develop NFTs and neuronal and memory loss in an age-dependent fashion[40]. Very little tau pathology exists at 1 mo of age, but hippocampal and cortical pre-tangle structures are detectable by 2.5 mo and argyrophilic tangles develop by 4-5.5 mo. By 5.5 mo of age, brain weight is significantly less and the total number of CA1 hippocampal neurons is decreased by about 60%[40]. Reduced hippocampal volume suggests a reduced capacity to handle stress. Neuronal loss may involve those cells that are critical to maintain the balance of corticosteroid receptors/responses in these regions, which together with other modulators control the final output of the stress response[52]. In rTg4510 mice treated with fluorogold, the abnormal behavior was observed at 2.5 mo of age, when no significant brain weight or neuron loss would be detectable[44]. Animals at this age, however, begin to show pre-tangles, an early sign of neuronal tau pathology, implying a reduced functional neuron capacity[40,53].

Interestingly, mortality occurred exclusively in the rTg4510 mice following the fluorogold injection and this did not correlate with either age or severity of tauopathy[44]: 17%-25% of the fluorogold injected rTg4510 mice died while all fluorogold injected wild type mice survived. Fourteen days after the fluorogold injection, in both rTG4510 and control mice, the fluorogold was well distributed and easily detected on the side of injection throughout the hippocampus and parietal cortex[44], both pivotal structures involved in the development of PTSD. Fluorogold deposition in the amygdala, hippocampus and primary and secondary motor cortices (which occurred *via* axonal transport whereas in the amygdala it occurred *via* direct injection) may have served as an enduring "traumatic event" which resulted in the abnormal behavior in the rTg4510 mice. Describing fluorogold injection as a "traumatic event" may be valid even though the amygdala did not exhibit significant caspase-3 immunoreactivity or terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling assay-measured neuronal death[44]. Because the distribution of fluorogold *via* axonal transport appeared similar in control and rTg4510 mice[44] (our unpublished data), the behavioral abnormalities expressed by the rTg4510 mice are, at least partially, attributable to a vulnerability associated with overexpression of human mutant tau.

Over-activation of excitatory pathways

Increased excitatory activity: The only FDA-approved treatments for AD involve pharmacological manipulation of the glutamatergic NMDA receptor pathway. One of those treatments is memantine, an adamantane derivative and NMDA receptor antagonist. NMDA receptors in the amygdala are thought to participate in the modulatory effect of glucocorticoids on the extinction of fear memories[54]. NMDA receptors are also involved in stress-induced anxiety: administration of the NMDA receptor antagonist, MK-801, before exposure to a predator prevented the increase in anxiety-like behaviors typically exhibited after that stress[55]. That same administration (*i.e.*, MK-801) increased the number of approaches to the predator[55]. Hippocampal-associated memory impairments after stress are likely influenced by stress-induced elevations in corticosteroid levels which modulate fast excitatory amino acid-mediated synaptic transmission and synaptic plasticity[56]. Relevant to our hypothesis here, the excitotoxin quinolinic acid can induce tau phosphorylation *via* NMDA receptor activation[57]. Tau-tubulin kinase-1 (TTBK1) levels were reported to be up-regulated in human AD brains compared with age-matched controls. Additionally, in TTBK1 transgenic mice, up-regulation of TTBK1 was associated with the aggregation of phosphorylated neurofilaments in brain and reduced expression of NMDA receptor types 2B and D[58], suggesting aberrant activities of NMDA receptors in these animals. Nevertheless, mechanisms underlying how over-expression of human mutant tau protein elicits over-activity of NMDA receptors or NMDA pathways remain unclear.

Reduced "inhibitory" activity associated with NMDA receptor activation: Neuronal activity often involves the NMDA receptor in the transfer of electrical signals and it is thought that the NMDA receptor ion channel must be open for it to be functional. Inhibition of NMDA receptors *via* NMDA antagonist treatment can induce an anesthetic state characterized by catalepsy, amnesia, and analgesia[59]. On the other hand, NMDA receptors can be modulated by various endogenous and exogenous molecules. Local ions, such as Mg^{2+} and Zn^{2+}, can block the NMDA receptor ion channel[60,61] and external and/or internal cellular Ca^{2+}, Na^{+} and K^{+} concentrations can modulate NMDA receptor activity[62-64]. Perhaps importantly, extracellular proton concentrations regulate NMDA channels[65]: the responses of NMDA receptors to glutamate can be down-regulated by increasing extracellular H^{+} ions[60], whereas under alkaline conditions, NMDA-evoked cytosolic calcium influxes can be increased[66]. Synaptically-evoked H^{+} shifts modulate NMDA receptor activity[67]. In addition, polyamines can modulate NMDA-induced depolarization[68]. Mechanistically, this modulation may depend upon on polyamine interactions with a proton-sensitive location on the extracellular N-terminal of the NR1 subunit[69]. In addition, polyamines can function as allosteric modulators of NMDA receptors *via* N-terminal interactions on NR2 subunits[70,71].

The vulnerability of rTg4510 mice to fluorogold treatment may be attributable to aberrant NMDA receptor function. Fluorogold may act on NMDA receptors in the transgenic mice differently than those in the normal/wild type controls. The active constituent of fluorogold is the weak base hydroxystilbamidine. Accordingly, fluorogold may affect NMDA receptor function by increasing the extracellular pH at the injection site. It has also been suggested that fluorogold crosses cell membranes in its uncharged form and then is trapped intracellularly in acidic cellular compartments due to a favorable pH gradient[72]. This action may then regulate NMDA receptor activity by changing intracellular pH. Second, because hydroxystilbamidine, as an aromatic diamidine, can inhibit the cellular uptake of polyamines[73,74], fluorogold may alter polyamine metabolism, thereby indirectly affecting NMDA receptor activity. Finally, fluorogold may cause imbalances in neurotransmitter concentrations at the injection site: micromolar concentrations of fluorogold inhibit dopamine release and fluorogold abolishes the dopamine release evoked by glutamate or Ca^{2+}[75].

FUTURE STUDIES

Two specific questions will be addressed in future studies. One aim of investigation will involve an examination of the hypothesis that expression of human mutant tau in amygdalar and hippocampal neurons enhances susceptibility to development of PTSD and that inhibition

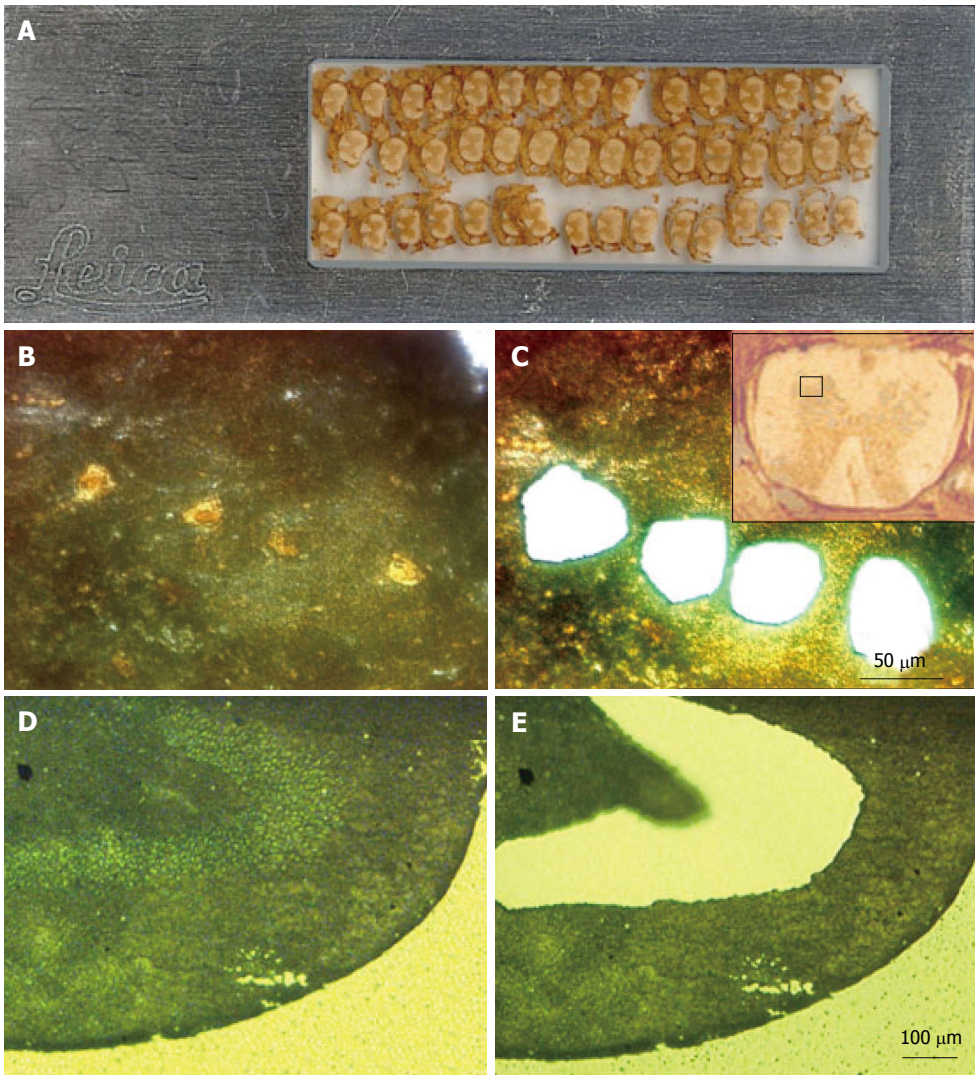


Figure 1 Examples of tissues following microdissection using a Leica DMLA laser-assisted microdissection system. A: A scanned image of a foil slide with multiple coronal sections of mouse spinal cord mounted. These sections are 16 μm thick; B: A high-power (× 40 objective, × 400 final magnification) image of the ventral gray region of mouse spinal cord that was immuno-stained for the NeuN neuronal marker. The yellow-brown profiles are motor neurons; C: The same view as in panel B, but after laser microdissection and collection of four motor neurons; C: A low-power (× 5 objective, × 50 final magnification) image of the spinal cord section (shown upside down), with the red square marking the region shown in panels B and C; D: A low-power view (× 10 objective, × 100 final magnification) of methyl green-stained rat dentate gyrus. The section is 10 μm thick; E: An image of the same section shown in panel D, but after microdissection and collection of the neurons in the granule cell layer.

of mutant tau expression will decrease this vulnerability. Young female rTg4510 and wild-type mice with/without doxycycline treatment could be subjected to PTSD modeling (such as electric foot-shock), followed by the measurement of anxiety-relevant behaviors, such as elevated plus maze behavior. If pathophysiological changes occur in the amygdala and hippocampus as a result of specific traumatic events (*e.g.*, foot shock), this may trigger the cascade needed to model PTSD. rTg4510 mice would be expected to exhibit increased vulnerability to foot shock which would be reflected in increased anxiety-like behavior as a result. It is highly likely that rTg4510 mice are also susceptible to other types of traumatic events due to their tau pathology burden and/or aberrant gene expression in neurons in the amygdala and hippocampus. Accordingly, lifelong suppression of tau gene expression by treatment with doxycycline may reverse this vulnerability in rTg4510 mice.

Another aim of investigation will test the hypothesis that intra-amygdala injection of fluorogold as a traumatic stimulus can produce animals with reliable and reproducible behavioral profiles reminiscent of PTSD. In addition, the fluorogold model could be used to optimize manipulations to decipher the molecular mechanisms underlying the susceptibility of the rTg4510 mouse to stress-induced abnormalities. Here, rTg4510 and wildtype mice would be unilaterally injected with fluorogold or vehicle into the amygdala and then subjected to footshock or sham-treatment. Subsequently, all mice would be assessed for anxiety-relevant behaviors. Mice would be sacrificed at various times following anxiety measures and brains harvested for evaluation. Neurons in the contralateral (*i.e.*, intact side) amygdala and hippocampus would be collected *via* laser-capture microdissection (LCM) or laser-assisted

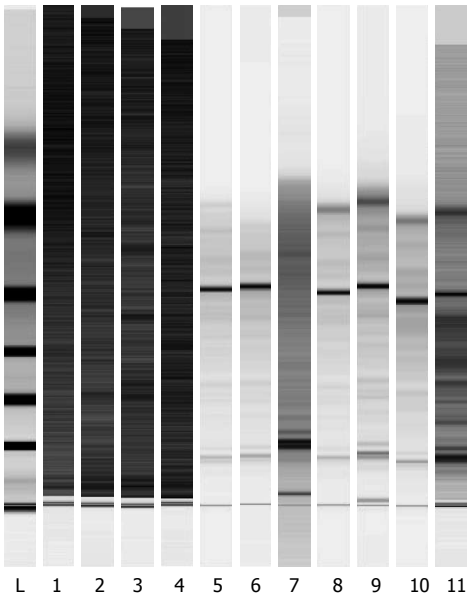


Figure 2 Ensuring quality of the microdissected neural RNA samples. For the 28S and 18S rRNA species/bands, a Bioanalyzer Model 2001 was used to examine the quality of the RNA sample derived from the neurons collectively harvested from the hippocampus (Figure 1D and E). Lane L: The RNA ladder; lanes 1-4: Non-sample controls; lanes 5-11: Electrophoretic profiles of RNA from multiple tubes of cells collected *via* laser-assisted microdissection; lane 7: The sample to be degraded and was excluded from further evaluation.

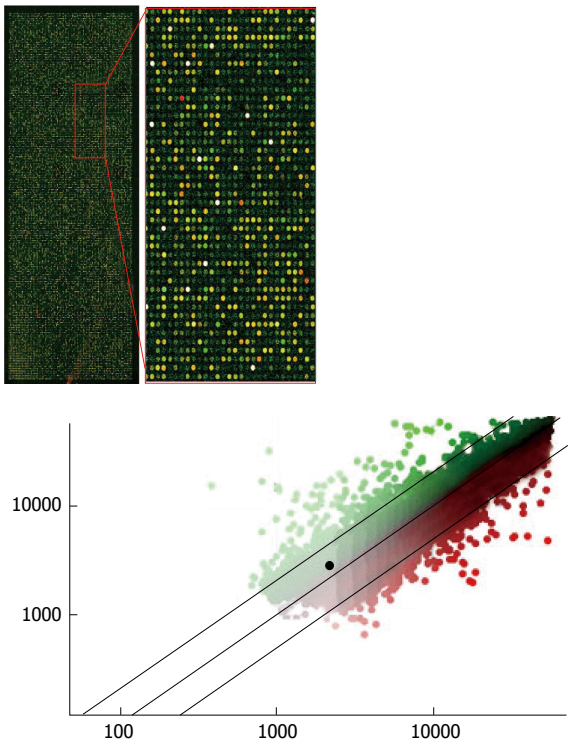


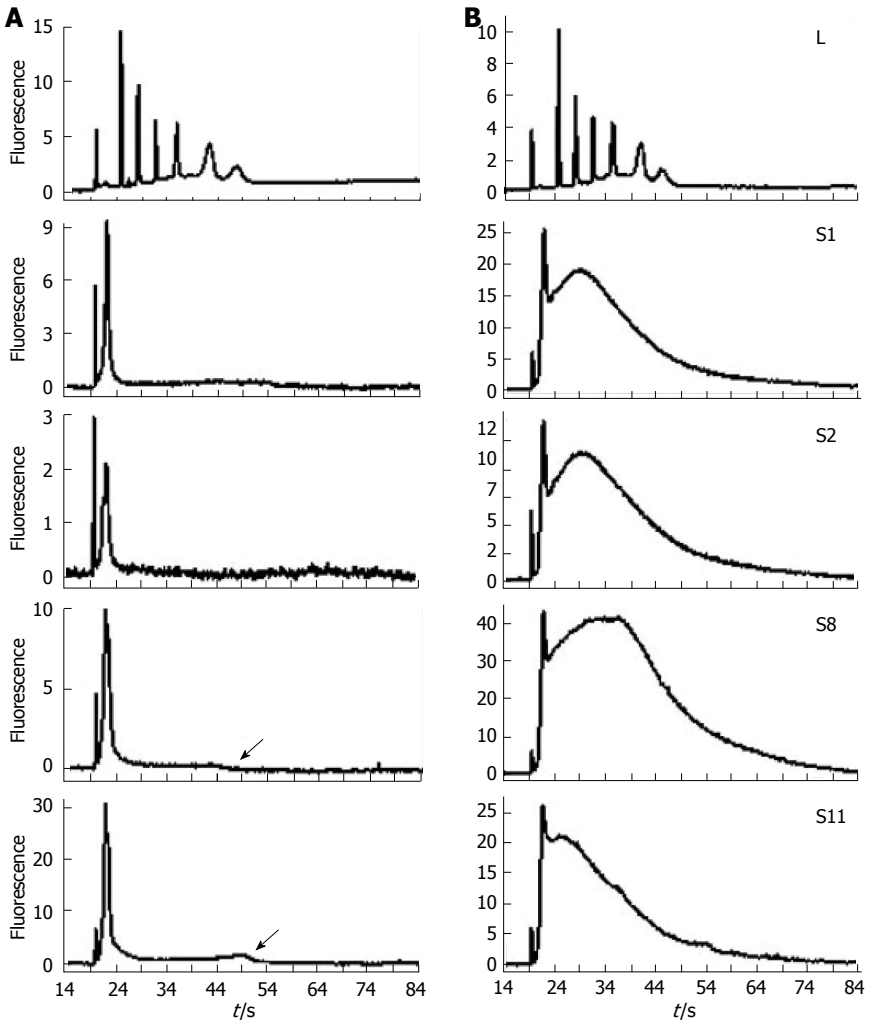
Figure 3 Example of an Agilent Rat Oligo microarray hybridized with probes from microdissected neurons. The upper, right-hand panel is an enlarged view of a portion of the microarray. The two probes used were made from RNA amplified through one stage of the T7 method, the Cy3-labeled probe was synthesized from total RNA extracted from several thousand CA1 pyramidal neurons and the Cy5-labeled probe was synthesized from several thousand dentate gyrus granule cells, after laser-assisted microdissection. The two probes are shown overlaid; the predominance of yellow spots indicates that most of the genes in the two samples were at or near equivalent levels. Only a few spots are saturated (white). Shown in the lower panel, the genes of interest can be identified on the scatterplot (CA1 neurons *vs* dentate granular neurons). An example of one gene of interest is the highlighted black spot, which represents caspase-3, a key apoptotic mediator.

microdissection (LAM). A T7 method (the Eberwine T7 protocol) that linearly increases mRNA copies could be used for mRNA signal amplification and RNA quantity and quality could be determined using microfluidic technology (*e.g.*, Bioanalyzer, Agilent Technologies, Palo Alto, CA, United States). Gene expression could then be profiled using genome-wide/pathway microarrays. Validation of microarray outcomes would be performed at the transcriptional and translational levels.

The molecular mechanisms underlying the pathology of PTSD are poorly understood. A traumatic event directly targeting the amygdala unilaterally may result in an animal model characterized by reliable and reproducible behavioral characteristics that are relevant to the study of PTSD. The contralateral (untreated) amygdala would remain "intact" and thus serve as a within-subject control, facilitating analyses of potential molecular mechanisms. Utilization of LCM/LAM to collect the targeted tissue for subsequent microarray analyses will allow for the evaluation of cell-specific gene expression. Validation of the information using independent molecular biological techniques will be important and may lead to the identification of new research and therapeutic and preventive strategies with direct relevance for PTSD.

Interestingly, there have been multiple studies using LCM/LAM and microarray analyses to define the genetics associated with the functional responses in the decisive components (neurons) within the amygdalar complex[76-78]. In addition, integrating LCM/LAM techniques with RNA amplification (PCR/quantitative PCR) has also been described in efforts to define changes in the targeted amygdalar gene(s) that may be responsible for the control of emotion or memory[79-83]. However, it appears that monitoring RNA quality before microarray/RNA amplification of microdissected neurons has not been properly addressed: the RNA quality in the cited references was either indirectly examined or was not verified at all; it is arguable, though, that the reproducibility of microarray data and/or the detectability of targeted genes provide evidence for a certain degree of reliability.

In our laboratory, neurons in the mouse spinal cord (NeuN-positive profiles) or the rat hippocampus (methyl green stained cells in the CA1 layer and dentate gyrus granule cell layer) were harvested either singly in the case of NeuN-stained motor neurons, or in groups in the case of methyl green-stained hippocampal cells (Figure 1) using a laser microdissection system (Version 4.0, Leica, Bannockburn, IL, United States) under a × 40 objective (final magnification × 400) for single cells or a × 10 objective (final magnification × 100) for groups of cells. A total of 37 tubes of microdissected cells from the hippocampus and dentate gyrus were collected. Each of these tubes contained variable numbers of cells, up to several thousand cells per tube. RNA extracts were then



	RNA Pico Lab chip Kit				RNA Nano Lab chip Kit	
	Toatal RNA(LAM)		aRNA(Round one)		aRNA(Round two)	
	RNA area	Conc.pg/μl	RNA area	Conc.pg/μl	RNA area	Conc.ng/μl
Ladder	121.70	1000	125.56	1000	85.06	150
S1	6.85	72	58.05	462	1250.68	2206
S2	8.27	68	20.82	166	770.75	1359
S8	19.70	70	86.57	689	3265.25	5758
S11	26.16	276	270.07	2149	1323.35	2333

Figure 4 Bioanalyzer profiles of aRNA products from microdissected mouse spinal cord motor neurons using the Arcturus PicoAmp kit. A and B: These are the standard views obtained from the Bioanalyzer of the Pico Chip and Nano Chip data, providing electrophoretic profiles in time (s). The ladder is shown in the uppermost profiles. Note that the quantity of ladder used in the Pico Chip (A, upper panel) was 1000 pg, but the quantity of ladder used in the Nano Chip (B, upper panel) was 150 ng (150000 pg). Thus, the scales for the Pico Chip (A) and Nano Chip (B) profiles are approximately 150-fold different. The samples shown (S1, S2, S8, and S11) are aRNA products obtained after the first round of PicoAmp amplification (A) and the second round of amplification (B). The black arrowheads in the Pico Chip data for S8 and S11 show visible points of maximal migration for RNA in these two samples; C: RNA concentrations before and after one- and two-rounds of amplifications in 4 representative samples of S1, 2, 8, and 11.

analyzed with a Pico Chip (Agilent Technologies, Palo Alto, CA, United States). A few of these samples exhibited noticeable degradation (*e.g.*, Figure 2 lane 7) although multiple, constrained, standard operating procedures were followed to ensure RNA quality used in profiling the 18S and 28S rRNAs with the Model 2001 Bioanalyzer (Agilent Technologies, Palo Alto, CA, United States). The amount of RNA in these tubes ranged from 193 pg/μL to 9475 pg/μL, RNA samples provided reasonable microarray data (Figure 3), while the RNA samples that did not qualify with the 18S and/or 28S rRNAs profiles were not analyzed. On the other hand, from approximately 1000 motor neurons that were individually harvested *via* microdissection from the anterior horn of the mouse spinal cord, the yield of RNA was 116 ± 68 pg/μL[84], which is beyond the recommended capability of the Bioanalyzer (limit of 200 pg/μL: below this value, the Bioanalyzer may not display the electrophoretic profiles including the 18S and/or 28S rRNAs). Thus, the RNA quality cannot be determined using the criteria used pre-

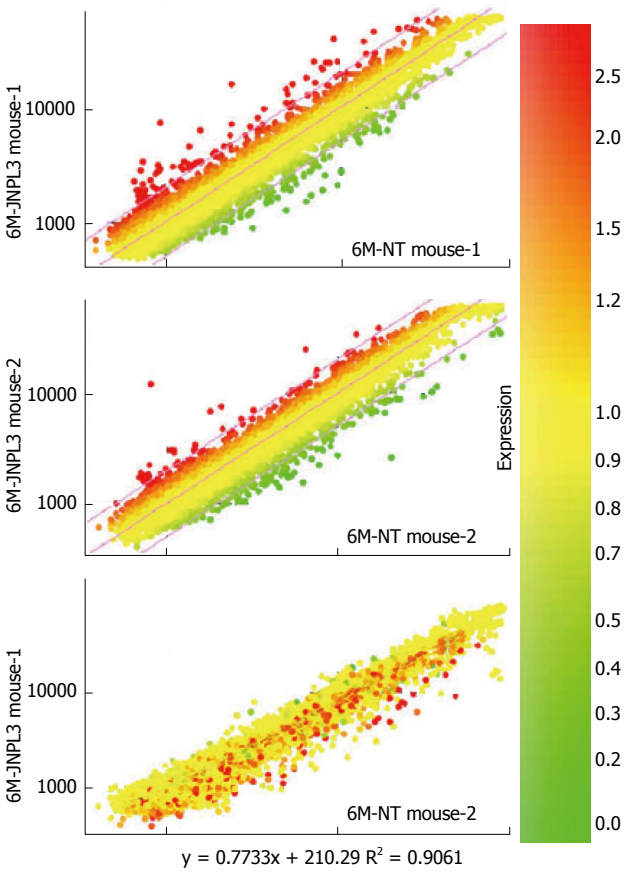


Figure 5 Examples of microarray data obtained from microdissected neurons. Two microarray experiments using spinal cord motor neurons from four mice were performed. Scatterplots of processed data generated using the program GeneSpring are shown. In each microarray experiment, a Cy3-labeled probe made from approximately 1000 motor neurons from a P301L transgenic mouse was co-hybridized with a Cy5-labeled probe made from approximately 1000 motor neurons from a non-transgenic littermate. The results are shown in the top two scatterplots. The colors of the plotted data are derived from the scale shown at right, indicating the expression fold change. To further examine the quality of the data, a derivative plot was made (bottom). The data from two non-transgenic littermates from the two microarray experiments were plotted against each other. The correlation coefficient of these two biological replicates was $r = 0.9$ (bottom panel).

viously for the 18S and 28S rRNAs. Forty-nine out of 50 sets of the 1000-neuron RNA samples were amplifiable using a T7 amplification method and the electropherograms (Figure 4) indicated that the aRNA sizes extended to well beyond 6000 nucleotides, providing an alternative measure: the number of nucleotides might be used as a type of criteria for addressing the quality of the aRNA. Actually, the aRNAs yielded reproducible microarrays with correlation coefficients of > 0.9 (Figure 5) between 2 microarrays that were randomly chosen. Practically, a subset of neurons that express the targeted proteins-such as excitatory glutamatergic neurons with CamKIIα as a marker or inhibitory GABAergic neurons with GAD67 as a marker[85]-can be selectively collected using an optimized immunohistochemical labeling technique followed by the LAM/LCM procedures. Presumably, the altered gene expressions in the excitatory and/or inhibitory neurons may indicate the signaling pathways accountable for the vulnerability to and onset of PTSD.

CONCLUSION

Susceptibility to PTSD may be related, in part, to aberrant tau expression in neurons of critical brain structures. This abnormal expression is postulated to interfere with the function of those central nervous system circuits that normally respond to traumatic stress, thus conferring vulnerability to PTSD development. Verification of the vulnerability of the brain to develop PTSD due to an overabundance of tau expression may require a model that does not employ direct intra-brain/amygdalar damage. On the other hand, modeling PTSD might be more feasible using this approach because the key PTSD brain structures, the amygdala and the hippocampus, could be directly targeted. Defining the molecular mechanism(s) underlying the expression of PTSD will be challenging. The integration of the LAM/LCM technique with gene expression analyses in neurons of brain structures critical to the development of PTSD seems a useful approach, provided that the quality of the RNA obtained using LAM/LCM can be demonstrated.

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EDITORIAL

Relevance of long QT syndrome in clinical neurology

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Abstract

Long QT syndrome (LQTS) is a cardiac conduction disorder that predisposes patients at potentially fatal cardiac events. Inherited conditions and acquired factors contribute to LQTS. A number of frequently prescribed CNS-active drugs prolong the QT interval. The clinical neurologist may encounter LQTS when initiating a pharmacotherapy or when increasing the dosage of drugs. The clinical neurologist may also encounter LQTS during the diagnostic work-up of patients with unexplained loss of consciousness, because LQTS may present as convulsive syncope. Some studies report an association of LQTS and stroke. Awareness of LQTS may help to recognize and prevent potentially fatal cardiac events associated with LQTS. This concise article highlights the clinically most relevant aspects of LQTS in the field of neurology.

Key words: Cardiac conduction disorder; Adverse drug effects; Antipsychotics; Antidepressants; Torsades de pointes; Syncope; Seizure disorder; Stroke

Core tip: Long QT syndrome (LQTS) is a potentially fatal condition. Considering the fact that many CNS-active drugs prolong the QT interval and considering the fact that diagnosis of LQTS may be missed in neurological patients with unexplained loss of consciousness, this concise article highlights the most relevant aspects of LQTS for clinical neurologists.

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INTRODUCTION

The long QT syndrome (LQTS) is characterized by an abnormally delayed repolarization of the heart. The delayed repolarization results in a prolonged QT interval that is detectable by electrocardiography. Inherited conditions (channelopathies, gender) as well as acquired factors (drugs, electrolyte imbalances, age) affect cardiac repolarization and can prolong the QT interval[1]. Different types of channelopathies that cause congenital LQTS have been described. Each channelopathy follows a distinct inheritance pattern, presents with distinct clinical symptoms and is associated with a distinct outcome. Irrespective of the affected type of ion-channel, the net result in LQTS is an abnormal repolarization of the heart muscle that predisposes at so-called early after depolarization which in turn can result in ventricular arrhythmias. The ancient discrimination between congenital LQTS and acquired LQTS seems to reflect insufficiently the underlying pathophysiology: so-called acquired LQTS may as well be unmasking of a clinically and electrocardiographically silent congenital LQTS, *i.e.,* exogenic factors (*e.g.,* hypokalemia) may be the trigger that a pre-existing (but so far clinically silent) channelopathy results in electrocardiographical changes and clinical symptoms.

SYMPTOMS OF LONG QT SYNDROME

Many patients with LQTS may not have any symptoms at

all. These patients may only be aware of their condition due to an incidental finding on the electrocardiogram or due to a family history of sudden cardiac death. If clinical symptoms are present in patients with LQTS, the spectrum of symptoms includes unspecific dizziness, fainting spells, paroxysmal tachycardia (torsades de pointes), ventricular fibrillation and sudden cardiac death. It has been reported that LQTS can also present with focal neurological signs (subsequent to a focal vascular lesion potentially caused by LQTS associated arrhythmia)[2]. Yet, a causal relationship between LQTS and a focal brain lesion remains disputable.

ANALYSING AND INTERPRETING THE QT INTERVAL

The QT interval is influenced by different factors, including gender, age and heart rate. Especially the heart rate has a major impact on the QT interval: the slower the heart rate, the longer the QT interval. It is therefore mandatory to use the frequency-corrected QT time (QT_c) when interpreting an electrocardiogram. Different formulas and standards tables are used to determine QT_c. All these formulas take into account that the (non-corrected) QT interval shortens with increasing heart rates and correct for this fact. There is a lack of unanimously accepted recommendations and guidelines concerning determination of corrected QT_c. The most often used formulas for the determination of QT_c are Bazett's formula (measured QT_c divided by the *square root* of the measured RR interval) and Fridericia's formula (measured QT_c divided by the *cube root* of the measured RR interval). The United States Food and Drug Administration (FDA) considers a QT_c < 430 ms for men and QT_c < 450 ms for women to be normal and QT_c > 450 ms for men and QT_c > 470 ms for women to be prolonged (source: http://www.fda.gov/ohrms/dockets/ac/01/slides/3746s_01_ruskin/sld023.htm).

QT INTERVAL AND CNS-ACTIVE DRUGS

A number of frequently prescribed CNS-active drugs confer the potential of prolonging the QT interval. The main mechanism by which drugs affect the QT interval is the blocking of the potassium outward current by interaction of these drugs with cardiac potassium channels. At present, more than 100 drugs have been shown to prolong the QT interval. The list of drugs discussed below does therefore not claim to be exhaustive but rather presents examples of drugs frequently used in neurological patients. More comprehensive and regularly updated lists of drugs that affect the QT interval are available one different websites (*e.g.* http://www.qtdrugs.org).

Many dopamine receptor antagonists affect the QT interval. The effect accounts for typical high-potency neuroleptic drugs (*e.g.* haloperidol, especially when injected intravenously)[3,4], low-potency neuroleptic drugs that are frequently used in the elderly due to their sedative effects, but also for atypical neuroleptic drugs[5]. Domperidone (a dopamine receptor antagonist with predominant antiemetic properties) is frequently used in patients under levodopa treatment to prevent levodopa-associated nausea. Also this antiemetic drug confers a substantial risk for LQTS with potential fatal outcome[6,7].

Another group of drugs that prolong the QT interval are antidepressants, especially some selective serotonin reuptake inhibitors (SSRI). Although there has been a number of reports concerning LQTS and SSRI, *e.g.,* citalopram[8], it is important to note that other groups of antidepressants, *e.g.,* tricyclics like amitriptyline may prolong the QT interval in a dose dependant manner as well[9].

Amantadine, a NMDA-receptor antagonist, is another potentially QT prolonging drug[10] that is frequently used in neurological patients. Amantadine is used in Parkinson's disease (due to its anti-glutamatergic properties) and sometimes also administered (off-label) for its vigilance-enhancing effects.

Besides the dugs mentioned above, polypharmacotherapy (and the resulting additive effects on the QT interval) as well as co-morbidities (*e.g.,* hypokalemia due to diuretics administered to treat hypertension or chronic heart failure) predispose the elderly neurological patient clientele to LQTS.

RECOMMENDATIONS, MANAGEMENT OF COMPLICATIONS

The clinical neurologist may prevent imminent harm to the patient by checking the patient's medication for drugs known to affect QT interval. In case a QT prolonging drug needs to be introduced or increased after careful risk-benefit assessment, the treating physician should obtain a baseline electrocardiogram in order to exclude a pre-existing prolonged QT interval. A control electrocardiogram should be obtained after introduction of the new drug and also after each dose adjustment (as QT prolonging effects are dose-dependent). In case of a significant increase in the QT interval, withdrawal of QT prolonging drugs needs to be considered. In addition, special attention should be given to prevent circumstances that may have additive effects on the QT interval (*e.g.,* hypokalemia, combination of different drugs with QT-prolonging potential). For patients with LQTS who develop torsades de pointes, the withdrawal of drugs that affect the QT interval and a cardiovascular monitoring are obligatory. Besides withdrawal of drugs that affect the QT interval, therapeutic options include the reduction of other pro-arrhythmogenic factors (*e.g.,* hypokalemia) and infusion of magnesium. Some patients with ventricular tachycardia may also require electrical cardioversion or electrical cardiac pacing. It is of importance to note that the anti-arrhythmic drug amiodarone is absolutely contraindicated in these patients due to its own QT-prolonging potential which will deteriorate the situation.

LONG QT SYNDROME PRESENTING AS "SEIZURE"

Besides the risk of inducing LQTS by pharmacotherapy, the clinical neurologist may encounter LQTS when patients with transient unexplained loss of consciousness are referred to the hospital: LQTS may present as (convulsive) syncopes that are misdiagnosed as epileptic seizures. There is a number of case reports about LQTS misdiagnosed as epileptic seizures (reviewed by Burghaus *et al*[11]). MacCormick *et al*[12] report a consecutive case series of 31 patients with genetically and electrocardiographically confirmed LQTS. Seizure disorder was the most common initial misdiagnosis in this cohort (5 of 31 patients). In some of these cases, the diagnosis of LQTS was missed despite prolonged QT intervals in the electrocardiogram. According to MacCormick's study, the misdiagnosis of LQTS as seizure disorder may be particularly fatal as the misdiagnosis "seizure disorder" resulted in a significantly longer diagnostic delay compared with other misdiagnoses (median delay 11.8 years compared to a median delay of 1 year for other misdiagnoses). Obtaining an electrocardiogram and evaluating the QT interval is therefore recommended in every patient with transient loss of consciousness, even in cases when an epileptic seizure is suspected. Gospe and Gabor stress the need to routinely and carefully assess the simultaneously recorded electrocardiogram for QT abnormalities in patients who undergo an electroencephalography[13].

Interestingly, there are also data that indicate that patients with some types of inherited LQTS-associated channelopathies have an increased risk of epilepsy[14,15]. The increased risk of epilepsy in these patients might be explained by the presence of the mutated channel proteins (and a consecutively altered channel activity) in brain tissue[14]. In conclusion, seizure disorder and inherited LQTS are not mutually exclusive conditions. In some cases, a detailed history taking and thorough diagnostic work-up (including implantable event recorders) may be necessary to distinguish between LQTS-associated syncope and seizure as the cause for a specific event.

LONG QT SYNDROME AND STROKE

There are reports that LQTS might be associated with an increased risk for stroke, even after correction for other cardiovascular risk factors[16]. The mechanisms that confer an increased risk for stroke in patients with LQTS remain speculative and may include arrhythmia-associated embolization and hypoperfusion[2]. Irrespective of the underlying causal relationship between LQTS and stroke, cardiac monitoring is obligatory in stroke patients given the increased mortality rates in these patients[17]. Stead *et al*[18] investigated the association between a prolonged QT_c and early mortality in acute ischemic stroke. The authors found a significantly increased early mortality rate in stroke patients who had a prolonged QT_c at the time of admission. In Stead's study this association was independent of other cardiovascular risk factors. The exact reasons for the increased mortality remain to be investigated. One explanation for the association of neurological disorders and LQTS (and other cardiac arrhythmias) might be cerebral arrhytmogenesis[19-21].

In summary, awareness for circumstances that can trigger LQTS and awareness that LQTS may present as seizure are important to prevent a potential fatal outcome in this condition. The studies on LQTS and stroke suggest that a prolonged QT_c is associated with a poor outcome in this patient population.

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REVIEW

Treatment with botulinum toxin: An update

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Abstract

Botulinum neurotoxin (BoNT) is a potent toxin produced by the anaerobic bacterium clostridium botulinum. It causes flaccid, long-lasting, local and reversible paralysis. In addition, BoNT inhibits the secretion of the exocrine glands and could have properties in the control of pain. Thus, BoNT is useful in the treatment of many neuromuscular conditions where an increase of muscle tone is associated with the pathogenic mechanism. Furthermore, BoNT is recommended in the treatment of some hypersecretion disorders of the exocrine gland and could play a role in the treatment of migraine and other chronic pain conditions. In the BoNT therapy adverse effects are usually mild and reversible. However, repeated injections of BoNT can lead to the development of neutralizing antibodies that can subsequently inhibit the biological activity of the toxin. In this sense, many factors can influence the immunogenicity of the BoNT, such as product-related factors, the dose of BoNT used, the frequency of injection and the previous exposure to the toxin. In this review, we are going to discuss the current clinical applications of BoNT with a special focus on evidence, doses, injection technique and adverse effects for those applications more frequently used in neurology, namely spasticity, blepharospasm, hemifacial spasm, cervical dystonia and other focal dystonias, as well as chronic migraine, tremor, sialorrhea, facial palsy, neurogenic bladder and many other neurological condition.

Key words: Botulinum toxin; Dystonia; Spasticity; Headache; Migraine; Pain; Tremor; Sialorrhea; Facial palsy; Neurogenic bladder

Core tip: In this review we are going to discuss evidence, doses, injection techniques and adverse effects of the botulinum neurotoxin therapy for those indications more frequently used in neurology.

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INTRODUCTION

Botulinum neurotoxin (BoNT) is a potent toxin produced by the anaerobic bacterium clostridium botulinum[1]. It causes flaccid, long-lasting and reversible paralysis by inhibiting exocytosis and can thus prevent acetylcholine release at the neuromuscular junction[2]. In addition, the effects of local injections of BoNT are usually limited to the applied area[2], although it is possible that BoNT could spread beyond the muscle injected causing adverse effects it is not frequent. Thanks to these attributes, BoNT has been found to be useful in the treatment of many neuromuscular conditions where an increase of muscle tone is associated with the pathogenic mechanism. Furthermore, BoNT could be helpful in the treatment of migraine and other chronic pain conditions. For these indications, the mechanism of action has not been fully elucidated, but could be due to the inhibition of the release of nociceptive inflammatory mediators, such as calcitonin gene-related peptide, glutamate and substance *P* from the peripheral termini of nociceptors. Inhibiting the release of these neurotransmitters prevents neurogenic inflammation and subsequent peripheral sensitization;

as a result, peripheral pain signals to the central nervous system are reduced. Thus, BoNT might indirectly block central sensitization for pain[3]. Finally, BoNT inhibits the release of presynaptic acetylcholine at the neurosecretory junctions[4]; therefore, it could play a role in the treatment of hypersecretion disorders of the exocrine glands.

BoNT is a protein complex consisting of a 150-kDa core neurotoxin and a number of associated non-toxic accessory proteins (NAPs) whose function is to stabilize and protect the core neurotoxin from low pH, enzymatic degradation an changes in temperature[5]. There are seven serotypes of BoNTs (termed A-G)[2], but only serotypes A (BoNT-A) and B (BoNT-B) are commercially available. Of these serotypes, the four most widely used formulations in clinical practice are three of the BoNT-A serotypes, named OnabotulinumtoxinA (A/Ona, Botox, Allergan, Irvine, CA, United States), AbobotulinumtoxinA (A/Abo, Dysport, Ipsen Biopharm, Wrexham, UK) and IncobotulinumtoxinA (A/Inco, Xeomin, Merz Pharmaceuticals, Frankfurt, Germany), and one BoNT-B serotype named RimabotulinumtoxinB (B/Rima, NeuroBloc/Myobloc, Eisai, Tokyo, Japan).

BoNT doses are expressed as mouse units for adults and as mouse units per kg body weight (units/kg bw) for children[6,7]. One mouse unit is defined as the amount that kills 50% of a group of mice when injected intraperitoneally[6]. Note that all BoNT formulations are distinct concerning their molecular structure and their manufacturing process[7]. Indeed, the methods used for determining biological activity are also different[7]. These pharmacological differences have significant implications for clinical use. Thus, individual dosages should be calculated independently for each BoNT formulation and therefore, fixed dose-conversion factors should not be used[7].

Repeated injections of BoNT can lead to the development of neutralizing antibodies that can subsequently inhibit the biological activity of the toxin[5]. Many factors can influence the immunogenicity of the BoNT. First, product-related factors, such as the manufacturing and storage processes, the toxin source and the antigenic protein load of each formulation, together with the excipients and the presence of NAPs can influence the immunogenicity[5]; Second, BoNT immunogenicity may be related to the dose that is injected. Indeed, the development of neutralizing antibodies is correlated to the increasingly cumulative doses[5]; Third, BoNT immunogenicity may be associated with the frequency of injection; injection intervals shorter than 2 mo may increase the risk for neutralizing antibody formation[5]; Finally, previous exposure to or vaccinations against BoNT may also affect the immunogenicity[5]. As a result, A/Inco, a new BoNT-A developed free from NAPs, has shown low rates of neutralizing antibodies and could have an improved immunogenicity profile[5,8].

Our aim is to review the current clinical applications of BoNT with a special focus on those applications more frequently used in neurology.

DATA SEARCH

The Cochrane Library and Medline databases were systematically searched with special focus in the last ten years. The search terms were "botulinum toxin" or "onabotulinumtoxina" or "incobotulinumtoxina" or "abobotulinumtoxina" or "rimabotulinumtoxinb" and "mechanism of action" or "targeting" or "neutralizing antibodies" or "dystonia" or "blepharospasm" or "hemifacial spasm" or "cervical dystonia" or "occupational dystonia" or "writer"s cramp" or "spasmodic dysphonia" or "oromandibular dystonia" or "spasticity" or "headache" or "myofascial pain syndrome" or "facial pain" or "neuropathic pain" or "migraine" or "tensional headache" or "cervicogenic headache" or "cluster headache" or "piriformis syndrome" or "temporomandibular dysfunction" or "trigeminal neuralgia" or "postherpetic neuralgia" or "sialorrhea" or "drooling" or "tremor" or "facial palsy" or "neurogenic detrusor overactivity" or "detrusor sphinter dyssinergia" or "tics" or "brachial plexus injury" or "stuttering" or "painful leg and moving toes" or "Parkinson´s disease" or "multiple sclerosis". Indeed, further references were obtained through their bibliographies. Finally, between all the references selected, one hundred and eighty five were considered relevant for the purpose of this review.

DYSTONIAS

BoNT therapy is often the treatment of choice for many focal or segmental dystonias. Blepharospasm, hemifacial spasm and cervical dystonia are those with stronger evidence of efficacy, although it could also be helpful in occupational dystonias, spasmodic dysphonia and other dystonias.

In the 1980's, blepharospasm was one of the first indications for BoNT treatment. Years of clinical use and many studies have subsequently shown the efficacy of BoNT in the treatment of blepharospasm[9-16]. However, evidence-based conclusions are variable between the different commercially available formulations due to the lack of well-designed and controlled trials (level A of recommendation for A/Ona and A/Inco, level B for A/Abo and level U for B/Rima)[17].

The treatment technique involves injection into the preseptal portion of the orbicularis oculi at four sites per eye, two in the upper lid (one medially and one laterally) and two in the lower lid (one at the lower lateral canthus and one near the middle of the lower lid)[18]. It is important to avoid injections into the medial two-thirds of the lower eyelid to prevent diplopia due to diffusion of the toxin to the inferior oblique muscle. It is also important to avoid injections close to the levator palpebrae muscle to prevent ptosis and to avoid injections into the central part of the lower lid to decrease entropion[18]. Pretarsal injections could be useful in refractory cases[19]. Electrically induced muscle activation does not increase the effectiveness of BoNT[20].

Table 1 Muscles and doses of botulinum neurotoxin more frequently used in the treatment of dystonias

	Muscles usually injected	Doses of botulinum toxin proposed			
		A/Ona	A/Inco	A/Abo	B/Rima
Blepharospasm	orbicularis oculi	20 u/e	20 u/e	40 u/e	1250 u/e
Hemifacial spasm	orbicularis oculi corrugator frontalis zygomaticus major buccinator depressor anguli oris platysma	10-34 u	10-34 u	53-160 u	1250-9000 u
Cervical dystonia	sternocleidomastoid splenius capitis trapezius levator scapulae scalenii semispinalis	100-300 u	100-300 u	500 u	2500-10000 u
Writer´s cramp	flexor digitorum superficialis flexor digitorum profundus flexor pollicis longus lumbricales extensor pollicis longus extensor indicis extensor digitorum comunis flexor carpi radialis flexor carpi ulnaris Pronator teres	Maximum of 160 u		Maximum of 250 u	
Spasmodic dysphonia	adductor spasmodic dysphonia: thyroarytenoid lateral cricoarytenoid interarytenoid supraglottic muscle complex	Bilateral injection: 0.9 u/vf Unilateral injection: 1.5 u			
	abductor spasmodic dysphonia: posterior cricoarytenoid	3.75 u unilaterally, additionally 0.6-2.5 u in the contralateral muscle			
Oromandibular dystonia	*jaw-closing* OMD: masseter medial pterygoid temporalis	masseter 25-50 u	25-50 u	100 u	2500 u
	jaw-opening OMD: lateral pterygoid submentalis complex	pterigoyds 15-30 u	15-30 u	30-60 u	1000 u
	deviating OMD: contralateral lateral pterygoid	temporalis 20-50 u	20-50 u	80-100 u	
	tongue-protrusion lingual dystonia: intrinsic tongue muscles	Intrinsin tongue muscles 10 u	10 u	30-40 u	

u: Units per session; u/e: Units per eye and per session; u/vf: Units per vocal fold and per session; OMD: Oromandibular dystonia.

The suggested doses of BoNT in the treatment of blepharospasm (per eye) are 20 units of A/Ona and A/Inco, 40 units of A/Abo and 1250 units of B/Rima[18] (Table 1). The duration of action ranges between 2 and 3.5 mo[21]. Adverse effects are transitory and include dry eye, ptosis, lagophthalmos and diplopia[18,21].

The efficacy of the BoNT therapy in hemifacial spasm is strongly supported by several studies[12,22-24] and by long-lasting daily clinical practice. However, due to the lack of well-designed and controlled trials, the available evidence only supports a level B recommendation and only for A/Ona[17].

The injection technique involves several facial muscles depending on the clinical examination[22]; the most frequently treated are the orbicularis oculi, corrugator, frontalis, zygomaticus major, buccinator, depressor anguli oris and platysma[21,25] (Table 1). Doses of BoNT range from 10 to 34 units for A/Ona (aproximately the same for A/Inco[8]), 53 to 160 units for A/Abo and 1250 to 9000 for B/Rima[26] (Table 1). The adverse effects are similar to those found in blepharospasm, including dry eye, ptosis, mild facial weakness, tearing and diplopia and, as in blepharospasm, they are usually transitory[21]. The mean duration of improvement ranged from 2.6 to 4 mo[21].

There is abundant evidence supporting the efficacy of BoNT therapy for cervical dystonia[27-34], which supports a level A of recommendation for A/Ona, A/Inco, A/Abo and B/Rima[17].

The optimal doses of BoNT for cervical dystonia are between 100 to 300 units of A/Ona[34] and A/Inco[8], 500 units of A/Abo[34] and between 2500 to 10000 units of B/Rima[34]. The average duration of maximum improve-

ment is 3.5 mo[35] (Table 1).

Hefter *et al*[36] proposed a useful algorithm to select the muscles to be injected for treating cervical dystonia. Otherwise, selection is usually based on clinical features, such as abnormal posture, muscle palpation, muscle hypertrophy and pain[37]. However, physical examination alone may not be sufficient to detect dystonic muscles in cervical dystonia in some cases. It has been reported that, firstly, 41% of dystonic muscles could be missed and 25% of inactive muscles would be judged dystonic only upon clinical examination[38] and, secondly, different combinations of muscle activated can lead to similar postural abnormalities[39,40]. This report is important because the inadequate selection of muscles may be one cause for non-responsiveness[37]. To resolve this issue, it has been proposed that EMG or PET/CT could be helpful for muscle selection, especially in complex forms of cervical dystonia and in non-responders[37].

The general consensus among most BTX experts is that the targeting of muscles can be based on clinical examination[35]. Nevertheless, extra guidance by EMG, ultrasound and/or CT could be helpful in some cases, such as when the muscles cannot be adequately palpated[35], when the patient develops adverse effects[41], when the patient does not obtain adequate relief of symptoms with conventional approach[35] or when the needle needs to be placed near an important structure[42], such as the internal carotid artery, vertebral artery, pharynx, spinal canal, brachial plexus or the base of the mouth.

The number of muscles injected usually ranges between 2 and 4 with 2 sites of injection per muscle[43]. The adverse effects are usually mild or moderate and transitory. The most frequently reported adverse effects were neck weakness, dysphagia, dry mouth and dysphonia[43].

In the field of writer's cramp and others occupational dystonias, some studies support the efficacy of BoNT treatment[44-48], although the evidence only provides a level B recommendations for A/Ona and A/Abo[17]. However, despite this reported benefit, the withdrawal rate in a long term follow-up was high[49]. This result was most likely due to the wide range of responses, which were frequently mild[50], and the disability due to weakness from injection[51].

With respect to the doses, no more than 160 units of A/Ona and 250 units of A/Abo have been proposed[52] (Table 1). The maximum improvement usually lasts approximately 3 mo[52].

Many muscle groups of the flexor and extensor compartment of the forearm may be involved in the development of the occupational dystonia[52] (Table 1). Thus, the strategy for the BoNT treatment should be the selection of those few dystonic muscles predominantly affected[51]. In this sense, clinical examination with a special focus to distinguish compensatory muscle activity from the real dystonic muscles is usually sufficient[53], although an EMG recording could be helpful in some complex cases[51].

The accuracy of the needle placement into the correct muscle could be poor in the forearm without any guidance[54]. Thus, EMG with or without electrical stimulation and/or ultrasound guidance should be considered in the muscles targeting[53,55].

Weakness is the most common adverse effect; other reported side effects include muscle atrophy, pain, bruising and numbness[52].

BoNT therapy could be helpful for treating the two main types of laryngeal dystonia: adductor or abductor spasmodic dysphonia. However, despite lengthy clinical experience[56], its efficacy is supported by only one controlled trial using A/Ona[57] (level C of recommendation[17]).

The muscles usually involved are the thyroarytenoid for adductor spasmodic dysphonia[56,58] and the posterior cricoarytenoid for abductor spasmodic dysphonia[56,59]. Additionally, in adductor spasmodic dysphonia, further muscles, such as the lateral cricoarytenoid[60], the interarytenoid[61] and the supraglottic muscle complex[62], have been proposed to be involved (Table 1).

Several injection strategies have been reported, but none of them have demonstrated to have a clear benefit over the others. Thus, the selected muscle can be approached either permucosally, through a channeled fiberoptic laryngoscope introduced trans-nasally under video screen visualization[63], or percutaneously, throughout the cricothyroid membrane guided by a laryngoscope[64], EMG[65] and/or by a "point-touch" technique based solely on laryngeal anatomy[66]. Finally, the injection can be uni- or bilateral[67].

There is no consensus on the dose of BoNT to be injected. In adductor spasmodic dysphonia, Blitzer reported an average dose of 0.9 units of A/Ona per vocal fold in the bilateral technique and 1.5 units in the unilateral one[56] (Table 1). In abductor spasmodic dysphonia, the same author suggested that 3.75 units should be injected unilaterally in the more dystonic posterior cricoarytenoid muscle with the option of an additional 0.6 to 2.5 units in the equivalent contralateral muscle[56] (Table 1).

The most common adverse effects reported are breathiness, weak voice, dysphagia and dyspnea[58]. Finally, the mean duration of benefit was over 11 wk[58].

Jaw-deviating, jaw-opening and, especially, jaw-closing oromandibular dystonia[9,68] (OMD) together with tongue-protrusion lingual dystonia[18,53] could also improve with BoNT therapy. The muscles usually injected are the masseter, the medial pterygoid and the temporalis in jaw-closing OMD[18,53], the lateral pterygoid and the submentalis complex in jaw-opening OMD[18,53] and the contralateral lateral pterygoid muscle in deviating OMD[18,53]. Furthermore, in deviating OMD, if it is associated with jaw protrusion, the ipsilateral external pterygoid muscle could additionally be injected[53] (Table 1). Lastly, the intrinsic tongue muscles are usually the target in tongue-protrusion lingual dystonia[18,53]. BoNT doses reported are 25-50/25-50/100/2500 units (A/Ona//A/Inco//A/Abo//B/Rima) for the masseter[18,53], 15-30/15-30/30-60/1000 units (A/Ona//

A/Inco//A/Abo//B/Rima) for the pterygoids[18,53], 20-50/20-50/80-100 units (A/Ona//A/Inco//A/Abo) for the temporalis[18,53] and 10/10/30-40 units (A/Ona//A/Inco//A/Abo) for the intrinsic tongue muscles[18,53] (Table 1). The most frequent side effects reported are dysphagia and dysarthria[68]. The mean duration of response is over 16 wk[68].

Finally, several reports have shown benefits with BoNT in the treatment of other focal dystonias, such as those associated with Parkinson's disease and other atypical parkinsonisms[69], with a special focus in "Off" painful dystonia[70].

SPASTICITY

Many studies have supported the efficacy of the BoNT therapy in the treatment of spasticity in both adults who have acquired brain injury[71-96] and children who have cerebral palsy[97-102], especially to improve muscle tone and passive function in adults and equinus varus deformity in children (level A of recommendation)[103]. Furthermore, other goals, such as the improvement of active function, the treatment of adductor, hamstrings and upper limb spasticity as well as the control of pain and spasms have been suggested[91-95,103,104]. In this sense, the choice of realistic individual treatment goals is very important for the success of the therapy[104-106].

The available formulations whose efficacy in the treatment of spasticity is the most widely supported are A/Ona and A/Abo[7,107]. In both of these formulations, the doses vary between adults and children and should be determined by the individual condition of the patient, the goal of treatment, the amount of spasticity and the muscle volume[104,107]. In this sense, the muscles most frequently injected and their recommended doses of BoNT can be found in several previous reports[84,104,105,108]. In addition, it is important to determine the safe maximum dosages of BoNT to be used without severe adverse events. In this sense, as confidence with BoNT has grown over the years, the maximum dose per session of BoNT recommended for the treatment of spasticity has increased[104]. The reason is that spasticity, especially in children with cerebral palsy, usually involves several muscle groups; therefore, a multi-level treatment strategy beyond the classic focal treatment is needed[104]. Thus, in adults, a maximum dose of 600 units per injection session and 50 units per injection site of A/Ona and a maximum of 1500 units per session and 125 units per site of A/Abo have been proposed[107], although other authors have gone to a maximum of 2000 units of A/Abo per session in the lower limbs, considering 1000 units per lower limb and 500 units per upper limb as an optimal dosage[108]. In children, the recommended safe doses per session are between 1 and 20 (a maximum of 25) units/kg bw for A/Ona and A/Abo[7]; other authors suggest doses up to a maximum of 14.29 units/kg bw for A/Ona and 37.5 units/kg bw for A/Abo[109]. In addition, the maximum doses proposed for children are 400-600 units per session and 10-50 units per site of A/Ona and 500-1000 units per session and 50-250 units per site of A/Abo[7]. In any case, several points should be considered. First, the adverse effects are dose-dependent; therefore, it is recommended to initiate BoNT therapy with doses as low as possible and to increase them according to the patient response[107]. Second, by distributing the total dose over multiple muscles and over multiple injection sites per muscle to avoid the saturation of the injection site, which could spread the toxin to the neighboring structures or into the systemic circulation, significant unwanted side effects can be avoided and are rare[104,105]. Third, it has been suggested that caution be taken for quadriplegic patients who have swallowing and/or respiration problems[104]. Finally, if spasticity is generalized and there are a considerable number of muscle groups involved, other therapies should be considered[105,110]. In this sense, BoNT for spasticity should always be given as part of an integrated and multidisciplinary rehabilitation program where other therapies, such us functional therapies, orthoses, oral medication, intrathecal baclofen and orthopedic surgery are available[7,104,107].

Because BoNT acts by blocking acetylcholine release at the neuromuscular junction, injection into the region of the motor end-plate should increase its efficacy[111]; therefore, it is recommended[107] in those muscles where these motor end-plates are well defined and can be located from external landmarks[112-115].

Clinical examination based on anatomical landmarks is often used for muscle targeting[104]. However, in a recent study, Schnitzler *et al*[116] showed that needle placement based solely in this technique was successful in only the 43% of the injections performed, even for large and superficial muscles. They concluded that muscle palpation and anatomical landmarks are insufficient to ensure the accuracy of muscle injection[116]. Thus, injection guidance with EMG, with or without electrical stimulation, and/or ultrasound should be considered[107]. Another point to consider is the dilution of the BoNT; in this sense, volumes between 1 mL per 100 units and 8 mL per 100 units have been compared and no significant differences in efficacy were found[101,117,118], although dilutions of 8 mL per 100 units could increase adverse effects in children[101]. Finally, local anesthesia, sedation or general anesthesia is recommended, especially in children with cerebral palsy[7].

Patients with spasticity usually require repeated injections of BoNT, although the frequency of sessions should not be more than once every 3 mo[104]. In this sense, sustained activity of the BoNT has been reported with repeated use[107], in addition to long-term benefits, such as a significant improvement in gait pattern and movement capacity[106] together with a delay and reduction of surgical orthopedic procedures[119]. Thus, in children with cerebral palsy, treatment should start as young as possible, when gait patterns and motor function are still flexible[104]. The optimal age often recommended is between 2 and 6 years old[104], although treatment in children under two years of age has also been suggested[109,120].

BoNT therapy for spasticity is safe[106], even in

Table 2 Method of injection of onabotulinumtoxinA for chronic migraine

Muscles injected	Injection sites (both sides)	Doses of A/Ona per muscle (both sides)
Frontalis	4	20 units
Corrugator	2	10 units
Procerus	1 (only one site in the midline of the forehead)	5 units
Temporalis	8	40 units
	Extra 2 optional sites	Extra 10 optional units
Occipitalis	6	30 units
	Extra 2 optional sites	Extra 10 optional units
Trapezius	6	30 units
	Extra 4 optional sites	Extra 20 optional units
Cervical paraspinal muscle group	4	20 units
Total sites range	31-39 total dose range	155-195 units

Adapted from the treatment paradigm based on the PREEMPT clinical program[132]: 155 units of onabotulinumtoxinA (A/Ona) every 12 wk administered to 31 injection sites across 7 specific head and neck muscle areas using a fixed-site, fixed-dose injection paradigm. Furthermore, up to 40 optional units could be administered using a "follow-the-pain" strategy. Each injection site = 0.1 mL = 5 units of A/Ona.

younger patients[109]. Local side effects, such as weakness or hematoma, as well as distant adverse events, such as tiredness or bladder dysfunction, could be present in one-third of injections[7,106], although they are usually mild[106]. The rate of severe adverse events is usually low, even with high doses on BoNT[106]. Nevertheless, a botulism-like syndrome with severe deterioration in respiratory and oromotor function has been reported and should be considered[109,120,121].

HEADACHE, MYOFASCIAL, FACIAL AND NEUROPATHIC PAIN

The role of BoNT in the treatment of headache is controversial[122,123]. Evidence only supports the use of BoNT being helpful in the prophylaxis of chronic migraine[124]. In contrast, the evidence that supports the use of BoNT in other clinical forms of headache, such as episodic migraine, as well as tensional, cervicogenic or cluster headaches, is poor or absent[124-128].

In this sense, several strategies of injection (fixed or "follow the pain") using a wide range of doses have been suggested for different forms of headache without conclusive results[129]. However, only one controlled trial has shown the efficacy of the BoNT therapy in headache[3]. In this study, which was designed for the prophylactic treatment of chronic migraine, patients received 155 units of A/Ona every 12 wk administered to 31 injection sites across 7 specific head and neck muscle areas using a fixed-site, fixed-dose injection paradigm[130] (Table 2). Furthermore, up to 40 optional units could be administered using a "follow-the-pain" strategy[130] (Table 2). The results showed a significant improvement over placebo-treatment in multiple headache symptom dimensions after 24 wk of follow-up, such as headache frequency, headache episodes, the rate of moderate/severe headaches per day and total cumulative hours of headache[3]. Furthermore, patients treated with BoNT used significantly less triptans and had a reduction of headache-related disability[3]. Adverse effects were usually transient, mild to moderate and occurred in fewer than 10% of patients[3]. Adverse effects that were more frequent were muscular weakness, ptosis, muscle tightness and local pain[3]. In summary, the authors concluded that A/Ona is an effective and safe prophylactic treatment for patients who have chronic migraine[3]. Finally, the same cohort was followed an additional 32 wk in an open-label phase. The results showed permanent long-term benefits after repeated injections of BoNT[131].

BoNT therapy has been suggested for the treatment of myofascial pain syndrome occurring at different muscular groups[132]. However, test trials have shown contradictory results; therefore, there are insufficient data to recommend its generalized use[132]. In any case, BoNT could be useful in some subgroups of patients where the evidence is stronger, namely patients with refractory myofascial pain[133], patients with temporomandibular disorders[134,135] and patients with piriformis syndrome[136].

Finally, although some studies have shown the efficacy of BoNT treatment in neuropathic pain syndromes, such as trigeminal neuralgia[137] or postherpetic neuralgia[138], there is insufficient evidence to provide a generalized recommendation for its use[139].

OTHERS INDICATIONS

BoNT could be a treatment option in many other neurological conditions. We are going to discuss the most relevant.

Sialorrhea and drooling are common and disabling manifestations in different neurological diseases, such as amyotrophic lateral sclerosis, Parkinson's disease or cerebral palsy. BoNT has been proposed as a treatment for these hypersalivation disorders because it inhibits the release of presynaptic acetylcholine at the neurosecretory junctions of the salivary glands[140-151]. In this sense, several injection techniques have been reported: first, either the parotid or the submaxillary salivary glands or both were injected[4]. Second, doses of BoNT per session were between 10 to 100 units of A/Ona (2 to 70 units in chil-

dren), between 20 to 450 units of A/Abo (5 to 40 units in children) and 2500 units (1500 to 3000 in children) of B/Rima[4]. Finally, the needle targeting was based on both ultrasound guidance and manual palpation[4].

The reported side effects included dysphagia, dry mouth, chewing difficulty and mandibular luxation[4]. Finally, the duration of therapeutic effects ranged from one and a half to 6 mo[4].

Several studies have shown the effectiveness of A/Ona in treating essential tremor, especially in the hand, head and voice[152-157]. However, the data available are limited[158], the reported benefits were usually modest[17] and the side effects, such as hand weakness, hoarseness and swallowing difficulties were, in some studies, too frequent[154,157]. Thus, BoNT therapy for treating essential tremor should be considered only in medically refractory cases[158].

Facial palsy may lead to unwanted permanent results, namely facial expression asymmetry, synkinesia, myokymia and hyperlacrimation. Several studies have suggested that injections of BoNT into the facial muscles could improve them[159-163].

Additionally, injections of A/Ona into the detrusor muscle or suburethrally *via* either flexible cystoscope under local anesthesia or rigid cystoscope under general anesthesia[164] has been reported as a treatment option in patients with both neurogenic detrusor overactivity and detrusor sphincter dyssynergia with good outcomes and minimal adverse effects[165-168].

In addition, A/Ona could be useful improving motor[169] and phonic[170] components of tics as well as to relieve premonitory sensations associated with them[171]. Furthermore, it could prevent spine disorders related to severe dystonic neck tics[172].

Finally, benefits have been reported when BoNT is used in the treatment of neurological disorders such as obstetric brachial plexus injury[173-177], camptocormia and Pisa syndrome in Parkinson´s disease[178,179], stuttering[180], painful legs and moving toes syndrome[181,182] and myokymia related to multiple sclerosis[183].

CONCLUSION

BoNT is useful and safe for a large number of neurological conditions. In this sense, many new indications are being suggested, not only for disorders where an increased muscle tone is associated with the pathogenic mechanism but also for chronic pain and other maladies. However, in some cases, although significant benefits are shown in daily clinical practice, the level of evidence is low due to the lack of controlled trials. This issue, together with the improvement of targeting techniques in some indications, as well as the problem of neutralizing antibodies, could be interesting goals for future studies.

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REVIEW

Advances in the molecular diagnosis of Charcot-Marie-Tooth disease

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Abstract

Charcot-Marie-Tooth (CMT) disease or hereditary motor and sensory neuropathy is the most common inherited neuromuscular disorder affecting at least 1 in 2500. CMT disease is pathologically and genetically heterogeneous and is characterized by a variable age of onset, slowly progressive weakness and muscle atrophy, starting in the lower limbs and subsequently affecting the upper extremities. Symptoms are usually slowly progressive, especially for the classic and late-onset phenotypes, but can be rather severe in early-onset forms. CMT is grouped into demyelinating, axonal and intermediate forms, based on electrophysiological and pathological findings. The demyelinating types are characterized by severely reduced motor nerve conduction velocities (MNCVs) and mainly by myelin abnormalities. The axonal types are characterized by normal or slightly reduced MNCVs and mainly axonal abnormalities. The intermediate types are characterized by MNCVs between 25 m/s and 45 m/s and they have features of both demyelination and axonopathy. Inheritance can be autosomal dominant, X-linked, or autosomal recessive. Mutations in more than 30 genes have been associated with the different forms of CMT, leading to major advancements in molecular diagnostics of the disease, as well as in the understanding of pathogenetic mechanisms. This editorial aims to provide an account that is practicable and efficient on the current molecular diagnostic procedures for CMT, in correlation with the clinical, pathological and electrophysiological findings. The most frequent causative mutations of CMT will also be outlined.

Key words: Charcot-Marie-Tooth disease; Charcot-Marie-Tooth; Neuropathy; Genetics; Molecular diagnosis

Core tip: Charcot-Marie-Tooth (CMT) disease is the most common neuromuscular disorder affecting at least 1 in 2500. CMT according to electrophysiological and pathological findings is categorised into demyelinating, axonal and intermediate forms and inheritance can be autosomal dominant, X-linked, or autosomal recessive. More than 30 causative genes have been identified. This editorial aims to present an efficient account of molecular diagnostic procedures for CMT, based on clinical, pathological and electrophysiological findings as well as summarize the most frequent causative mutations.

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INTRODUCTION

Charcot-Marie-Tooth (CMT) disease, also known as Charcot-Marie-Tooth neuropathy, hereditary motor and sensory neuropathy (HMSN) and Peroneal Muscular Atrophy was first described by Charcot *et al*[1] and Tooth[2] in 1886. CMT is the most common inherited neuromuscular disorder, with a prevalence of 17-40 per 100000 in-

dividuals[3,4]. Clinically, it is characterized by a variable age of onset and a variable phenotype. The main features of CMT comprise, a slowly progressive symmetric weakness and muscle atrophy of the peroneal and distal muscles of the lower limbs, sensory loss, foot deformities (pes cavus and hammer toes), and decreased or absent tendon reflexes. Hands and forearms are affected later in life. Bilateral pes cavus is almost invariably present with substantial variation in the level of sensory symptoms and signs[5]. According to electrophysiological and pathological findings CMT is classified into demyelinating, axonal and intermediate forms. The demyelinating types (CMT1 or HMSN I) are characterized by severely reduced motor nerve conduction velocities (MNCVs) (median nerve MNCV < 38 m/s) and mainly by myelin abnormalities on nerve biopsy including onion bulbs[6]. The axonal types (CMT2 or HMSN II) with primarily axonal degeneration are characterized by normal or slightly reduced motor nerve conduction velocities (median nerve MNCV > 38 m/s) but reduced amplitudes. Normal amplitudes are usually more than 4-6 μV, however this also differs between nerves[7-9]. Intermediate types include CMT patients that have features of both demyelination and axonopathy with median nerve MNCVs ranging from 25 to 45 m/s[10]. Inheritance in CMT can be autosomal dominant (AD)[11], X-linked[12,13], or autosomal recessive[14,15]. More than 50 loci and more than 30 CMT genes have thus far been identified (Table 1, http://neuromuscular.wustl.edu/time/hmsn.html).The majority of CMT patients worldwide have been characterized at the molecular genetic level. More than 70% of all CMT patients have mutations in one of four genes: *PMP22*, *GJB1*, *MPZ*, and *MFN2*. Approximately 25% of CMT patients are still pending molecular diagnosis, the great majority of them with CMT2[16]. Despite major advances in the identification of causative CMT genes, the related pathogenic mechanisms still remain to be clarified[17,18].

CMT CLASSIFICATION

CMT is classified into the following 3 types, based on clinical and neurophysiological findings, the inheritance pattern and associated gene mutations.

CMT1 (HMSNI): Autosomal dominant demyelinating CMT

CMT1 is the most common type of CMT and is divided into different subtypes based on molecular genetic findings.

CMT1A is the most common subtype (70% of demyelinating CMT and 40% of all CMT patients)[16,19,20]. CMT1A is caused by the duplication of a 1.4 Mb region on chromosome 17p12 that contains the peripheral myelin protein 22 (*PMP22*) gene[16,21-24]. *De novo PMP22* duplication mutations occur in 6.5% of CMT1A patients. PMP22 protein is a hydrophobic 22 kDa glycoprotein that is expressed mainly in myelinating Schwann cells and plays an important role in myelination, proliferation and differentiation[25,26]. Overexpression of *PMP22* gene dosage reduces the proliferation of Schwann cells and may affect the intracellular degradation of membrane components[3,27]. The onset of clinical symptoms is in the first or second decade of life, usually in childhood, characterized by a typical CMT phenotype, and usually a mild disease progress. However, disease severity is variable, even in individuals of the same family. In many cases there is also

Table 1 Charcot-Marie-Tooth classifications

Type	Locus	Gene	OMIM	Ref.
CMT1: Dominant demyelinating				
CMT1A	17p12	*PMP22*	*601097	[21,22]
CMT1B	1q22	*MPZ*	*159440	[28]
CMT1C	16p13	*LITAF* (*SIMPLE*)	*603794	[171]
CMT1D	10q21.3	*EGR2*	*129009	[37]
CMT1E	17p12	*PMP22*	*601097	[21,22]
CMT1F	8p21	*NEFL*	*162280	[105]
CMT1	14q32	*FBLN5*	*604580	[47]
CMT2: Dominant axonal				
CMT2A	1p36.22	*MFN2*	*608507	[51,52]
CMT2B	3q21.3	*RAB7*	*602298	[55]
CMT2C	12q24.11	*TRPV4*	*605427	[61]
CMT2D	7p15	*GARS*	*600287	[64]
CMT2E	8p21	*NEFL*	*162280	[66]
CMT2F	7q11.23	*HSPB1* (*HSP27*)	*602195	[69]
CMT2G	12q12-13.3	*Unknown*		
CMT2H/2K	8q21.11	*GDAP1*	*606598	[76]
CMT 2I/2J	1q22	*MPZ*	*159440	[80,81]
CMT2L	12q24.3	*HSPB8* (*HSP22*)	*608014	[83]
CMT2M	19p13	*DNM2*	*602378	[84]
CMT2N	16q22.1	*AARS*	*601065	[89]
CMT2O	14q32.31	*DYNC1H1*	*600112	[93]
CMT2P	9q33	*LRSAM1*	*610933	[95,96]
CMT2Q	10p14	*DHTKD1*	*614984	[100]
DI-CMT: Dominant intermediate				
DI-CMTA	10q24.1-q25.1	*Unknown*		
DI-CMTB	19p13	*DNM2*	*602378	[84]
DI-CMTC	1p35.1	*YARS*	*603623	[106]
DI-CMTD	1q22	*MPZ*	*159440	[10]
DI-CMTE	14q32.33	*IFN2*	*610982	[107]
DI-CMTF	3q26.33	*GNB4*	*610863	[108]
CMT4: Recessive demyelinating				
CMT 4A	8q21.11	*GDAP1*	*606598	[78]
CMT 4B-1	11q22	*MTMR2*	*603557	[112]
CMT 4B-2	11p15	*MTMR13* (*SBF2*)	*607697	[117]
CMT 4C	5q23-33	*SH3TC2*	*608206	[119]
CMT 4D	8q24.3	*NDRG1*	*605262	[125]
CMT 4E	10q21.3	*EGR2*	*129010	[37]
CMT 4F	19q13.2	*PRX*	*605725	[43,129]
CMT 4G	10q23.2	*HK1*	*142600	[134]
CMT 4H	12p11.21	*FGD4*	*611104	[137,138]
CMT 4J	6q21	*FIG4*	*609390	[141]
AR-CMT2: Recessive axonal				
AR CMT 2A	1q22	*LMNA*	*150330	[144]
AR CMT 2B	19q13.3	*MED25* (*ACID1*)	*610197	[146]
AR-CMT 2C	8p21	*NEFL*	*162280	[66,150]
AR CMT2D	8q21.11	*GDAP1*	*606598	[76]
AR CMT2E	9q33	*LRSAM1*	*610933	[95,96]
CMTX: X-linked				
CMTX1	Xq13.1	*GJB1*	*304040	[153]
CMTX2	Xp22.2	*Unknown*		
CMTX3	Xq26.3-q27.1	*Unknown*		
CMTX4	Xq26.1	*AIFM1*	*300169	[161]
CMTX5	Xq22.3	*PRPS1*	*311850	[165]
CMTX6	Xp22.11	*PDK3*	*602526	[166]

OMIM: Online endelian inheritance in man; CMT: Charcot-Marie-Tooth; DI: Dominant intermediate; AR: Autosomal recessive.

nerve hypertrophy (25%) and in some hearing loss (5%)[3]. The MNCVs are reduced (< 38 m/s) in the early stages of the disease.

CMT1B is caused by mutations in the myelin protein zero (*MPZ* or *P0*) gene, located on chromosome 1q22-q23 and account for about 5% of CMT1 cases[3,16,28]. MPZ protein is a 28 kDa glycoprotein that is located in Schwann cells. This protein is necessary for normal myelin structure and function and is found in abundance in the myelin of peripheral nerve tissues and is completely absent from the myelin of the central nervous system[27,29]. CMT1B is characterized by an early onset (usually first decade) and most *MPZ* mutations cause a classical CMT1 phenotype, however, some mutations cause a more severe Dejerine-Sottas syndrome (DSS-CMT3B) or congenital hypomyelination neuropathy (CHN)[30,31]. Patients with an early onset have reduced MNCVs and patients with a late onset have normal or slightly reduced MNCVs[32].

CMT1C is caused by mutations in the lipopolysaccharide-induced tumor necrosis factor (*LITAF*)/small integral membrane protein of lysosome late endosome (*SIMPLE*) gene. The gene is located on chromosome 16p13.1-p12.3 and plays an important role in protein degradation[33]. *LITAF* mutations account for less than 1% of CMT patients[34]. The first clinical symptoms in patients with CMT1C appear in the second decade with a typical CMT1 phenotype and conduction velocities around 16-25 m/s[3,33,35,36].

CMT1D accounts for less than 1% of CMT patients and is caused by mutations in the early growth response element 2 (*EGR2*) gene, that is located on chromosome 10q21.3[37]. *EGR2* is a transcription factor that is involved in the regulation of myelin genes[38,39]. Most patients have very early onset CMT1 or the most severe DSS (DSS-EGR) or CHN phenotypes, however, patients with late onset and a milder phenotype have been described[37,40-43]. Patients with cranial nerve deficits, including diseases of the pulmonary system, respiratory failure, diplopia and vocal cord paresis have been reported[3,34]. Motor nerve conduction velocities are slightly to severely reduced (9-42 m/s)[3].

CMT1E is caused by point mutations in the *PMP22* gene[21-23]. Patients with *PMP22* point mutations have more severe symptoms than patients with CMT1A and usually have slower NCV[44]. Point mutations of *PMP22* may cause various other phenotypes such as HNPP, DSS (CMT3A) or CHN[45].

CMT1F is caused by mutations in the neurofilament light chain (*NEFL*) gene located on chromosome 8q21. The encoded protein plays a role in intracellular transport of axons and dendrites[46]. The first symptoms of the disease appear in the first decade of life and are usually severe, with severely reduced MNCVs (15-38 m/s)[3]. *NEFL* mutations also cause CMT2E and CMT4C2.

CMT1G has been recently described and is caused by mutations in the fibulin-5 (*FBLN5*) gene on chromosome 14q32[47]. *FBLN5* is located in an extracellular matrix and is a calcium-binding glycoprotein that plays an important role in elastic fiber assembly and in endothelial cell adhesion[48]. The age of onset, the phenotype and the MNCVs of the disease vary[47].

CMT2 (HMSNII): Autosomal dominant axonal CMT

CMT2 accounts for 20% of all CMT patients and is characterized by normal or slightly reduced MNCVs[16,35].

CMT2A is the most common form of CMT2 and accounts for 20% of CMT2 patients[49,50]. It is caused by mutations in the mitofusin 2 (*MFN2*) gene, located on chromosome 1q36.22. A mutation in the kinesin motor protein 1B (*KIF1B*) gene has been reported in a Japanese family, but mutations in the *KIF1B* have not thus far been confirmed in any other family[51]. MFN2 is a large dynamin-like GTPase protein that plays an important role in the fusion of mitochondria. When this protein is modified as a result of gene mutations, it leads to an insufficient protein transfer between mitochondria and the axons of peripheral nerves[3]. Most patients (80%) have an early onset of symptoms (< 10 years old) with a severe phenotype and usually become wheelchair bound by 20 years of age. The remaining 20% of patients have a later onset of symptoms (10-50 years old) and a milder phenotype[52]. CMT2A patients with optic atrophy, hearing loss, cerebral white matter abnormalities and diabetes mellitus have been described[16,49,50,53,54]. Motor nerve conduction velocities are typically normal, however, the amplitudes are slightly to severely reduced or absent[54].

CMT2B is caused by mutations in the RAS-associated GTP-binding protein (*RAB7*) gene located on chromosome 3q21.3[55]. RAB7 is a GTPase protein that localizes to late endosomes and lysosomes and is involved in the regulation of late endocytic traffic[56,57]. Clinical symptoms appear between the second and fourth decades and include typical CMT phenotype and mild to moderate sensory loss, that often leads to foot ulcerations and subsequently infections and amputations[58,59]. MNCVs are normal to slightly reduced with usually reduced amplitude.

CMT2C is caused by mutations in the transient receptor potential vanilloid 4 (*TRPV4*) gene located on chromosome 12q24.11[60,61]. The TRPV4 protein is a cation channel (Ca^{2+} channel) that activates pathways leading to the regulation of systemic osmotic pressure[62]. CMT2C is characterized by weakness of proximal muscles, vocal cord, diaphragmatic paresis and occasionally a fatal outcome. Some other features have been reported including sensorineural hearing loss, raspy voice, bladder urgency and incontinence[7,61,62]. The age of onset is between the second and fifth decades of life and MNCVs are normal (> 50 m/s)[61,62].

CMT2D is caused by mutations in the glycyl-tRNA synthetase (*GARS*) gene located on chromosome 7p15[63,64]. The encoded protein plays an important role in translation processes and *GARS* gene mutations affect protein synthesis, which is important for the normal function of the motor nerve[3]. The first symptoms appear between first and forth decade and primarily affect the upper extremities and then to a lesser extent the

lower extremities[64,65]. MNCVs are normal.

CMT2E is caused by mutations in the *NEFL* gene[66]. *NEFL* mutations also cause CMT1F and CMT4C2[66,67]. CMT2E is clinically similar to CMT1F, although typically it is less severe, with normal or slightly reduced MNCVs.

CMT2F is caused by mutations in the heat shock protein B1 (*HSPB1*, also known as *HSP27*) gene located on chromosome 7q11.23[68,69]. The HSPB1 protein protects the structure of other proteins (bind and prevent misfolding and aggregation of nascent proteins) and also interacts with the NEFL protein and protects motor neurons[57,70]. Symptoms progression is slow and they begin with symmetrical weakness of the lower extremities resulting in foot drop, foot deformities, and sensory dysfunctions and then progresses slowly to the upper extremities[71]. The age of onset of the disease is in the first or second decade of life and the MNCVs[72].

CMT2G maps to chromosome 12q12-q13.3 but associated gene mutations are still unknown[73,74]. The age of onset varies from the first to the eighth decade, although most patients developed symptoms in the second decade. Clinical symptoms include foot deformity and difficulty in walking, with very slow progression and absent ankle reflexes[73]. MNCVs are normal or mildly decreased.

CMT2H and CMT2K are caused by mutations in the ganglioside induced differentiation associated protein 1 (*GDAP1*) gene on chromosome 8q21.11[75,76]. *GDAP1* mutations also cause axonal recessive CMT (CMT4C4) or demyelinating recessive CMT (CMT4A)[76-78]. The clinical symptoms in CMT2H/K appear in the second decade with a mild to moderate and slowly progressive phenotype with vocal cord paralysis and occasionally with optic nerve atrophy and normal or slightly reduced MNCVs[79].

CMT2I and CMT2J are caused by mutations in the *MPZ* gene[80,81]. The first symptoms appear very late (between 45 and 60) with a typical CMT2 phenotype, although there are some patients with pupillary abnormalities, deafness and sensory disturbances[16,81]. MNCVs are normal or slightly reduced (> 38 m/s), but during the progress of the disease they are decreased (< 38 m/s)[3].

CMT2L is caused by mutations in the heat shock 22 kDa protein 8 (*HSPB8*) gene, also known as heat shock protein 22 (HSP22) on chromosome 12q24[82,83]. HSPB8 is highly expressed in the spinal cord and in the motor and sensory neurons and is mainly localized to the plasma membrane. Also it possesses chaperone-like activity and inhibits protein aggregation and degrades misfolded proteins[57]. Clinical symptoms start between 15-35 years old and include distal muscle weakness and atrophy, mild sensory loss and scoliosis present in some patients. MNCVs are normal or near-normal.

CMT2M is caused by mutations in the dynamin 2 (*DNM2*) gene located on chromosome 19p13[84,85]. DNM2 is a large GTPase protein involved in membrane trafficking and endocytosis[86]. CMT2M is characterized by distal muscle weakness and atrophy of the lower extremities, mild weakness of upper extremities and foot deformities, including pes cavus and toe clawing[87,88]. The symptoms appear between the age of 20-55 years and the MNCVs are normal to slightly reduced.

CMT2N is caused by mutations in alanyl-tRNA synthetase (*AARS*) gene on chromosome 16q22.1[89]. AARS protein is an aminoacyl-tRNA synthetase (ARS). ARSs are ubiquitously expressed, essential enzymes that ligate amino acids to produce tRNAs needed for global protein synthesis[90]. Clinical features include mild to moderate weakness of lower limbs and milder or absent weakness of the upper limbs. Some patients had foot drop, pes cavus, hammer toes, absent ankle reflexes and hyporeflexia[89,91,92]. The age of onset is varying (6-54 years old) and MNCVs are normal.

CMT2O is caused by mutations in the dynein cytoplasmic 1 heavy chain 1 (*DYNC1H1*) gene on chromosome 14q32.31[93]. Dyneins are a group of ATPases that help to convert chemical into mechanical energy. Cytoplasmic dynein is a large motor protein complex that is involved in intracellular functions, including reversing axonal transport in neurons[93,94]. Clinical features include progressive distal lower limb weakness, pes cavus, variable sensory loss and in some patients proximal weakness and waddling gait[93]. The first symptoms occur in childhood and the MNCVs are normal.

CMT2P is caused by mutations in the leucine rich repeat and sterile alpha motif containing 1 (*LRSAM1*) gene on chromosome 9q33.3. The inheritance can be autosomal recessive (AR-CMT2)[95] or autosomal dominant (CMT2P)[96,97]. LRSAM1 is a multifunctional RING finger E3 ubiquitin ligase that plays an important role in endocytosis and in neuronal cells adhesion[98,99]. The first symptoms for CMT2P appear between the second and fifth decade of life and include distal weakness in the lower limbs and in some patients also present in the upper limbs. Other features have been reported in some patients including episodic cramps, bilateral pes cavus, foot drop, absent tendon reflexes, severe loss of sensation in feet and legs and mild loss of sensation on fingertips, sensory and motor dysfunction[95-97]. MNCVs are normal to slightly reduced.

CMT2Q is caused by mutations in the dehydrogenase E1 and transketolase domain-containing 1 (*DHTKD1*) gene on chromosome 10p14[100]. This gene encodes a mitochondrial 2-oxoglutarate-dehydrogenase-complex-like protein that is involved in the degradation of several amino acids pathways[100,101]. The age of onset is in the first and second decade and the phenotype is typical CMT2, including distal muscle weakness of the lower limbs, decreased or absent tendon reflexes, and mild to moderate sensory loss[100]. MNCVs are normal (> 40 m/s).

Late onset CMT2 is caused by mutations in the methionyl-tRNA synthetase (*MARS*) gene[102]. This type has recently been identified in one CMT2 family. Clinically it is characterized by late onset (> 50 years old) and a mild CMT2 phenotype[102]. MNCVs studies confirmed an axonal neuropathy.

DI-CMT: Autosomal dominant intermediate CMT

Dominant intermediate CMT types (DI-CMT) are characterized by intermediate MNCVs (25-45 m/s) and the

clinical symptoms are moderate to severe. Electrophysiological and pathological features include both axonal and demyelinating types.

DI-CMTA has been mapped to chromosome 10q24.1-q25.1, but the responsible gene is unknown[103,104]. The phenotype is typical CMT and the MNCVs are moderately reduced[103].

DI-CMTB is caused by mutations in the *DNM2* gene that also cause axonal dominant CMT (CMT2M). Patients present with a classic CMT phenotype at the age of 2-50 years old. MNCVs are ranging from 26 to 54 m/s.

DI-CMTC is caused by mutations in the tyrosyl-tRNA synthetase (*YARS*) gene located on chromosome 1p35.1[105,106]. YARS plays an important role in protein synthesis and in signal transmission from nerves to muscles[106]. The age of onset is between the first and sixth decades with a classic CMT phenotype and numbness in some patients[106]. MNCVs are from 30-40 m/s.

DI-CMTD is caused by mutations in the *MPZ* gene[10]. MPZ mutations are also associated with CMT1B and CMT2I/2J. DI-CMTD is characterized by a variable severity, distal muscle atrophy, weakness, and sensory loss in the lower and upper limbs. MNCVs are 30-40 m/s.

DI-CMTE is caused by mutations in the inverted formin-2 (*IFN2*) gene on chromosome 14q32.33[107]. The encoded protein may function in polymerization and depolymerization of actin filaments. INF2 mutations disrupt actin dynamics in peripheral Schwann cells, leading to disturbed myelin formation and maintenance resulting in CMT[107]. The clinical phenotype is typical CMT, including distal muscle weakness and atrophy and distal sensory loss, with focal segmental glomerulonephritis (FSGS) including proteinuria that progresses to renal disease. The first symptoms appear between the first to third decade and MNCVs are normal to moderately reduced (23-45 m/s).

DI-CMTF is caused by mutations in the guanine nucleotide binding protein, beta polypeptide 4 (*GNB4*) gene[108]. GNB4 protein may play a role in peripheral nerve regeneration. DI-CMTF is characterized by slowly progressive distal muscle atrophy and weakness, and atrophy of the upper and lower limbs, steppage gait and distal sensory loss with decreased reflexes with onset around adolescence. MNCVs are between 16 to 45 m/s.

CMT4: Autosomal recessive demyelinating CMT

CMT4 is a demyelinating type of hereditary polyneuropathy with autosomal recessive inheritance[109].

CMT4A is caused by mutations in the *GDAP1* gene. Clinically severe motor disturbances and progressive scoliosis are observed[110]. CMT4A is characterized by an early age of onset and reduced MNCVs (25-35 m/s).

CMT4B1 is caused by mutations in the myotubularin-related protein 2 (*MTMR2*) gene on chromosome 11q22[111,112]. MTMR2 protein has phosphatase activity and influences transcription and cell proliferation[112]. The phenotype of the disease is severe CMT1 and diaphragmatic and facial weakness may occur, as may scoliosis in adult patients[113-115]. Onset is usually in childhood and MNCVs are severely reduced (10-25 m/s).

CMT4B2 is caused by mutations in the set binding factor 2 (*SBF2*) or myotubularin related protein 13 (*MTMR13*) gene located on chromosome 11p15[116,117]. The encoded protein is a pseudophosphatase that is involved in membrane trafficking[118]. The clinical phenotype, age of onset and MNCVs are similar to CMT4B1[116,119].

CMT4C is caused by mutations in the SH3 domain and tetratricopeptide repeat domain 2 (*SH3TC2*) gene that is located on chromosome 5q32[119,120]. SH3TC2 protein is expressed in Schwann cells of the peripheral nerves and localizes to the membrane with a possible function in myelination and in regions of axoglial interactions[121]. CMT4C is characterized by early-onset, distal weakness, foot deformities, walking difficulty, scoliosis and occasionally facial and bulbar weakness, sensorineural deafness and respiratory insufficiency[119,122-124]. MNCVs are reduced (10-35 m/s).

CMT4D (HMSN-L) is caused by mutations in the N-myc downstream-regulated gene 1 (*NDRG1*) on chromosome 8q24.3[125]. NDRG1 protein appears to play a role in growth arrest and cell differentiation[126]. CMT4C is characterized by distal muscle wasting and atrophy, foot and hand deformities, absent tendon reflexes, and sensory loss. The age of onset is between the first and second decade. Deafness is an invariant feature of the phenotype and usually develops in the third decade.

CMT4E (Congenital Hypomyelinating Neuropathy-CHN) is caused by mutations in the *EGR2* gene[37]. EGR2 mutations are also associated with CMT1D. CMT4E is characterized clinically by an early age of onset, hypotonia, absent of reflexes, distal muscle weakness, and extremely reduced MNCVs (< 10 m/s).

CMT4F is caused by mutations in the periaxin (*PRX*) gene located on chromosome 9q13.2[127-129]. PRX is a Schwann cell protein that plays an important role in axon-glial interactions and is needed for the maintenance of peripheral nerve myelin and regenerating axons[130,131]. CMT4F is characterized, by distal muscle weakness and atrophy, affecting the lower more than the upper limbs, by distal sensory loss and occasionally sensory ataxia. The age at onset is variable, from first to fifth decade and the MNCVs are severely reduced (< 15 m/s)[128,132]. *PRX* mutations also cause DSS (CMT3D)[128].

CMT4G or CMT-Russe (HSMNR) is caused by mutations in the Hexokinase 1 (*HK1*) gene that is located on chromosome 10q23.2[133,134]. HK1 protein is involved in the controlled production of ATP and in the regulation of cell survival. Also HK1 is highly expressed in the nervous system and it is involved in NGF-mediated neurite outgrowth[57,134]. Clinically it is characterized by an early age of onset (5-16 years old), distal muscle weakness progressing to severe on lower limbs, prominent sensory loss, hand and foot deformities[133,135]. MNCVs are mildly reduced (30-35 m/s).

CMT4H is caused by mutations in the FYVE, RhoGEF and PH domain containing 4 (*FGD4*) gene, on chromosome 12p11.21[136,137]. FGD4 (or Frabin) protein

is involved in the myelination process, although the molecular mechanisms by which *FGD4* mutations cause CMT4H are completely unknown[57,137,138]. CMT4H is characterized by distal muscle weakness and atrophy, areflexia, sensory loss, foot abnormalities and occasionally scoliosis, hypotrophy of thenar and hypothenar muscles[136,139,140].

CMT4J is caused by mutations in the FIG4 homolog, SAC1 lipid phosphatase domain containing (*FIG4*) gene, located on chromosome 6q21[141]. FIG4 protein has been shown to possess phosphoinositide phosphatase activity and plays a key role in intracellular transport vesicles[141,142]. The clinical phenotype is severe CMT1 with early onset (childhood but sometimes adult onset) and severely reduced MNCVs (< 10 m/s)[3].

AR-CMT2: Autosomal recessive axonal CMT

AR-CMT2 also called CMT4C is a recessive axonal hereditary neuropathy that is very rare.

AR-CMT2A or CMT2B1 or CMT4C1 is caused by mutations in the lamin A/C (*LMNA*) gene on chromosome 1q22. Lamin A/C is an intermediate filament protein that forms the nuclear lamina[57]. Lamin proteins are involved in nuclear stability, chromatin structure and gene expression, and also the A-type lamins are important in the protection of the cell from mechanical damage[29,143]. Clinical symptoms usually appear in the second decade (onset between 5-25 years old) with a severe CMT phenotype including proximal muscle involvement although some have a milder phenotype. *LMNA* mutations have also been associated with other phenotypes including Emery-Dreifuss muscular dystrophy, cardiomyopathy and Dunnigan-type familial partial lipodystrophy[144,145]. MNCV are normal or just slightly reduced.

AR-CMT2B or CMT2B2 or CMT4C3 is caused by mutations in the mediator complex subunit 25 (*MED25*) gene, also known as *ACID1*, that is located on chromosome 19q13.33[146-148]. The encoded protein is a component of the Mediator complex that plays a role in gene transcription and also is important in myelination[146,149]. Clinical phenotype is typical CMT2 with late onset, in the third to fifth decade of life, and normal or mildly decreased MNCVs[147,148].

AR-CMT2C or CMT4C2 is caused by mutations in the *NEFL* gene. The clinical phenotype is severe CMT2 with early onset (< 2 years old) and severely reduced MNCVs (10-25 m/s)[150].

AR-CMT2D or CMT4C4 is caused by mutations in the *GDAP1* gene. The clinical phenotype is more severe than CMT2H/K, with early onset and normal MNCVs[76,151,152].

AR-CMT2E is caused by mutations in the *LRSAM1* gene with a more severe clinical phenotype than CMT2P and earlier age of onset (first and second decade)[95-97].

CMTX: X-linked CMT

CMTX is an X-linked CMT with dominant or recessive inheritance. Clinically heterozygous females are more mildly affected (or asymptomatic) than hemizygous males[153].

CMTX1 is the second commonest form of demyelinating CMT with a frequency of 12% among all CMT patients[16,20]. CMTX1 has both demyelinating and axonal features and is caused by mutations in the gap junction binding 1 (*GJB1*) gene that is located on chromosome Xq13.1[154]. The encoded protein CX32 (connexin 32) is a transmembrane protein that forms gap junction channels that allow the transfer of small molecules between cells[153]. Clinically males have more severe symptoms, than females. Symptoms in males appear in childhood and later in females[35,155]. MNCVs are slightly reduced, between 30-40 m/s in affected males and 30-50 m/s in affected females[156]

CMTX2 has been mapped to chromosome Xp22.2 and the associated gene mutations are still unknown[157,158]. CMTX2 also has both demyelinating and axonal features and the clinical phenotype is characterized by onset in infancy, weakness and atrophy of the lower limbs, absent reflexes and pes cavus in males. MNCVs are normal to slightly reduced.

CMTX3 has been mapped to chromosome Xq26 and the associated gene mutations are also still unknown[159]. The disease onset is in first and second decade are patients and clinically characterized by progressive weakness of lower limbs and decreased tendon reflexes[157,158]. Electrophysiological findings are compatible with both axonal and demyelination features with MNCVs in the range of 25-57 m/s.

CMTX4 (Cowchock syndrome) is caused by mutations in the apoptosis-inducing factor, mitochondrion-associate 1 (*AIFM1*) gene on chromosome Xq26.1[160,161]. CMTX4 is characterized by an early childhood onset, distal muscle weakness and atrophy, sensory loss, areflexia and in some patients (approximately 60%) deafness and mental retardation[160]. Heterozygous females are asymptomatic[162]. MNCVs are normal to slightly reduced (33-56 m/s) with decreased sensory conduction velocities.

CMTX5 is caused by mutations in the phosphoribosylpyrophosphate synthetase 1 (*PRPS1*) gene located in chromosome Xq22.3[163-165]. PRS1 protein is an enzyme critical for nucleotide biosynthesis[57]. Symptoms appear in the first decade of life and the phenotype is characterized by severe peripheral neuropathy with sensorineural deafness, and optic atrophy[165]. MNCVs are normal (43-51 m/s). Heterozygous females are asymptomatic.

CMTX6 is caused by mutations in the pyruvate dehydrogenase kinase isoenzyme 3 (*PDK3*) gene on chromosome Xp22.11[166]. PDK3 is involved in the regulation of the pyruvate dehydrogenase complex (PDC). PDC catalyzes the oxidation of pyruvate to acetyl-CoA that is a key enzyme involved in the Krebs cycle and lipogenic pathways[166,167]. Disease onset is in the second decade and includes progressive moderate-to-severe wasting below the knees, minimal weakness of the hand muscles, foot deformity, steppage gait, absent ankle reflexes distal lower limb weakness and sensory abnormalities[166]. MNCVs are normal (> 38 m/s).

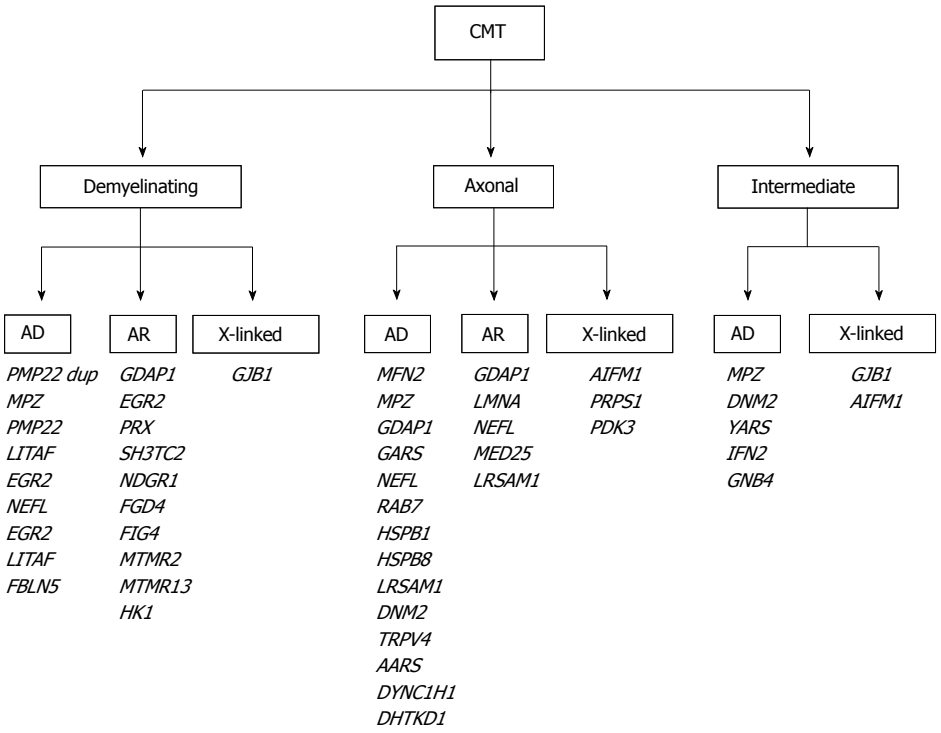

Figure 1 Molecular genetic analysis algorithm for Charcot-Marie-Tooth diagnostics. CMT: Charcot-Marie-Tooth; AD: Autosomal dominant; AR: Autosomal recessive.

MOLECULAR GENETIC TESTING

As outlined above the CMT group of disorders is characterized by a high variability in the clinical phenotype, and great differences exist in the age of the onset, disease progression and severity. There are many types of CMT and a large number of causative genes. As a result of this complexity and the considerable cost of molecular studies, it is useful to establish an algorithm for targeted molecular analysis. Several studies have been published that suggest particular methodologies for performing genetic analysis based on clinical and electrophysiological findings, age of onset of symptoms, family history and relative frequencies of gene mutations[19,20,34,35,145,168,169]. However, some laboratories are currently switching to new screening methods, such as the next generation sequencing (NGS) technology for whole exome and whole genome analysis, slowly replacing the more traditional Sanger sequencing based screening methods. The established flowchart is based on the different CMT types that are determined following the clinical and electrophysiological evaluation of the patient and the existing frequency of causative mutations (Figure 1). PMP22 duplication is the most frequent genetic abnormality and accounts for about 40% of CMT patients, GJB1 for 15%, MFN2 for 10%, MPZ for 5%, PMP22 point mutation for 2.5%; mutations in each of the other CMT genes account for less than 1%[16,19,35,170].

FUTURE STRATEGIES

Traditional Sanger sequencing based screening methods are important tools in genetic research. However, the NGS technology already used as a diagnostic tool in some centers will provide new potential capabilities in molecular diagnostic services. NGS is a high throughput technique with low cost and enables sequencing of multiple known and unknown genes in a single run. Additionally, NGS, in combination with other new technologies, such as proteomics and cellular reprogramming may play an important role in the effort to elucidate the pathogenic mechanisms of the disease and lead to the discovery of new therapeutic approaches in CMT and other diseases.

CONCLUSION

Despite the progress in molecular genetics and the development of new techniques, molecular diagnosis of patients with CMT is still challenging. New genes causing CMT continue to be identified and there exist many more that need to be identified. Increased understanding of the biological processes involved in CMT will enable better understanding of the CMT neuropathy pathogenetic mechanisms and contribute further towards the goal of inventing more effective therapeutic strategies.

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Variation in experimental autoimmune encephalomyelitis scores in a mouse model of multiple sclerosis

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Core tip: Multiple sclerosis (MS) is a common demyelinating central nervous system disease associated with progressive physical impairment. Experimental autoimmune encephalomyelitis (EAE) is often used as an animal model to study MS. EAE can be induced in various species by introducing specific antigens, and the severity of the paralysis is indicated using the EAE score. The score is simple and easy to use, however, its application varies between laboratories, and the scoring is dependent on the subjective bias of the researchers. We described the criteria used for the EAE scoring systems in various laboratories, to facilitate the study of MS.

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Abstract

Multiple sclerosis (MS) is a common demyelinating central nervous system disease associated with progressive physical impairment. To study the mechanism underlying disease pathogenesis and develop potential treatments, experimental autoimmune encephalomyelitis (EAE) is often used as an animal model. EAE can be induced in various species by introducing specific antigens, which ultimately result in motor dysfunction. Although the severity of the paralysis is indicated using the EAE score, there is no standard scoring system for EAE signs, and there is variability between research groups with regard to the exact EAE scoring system utilized. Here, we describe the criteria used for EAE scoring systems in various laboratories and suggest combining EAE score with another quantitative index to evaluate paralysis, such as the traveled distance, with the goal of facilitating the study of the mechanisms and treatment of MS.

Multiple sclerosis (MS) is an inflammatory demyelinating disorder of the brain and spinal cord. More than 100 years have passed since the clinical and pathological characteristics of MS were first described in the medical literature[1]. Despite extensive research since then, the pathogenesis underlying MS is still not fully understood. There are more than 2500000 patients with MS worldwide, and the prevalence is approximately 4 to 150 per 100000 in the population[2,3]. The incidence of MS varies across the world; it is quite high in northern Europe but lower in Asian and African countries[3,4]. MS typically manifests in young adulthood, primarily between the late twenties and early forties. Although the clinical course and prognosis of the disease demonstrate individual dif-

ferences, 50% of patients need help walking, or in some cases require a wheelchair within 15 years of the initial disease onset[4]. The demyelinating lesion of MS has been described as "disseminated in time and space"[5]. The clinical course of the disease is characterized by four major subtypes: relapse-remitting, secondary progressive, primary progressive, and progressive-relapsing MS[1,4,6]. Approximately 80% of all patients initially manifest with relapsing-remitting type MS. Symptoms and signs typically become aggravated over a period of several days, and the condition then gradually stabilizes. Patients often improve spontaneously or in response to treatment within weeks. Eventually, approximately 65% of patients with relapsing-remitting MS enter the secondary progressive phase[4]. Moreover, in 20% of all patients, the illness gradually worsens after onset, which is defined as primary progressive MS[1,4]. There is no consensus definition in progressive-relapsing MS[6].

Because the opportunity to obtain central nervous system tissue from individual patients is rare, animal models of MS have been developed to investigate the pathogenesis and treatment of the disease. Experimental autoimmune encephalomyelitis (EAE) is the most popular animal model of MS[7-11]. EAE is characterized by inflammatory infiltrates consisting of T-lymphocytes, B-lymphocytes, macrophages, and focal demyelinating plaques in the CNS; these features are also observed in MS. EAE is induced in various species, including rodents and primates, either by active immunization using a myelin antigen in adjuvant (active EAE) or by the adoptive transfer of encephalitogenic T cells (passive EAE)[10,11]. In addition, T cell receptor transgenic mice have been generated as a spontaneous EAE model. The characteristics of these mice are variable, and thus, most of the spontaneous EAE mice are also defined as atypical EAE[8,12,13].

Commonly used murine EAE models manifest motor dysfunction as ascending flaccid paralysis, beginning with a flaccid or limp tail[7,10,11]. The paralysis progresses from the hind limbs to the fore limbs and is occasionally followed by urinary incontinence and fecal impaction (classical EAE models)[7,10]. Lesions are predominantly localized to the spinal cord in classical EAE. The standard EAE mouse model is induced using myelin proteolipid protein (PLP) peptide (amino acids 139-151), which causes relapse-remitting EAE in SJL mice. In addition, the myelin oligodendrocyte glycoprotein (MOG) peptide (amino acids 35-55) causes monophasic EAE with an incomplete recovery in C57BL/6 mice[8]. Several reports have demonstrated real primary progressive or secondary progressive EAE, in which mice die as a result of disease progression[14]. However, variations from the classical EAE phenotype, such as ataxia or the head rolling phenomenon rather than limb paralysis, have been described and are referred to as atypical EAE[15-17]. The clinical signs observed in atypical EAE models reflect an increase in inflammation in the brain compared to classical EAE models. The characteristics of spontaneous EAE mice are also defined as atypical EAE.

In the active EAE model, the mice are immunized by subcutaneous injection of the myelin antigen with complete Freund's adjuvant (that is, the antigen is emulsified in paraffin oil containing inactive *Mycobacterium tuberculosis*). Intravenous or intraperitoneal injection of pertussis toxin is required to increase the incidence of EAE induction. Although the signs of motor dysfunction depend on the type of EAE model, paralysis usually begins within 9 to 14 d after sensitization[10]. Passive induction of EAE in naïve mice is achieved by the adoptive transfer of T cells isolated from active EAE mice that have been primed with myelin antigens. The day of onset of visible EAE signs varies, and depends on the model; however, signs usually appear between 10 and 15 d after induction[11].

In this review, unless otherwise noted, we describe the development of the signs of classical EAE and the evaluating system used by researchers. Many researchers misuse terms for evaluating EAE signs; for example, "EAE symptom" or "clinical assessment of EAE." The term "symptom" and "clinical" should only be used in the context of humans; and thus, the term "signs" must be used instead of "symptom." In addition, "clinical" must not be used in EAE studies. The severity of EAE is generally evaluated using an EAE score (occasionally referred to as the EAE scale or grade). Mice are scored daily after the day of sensitization to precisely detect the time of disease onset and to investigate the progression of EAE. The commonly used EAE scores are 0 to 5 or 0 to 6 point scales (Tables 1-3)[10,18-29]; however, there are problems using this method. First, each laboratory has its own method for evaluating the severity of EAE; these methods have not been standardized between laboratories. In most laboratories, a loss of tail tone is recognized as a score of 1, which is designated as a "loss of tail tonicity"[18,20,23,26,29], "flaccid tail"[19,22], "limp tail"[25,28], "tail weakness"[24] and "tail atony"[27]. In particular, a complete loss of tone has been required in previous studies[25-29]. In contrast, Sobel *et al*[21] described a score of 1 as "decreased tail tone or slightly clumsy gait". A score of 2 is identified by symptoms of paralysis/weakness of the hind limbs, impairment of the righting reflex (the mice have difficulty turning over after being laid down on their back, but there are no observed locomotor difficulties), tail paralysis and gait disturbance. The term "paralysis" indicates a complete or partial loss of voluntary movement. The prefix "para" means "both" and "plegia" means severe weakness. The term "paraplegia" is defined as a severe symmetrical muscle weakness of both lower limbs. "Paraparesis" commonly means slight or partial paralysis of both lower limbs; however, the definition of these terms is subjective and indistinct. These symptoms have been described as "mild hind limb or unilateral paralysis"[19,22,26,28] or "hind limb weakness"[18,27], "impaired or poor righting reflex"[20,21,29] or "loss of the righting reflex"[25], "tail atony or paralysis"[21,24] or "flaccid tail"[23], and "moderately clumsy gait"[21] or "abnormal gait"[29]. Most laboratories define a score of 3 as hind limb paralysis[18-20,23,25-29] or weakness[21,22]; however, Pollak *et al*[24] included "loss of

Table 1 Commonly used classical experimental autoimmune encephalomyelitis score

Score	EAE signs	Observation
0	No signs of EAE	Hind legs are extended; tail extends up and moves; tail wraps around cylindrical object; normal gait
0.5	Partially limp tail	Hind legs are extended; tip of tail droops and/or does not wrap around cylindrical object; normal gait
1	Paralyzed tail	Hind legs are extended; tail droops and does not wrap around cylindrical object; normal gait
2	Loss in coordinated movement; hind limb paralysis	Hind legs contract when held at the base of tail; mouse walks with uncoordinated movement; hind limbs reflex when toes are pinched; limp tail
2.5	One hind limb paralysis	Mouse drags one hind limb; one hind limb does not respond to pinch; limp tail
3	Both hind limbs paralysis	Mouse drags both hind limbs; both hind limbs do not respond to toe pinch; limp tail
3.5	Hind limb paralysis; weakness in forelimbs	Mouse drags hind limbs but has difficulty using forelimbs to pull body; forelimbs respond to toe pinch; limp tail
4	Forelimbs paralysis	Mouse cannot move; forelimbs do not respond to toe pinch; limp tail
5	Moribund	No movement; cold to the touch; altered breathing

Note there are no scores denoted 1.5 and 4.5. The data available from reference 10. EAE: Experimental autoimmune encephalomyelitis.

Table 2 Differences in experimental autoimmune encephalomyelitis scores among researchers

	Swanborg[18]	Matsumoto *et al*[19]	Yamamura *et al*[20]	Sobel *et al*[21]	Kalyvas *et al*[22]	Mendel *et al*[23]	Pollak *et al*[24]	Takeuchi *et al*[25]
Strain	Lewis rat	Lewis rat	C57BL/6J mice	SJL/J mice	C57BL/6J mice	C57BL/6J mice	SLJ/J mice	C57BL/6J mice
Antigen	MBP	MBP	MOG_{35-55}	$PLP_{131-151}$	MOG_{35-55}	MOG	$PLP_{131-151}$	MOG_{35-55}
0	ND	ND	No clinical signs	No disease	Normal	No clinical signs	No neurological signs	No detectable signs of paralysis
1	Loss of tail tonicity	Flaccid tail	Loss of tail tonicity	Decreased tail tone or slightly clumsy gait	Flaccid tail	Loss of tail tonicity	Tail weakness	Completely limp tail
2	Definite hind quarter weakness	Mild paraparesis	Impaired righting reflex	Tail atony and/or moderately clumsy gait and/or poor righting ability	Mild hindlimb paralysis	Flaccid tail	Tail paralysis	Loss of the righting reflex
3	Hind leg paralysis	Severe paraparesis	Partial hindlimb paralysis	Limb weakness	Severe hindlimb weakness	Hind leg paralysis	Loss of righting reflex	Partial hind limb paralysis
4	ND	Moribund condition	Total hindlimb paralysis	Limb paralysis	Hindlimb paralysis	Hind leg paralysis with hind body paresis	Hind limb paresis/paralysis	Complete hind limb paralysis
5	ND	ND	ND	Moribund	Hindlimb paralysis and forelimb weakness or moribund	Hind and fore leg paralysis	Quadriplegia (immobility)	Total paralysis of allfour limbs
6	ND	ND	ND	ND	ND	Death	Death	Death

ND: Not defined.

the righting reflex". Thus, the same EAE score may indicate different conditions in the EAE mice. Furthermore, the score is not a quantitative analysis. Some researchers use "in-between" scores (0.5 point) when the symptom lies between the two defined scores (Table 3). Tsunoda *et al*[26] and Storch *et al*[27] only showed an in-between score of between 0 and 1 (0.5 score) but not between other intervals. In contrast, there were in-between scores for all intervals except for a score of 4.5 in a method used by Greter *et al*[28]. Most researchers judge the statistical significance of EAE signs by comparing the scores of two groups of EAE mice: *i.e.*, wild type EAE mice and genetically modified EAE mice, or treated EAE mice and non-treated EAE mice. Furthermore, the method of assessing an EAE score depends on subjective observation. For example, researchers hold the base of the mouse tail to judge tail limpness. In addition, they touch or perform a toe pinch to evaluate the gait condition of the mice[10,11]. Finally, designation of the sign as "mild" or "severe" is ill-defined; thus, we need to eliminate the observer bias in the judgment of EAE scores. In addition, the cumulative score is obtained by the sum of the daily EAE score using previously described methods.

Not only is a standard behavior scoring system for EAE needed, but a different method for evaluating EAE progression is also required. After sensitization, in general, the mice are weighed regularly and scored for EAE signs. Body weight loss is a common feature of EAE that

Table 3 Experimental autoimmune encephalomyelitis scores using the 0.5 point scale

	Tsunoda *et al*[26]	Storch *et al*[27]	Greter *et al*[28]	Kihara *et al*[29]
Strain	SJL/J mice	BN and DA rat	C57/BL mice	C57/BL mice
Antigen	PLP	MOG_{1-125}	MOG_{35-55}	MOG_{35-55}
0	No clinical disease	ND	No detectable signs of EAE	No signs
0.5	Loss of tonicity of the distal half of the tail	Partial loss of tail tone	Distal limp tail	Mild loss of tail tone
1	Complete loss of tail tonicity	Complete tail atony	Complete limp tail	Complete loss of tail tone
1.5	ND	ND	Limp tail and distal limb weakness	Mildly impaired righting reflex
2	Mild hind leg paresis	Hind limb weakness	Unilateral partial limb paralysis	Abnormal gait and/or impaired righting reflex
2.5	ND	ND	Bilateral partial hind limb paralysis	Hind limb paresis
3	Moderate hind leg paralysis	Hind limb paralysis	Complete bilateral hind limb paralysis	Hind limbs paralysis
3.5	ND	ND	Complete bilateral hind limb paralysis and unilateral forelimb paralysis	Hind limb paralysis with hind body paresis
4	Complete paraplegia	Tetraplegy, moribund state	Total paralysis of fore and hind limbs	Hind and fore limb paralysis
4.5	ND	ND	ND	Moribund
5	quadriplegia, moribund state or death	Death	Death	Death
5.5	ND	ND	ND	ND
6	ND	ND	ND	ND

ND: Not defined.

usually precedes paralysis, and low body weight remains during the recovery phase[12,30,31]. Body weight begins to increase during the chronic phase of the disease; thus, weight loss is an important sign during the acute stage of EAE. Jones *et al*[32] observed the relationship between the EAE score and rotarod performance, the grip strength test of both the fore limbs and hind limbs, and the open field test. In the open field test, gait and rearing events of natural exploratory behavior could be detected. A drawback of this method is that severe (and, in some cases, moderate) paralysis prevents animals from performing the rotarod and grip strength tests. The Basso, Beattie, and Bresnahan (BBB) score is a well-established technique for evaluating spinal cord injury in animal models[33,34]; thus, this score may also be used in EAE studies[35,36]. To determine the BBB score, mice are placed in an open field area with two observing researchers. The BBB sub-components include limb movement, trunk position, paw placement, walking, predominant paw position, trunk instability and tail position. BBB is rated on a scale from 0 (no observable hind limb movements) to 21 (consistent coordinated gait, consistent toe clearance, predominant paw position is parallel at initial contact and lift-off, tail consistently up, and consistent trunk stability), which represents the sequential recovery of spinal cord injury[33]. Kerschensteiner *et al*[36] reported that the BBB score is predictive of the site and extent of the pathological lesion and is more sensitive for assessing the development of EAE than are EAE scores. However, the BBB score must be determined in precisely 21 stages by two proficient observers. Thus, a simple, universal and clear-cut method is needed for evaluating motor dysfunction in EAE. Our recent study suggests that the traveled distance is a sensitive and accurate marker of motor dysfunction in a MS mouse model (unpublished).

In conclusion, the EAE score is simple and easy to use; however, its application varies between laboratories, and scoring is dependent on the subjective bias of the researchers. To achieve the standard scoring system in EAE, it is necessary to define the terms for signs and to clarify the criteria for the signs in the EAE score. Furthermore, ambiguous representation, *e.g.*, weak or strong weakness of the hind limb, must be eliminated. We suggest that a standardized EAE scoring system should be implemented and combined with another quantitative index, such as the distance traveled in the open field test, which would provide a substantial advantage over the current conventional EAE scoring methods.

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BRIEF ARTICLE

Human obturator nerve: Gross anatomy

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Abstract

AIM: To study the anatomy (formation, course, relationships and branching pattern) of the obturator nerve in detail.

METHODS: The study was based on 500 adult human formalin-embalmed cadavers, 342 males and 158 females. We studied the anatomical formation, course and relationships of the obturator nerve within the lesser pelvis before the obturator canal. Finally, the whole course of the obturator nerve was examined.

RESULTS: We found numerous anatomical variations about the formation of the obturator nerve, its division into two main branches, its articular branches, its intrapelvic branches for the periosteum of the pubic bone, and also the number of its muscular divisions and its anatomical relationship to the obturator externus muscle and obturator artery. We found that fibers from the L3 and L4 spinal nerves are standard components of the obturator nerve. The main trunk of the obturator nerve divides into anterior and posterior branches, within the pelvis in 23.30%, within the obturator canal in 52.30% and extrapelvic in 24.35% of cases. The anterior branch of the obturator nerve supplies three muscular branches in 67.10%, two muscular branches in 28.94% and four muscular branches in 3.94% of the cases. The posterior branch of the obturator nerve supplies two muscular branches in 60.52%, three muscular branches in 19.07%, one muscular branch in 14.47% and four muscular branches in 5.92% of cases.

CONCLUSION: We present a gross anatomical study of the human obturator nerve based on a remarkably large number of cases as well as potential clinical applications of our findings.

Key words: Anatomy; Articular branches; Lumbar plexus; Muscular branches; Obturator nerve

Core tip: Our purpose was to study the anatomy of the obturator nerve in detail. The study was based on 500 adult human formalin-embalmed cadavers, 342 males and 158 females. We studied the anatomical formation, course and relationships of the obturator nerve within the lesser pelvis before the obturator canal. We found numerous anatomical variations about the formation of the obturator nerve, its division into two main branches, its articular branches, its intrapelvic branches for the periosteum of the pubic bone, and also the number of its muscular divisions and its anatomical relationships to the obturator externus muscle and obturator artery.

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INTRODUCTION

The obturator nerve is a peripheral nerve, a branch of the lumbar plexus. It usually arises from the ventral divisions of L2-L4 spinal nerves and innervates the medial thigh adductor muscles: gracilis, pectineus, adductor longus, brevis and magnus, as well as obturator externus. The obturator nerve pierces the medial border of the psoas muscle entering the pelvic cavity along the wall in

the retroperitoneum within the obturator fossa. It is the only motor nerve arising from the lumbar plexus which passes through the pelvis without innervating any pelvic structures. The nerve then leaves the pelvis *via* the obturator foramen accompanied by the obturator artery and vein[1].

A good working knowledge of the location and anatomy of the nerves of the lumbar plexus on the posterior abdominal wall is necessary for the surgeon who operates in this region[2]. The obturator nerve belongs to these nerves and its detailed anatomy, together with its variations, is necessary information, not only for surgical specialists who may intervene in this area, but also for anesthesiologists applying regional anesthesia techniques. However, anatomical studies of the obturator nerve are infrequent and the available data are based on restricted numbers of specimens.

MATERIALS AND METHODS

This anatomical study of the obturator nerve was based on 500 adult human formalin-embalmed cadavers, 342 males and 158 females, which we had over several years in the dissection room of our department from cadaver donors for students' education.

Our study, which lasted for more than 15 years (1970-1985), focused on the following aspects: (1) the formation (nerve fibers) of the obturator nerve (on 88 lumbar plexuses); (2) its course and anatomical relationships (on 1000 lumbar plexuses); (3) the level of its division into two main branches (on 390 lumbar plexuses); (4) the relationships of its main trunk and main branches with the obturator externus muscle (on 1000 lumbar plexuses); (5) its articular branches (on 168 lumbar plexuses); (6) its intrapelvic branches for the periosteum of the pubic bone (on 56 lumbar plexuses); and finally (7) the muscular divisions of its anterior and posterior branches after it exits the obturator canal (on 152 lumbar plexuses).

There are different numbers of each aspect of the study because not all the aspects were included for our study's purposes from the beginning and not all the studied branches were able to be examined in all our specimens.

All cadavers were carefully studied bilaterally using the same methodology. First, we carried out gross anatomical examination of the structures (*e.g.*, femoral fascia) of the anterior and internal thigh after removal of the skin and subcutaneous tissues. We identified and examined anatomically the local sensory nerves as well as the obturator nerve as it exits the obturator canal and the course and distribution of its main branches (anterior, posterior). Then we opened the abdominal wall and carried out gross anatomical examination of the retroperitoneal organs, especially the lumbar plexus. We studied the anatomical formation, course and relationships of the obturator nerve within the lesser pelvis before the obturator canal. Finally, the whole course of the obturator nerve (from the abdomen to the thigh) was examined.

Table 1 Formation (spinal nerves) of the obturator nerve studied on 88 lumbar plexuses

Spinal nerves (fibers)	Number of lumbar plexuses				Total (n = 88)
	Left lumbar plexuses		Right lumbar plexuses		Males + Females
	Males	Females	Males	Females	
L1, L2, L3, L4	1	2	1	1	5 (5.64%)
L2, L3, L4	19	9	17	11	56 (63.60%)
L3, L4	4	4	4	2	14 (15.90%)
L2, L3, L4, L5	4	1	6	2	13 (14.72%)

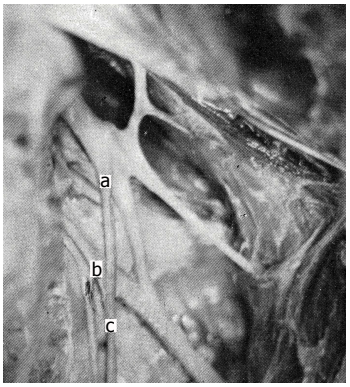

Figure 1 The left obturator nerve arising from the ventral divisions of the L3 and L4 spinal nerves. The contribution of the ventral division of the L2 spinal nerve is absent. a: Ventral division of the L3 spinal nerve; b: Ventral division of the L4 spinal nerve; c: Obturator nerve.

RESULTS

We found numerous anatomical variations regarding: (1) the formation of the obturator nerve (nerve fibers); (2) its division into two main branches; (3) its articular branches; (4) its intrapelvic branches for the periosteum of the pubic bone; (5) the number of its muscular divisions; and (6) its anatomical relationships with the obturator externus muscle and obturator artery.

Formation, course and division

The obturator nerve includes nerve fibers from the ventral divisions of the L2, L3, L4 spinal nerves in 63.60%, of the L3, L4 in 15.90% (Figure 1), of the L2, L3, L4, L5 in 14.72% and of the L1, L2, L3, L4 in 5.64% of specimens (Table 1). Thus we found that fibers from the L3 and L4 spinal nerves are standard components of the obturator nerve. The distribution between sides and gender of these findings are shown on Table 1.

Even though it is well established[3], we report our observations that the obturator nerve descends through the fibers of the psoas major muscle and emerges from its medial border. It runs along the lateral wall of the lesser pelvis, above and in front of the obturator vessels, to the upper part of the obturator foramen. Passing through the obturator canal, the nerve extends to the thigh.

The main trunk of the obturator nerve divides into anterior and posterior branches, within the pelvis in 23.30%, within the obturator canal in 52.30% and extra-

Table 2 Muscular branches of the obturator nerve studied on 152 lumbar plexuses

Number of branches	Anterior branch	Posterior branch
1	-	14.47%
2	28.94%	60.52%
3	67.10%	19.07%
4	3.94%	5.92%
Muscles innervated	Gracilis (70.10%) Adductor longus (70.10%) Adductor brevis (25.90%) Pectineus (4.00%)	Adductor magnus (85.53%) Adductor longus (85.53%) Adductor brevis (25.00%) Obturator externus (6.00%)

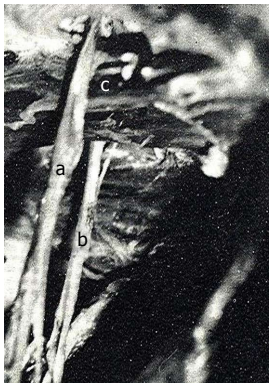

Figure 2 A muscular mass of the left obturator externus muscle intervening between obturator nerve's anterior and posterior branches, as the latter exits the obturator canal. a: Anterior branch of the obturator nerve; b: Posterior branch of the obturator nerve; c: Obturator externus muscle.

pelvic in 24.35% of cases (n = 390). The level of division of the main trunk was similar between male and female cadavers.

As the obturator nerve exits the obturator canal, a muscular mass of the obturator externus muscle intervenes between its anterior and posterior branches in 25.70% of cases (n = 1000) (Figure 2).

Articular branches

The obturator nerve supplies articular branches to the human hip joint. These branches originate most frequently from the common obturator trunk in 68.70% and rarely from either the anterior or the posterior branch of the obturator nerve. These articular branches, one to three (but usually one with similar proportions between male and female cadavers), arise within the pelvis in 47.60% (44.40% in male cadavers, 53.30% in female cadavers), within the obturator canal in 32.14% (35.18% in male cadavers, 26.60% in female cadavers) and finally extrapelvic in 20.23% (20.30% in male cadavers, 20.00% in female cadavers) of specimens (n = 168).

Muscular branches

The anterior branch of the obturator nerve supplies three muscular branches in 67.10% (slightly more frequent in female cadavers), two muscular branches in 28.94% (more frequent in male cadavers) (in some cases these branches look like the two main branches of the obturator nerve's trunk) and four muscular branches in 3.94% of the cases (Table 2). We found no remarkable difference between the two sides. These branches innervate the gracilis and adductor longus muscles in 70.10%, the adductor brevis muscle in 25.90% and the pectineus muscle in 4% of the cases (n = 152).

The posterior branch of the obturator nerve supplies two muscular branches in 60.52%, three muscular branches in 19.07%, one muscular branch in 14.47% (more frequent in male cadavers) and four muscular branches in 5.92% (more frequent in female cadavers) of cases (Table 2). We found no remarkable difference between the two sides, although there was noticeably higher variability in comparison with the anterior branch's muscular divisions. These branches innervate the adductor magnus and adductor longus muscles in 85.53%, the adductor brevis muscle in 25.00% and obturator externus muscle in 6.00% of cases (n = 152).

Other branches

The obturator nerve supplies one to three intrapelvic branches for the periosteum of the posterior surface of the pubic bone in 61.36% of cases (n = 56). In both sides and gender, the most frequent branching pattern for the pubic bone's periosteum was the presence of a single intrapelvic branch, with a proportion of about 60% (ranging from 55.50% to 62.50%).

DISCUSSION

Formation, course and division

As shown in Table 1, the anatomical formation of the obturator nerve is remarkably variable. We noticed that, apart from our results, the obturator nerve has been also described to be formed from the union of the L1, L2 and L3 spinal nerves[4]. In combination with our findings, it is obvious that L3 is the only standard spinal nerve in forming the obturator nerve.

A recent study by our department based on 84 cadavers revealed similar results about the obturator nerve's level of division: within the obturator canal 51.78%, in the medial thigh 25% and finally intrapelvic 23.22% of cases[3]. High anatomical variability in the obturator nerve divisions and subdivisions does exist and explains the difficulty frequently encountered in the application of regional anesthetic techniques[3].

The obturator nerves lie outside the psoas muscle at the L5 spinal vertebrae[5]. Within the psoas major, the obturator nerve is often separated from the lateral femoral cutaneous and femoral nerves by a muscular fold, with commonly presenting anatomical variations[6].

Articular branches

In the mentioned recent study by our department[3], we found a high variability of the proportion of cases where the articular branches arose from the common obturator trunk. This proportion was 76.92% in cases

where only one articular branch was observed. This proportion was 47.05% in cases where two articular branches were observed and 53.33% in cases with three articular branches[3].

The hip joint is innervated by articular branches of the obturator, femoral, superior gluteal and sciatic nerves. The responsible nerve can be determined by a diagnostic nerve block in cases of hip joint pain. Then, radiofrequency ablation of the identified articular branches of the hip can be applied in order to provide hip pain relief[7]. They vary in location over a wide area, especially for the articular branches of the obturator nerve. So radiofrequency denervation of these branches should be accomplished by multiple lesions to account for the location of the target nerves and their variability[8]. Our study orientates the clinician as to where to search for these branches.

Moreover, regarding the hip joint capsule, there is a separation between its anterior and posterior sensory innervation. The anteromedial innervation has been found to be determined by the articular branches of the obturator nerve. Consequently, the obturator nerve block is insufficient for the treatment of hip pain[9].

Muscular branches

The importance of the obturator nerve in the innervation of the adductor muscles is well known[10] and also confirmed by our findings (innervation of adductor magnus, longus and brevis muscles). In our department's recent study, we found similar proportions of numbers of muscular divisions of the obturator nerve's anterior and posterior branches[3].

It has been found that the obturator nerve is responsible for the majority of cases with loss of adduction strength, with following the sacral plexus and the femoral nerve, respectively. The obturator nerve plays a major role in the adduction of the lower limbs. Furthermore, assessing the adductor strength is the only way to effectively evaluate the obturator nerve function[10].

In general, the adductor magnus is innervated by branches of the obturator nerve that enter its anterior surface[11]. We found that these muscular branches come from the posterior branch of the obturator nerve. A twig of a branch from the quadratus femoris nerve can communicate with a twig of the obturator nerve within this muscle. It has been reported that the root of the branches to the adductor magnus occupies the caudal most region of the obturator nerve and frequently forms a common trunk with the root of the quadratus femoris nerve[11].

The human adductor brevis (specific adductor brevis) muscle, found in 23% according to the study of Miura *et al*[12] (1994), is innervated dually by the anterior and posterior branches of the obturator nerve. Specifically, its anterior and posterior surfaces receive twigs from the anterior branch of the obturator nerve and filaments from the posterior branch respectively. Based on our findings, we confirm that this muscle can be innervated by the anterior and posterior branches of the obturator nerve.

The gracilis muscle is, according to our results, innervated by muscular branches of the obturator nerve's anterior branch. The gracilis perforator flap is used in plastic surgery. Occasionally a sensory branch of the anterior obturator nerve is found to accompany the perforators. It can be used as a sensory flap in these few cases of the existence of nerve supply by a sensory branch of the obturator nerve[13].

Other branches

In some people there is also cutaneous contribution of the obturator nerve[10]. Branches derived from the anterior branch of the obturator nerve have been reported to pierce the vastoadductor membrane en route to the skin of the medial thigh[14]. Cutaneous innervation of the obturator nerve to the medial aspect of the knee has also been reported but is highly variable and sometimes missing[10]. The anatomy of these important branches will be the object of a future study by our department.

Nerve injury

Obturator nerve injury could happen due to disease or procedures in obstetrics and gynecology. It has been reported primarily in patients with endometriosis, malignancy and obstetrical forceps injury. Gynecological oncology patients undergoing lymph node dissection or extensive retroperitoneal surgery at the sidewall in the obturator fossa are at an increased risk for injury[1].

Obturator nerve injury, in the form of neurapraxia, axonotmesis and neurotmesis, causes morbidity in the form of pain, sensory loss to the medial thigh and inconstant motor loss to the adductor muscle group. Therapy should be directed at correcting the condition and surgical repair by an epineurial approach should be performed if nerve division is noted during surgery. In contrast to surgical division of other complex pelvic nerves, obturator nerve injury is a highly treatable condition[1].

Limitations

The fact that there are different numbers of each aspect of the study (for reasons already explained in our methodology) could be considered as a major limitation (potential selection bias).

In conclusion, there is high anatomical variability in the human obturator nerve divisions and subdivisions[3] and just a few already published data about its detailed gross anatomy. We presented a gross anatomical study of the human obturator nerve based on a large number of specimens. We believe that our findings could be useful for clinicians, especially those who intervene at the obturator nerve's area, such as anesthesiologists, urologists, gynecologists, orthopedic surgeons, neurosurgeons, plastic surgeons and others.

COMMENTS

Background

The obturator nerve is a peripheral nerve, a branch of the lumbar plexus. It

usually arises from the ventral divisions of L2-L4 spinal nerves and innervates the medial thigh adductor muscles: gracilis, pectineus, adductor longus, brevis and magnus, as well as obturator externus. Anatomical studies of the obturator nerve are infrequent and the available data are based on restricted numbers of specimens.

Research frontiers

The detailed anatomy of the obturator nerve, together with its variations, is necessary information, not only for surgical specialists who may intervene in this area, but also for anesthesiologists applying regional anesthesia techniques.

Innovations and breakthroughs

The authors found numerous anatomical variations about the formation of the obturator nerve, its division into two main branches, its articular branches, its intrapelvic branches for the periosteum of the pubic bone, and also the number of its muscular divisions and its anatomical relationship to the obturator externus muscle and obturator artery. The authors found that fibers from the L3 and L4 spinal nerves are standard components of the obturator nerve.

Applications

These findings could be useful for clinicians, especially those who intervene at the obturator nerve's area, such as anesthesiologists, urologists, gynecologists, orthopedic surgeons, neurosurgeons, plastic surgeons and others.

Peer review

This study may have reasonable scientific merit.

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BRIEF ARTICLE

Thickness-based correlations of cortical areas involved in senses, speech and cognitive processes

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Abstract

AIM: To explore the existence of potential correlations of cortical thickness between different functional brain areas.

METHODS: Our material consisted of 38 formalin-fixated human cerebral hemispheres from twenty males and three females, cadaver donors for students' education. We selected the following cortical areas at each hemisphere to examine: supramarginal gyrus (S), angular gyrus (A), area of colors recognition (F), area of names recognition (N), area of auditory attention (H), area of place memory (M), cortex of the superior wall of the calcarine sulcus (V1) and cortex of the inferior wall of the calcarine sulcus (V2). We measured the thickness of each cortical area and statistically analyzed our data.

RESULTS: We found a significant difference of the mean value of the V1 ($P < 0.05$) between right and left hemispheres, as well as very significant correlations ($P < 0.001$) between the following cortical areas: N and F, A and F, S and F, A and S, A and N, S and N. We also found significant correlations ($P < 0.01$) between the following areas: S and M, S and H, N and H, as well as between the following areas ($P < 0.05$): V1 and V2, M and F, M and N, A and H.

CONCLUSION: Our results suggest that there could be a potentially stronger impact for objects placed in the left inferior quarter of the visual field. Our study revealed several thickness-based correlations among different functional cortical areas. Most of them seem to have a more or less rational explanation.

Key words: Anatomy; Cerebral cortex; Correlations; Cortical thickness; Functional areas

Core tip: Our primary purpose was to explore the existence of potential correlations of cortical thickness between different functional areas of the human brain. Our material consisted of 38 formalin-fixated cerebral hemispheres. We examined eight specific cortical areas at each hemisphere. We found several statistically significant correlations. Our results suggest that there could be a potentially stronger impact for objects placed in the left inferior quarter of the visual field. Our study also revealed several thickness-based correlations among different functional cortical areas. Most of them seem to have a more or less rational explanation.

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INTRODUCTION

The human brain is the most mysterious organ of our body. Despite the significant research progress on neurosciences during the last century, there is still a lot to

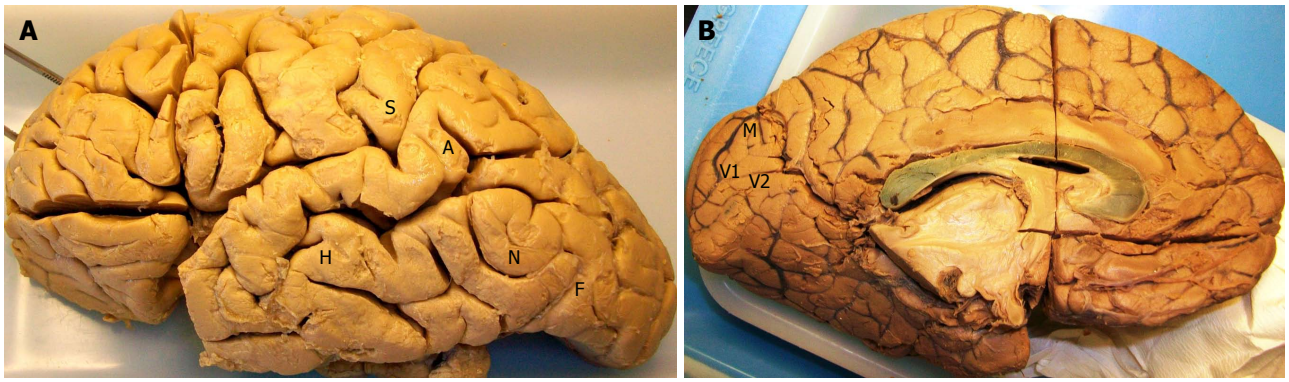

Figure 1 Human brain from a middle-aged male, left hemisphere. A: External surface; B: Internal surface. S: Supramarginal gyrus (Brodmann area 40); A: Angular gyrus (Brodmann area 39); F: Area of colors recognition (Brodmann area 19); N: Area of names recognition (Brodmann area 37); H: Area of auditory attention (effort to listen) (Brodmann area 21); M: Area of place memory (Brodmann area 19); V1: Cortex of the superior wall of the calcarine sulcus (Brodmann area 17); V2: Cortex of the inferior wall of the calcarine sulcus (Brodmann area 17).

learn. Although functional neuroimaging methods, mainly the functional magnetic resonance imaging (fMRI), offered a window with a magnificent view to brain functions, there are still well kept secrets regarding these complex functions. The knowledge that specific cortical areas of the brain are correlated with specific functions, known at least since the 19th century, resulted in several functional maps of the cerebral cortex as well as the famous motor and sensory homunculi. Modern functional neuroimaging techniques allowed this knowledge to evolve, revealing, however, the complexity of such correlations.

In human anatomy, information regarding structure can indirectly provide us with information regarding function. Neuroanatomical studies are traditionally the 'gold standard' method of exploring the brain structure and they offer information indirectly about its function. But could anatomical studies, focused on the cerebral cortex, offer indirect information regarding cortical function? That is what we aimed to do with the present study. Our primary purpose was specifically to explore the existence of potential correlations of cortical thickness between different functional cortical areas of the human brain, as well as to compare such thickness between sides.

MATERIALS AND METHODS

Our material consisted of 38 formalin-fixated normal human cerebral hemispheres (23 left, 15 right) in our department. They came from twenty males (32 hemispheres) and three females (6 hemispheres), predominately middle-aged cadaver donors (with cause of death irrelevant to brain pathology) for students' education. We selected the following cortical areas[1] at each hemisphere to examine (Figure 1).

External surface

S = supramarginal gyrus (auditory center of speech at the dominant hemisphere[2]) (Brodmann area 40); A = angular gyrus (visual center of speech at the dominant hemisphere[2]) (Brodmann area 39); F = area of colors recognition (Brodmann area 19); N = area of names understanding (Brodmann area 37); H = area of auditory attention (Brodmann area 21).

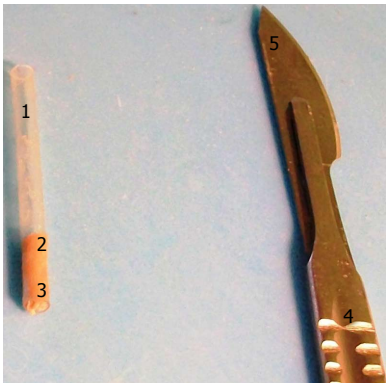

Figure 2 Method of cortical thickness measuring. 1: Plastic tube with brain sample; 2: Cerebral cortex within the tube; 3: Subcortical white matter within the tube; 4: Scalpel; 5: Scalpel blade.

Internal surface

M = area of place memory (Brodmann area 19); V1 = cortex of the superior wall of the calcarine sulcus (primary visual cortex, Brodmann area 17); V2 = cortex of the inferior wall of the calcarine sulcus (primary visual cortex, Brodmann area 17).

After removing the choroid meninge, we measured the thickness of each of these eight cortical areas. Methodologically, we used a transparent plastic tube approximately 25 mm long and 2 mm wide which was inserted perpendicularly to the cerebral surface in order to remove a cylindrical piece of brain tissue from each selected area. The transparency of the tube allowed the identification of the cortex-white matter limit and hence the measuring of the cortical thickness without even removing the sample from the tube. A scalpel was occasionally used to help the removal of the brain tissue piece by cutting at its bottom (Figure 2).

Our results were statistically analyzed by calculating

each thickness' mean value (MV) and standard deviation (SD), as well as by applying student's *t*-test among each side's MVs. We also applied Pearson's correlation coefficient (*r*) among different areas' thicknesses.

RESULTS

Table 1 shows the measured data with their MVs and SDs and the measured data separately for right and left hemispheres (hemisphere column numbers are for specimen identifying purposes). As it can be seen on these tables: (1) The S varied from 0.9 mm to 4.0 mm and its MV ± SD was S = 2.20 ± 0.76 mm (n = 34). For right hemispheres we found S = 2.15 ± 0.82 mm (n = 13) and for left S = 2.23 ± 0.74 mm (n = 21); (2) The A varied from 1.0 mm to 4.5 mm and its MV ± SD was A = 2.23 ± 0.76 mm (n = 34). For right hemispheres we found A = 2.14 ± 0.90 mm (n = 13) and for left A = 2.29 ± 0.68 mm (n = 21); (3) The F varied from 1.0 mm to 4.0 mm and its MV ± SD was F = 2.03 ± 0.62 mm (n = 37). For right hemispheres we found F = 2.12 ± 0.73 mm (n = 15) and for left F = 1.97 ± 0.54 mm (n = 22); (4) The N varied from 0.9 mm to 4.2 mm and its MV ± SD was N = 2.14 ± 0.71 mm (n = 37). For right hemispheres we found N = 2.13 ± 0.79 mm (n = 15) and for left N = 2.14 ± 0.68 mm (n = 22); (5) The H varied from 0.9 mm to 3.9 mm and its MV ± SD was H = 2.11 ± 0.70 mm (n = 34). For right hemispheres we found H = 2.00 ± 0.66 mm (n = 15) and for left H = 2.20 ± 0.74 mm (n = 19); (6) The M varied from 1.4 mm to 4.0 mm and its MV ± SD was M = 2.16 ± 0.65 mm (n = 30). For right hemispheres we found M = 2.37 ± 0.65 mm (n = 11) and for left M = 2.04 ± 0.63 mm (n = 19); (7) The V1 varied from 1.2 mm to 4.2 mm and its MV ± SD was V1 = 2.07 ± 0.64 mm (n = 30). For right hemispheres we found V1 = 2.42 ± 0.80 mm (n = 11) and for left V1 = 1.87 ± 0.43 mm (n = 19); and (8) The V2 varied from 1.3 mm to 4.0 mm and its MV ± SD was V2 = 2.13 ± 0.65 mm (n = 30). For right hemispheres we found V2 = 2.28 ± 0.65 mm (n = 11) and for left V2 = 2.04 ± 0.65 mm (n = 19).

The statistical analysis of our measurements revealed no statistically significant difference between sides regarding the S, A, F, N, H, M and V2. It also revealed a statistically significant difference of the V1 MV between right (2.42 ± 0.80 mm) and left (1.87 ± 0.43 mm) hemispheres (t = 2.11, degrees of freedom = 28, P < 0.05), as well as the following correlations (beginning from the most powerful): (1) Statistically significant correlation between the N and F (r = 0.711, degrees of freedom = 35, P < 0.001); (2) Statistically significant correlation between the A and F (r = 0.698, degrees of freedom = 32, P < 0.001); (3) Statistically significant correlation between the A and S (r = 0.680, degrees of freedom = 32, P < 0.001); (4) Statistically significant correlation between the A and N (r = 0.586, degrees of freedom = 32, P < 0.001); (5) Statistically significant correlation between the S and F (r = 0.579, degrees of freedom = 32, P < 0.001); (6) Statistically significant correlation between the S and N (r = 0.579, degrees of freedom = 32, P < 0.001); (7) Statistically significant correlation between the S and M (r = 0.558, degrees of freedom = 26, P < 0.01); (8) Statistically significant correlation between the S and H (r = 0.530, degrees of freedom = 30, P < 0.01); (9) Statistically significant correlation between the N and H (r = 0.514, degrees of freedom = 32, P < 0.01); (10) Statistically significant correlation between the V2 and V1 (r = 0.447, degrees of freedom = 28, P < 0.05); (11) Statistically significant correlation between the M and F (r = 0.441, degrees of freedom = 27, P < 0.05); (12) Statistically significant correlation between the M and N (r = 0.431, degrees of freedom = 27, P < 0.05); (13) Statistically significant correlation between the A and H (r = 0.394, degrees of freedom = 30, P < 0.05); (14) Statistically ambiguous correlation between the V1 and F (r = 0.340, degrees of freedom = 27, P < 0.1); (15) Statistically ambiguous correlation between the N and V2 (r = 0.326, degrees of freedom = 27, P < 0.1); and (16) Statistically ambiguous correlation between the H and F (r = 0.303, degrees of freedom = 32, P < 0.1).

DISCUSSION

As shown on Table 1, from the eight areas studied, area A was the thickest one in left hemispheres and area V1 in right hemispheres. The latter area was in contrast the thinnest one in left hemispheres, whereas the thinnest in right hemispheres was area H. The absence of significant difference between sides regarding areas S and A is somehow surprising, given the location of Wernicke's area at the dominant hemisphere.

The significantly thicker V1 for right hemispheres is an interesting finding. This cortical area receives visual pathway information from the left inferior quarter of the visual field, while the significantly thinner left V1 area receives visual pathway information from the right inferior quarter of the visual field. Could this finding mean that there is a potentially stronger impact for objects placed in the left inferior quarter of our visual field? Further research is needed to determine whether this difference reflects real functional differences between the right and left V1 cortical fields. It is also interesting that there was no such difference observed regarding the V2 areas (right and left) of the visual cortex which receive visual pathway information from the superior half of the visual field.

Considering the correlations (very significant, significant) we found, it seems quite difficult to find a probable explanation. However, we suggest the following potential explanations and interpretations:

Very significant correlations

N and F: Our mind possibly correlates names with color images (of the respective people or objects *etc.*), mainly based on our previous experience.

A and F, S and F: When we perceive (hear or see) the name of the color, the image of the color comes up into our mind.

Table 1 Cortical thickness of eight functional areas of the human cerebral cortex, right hemispheres and left hemispheres

Hemisphere		S (mm)	A (mm)	F (mm)	N (mm)	H (mm)	M (mm)	V1 (mm)	V2 (mm)
Human cerebral cortex									
1	R3	2.5	2.8	1.7	2.6	3.5	2.9	2.2	2.9
2	R7	4	4.5	4	4.2	2.5	3.4	2.6	3.1
3	L8	2.7	3.3	2	2.9	2.9	1.9	2.3	4
4	L11	-	-	-	-	-	2	1.9	2.1
5	L9	2	1.9	2.3	2.3	2.6	2.2	1.5	1.6
6	L12	3.5	3.9	2.3	1.7	2.9	1.9	1.8	1.7
7	L10	3.9	3	2	2.9	2	2.5	2	1.8
8	L2	2.5	2	2	2.5	3.9	3	1.5	2.5
9	R2	2.5	3.1	3	2.4	2.5	2.4	3	1.9
10	L3	3.1	2.7	3.1	2.8	3.1	2.1	3	3.1
11	R5	3.3	1.5	2	2.3	2.4	2.8	2.5	3
12	R1	2	2.4	2.8	2.5	2	2.9	4.2	2.2
13	L6	1.5	2	1.9	2.1	1.8	1.5	1.8	1.6
14	R6	1.2	1.6	2.4	2.2	1.3	1.9	1.4	1.8
15	R4	1.6	1.5	1.5	2	2.3	1.6	1.5	1.6
16	L7	1.5	2	1.2	1	2.2	1.9	2	2
17	L1	2	2.1	2.5	2.6	2.1	1.8	1.5	2.7
18	L4	2.9	2.1	2.5	2.9	2.5	4	1.2	1.3
19	L5	2	2.3	2	2	-	2.1	2.2	2
20	L15	2	2.1	2.1	2	2.2	1.7	1.7	1.5
21	L16	3	2.9	2.2	3	2.5	2.5	2	1.4
22	L13	2.4	2.6	2	2.5	2.4	1.5	1.7	1.9
23	L14	1.9	1.2	1.2	0.9	-	1.5	2.3	1.9
24	R8	2	1.7	2.2	2	2.2	2.5	1.9	1.6
25	R9	1.9	2	2.3	3	2.3	1.4	2.4	2.6
26	L18	1.6	1.9	1.6	1.4	1.6	1.5	1.5	2
27	R17	-	-	1.9	2	1.9	-	-	-
28	L25	-	-	1.6	1.8	-	-	-	-
29	R10	-	-	1.9	1.6	1.5	1.6	1.9	1.4
30	L17	1.6	2.1	2	3.2	2	1.6	2.3	1.9
31	R13	2.1	1.4	1.5	1	1.8	-	-	-
32	L21	2.5	3	3	2	0.9	-	-	-
33	R15	2.2	2.3	2.1	1.4	1.9	-	-	-
34	L23	1.1	2.3	1.6	1.2	1	-	-	-
35	R14	0.9	1.5	1.2	1.4	1	-	-	-
36	L22	1.8	1	1	1.5	1.2	-	-	-
37	R16	1.8	1.5	1.3	1.4	0.9	2.7	3	3
38	L24	1.4	1.7	1.3	1.9	2	1.5	1.3	1.7
MV		2.2	2.23	2.03	2.14	2.11	2.16	2.07	2.13
SD		0.76	0.76	0.62	0.71	0.7	0.65	0.64	0.65
Human cerebral cortex, right hemispheres									
1	R3	2.5	2.8	1.7	2.6	3.5	2.9	2.2	2.9
2	R7	4	4.5	4	4.2	2.5	3.4	2.6	3.1
3	R2	2.5	3.1	3	2.4	2.5	2.4	3	1.9
4	R5	3.3	1.5	2	2.3	2.4	2.8	2.5	3
5	R1	2	2.4	2.8	2.5	2	2.9	4.2	2.2
6	R6	1.2	1.6	2.4	2.2	1.3	1.9	1.4	1.8
7	R4	1.6	1.5	1.5	2	2.3	1.6	1.5	1.6
8	R8	2	1.7	2.2	2	2.2	2.5	1.9	1.6
9	R9	1.9	2	2.3	3	2.3	1.4	2.4	2.6
10	R17	-	-	1.9	2	1.9	-	-	-
11	R10	-	-	1.9	1.6	1.5	1.6	1.9	1.4
12	R13	2.1	1.4	1.5	1	1.8	-	-	-
13	R15	2.2	2.3	2.1	1.4	1.9	-	-	-
14	R14	0.9	1.5	1.2	1.4	1	-	-	-
15	R16	1.8	1.5	1.3	1.4	0.9	2.7	3	3
MV		2.15	2.14	2.12	2.13	2	2.37	2.42	2.28
SD		0.82	0.9	0.73	0.79	0.66	0.65	0.8	0.65
Human cerebral cortex, left hemispheres									
1	L8	2.7	3.3	2	2.9	2.9	1.9	2.3	4
2	L11	-	-	-	-	-	2	1.9	2.1
3	L9	2	1.9	2.3	2.3	2.6	2.2	1.5	1.6
4	L12	3.5	3.9	2.3	1.7	2.9	1.9	1.8	1.7
5	L10	3.9	3	2	2.9	2	2.5	2	1.8
6	L2	2.5	2	2	2.5	3.9	3	1.5	2.5
7	L3	3.1	2.7	3.1	2.8	3.1	2.1	3	3.1

8	L6	1.5	2	1.9	2.1	1.8	1.5	1.8	1.6
9	L7	1.5	2	1.2	1	2.2	1.9	2	2
10	L1	2	2.1	2.5	2.6	2.1	1.8	1.5	2.7
11	L4	2.9	2.1	2.5	2.9	2.5	4	1.2	1.3
12	L5	2	2.3	2	2	-	2.1	2.2	2
13	L15	2	2.1	2.1	2	2.2	1.7	1.7	1.5
14	L16	3	2.9	2.2	3	2.5	2.5	2	1.4
15	L13	2.4	2.6	2	2.5	2.4	1.5	1.7	1.9
16	L14	1.9	1.2	1.2	0.9	-	1.5	2.3	1.9
17	L18	1.6	1.9	1.6	1.4	1.6	1.5	1.5	2
18	L25	-	-	1.6	1.8	-	-	-	-
19	L17	1.6	2.1	2	3.2	2	1.6	2.3	1.9
20	L21	2.5	3	3	2	0.9	-	-	-
21	L23	1.1	2.3	1.6	1.2	1	-	-	-
22	L22	1.8	1	1	1.5	1.2	-	-	-
23	L24	1.4	1.7	1.3	1.9	2	1.5	1.3	1.7
MV		2.23	2.29	1.97	2.14	2.2	2.04	1.87	2.04
SD		0.74	0.68	0.54	0.68	0.74	0.63	0.43	0.65

S: Supramarginal gyrus (Brodmann area 40); A: Angular gyrus (Brodmann area 39); F: Area of colors recognition (Brodmann area 19); N: Area of names recognition (Brodmann area 37); H: Area of auditory attention (move to listen) (Brodmann area 21); M: Area of place memory (Brodmann area 19); V1: Cortex of the superior wall of the calcarine sulcus (Brodmann area 17); V2: Cortex of the inferior wall of the calcarine sulcus (Brodmann area 17); R: Right; L: Left; MV: Mean value; SD: Standard deviation.

A and S: It seems expected as they constitute the Wernicke's area.

A and N, S and N: The functions of the auditory and visual centers of speech are obviously crucial for a name understanding process. When we read or hear the name of a person (or object, place *etc.*) we know, this particular person usually comes to our mind. Moreover, we can sometimes recognize somebody from the style they write or the way they speak.

Significant correlations

S and M: The auditory perception of particular phrases regarding places we know can bring relative images from the past to our mind.

S and H, A and H: When we perceive somebody's speech, they usually attract our attention, at least initially. We might even move towards them to listen better.

N and H: A simple example of the usefulness of such a correlation is that, when we hear our name, we focus our attention on the particular person talking to us.

V2 and V1: It seems expected as they both consist the primary visual cortex.

M and F: The images of places usually include several types of color.

M and N: Many places have specific names or are subconsciously connected with specific people.

Cortical thickness measuring methods

Falk *et al*[3] described a method for obtaining clear 3D MRIs of the cortical surface of the brain in living human subjects. By combining volume composite and depth encoded images, they obtained surface coordinate data that resulted in highly repeatable measurements of sulcal lengths and cortical surface areas in eight normal adult volunteers. Sulcal lengths were determined for specific parts of the Sylvian fissure, central sulcus and frontal operculum. They observed previously unrecognized directional asymmetries in the length of the anterior limb of the pars triangularis, length of the ascending limb of the posterior Sylvian fissure, and position of the lateral end of the central sulcus. They attributed the finding of three new directional asymmetries for the human cortex, as well as the high repeatability of their measurements, to the sensitivity and accuracy of the 3D MRI technology[3].

The analysis of the human cerebral cortex and the measurement of its thickness based on MRI data can provide insight into normal brain development and neurodegenerative disorders. Accurate and reproducible results of the cortical thickness measurement are desired for sensitive detection[4]. Lüsebrink *et al*[4] compared ultra-high resolution data acquired at 7 Tesla (T) with 3T data for determination of the cortical thickness of the human brain. At identical resolution, the cortical thickness determination yielded consistent results between 3T and 7T, confirming the robustness of the acquisition and processing against potential field strength related effects. However, the ultra-high resolution 7T data resulted in significantly reduced values for the cortical thickness estimation compared to the lower resolution data. The reduction in thickness amounts to approximately one sixth to one third, depending on the processing algorithm and software used. This suggests a bias in the gray matter segmentation due to partial volume effects and indicates that true cortical thickness is overestimated by most current MR studies using both a voxel-based or surface-based method and can be more accurately determined with high resolution imaging at 7T[4].

Cortical thickness findings on aging and clinical conditions

Westlye *et al*[5] reported that cortical thickness decreases from childhood throughout life, as estimated by MRI. This monotone trajectory does not reflect the fundamentally different neurobiological processes underlying

morphometric changes in development versus aging. The spatial pattern of intracortical neurodevelopment followed a posterior-anterior gradient, with earliest maturation of occipital visual cortices and most protracted in superior frontal regions[5].

Lyoo *et al*[6] reported that subjects with bipolar disorder exhibited significantly decreased cortical thickness in the left cingulate cortex, left middle frontal cortex, left middle occipital cortex, right medial frontal cortex, right angular cortex, right fusiform cortex and bilateral postcentral cortices, compared to healthy subjects (all $P < 0.001$). Duration of illness in bipolar subjects was inversely correlated with the cortical thickness of the left middle frontal cortex. Cortical thinning was present in multiple prefrontal cortices in bipolar disorder. There was also cortical thinning in sensory and sensory association cortices, which has not been reported in previous studies using region-of-interest or voxel-based morphometry methods. They proposed that cortical thinning may be related to impairment of emotional, cognitive and sensory processing in bipolar disorder but longitudinal studies will be necessary to test this hypothesis[6].

Developmental and psychiatric disorders, including schizophrenia, may be associated with altered cortical thickness and folding. Reduced cell density has been reported in the fundi of some sulci in the temporal lobe in schizophrenia[7]. Moreover, functional imaging studies suggested changes in primary visual cortex activity in subjects with schizophrenia. Interestingly, postmortem studies of subjects with schizophrenia reported an increased density of neurons in the primary visual cortex (Brodmann's area 17)[8]. Dorph-Petersen *et al*[8] estimated the total volume and neuron number of Brodmann's area 17 in postmortem brains from 10 subjects with schizophrenia and 10 matched normal comparison subjects. In addition, they assessed cortical thickness. They found a marked and significant reduction in total neuron number (25%) and volume (22%) of Brodmann's area 17 in the schizophrenia group compared to the normal subjects. Subjects with schizophrenia therefore have a smaller cortical area allocated to primary visual perception. This finding suggests the existence of a schizophrenia-related change in cortical parcellation[8].

Brain atrophy is common in subcortical ischemic vascular disease but the underlying mechanisms are poorly understood[9]. Duering *et al*[9] provided *in vivo* evidence for secondary cortical neurodegeneration after subcortical ischemia as a mechanism for brain atrophy in cerebrovascular disease.

Cortical asymmetries

The use of computational approaches in the analysis of high resolution MRI of the human brain provides a powerful tool for *in vivo* studies of brain anatomy[10]. Watkins *et al*[10] reported results obtained with a voxel-wise statistical analysis of hemispheric asymmetries in regional 'amounts' of gray matter, based on MRI scans obtained in 142 healthy young adults. The voxel-wise analysis detected the well-known frontal (right > left) and occipital (left > right) petalias. Their analysis confirmed the presence of left-greater-than-right asymmetries in several posterior language areas, including the planum temporale and the angular gyrus; no significant asymmetry was detected in the anterior language regions. They also confirmed previously described asymmetries in the cingulate sulcus (right > left) and the caudate nucleus (right > left). In some brain regions they observed highly significant asymmetries that were not reported before, such as in the anterior insular cortex (right > left)[10].

Brain asymmetry has been observed in animals and humans in terms of structure, function and behavior. This lateralization is thought to reflect evolutionary, hereditary, developmental, experiential and pathological factors[11]. Toga and Thompson[11] reviewed the diverse literature describing brain asymmetries, focusing primarily on anatomical differences between the hemispheres and the methods that have been used to detect them. Brain-mapping approaches in particular can identify and visualize patterns of asymmetry in whole populations, including subtle alterations that occur in disease, with age and during development. These and other tools show great promise for assessing factors that modulate cognitive specialization in the brain, including the ontogeny, phylogeny and genetic determinants of brain asymmetry[11].

Findings on functional cortical areas interconnections

Numerous studies measuring field potentials or functional imaging signals have reported auditory cortex activations to either visual or somatosensory stimuli and together with neuroanatomical studies provide compelling evidence for the presence of synaptic inputs from other sensory modalities to the auditory cortex. Visual and somatosensory stimuli can modulate low frequency oscillations in the auditory cortex and by doing so, shape the excitability of local networks and mediate stimulus selection. Multisensory influences also interact with attentional selection, suggesting the existence of a shared substrate for both mechanisms of selective stimulus enhancement[12].

The study of Lyttelton *et al*[13] revealed several new findings, including a striking leftward increase in surface area of the supramarginal gyrus (peak effect 18%) compared with a smaller areal increase in the left Heschl's gyrus and planum temporale region (peak effect 8%). A second finding was a rightward increase in surface area (peak effect 10%) in a band around the medial junction between the occipital lobe and parietal and temporal lobes[13].

According to the results of Rosenthal *et al*[14], the right angular gyrus had a critical role in perceptual sequence learning, whereas the primary motor cortex had a causal role in developing experience-dependent functional attributes relevant to conscious knowledge on manual but not perceptual sequence learning[14].

Sakurai *et al*[15] aimed to determine the features of alexia or agraphia with a left angular or supramarginal

gyrus lesion. They assessed the reading and writing abilities of three patients using kanji (Japanese morphograms) and kana (Japanese syllabograms). They found that alexia occurs as "angular" alexia only when the lesion involves the adjacent lateral occipital gyri. Transposition errors suggest disrupted sequential phonological processing from the angular and lateral occipital gyri to the supramarginal gyrus. Substitution errors suggest impaired allographic conversion between hiragana (one form of kana) and katakana (another form of kana) attributable to a dysfunction in the angular/lateral occipital gyri[15].

Considerations

As limitations of our study we should mention the lack of functional data (it is a purely anatomical study), the absence of gender balance, the formalin effect on the specimens and the potential cerebral atrophy. However, the last two limitations could potentially affect the absolute cortical thickness values but not the correlations we found. Furthermore, potentially useful details concerning the manual dominance or potential neurological history of the donors were unfortunately not available. On the other hand, thickness overestimation (observed in MRI studies[4]) was not an issue in our study due to the anatomical method of cortical thickness measuring used.

Conclusions

Our results could be parallelized with a glimpse of our brain's mysteries. Their interpretation is anything but easy. However, our results suggest that there could be a potentially stronger impact for objects placed in the left inferior quarter of the visual field. Our study also revealed several thickness-based correlations among different functional cortical areas. Most of them seem to have a more or less rational explanation. Furthermore, we believe that functional imaging of the cerebral cortex could be able to provide us with a more documented approach to the interpretation of such findings, especially when combined with clinical data.

COMMENTS

Background

The knowledge that specific cortical areas of the brain are correlated with specific functions, known at least since the 19th century, resulted in several functional maps of the cerebral cortex as well as the famous motor and sensory homunculi. Modern functional neuroimaging techniques allowed this knowledge to evolve, revealing, however, the complexity of such correlations.

In human anatomy, information regarding structure can indirectly provide us with information regarding function.

Research frontiers

Despite the significant research progress on neurosciences during the last century, there is still a lot to learn. Although functional neuroimaging methods, mainly the functional magnetic resonance imaging, offered a window with a magnificent view to brain functions, there are still well kept secrets regarding these complex functions.

Innovations and breakthroughs

The authors found a significant difference of the mean value of the cortical thickness of the superior wall of the calcarine sulcus between right and left hemispheres, as well as very significant correlations among several functional cortical areas.

Applications

The authors' results suggest that there could be a potentially stronger impact for objects placed in the left inferior quarter of the visual field. The study also revealed several thickness-based correlations among different functional cortical areas.

Peer review

The question of the study is interesting. The authors reveal several thickness-based correlations among different functional cortical areas. This is important research to help us enhance our understanding of how functional brain areas are associated with their structural substrates.

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CASE REPORT

Low grade spinal malignant triton tumor with mature skeletal muscle differentiation

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Abstract

Malignant peripheral nerve sheath tumor (MPNST) is an uncommon sarcoma which arises from pluripotent stem cells of the neural crest and differentiates predominantly towards Schwann cells. Low grade spinal MPNST with skeletal muscle differentiation (malignant triton tumor) is vanishingly rare. In this study, we report a case of a 53-year-old female with a homogenously enhancing C2-C4 extradural lesion. The lesion demonstrated a biphasic histologic pattern with a diffusely infiltrating, atypical spindle cell component strongly positive for vimentin and focally positive for S-100. The second component consisted of diffusely scattered clusters of mature skeletal muscle cells which were positive for desmin, fast myosin and muscle specific actin but negative for Myogenin and MyoD-1. The Ki-67 labeling index was low (< 1%) and no necrosis was identified. The present case is remarkable because of its rare location, low grade histology and unusual immunophenotype of the skeletal muscle component, which were not previously described.

Key words: Malignant triton tumor; Cervical spine; Mature skeletal muscle differentiation; Prognosis

Core tip: Malignant peripheral nerve sheath tumor (MPNST) is an uncommon sarcoma with dismal prognosis. Low grade MPNST with skeletal muscle differentiation (malignant triton tumor) in cervical spine is vanishingly rare. The present case is remarkable because of its rare location, low grade histological features and unusual immunophenotype of the mature skeletal muscle component, which were not previously described. Thus, our study expands the morphological spectrum and the clinicopathological significance of this uncommon tumor. These novel findings should be interesting to the neuropathologists, neurologists and neurosurgeons.

Zhang M, Weaver M, Khurana JS, Mukherjee AL. Low grade spinal malignant triton tumor with mature skeletal muscle differentiation. *World J Neurol* 2013; 3(3): 75-78 Available from: URL: http://www.wjgnet.com/2218-6212/full/v3/i3/75.htm DOI: http://dx.doi.org/10.5316/wjn.v3.i3.75

INTRODUCTION

Malignant peripheral nerve sheath tumor (MPNST) is an uncommon soft tissue sarcoma which arises from pluripotent stem cells of the neural crest and differentiates towards Schwann cells[1]. Spinal MPNSTs are rare and have dismal prognosis[2]. MPSNT with coexistence of rhabdomyosarcomatous elements are termed malignant triton tumor (MTT)[3] and have been rarely reported in the spinal cord[4]. Typically, they are high grade with

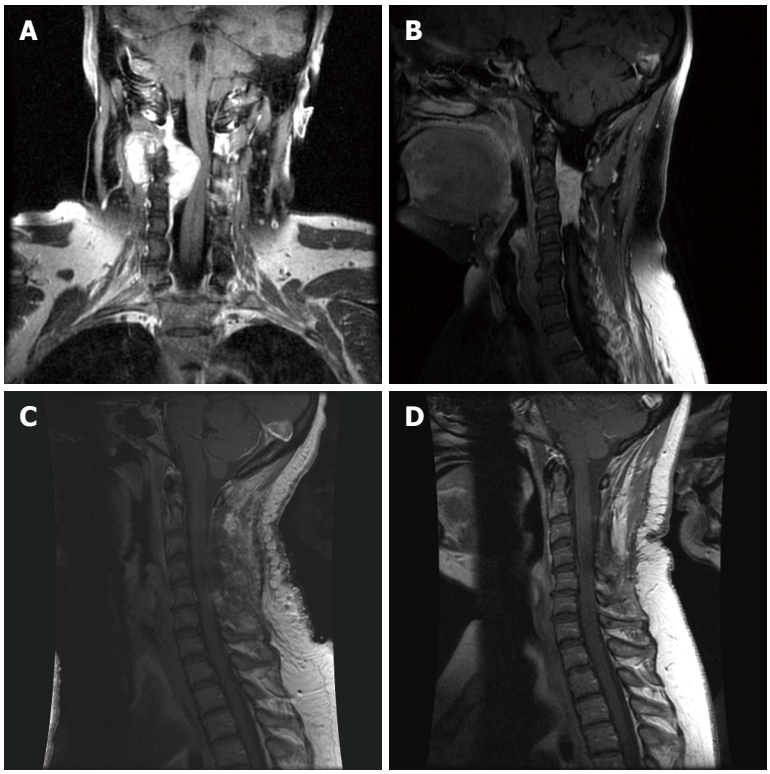

Figure 1 Pre-operative magnetic resonance imaging of the cervical spine. On coronal (A) and sagittal (B) view showed an extradural soft tissue mass at C2-C4 levels. After debulking of the mass (C) and at 10 mo post-operative follow up (D) showed markedly decreased mass effect on the cervical cord.

worse prognosis compared to conventional MPNST[5,6]. Low grade spinal MTT is vanishingly rare with a single case reported in literature[2]. A low grade spinal MTT with cytologically benign and immunophenotypically mature skeletal muscle component has not been previously described.

CASE REPORT

Clinical history

A 53-year-old female without family history or stigmata of neurofibromatosis presented with worsening neck pain and right sided neck stiffness. The pain is present in the left side (buzzing in left hip and left heel). The quality of the pain is described as aching and shooting (pulling, popping) and the severity of the pain is at 6/10. The symptoms are aggravated by position and twisting. The pain is worse during the night while the stiffness is present all day. Physical examination is unremarkable except for right facial nerve weakness nerve. MRI showed a homogenously enhancing C2-C4 intraspinal extradural lesion exiting out the foramen at C2-C3 in a dumbbell fashion (Figure 1A and B). A C2-C4 laminectomy was performed by using a combination of Leksell rongerus, Kerrisons and curettes. A tannish shear mass was identified in the epidural space from C2 to the top of C4. The mass was debulked from the inside using the combination of pituitary rongeurs as well as cavitron ultrasonic surgical aspirator debulking and bipolar electrocautery. The post-operative MRI showed markedly decreased mass effect on the cervical cord (Figure 1C). Bone stimulator and cervical collar were placed after surgery. The patient is treated with Percocet for analgesia and managed by physical therapy. No adjuvant treatment no radiation treatment were started. She is currently under regular follow up every six month (Figure 1D, 10 mo post-operative MRI), without evidence of recurrence (23 mo post surgery).

Pathological findings

The tumor was received as tan soft tissue aggregates measuring 5.0 cm × 3.4 cm × 0.8 cm. Microscopic examination demonstrated a distinctly biphasic neoplasm with an infiltrating, moderately atypical amitotic spindle cell component (Figure 2A). This component had hypercellular areas that alternate with paucicellular highly collagenized areas containing small-spindled nuclei with a wavy appearance, suggestive of a low grade malignant peripheral nerve sheath tumor[7]. The second component consisted of diffusely scattered clusters of mature appearing amitotic small skeletal muscle cells (Figure 2B). The immunohistochemical studies showed the spindle cell component to be strongly positive for vimentin (Figure 3A) and focally positive for S-100 (Figure 3B), CD57. Rare cells were positive for CD34 and CD117. The neoplastic cells were negative for smooth muscle actin, estrogen receptor, EMA, AE1/AE3 and p53. The small round cells with skeletal muscle morphology showed a mature skeletal muscle phenotype with expression of desmin, fast myosin and muscle specific actin (Figure 3C). However, they were negative for rhabdomyoblastic markers Myogenin and MyoD-1. Neurofilament protein immunostain did not show any entrapped axons in the lesion. The Ki-67 labeling index was low (< 1%) (Figure 3D) and no necrosis was identified. The immunomorphological features were interpreted to be consistent with unusual low grade variant of MPNST with mature

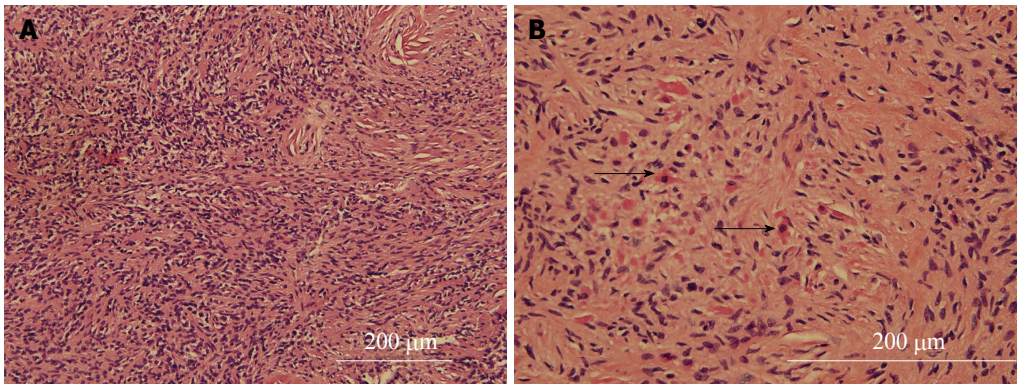

Figure 2 Histopathology of the tumor. A: High-power micrographs show a spindle cell lesion with focally increased cellularity and nuclear atypia; B: Scattered clusters of mature-appearing, small round cells with moderately abundant eosinophilic cytoplasm (arrow head) resembling a skeletal muscle differentiation.

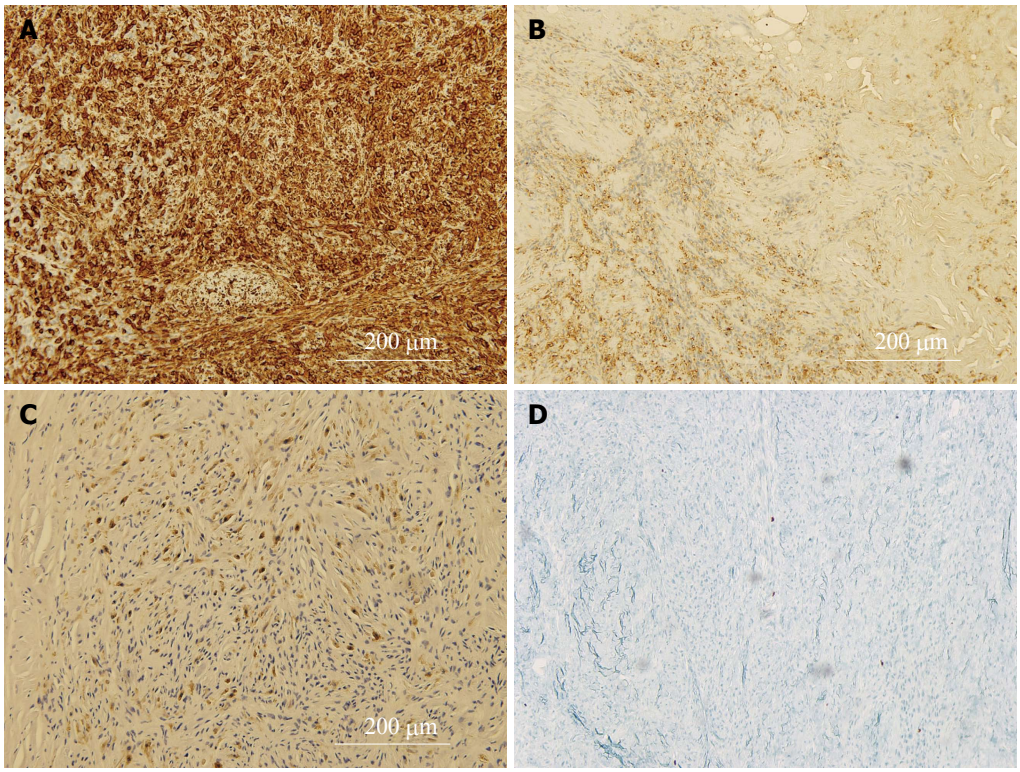

Figure 3 Immunohistochemistry. The spindle cells are diffusely and strongly positive staining for vimentin (A) and focally positive for S-100 protein (B); The small round cells with skeletal muscle morphology showed positivity of muscle specific actin (C); The Ki-67 immunostaining shows low labeling index (D).

skeletal muscle differentiation. Neurofibroma and cellular schwannoma were also considered in the differential diagnosis. But hypercellularity, nuclear enlargement (three times the size of neurofibroma nucleus), infiltrative growth pattern, lack of neurofilament immunostaining and focal S-100 positivity favored the diagnosis of low grade MPNST. Of note, cellular schwannoma is typically strongly and diffusely positive for S-100 and neurofibromas shows entrapped neurofilament positive axons.

DISCUSSION

The present case is remarkable because of its location, low grade histology and unique phenotype of the skeletal muscle component. A literature review of the spinal MPNSTs revealed a total of 59 reported cases with only one case showing exclusive extradural location in cervical spine[2,8]. Exclusively extradural cervical spinal location of our case is unusual. Spinal MPNSTs, reported in the literature typically show high grade histology (necrosis, high mitotic rate and Ki-67 labeling index) and have worse prognosis compared to conventional MPNST[5,8,9]. Only a small number of MPNSTs are low grade (15%) and they can have diverse histopathology, location and growth patterns[7,9]. Smooth muscle differentiation has been reported in a case of low grade MPNST[10]. However, mature skeletal muscle component in a low grade MPNST has not been previously reported.

The skeletal muscle component was diffuse and seemed an integral part of the tumor. The diffuse nature of the skeletal muscle component excluded the possibility that this was entrapped muscle at the edge of the tumor. Malignant triton tumors typically have a rhabdomyosarcomatous component[3]. Benign triton tumor usually refers to neuromuscular hamartomas, where mature skeletal muscle replaces nerve fascicles[11]. In contrast to conventional MTT which are positive for rhabdomyoblastic markers Myogenin and MyoD-1, both markers are negative in our case. MyoD-1 and myogenin are members of the Myogenic transcriptional regulatory protein, are the

most widely used markers to express the early in skeletal muscle differentiation. They are very sensitive and specific for the diagnosis of immature skeletal muscle in rhabdomyosarcoma. Mature skeletal muscle component in a diffusely infiltrating peripheral nerve sheath tumor with hypercellularity and nuclear atypia is very unusual. Low grade MTT has been reported in the oral cavity[12] and recently in lumber spine[2]. In both the cases, immunophenotypic maturity of the skeletal muscle component was not evaluated by MyoD-1 and Myogenin immunostain. In the only reported low grade spinal MTT, the patient is alive after 18 mo with no evidence of disease following total surgical removal and radiotherapy[2]. Although the clinical indication of such features is still undetermined, the mature skeletal muscle differentiation might indicate a better prognosis than the conventional MTT, evidenced by the prolonged disease free interval of our patient (23 month post surgery without chemotherapy and radiation therarpy).

In conclusion, here we have reported a case of low-grade MTT with unusual location and distinctive mature skeletal muscle differentiation, which expands the morphological spectrum of this rare tumor.

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CASE REPORT

Complete recovery of severe tetraparesis after excision of large C1-osteochondroma

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Abstract

Severe tetraparesis resulting from cervical cord compression due to osteochondroma of the Atlas is a rare condition, especially in young children. In this report, the author discusses the clinical presentation, and outcome of surgical excision of a large C1 osteochondroma presenting with severe myelopathic tetraparesis, in a 10-year-old girl. Computed tomography and magnetic resonance images revealed a large bony lump arising from the posterior arch of atlas, filling most of the spinal canal, and compressing the cervical spinal cord. Another histologically proven exostosis was incidentally found at the spinous process of T1. There was no history of exostosis in the family, and the patient improved dramatically after removal of the C1 osteochondroma.

Key words: Osteochondroma; Exostosis; Atlas; Tetraparesis; Myelopathy; Recovery

Core tip: Solitary cervical spine osteochondroma affects C1, whereas multiple exostoses involve C2 more. Osteochondroma usually originates from the posterior element, and continues to grow slowly until puberty. It appears in computed tomography as an extradural mass, and spinal cord changes are better seen in magnetic resonance imaging. The author highlighted this case of a child with multiple exostoses arising from the posterior arch of C1 and a smaller one at the spinous process of C7, because the associated severe neurological symptoms are more pronounced than reported previously, and adequate decompression by total excision was associated with significant neurological recovery.

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INTRODUCTION

Spinal exostosis causing severe spinal cord compression is a rare form of bone dysplasia representing 4% to 7% of all primary benign spinal tumors, which generally becomes symptomatic during the second and third decades of life[1-3]. Most spinal exostoses grow from the external parts of the lamina and occasionally arise from the posterior arch of the atlas, causing cord compression and subsequently quadriparesis[4].

Spinal exostoses are better visualized by computed tomography (CT) than by magnetic resonance imaging (MRI). However, spinal cord compression is best studied by MRI, as is the size of the cartilaginous cap, which assists in differentiating exostoses from chondrosarcoma[1-5].

Neurological recovery after surgical excision of intraspinal exostoses and decompression of the spinal cord is excellent, and the recurrence rate is low[2-5]. The author reports complete recovery of severe tetraparesis after excision of a large intraspinal C1 osteochondroma in a 10 year old girl who also had an T1 spinous process exostosis, found incidentally.

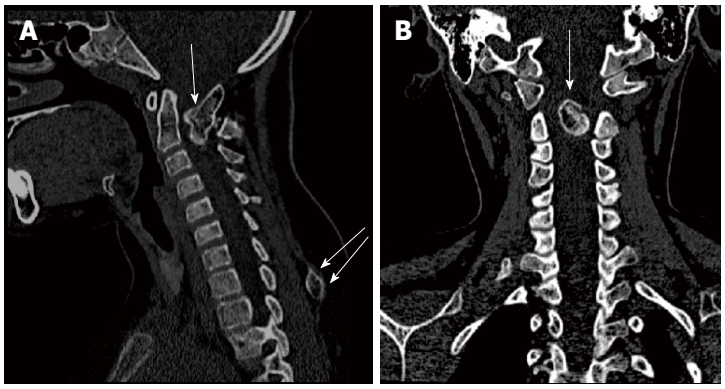

Figure 1 Computed tomography scan of the cervical spine. A: Sagittal; B: Coronal reconstruction showing a bony outgrowth filling the spinal canal, arising from the inner posterior arch of C1 at the left site, growing anteriorly and obliterating most of the spinal canal (top single arrow). Another exostosis was incidentally found at the spinous process of T1 (bottom double arrows).

CASE REPORT

A 10 years old girl, the product of uneventful pregnancy for a non-consanguine couple was born at term by caesarian section. She presented with a 7 d history of left leg pain, and frequent falls at school. She further complained of moderate neck pain and swelling at the back of the neck which was thought to be related to the fall. She admitted that she has been troubled by unsteadiness and had noticed weakness and stiffness of both upper and lower limbs, particularly on the left side, over the previous year. She had a history of iron-deficiency anaemia in early childhood, and there was no family history of neurologic or musculoskeletal disorder.

On examination, the patient's neck was tilted to the right side and a hard swelling was felt just below the vertebra prominens at the T1 spinous process. She was able to move her neck in all directions, with some limitations due to stiffness. She was found to have G2/5 power in the left arm and leg and G3/5 power in the right upper and lower limbs, along with myelopathic manifestations affecting all 4 limbs but mainly the left side, with hypertonia, brisk reflexes, unsustained left ankle clonus, upgoing planters, and positive Hoffman's sign bilaterally. Pin prick sensation was impaired with a sensory level at the root of the neck, but deep sensation was preserved.

Radiological investigations, including CT and MRI scans, revealed an irregular bony outgrowth arising from the posterior arch of the atlas extending into the spinal canal, the posterior part forming a pseudo joint with the posterior arch of the axis. Findings were suggestive of a congenital anomalous bony outgrowth from C1 (Figure 1). This bony overgrowth was mainly at the left side, extending anteriorly into the spinal canal and causing significant compression, flattening and displacement of the cord to the right side. There was no evidence of alteration of cord signal intensity (Figure 2). Prominence of the T1 spinous process with a rounded bony outgrowth was found incidentally on screening the rest of the patient's spine (Figure 2). Skeletal survey excluded similar bony lesions.

The patient underwent excision of the masses through a posterior approach. The bony lump was composed mainly of cancellous bone lined by a thin cortical layer. Drilling of the posterior arch of C1 and piecemeal removal of the cancellous content, extended deep into the spinal canal and a cap which was found adhering to the underlying dura was removed. The mass arising at the spinous process of T1 was removed through a separate incision.

The patient tolerated the procedure well and started to recover immediately following surgery. Postoperative CT and MRI scans showed widening of the spinal canal, relief of the neural compression, and improvement of the spinal cord deformation (Figure 2). Histological examination of both specimens proved them to be osteochondromas.

The patient continued to improve with the help of physiotherapy until she was back to her normal self at 6 mo follow-up after surgery. Physical examination showed no muscle weakness, normal sensation, improved hyperreflexia, and no lower limb clonus.

DISCUSSION

Osteochondromas are considered the most common benign skeletal tumor. It has been reported that about 3% occur in the spine. However, spinal exostoses are probably under-reported in relation to their true incidence because most are asymptomatic[5,6]. Most solitary and hereditary osteochondromas tend to occur in the cervical spine, while the most common site for solitary lesions is C1. In hereditary multiple exostosis (HME), it the C2 vertebra seems to be the most commonly affected[4-7].

In a review of 165 cases of spinal exostoses reported in the literature, 89 of them (53%) were affected by solitary lesions, with the cervical spine the most affected site (54%), and C1 the most common level (10%). On the other hand, lesions associated with HME also occurred most commonly in the cervical spine (57%), most commonly at C2 level (19%)[8]. The results of Bess *et al*[8] also indicated that the majority of cervical spine exostoses were solitary.

Spinal osteochondromas usually arise from the posterior elements (the secondary centers of ossification), most commonly near the tips of the spinous processes[6]. Of the 121 exostoses whose origins were reported, 106 (88%) emanated from the posterior elements. The remaining 15 exostoses (12%) originated from the vertebral body. Solitary and HME exostoses had similar distributions of vertebral origin[8].

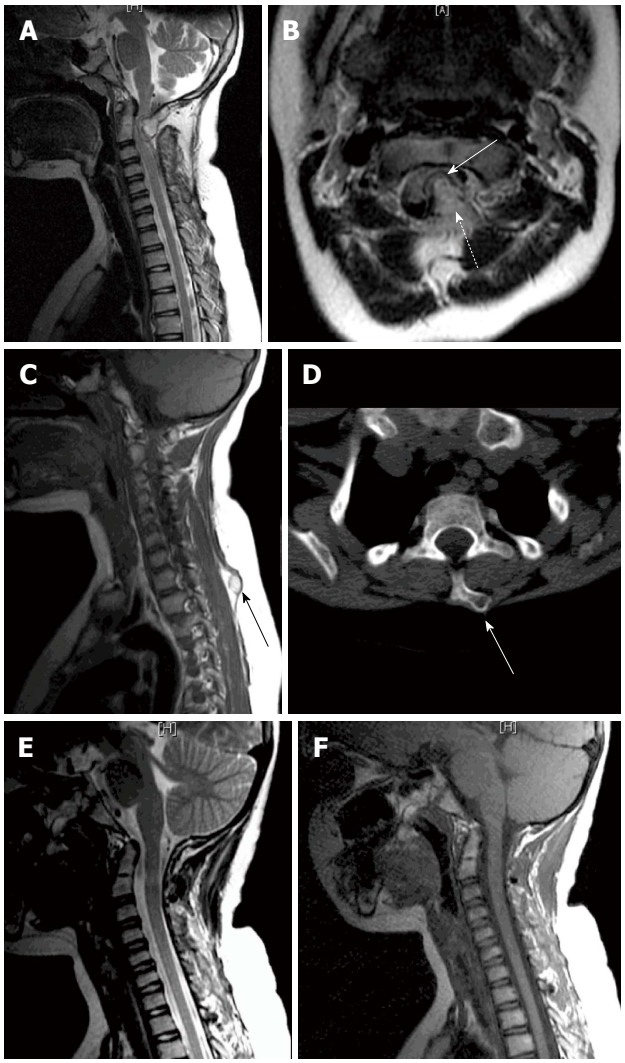

Figure 2 T2, T1-weighted magnetic resonance imaging. A: T2-weighted magnetic resonance imaging (MRI) sagittal image; B: Axial cut, demonstrating a C1 posterior mass (solid arrows) severely compressing the cord. The mass is hyperintense, whereas the cortical margin is hypointense (dotted arrow); C: T1-weighted MRI sagittal image; D: Bone window computed tomography scan showing T1 spinous process exostosis; E: Postoperative sagittal T2-weighted; F: Postoperative sagittal T1-weighted MRI showing adequate decompression of the spinal cord and no visible lesion.

Neurological damage is usually caused by the progressive encroachment of the slowly expanding lesion. However, the reported incidence of spinal cord or nerve root compression is 0.5% to 1.0%, and a higher incidence of symptomatic exostoses was found with lesions associated with HME, especially in young patients[9,10].

Osteochondromas cease to grow after skeletal maturity and do not develop in postpubertal individuals. However, in the majority of children, the tumor remains asymptomatic until skeletal maturity. The clinical presentation in the present case started at the age of nine and progressed relentlessly over the course of a year, resulting in myelopathy affecting all extremities by one week before presentation[11].

In osteochondroma a CT scan is the diagnostic imaging modality of choice. CT shows the extent of the cartilaginous and osseous components, and their relationship to the vertebral and neural elements of the spine[8]. MRI is more useful than CT in defining the extradural component and is the preferred method for examining the spinal canal and the effect of pressure on the spinal cord[1-5].

The incidence of malignant transformation of osteochondroma into sarcoma is between 5% and 11%, and is associated with sudden pain or neurological deterioration[5,12]. In this case, rapid neurological deterioration was probably due to sudden decompensation of the compressed spinal cord and not to malignant transformation, as confirmed by histological examination.

The management of this case was aimed at relieving neurological symptoms, by excision of the lump and decompressing the spinal cord. Improvement and satisfactory recovery of function after resection of the lesion are to be expected in most cases[13]. This patient's favorable prognostic factors included her young age, short period of rapid symptom progression, and absence of signal cord changes in the MRI. Although spinal cord compression was severe, the patient showed such rapid and satisfactory recovery that she was back to almost her normal self, within 6 mo after surgery.

Albrecht *et al*[14] emphasized that neurological recovery after surgical treatment of intraspinal exostoses causing spinal cord compression is excellent, and the recurrence rate is low. They found that 89% of symptomatic patients treated operatively reported improvement of symptoms. All patients except two in the Bess *et al*[8] series surgically treated for intraspinal exostoses, had eventual resolution of presenting symptoms after surgery.

This patient had no family history of similar condition, and no family screening was performed. However, having multiple spinal exostosis affecting C1 and C7, this case could be the first in the family to develop HME. It is an autosomal dominant disorder, with full penetrance and has an equal gender prevalence[15].

In conclusion, Spinal exostosis occurs most commonly in the cervical spine, and the associated neurological symptoms are more common than reported previously. Multiple exostoses tend to affect younger patients and have a higher incidence of neurological symptoms than solitary lesions.

Although MRI is the preferred method for examining the spinal canal and the effect of pressure on the spinal cord, CT scan can be more specific and is the imaging modality of choice. Total excision of the exostosis is associated with a high probability of neurological recovery.

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CASE REPORT

Hypertensive brain stem encephalopathy with pontine hemorrhage: A case report

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Abstract

Hypertensive brain stem encephalopathy (HBE) is a rare, under diagnosed subtype of hypertensive encephalopathy (HE) which is usually reversible, but with a potentially fatal outcome if hypertension is not managed promptly. To the best of our knowledge, only one case of HE with brain stem hemorrhage has been reported. We report a case of HBE with pontine hemorrhage in a 36-year-old male patient. The patient developed severe arterial hypertension associated with initial computed tomography showing the left basilar part of pons hemorrhage, fluid-attenuated inversion-recovery showing hyperintense signals in the pons and bilateral periventricular, anterior part of bilateral centrum ovale. The characteristic clinical findings were walking difficulty, right leg weakness, and mild headache with nausea which corresponded to the lesions of MR imagings. The lesions improved gradually with improvements in hypertension, which suggested that edema could be the principal cause of the unusual hyperintensity on magnetic resonance images.

Key words: Hypertensive brain stem encephalopathy; Pontine hemorrhage

Core tip: Hypertensive brain stem encephalopathy (HBE) is a rare, underdiagnosed subtype of hypertensive encephalopathy. The disease is still not recognized by clinical doctors. The prompt diagnosis and treatment is crucial. Here, we report a case of HBE with pontine hemorrhage in a 36-year-old man. The case will help clinicians to further know about the disease.

Zhou ZH, Qu F, Chen HS. Hypertensive brain stem encephalopathy with pontine hemorrhage: A case report. *World J Neurol* 2013; 3(3): 83-86 Available from: URL: http://www.wjgnet.com/2218-6212/full/v3/i3/83.htm DOI: http://dx.doi.org/10.5316/wjn.v3.i3.83

INTRODUCTION

Hypertensive brain stem encephalopathy (HBE) is a rare, under diagnosed subtype of hypertensive encephalopathy (HE) which is usually reversible, but with a potentially fatal outcome when hypertension is not treated promptly[1]. The typical feature on magnetic resonance imaging (MRI) of HE are signals of increased intensity in the white matter of both occipital regions consistent with edema. The term "reversible posterior leukoencephalopathy syndrome" or "posterior reversible encephalopathy syndrome" has been used to describe the characteristic neuroimaging features in HE[2,3]. Involvement of the brainstem in addition to the supratentorial lesions has been rarely reported, and is termed HBE. The typical imaging features of HBE are hyperintense lesions on T2-weighted MRI and swelling of the brainstem ,which may be misdiagnosed as brainstem infarction, pontine glioma, central pontine myelinolysis and infective encephalitis[1,4,5]. To the best of our knowledge only one case of HE with brain stem hemorrhage has been re-

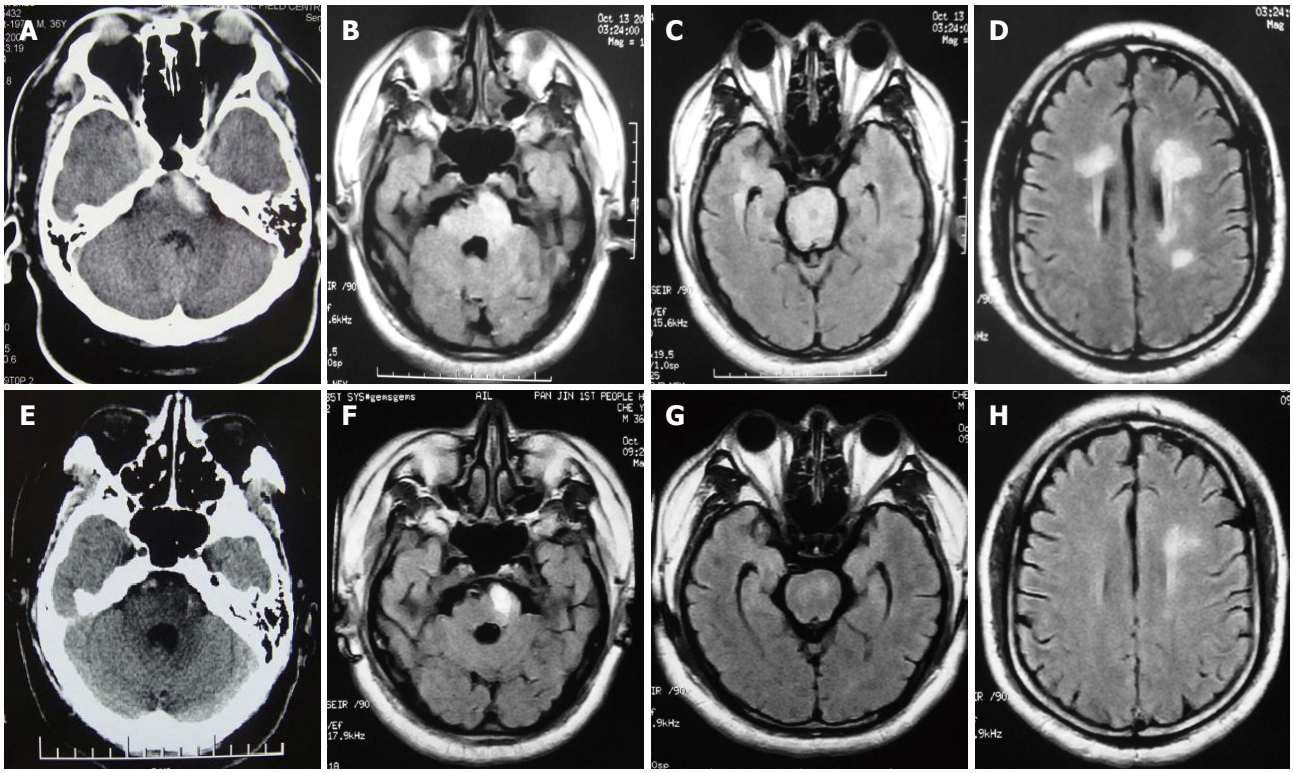

Figure 1 The imagings before admission and after 17 d. A: Initial computed tomography (CT) shows the left basilar part of pons hemorrhage; B, C: Initial fluid-attenuated inversion-recovery (FLAIR) show hyperintense signals in the pons and bilateral periventricular, anterior part of bilateral centrum ovale; D: Follow-up CT 17 d after initial imaging, showing low-density signal changes in the left basilar part of pons, indicating pontine hemorrhage has been almost absorbed (E); F, G: Follow-up FLAIR 17 d after initial imaging, showing a nearly complete resolution of hyperintensity except hemorrhage in the pons and bilateral periventricular, anterior part of bilateral centrum ovale (H).

ported[6]. We report a case of HBE with pontine hemorrhage in a 36-year-old male patient.

CASE REPORT

A 36-year-old man with a history of poorly controlled hypertension presented with 2 d history of difficulty walking, right leg weakness, and mild headache with nausea. His blood pressure was 220/160 mmHg. The cranial nerve functions were intact, including visual function and an ophthalmologic examination. Clinical examination revealed Medical Research Council grade 4/5 strength in the right leg. Deep tendon reflexes were brisk with bilateral extensor plantar responses and positive Babinski sign.

A laboratory evaluation on admission including renal function, hepatic function, serum electrolyte concentrations was unremarkable. Immunologic studies of the following were negative: antinuclear factor, antibodies to native DNA, antibodies to ribonucleoprotein, antibodies to smooth muscle, anti-Ro antibody (SS-A), anti-La antibody (SS-B), serum immunoglobulin (Ig)G, IgA, or IgM, C3, C4, and serum immunocomplex. An antihuman immunodeficiency virus antibody was negtive. An electroencephalogram was normal. A CT showed high-density signal changes in the left basilar part of pons and mild swelling in the brainstem (Figure 1A). The T2-weighted and fluid-attenuated inversion-recovery (FLAIR) images showed high-intensity signal changes in the whole pon, bilateral periventricular, anterior part of bilateral centrum ovale (Figure 1B-D). The T1-weighted images showed moderate hyperintense signals and diffused-weighted images showed hyperintense signals in the left basilar part of pons where CT showed high-density signal changes. However, despite the presence of extensive neuroimaging brainstem lesions, there were no symptoms or signs of brainstem dysfunction.

Immediate treatment with pumped nitroprusside (3 μg/kg/min) led to gradual improvement in right leg weakness ,disappearance of the headache and rapid control of the BP, which decreased to 160/110 mmHg after 4 h. He was prescribed amlodipine and ramipril on the 3rd day. Ten days later, the systolic and diastolic pressures stabilized at 130-150 and 80-100 mmHg respectively. A cerebral CT performed two weeks later, showed low-density signal changes in the left basilar part of pons, suggesting absorption of the pontine hemorrhage (Figure 1E). The brainstem and supratentorial lesions except hemorrhage almost resolved as shown in FLAIR (Figure 1F-H) and T1-weighted images, after stabilization of his blood pressure.

At the time of discharge, 18 d later, the neurological examination revealed no abnormalities except for a right positive Babinski sign.

DISCUSSION

The BHE is characterized by very high blood pressure with marked clinicoradiologic dissociation. Patients typically have mild clinical and neurologic symptoms, prominent brainstem involvement and relatively mild supratentorial lesions with rapidly improved MR findings after controlling hypertension[7].

The clinical features of our case presented here were strongly suggestive of BHE, with severe hypertension, mild right leg weakness, headache, severe predominantly brainstem lesions and and subsequent improvement after a reduction in blood pressure.

In our patient, the diagnosis of BHE was difficult at first because of the co-existence of pontine hemorrhage and the whole brain edema. The differential diagnosis in these patients is: hypertensive pontine hemorrhage and hemorrhagic brain stem infarction. In both of these pathologies, patient would be in clinically grave situation and a poor prognosis based on the neuroimaging.

Brainstem infarction can be ruled out by the lack of major brainstem signs, rapid clinical recovery, predominant white matter involvement in T2-weighted and FLAIR images, and absence of signal changes in diffusion-weighted MRI except pontine hemorrhage.

Other differential diagnosis include central pontine myelinolysis (CPM), infectious brainstem encephalitis, acute disseminated encephalomyelitis (ADEM), neuro-Behcet disease, and tumor. CPM may produce a similar radiologic picture, but the absence of abnormal serum sodium levels, the clinical recovery, and resolution of the MRI lesions made the possibility of CPM quite unlikely. The rapidity of the clinical evolution should help differentiate their condition from tumors. The patient lacked skin and eye symptoms, aphthous stomatitis, and definitive brain stem symptoms, so we excluded neuro-Behcet disease. Infectious brainstem encephalitis or ADEM was unlikely because of mild clinical and neurologic symptoms, normal electroencephalogram, and rapid recovery without specific treatment although cerebrospinal fluid testing was not performed.

Intracerebral hemorrhage associated with HE is generally considered an atypical finding. Although the incidence of hemorrhage was 15.2%, brain stem hemorrhage is rare and only one case has been reported in the literature[6]. Intracerebral hemorrhage associated with HBE is much less frequent with 2 of 26 HBE patients reported in a reviews having a small thalamic hemorrhage simultaneously[8]. Our patient had HBE with pontine hemorrhage. No case of brain stem hemorrhage associated with HBE has been reported yet.

The proposed mechanism underlying HE involves the breakdown of autoregulation, resulting in dilatation of cerebral arterioles, the disruption of the blood-brain barrier, and the breakthrough accumulation causing vasogenic edema[9]. As the vertebrobasilar system and posterior cerebral arteries are sparsely innervated by sympathetic nerves[10], accounting for the susceptibility of the brain stem and other posterior brain regions to this breakdown of autoregulation.

The hemorrhage in our patient does not seem to be due to hypertensive rupture of small artery based on clinical presentation and rapid improvement of symptoms. It is thought to be due to a breakthrough of autoregulation by regional hyperperfusion and increased intracranial pressure due to the elevated blood pressure[11].

In conclusion, clinical recognition of HBE may be difficult. The features of a lack of correlation between the severity of the radiological abnormality and the clinical status, combined with the rapid resolution following antihypertensive treatment, should suggest the diagnosis. It is important for the radiologist to be familiar with the imaging abnormalities (especially with hemorrhage) of this life-threatening, but treatable condition. Rapid identification and appropriate diagnostics are essential, as prompt treatment usually results in reversal of symptoms; permanent neurologic injury or death can occur with treatment delay.

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Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/2218-6212/g_info_20100723222255.htm.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*
Biology: *H. pylori*, *E coli*, *etc.*

Examples for paper writing

All types of articles' writing style and requirement will be found in the link: http://www.wjgnet.com/esps/NavigationInfo.aspx?id=15

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