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Editorial Board Member of *World Journal of Translational Medicine*, Wen-Bin Ou, PhD, Department of Biotechnology and Biomedicine, Yangtze Delta Region Institute of Tsinghua University, Chuangxin Building, RM1003, Yatailu 705, Jiaxing 314006, Zhejiang Province, China

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World Journal of Translational Medicine (*World J Transl Med*, *WJTM*, online ISSN 2220-6132, DOI: 10.5528) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJTM publishes articles that report the results of translational medicine-related applied and basic research in fields such as immunology, physiopathology, cell biology, pharmacology, medical genetics, and pharmacology of Chinese herbs. Priority publication will be given to articles concerning diagnosis and treatment of translational medicine diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

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REVIEW

Obesity research: Status quo and future outlooks

Alexander T El Gammal, Anna Dupree, Stefan Wolter, Jens Aberle, Jakob R Izbicki, Cenap Güngör, Oliver Mann

Alexander T El Gammal, Anna Dupree, Stefan Wolter, Jakob R Izbicki, Cenap Güngör, Oliver Mann, Department of General, Visceral, and Thoracic Surgery, University Medical Center Hamburg-Eppendorf, 20246 Hamburg, Germany
Jens Aberle, Department of Endocrinology and Diabetology, University Medical Center Hamburg-Eppendorf, 20246 Hamburg, Germany
Author contributions: El Gammal AT, Dupree A and Wolter S contributed equally to the presented work and therefore share first authorship; Aberle J and Izbicki JR contributed to the manuscript; Güngör C and Mann O contributed equally to the presented work and therefore share senior authorship.
Correspondence to: Cenap Güngör, PhD, Department of General, Visceral, and Thoracic Surgery, University Medical Center Hamburg-Eppendorf, Martinistrasse 52, 20246 Hamburg, Germany. c.guengoer@uke.de
Telephone: +49-40-741051956 Fax: +49-40-741053496
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Abstract

Obesity is a multifactorial disease showing a pandemic increase within the last decades in developing, and developed countries. It is associated with several severe comorbidities such as type Ⅱ diabetes, hypertension, sleep apnea, non-alcoholic steatosis hepatis and cancer. Due to the increasing number of overweight individuals worldwide, research in the field of obesity has become more vital than ever. Currently, great efforts are spend to understand this complex disease from a biological, psychological and sociological angle. Further insights of obesity research come from bariatric surgery that provides new information regarding hormonal changes during weight loss. The initiation of programs for obesity treatment, both interventional and pharmaceutical, are being pursued with the fullest intensity. Currently, bariatric surgery is the most effective therapy for weight loss and resolution of comorbidities in morbid obese patients. Reasons for weight loss and remission of comorbidities following Roux-en-Y-Gastric Bypass, Sleeve Gastrectomy, and other bariatric procedures are therefore under intense investigation. In this review, however, we will focus on obesity treatment, highlighting new insights and future trends of gut hormone research, the relation of obesity and cancer development *via* the obesity induced chronic state of inflammation, and new potential concepts of interventional and conservative obesity treatment.

Core tip: This review focuses on the latest obesity research breakthroughs, current therapy options, future outlooks, also from a view of a surgeon as well as recently identified molecules that promote obesity and its comorbidities, outlining their great potential as new target molecules in the fight against the global pandemic, called "obesity".

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INTRODUCTION

Obesity is a multifactorial disease caused by an energy sparing lifestyle on a predisposed polygenetic background. An obese person is defined as having a body mass index (BMI) greater than 30 kg/m². Within the last decades, there has been an extraordinary increase in the worldwide prevalence of obesity becoming a major human health threat especially in developing and developed countries with a tendency to rise. Being referred to as a global pandemic[1], the number of overweight or obese

individuals increased up to 2.1 billion worldwide. Unfortunately, no single country announced decreasing numbers of obese individuals during the last three decades[2]. Obesity is associated with several severe comorbidities (Figure 1) such as type Ⅱ diabetes mellitus (T2DM), hypertension, sleep apnea, non-alcoholic steatosis hepatis (NASH) and cancer. Obesity-related diabetes can lead to coronary heart disease, apoplex or kidney failure. Over 80% of all patients with type Ⅱ diabetes in the United States are overweight and up to 20% of United States health expenditures are estimated to be spent on treating obesity-related diseases[3].

It is expected that NASH will be the leading cause of liver transplantation within the next years[4]. Additionally, obesity is associated with an increased risk of developing various cancer entities such as colorectal-, esophageal-, liver- and breast cancer[5]. Visceral-, orthopedic or cardiac surgical treatment of obese patients is associated with higher complication rates[6-9]. Subsequently, obesity is the origin of a wide spectrum of diseases and a cofounding factor hindering adequate treatment. Due to this reasons, obesity and overweight are associated with an increased risk of death. Thus, therapy for obesity should be individually tailored and various factors such as sex, obesity degree, individual health risks should be taken into account[10,11].

Secondary causes for obesity like endocrine disorders (*e.g.*, hypothyroidism, cushing disease), drug-induced obesity (*e.g.*, glucocorticoids, psychoactive drugs), inherited syndromes (*e.g.*, Prader-Willi syndrome, Bardet-Biedl syndrome) or monogenetic disorders (leptin receptor, melanocortin receptor) play a minor role or are cofactors in causation of obesity in daily practice. Therefore, identifying single reasons for obesity is a complex task. Intervention strategies for weight loss and maintenance at the individual and community level are strongly needed to reduce general health risks as well as health expenditures.

STATUS QUO

Due to the increasing number of overweight individuals worldwide, research in the field of obesity has become more vital than ever. As a multifactorial disease, research is conducted at a wide variety of areas. Currently, great efforts are spend to understand this complex disease from a biological, psychological and sociological angle. Further insights of obesity research come from bariatric surgery, which display new information regarding the hormonal changes during weight loss. The initiation of programs aiming to treat obesity, both interventional and pharmaceutical, are being pursued with the fullest intensity. There are various scopes of possible research activities. In this review, however, we will focus on obesity treatment, highlighting new insights into gut hormones and the relation of obesity and cancer development.

Multidisciplinary Treatment Modalities-or, how to lock the stable door after the horse had bolted

Among physicians there is consensus, to treat obese patients multidisciplinary. After diagnosis, the patient

Figure 1 Obesity related diseases. T2DM: Type 2 diabetes mellitus; NASH: Non-alcoholic steatosis hepatis.

should undergo a multimodal therapy concept based on individualized dietary education focusing on reducing energy intake, physical exercising, pharmacological therapy and psychological attendance with behavioral therapy. For the latter, many efforts to modify the behavior of obese individuals through encouragement of changes in dietary intake along with physical activity have not declined the obesity epidemic, unfortunately. The primary causes are high rates of therapy abandonment and poor patient compliance.

Patients who completed a comprehensive program including a low-calorie diet are able to lose approximately 15%-25% of their initial body weight during 3 to 6 mo of treatment. After therapy, most patients maintain a weight loss of 8% one year after treatment, 7% three years after treatment, and 5% four years after treatment[12]. These results represent the best-case scenario, excluding patients who dropped out of their programs. It was already shown that patients who have completed structured weight loss programs, maintained their weight loss of less than 3 kg on average after 5 years; patients who accomplished more radical low-calorie diets had significantly higher weight loss of up to 20 kg and maintained more weight loss over time[13]. In a randomized study, Jeffery *et al*[14] evaluated the efficacy of long-term weight loss comparing one group with behavior therapy and an energy expenditure goal of 1000 kcal per week to a group of patients with high physical activity treatment and an energy expenditure goal of 2500 kcal per week. The high activity group showed significant higher weight loss and long term weight loss maintenance, reflecting that mobility is of high importance[14].

Is bariatric surgery the best choice for long-term weight loss accompanied by remission of comorbidities in severe obese (BMI > 40 kg/m²) patients?

Bariatric surgery is more effective for weight loss and

Figure 2 Bariatric surgical procedures. A: Roux-Y gastric bypass; B: Sleeve gastrectomy; C: Biliopancreatic diversion with duodenal switch; D: Gastric band.

resolution of comorbidities than conventional medical treatment modalities[15,16]. A variety of procedures are described in the literature but only Roux-Y gastric bypass (RYGB) (46.6% of all bariatric procedures worldwide), sleeve gastrectomy (SG) (27.8%), adjustable gastric banding (GB) (17.8%) and in a smaller proportion biliopancreatic diversion with duodenal switch (BPD/DS) (2.2%) are performed in a notable quantity[17].

Gastric bypass was first performed in the 1960s by Mason *et al*[18]. It was modified to a RYGB in the 1970s[19]. It is still the most common bariatric surgical procedure. The combination of food-intake restriction by a small pouch and malabsorption through the smaller common channel (Figure 2A) leads to long-term weight loss[15]. Also, hormonal changes after surgery may have a great impact on weight loss and diabetes remission.

SG (Figure 2B), includes the resection of the greater curve of the stomach. It is the first step of the BPD/DS (Figure 2C). BPD/DS can be performed by a two step procedure; a minority of patients do not need the second surgical step for weight loss[20]. While SG is described as a primary restrictive procedure, including minor hormonal changes, BPD/DS causes malabsorption and leads to a higher rate of deficiencies[21]. GB, which was developed in the 1970s, restricts food-intake by an inflatable, adjustable gastric band resulting in a small gastric pouch (Figure 2D). Since it is based on restriction only, it is the most insufficient bariatric surgical procedure regarding long-term weight loss[22].

Overall, a preoperative multidisciplinary program is recommended. Our own clinical experiences and a review of the literature revealed that bariatric surgery for severe obese patients results in extensive weight loss and long-term comorbidity remission in a very short time frame.

There is no evidence for conventional treatment leading to sufficient excess weight loss in severe obese patients.

Padwal *et al*[23] performed an observational study of 500 patients with a two years follow-up. Three patient cohorts were included in which 200 patients received medical treatment, 150 patients received bariatric surgical treatment, and 200 patients received no therapy and were grouped as being waitlisted. Medically treated patients received individualized and intensive medical management consisting of a 24-36 wk life style counseling (diet education, physical exercise, and behavioral therapy) and were observed by a multidisciplinary staff which is mandatory before bariatric surgery. Mean weight loss in the waitlisted group was 0.9%, 1.8% in the medically treated group and 22% in the surgery group. The proportion of patients who achieved at least 5% weight loss was 17% in the waitlisted group, 32% in the medically treated group and 75% in the surgery group. The prevalence of hypertension, diabetes and dyslipidemia was reduced in the surgical group, but remained unchanged or increased in the medically treated and waitlisted group[23].

A large meta-analysis included 164 studies (37 randomized controlled trials and 127 observational studies). A total of 161756 patients were analyzed regarding effectiveness and outcome after bariatric surgery.

One year after surgery the patients showed 60% excess weight loss (EWL), and 57% EWL after 3 years. T2DM remission after surgery was 92%, hypertension remission was 75%, dyslipidemia remission was 76%, cardiovascular diseases remission was 58% and remission of sleep apnea was 96%, reflecting that surgical intervention may increase the long-term quality of life[24].

Interestingly, 75.3% of patients that received bariatric surgery showed excess weight loss, whereas patients that had received conventional therapy showed only 11.3% excess weight loss. Moreover, remission of T2DM was reported in 63.5% of cases in surgery group, compared to 15.6% of patients in the conventional therapy group[25]. Subsequently, there is no evidence for conventional treatment leading to sufficient EWL in obese patients with a BMI greater 40 kg/m². In fact, the only efficient treatment showing results in EWL and release of obesity associated diseases results from bariatric surgery. However, there is a strong recommendation to include the patients to a perioperative multidisciplinary medical treatment consisting of dietary changes, exercising and behavioral therapies. There is evidence that preoperative multidisciplinary preparation and education may lead to better long-term effects of bariatric surgery.

In sum, bariatric surgery is currently the only effective treatment for morbid obesity[26]. Reasons for weight loss

Table 1 Gut hormones and their clinical relevance

Peptide	Production site	Effect	After bariatric surgery	Potential pharmaceutical intervention
Ghrelin	Stomach, mainly fundus	Appetite stimulating Growth hormone releasing	↓	Receptor antagonists GOAT inhibition Vaccination
GLP-1	L-cells of the distal small bowel	Postprandialinsuline secretion Suppresses glucagon secretion Delays gastric emptying Suppresses appetite	↑	Weight loss in patients with diabetes Off-label use in obese patients
GIP	Duodenum, jejunum	Postprandial insulin secretion Energy expenditure	↓	GIP receptor antagonist
CCK	Duodenum, jejunum	Delays gastric emptying Suppresses appetite	↑	CCK analogue substance
PYY	Distal small bowel	Delays gastric emptying Suppresses appetite	↑	Long-acting analogue substance
PP	Distal small bowel	Suppresses appetite	↔	PP analogue substance
OXM	L-cells of the distal small bowel	Delays gastric emptying Suppresses appetite Increase energy expenditure	↑	Receptor agonist

Modified according to Kim *et al*[135]. GLP-1: Glucagon-like peptide-1; GIP: Glucose-dependent insulinotropic polypeptide; CCK: Cholecystokinin; PYY: Peptide YY; PP: Pancreatic polypeptide; OXM: Oxyntomodulin; GOAT: Ghrelin O-acyltransferase.

and remission of comorbidities following RYGB, SG, and other bariatric procedures are therefore in a strong research focus.

Gut hormones and their impact on weight loss

Alterations of gut hormone serum levels after RYGB influence appetite, satiety, energy expenditure, and glucose homeostasis[27-29]. Several hormones and peptides are considered to be involved in weight loss in bariatric patients (Table 1).

Incretins are gut-derived peptides that increase pancreatic insulin secretion. The Glucagon-like peptide (GLP-1) and Glucose-dependent insulinotropic polypeptide (GIP) are well explored. GLP-1 and its analogues are used to treat diabetes. Beside its stimulating effects on β-cells of pancreatic Langerhans' islets, GLP-1 also suppresses glucagon secretion, delays gastric emptying and suppresses appetite[30,31]. Therefore, GLP-1 is currently under intense discussion to become a potential therapeutic drug for obesity treatment[32].

Ghrelin is mainly produced in the fundus of the stomach and plays an important role in satiety. When administered to humans, it increases food intake. Several studies showed that postprandial reduction of Ghrelin after bariatric surgery led to weight loss and T2DM remission[33,34]. Therefore, lowering of Ghrelin plasma levels by non-surgical interventions might be a useful approach for obesity treatment. Different approaches already exist in the development of anti-obesity drugs. Pharmacological molecules like Ghrelin antagonists or Ghrelin receptor antagonists showed heterogeneous results in food intake reduction[35]. Other strategies are the inhibition of Ghrelin O-acyltransferase (GOAT) that is required for activation of Ghrelin[36] or lowering body weight by a vaccination targeting Ghrelin[37].

Administration of Oxyntomodulin (OXM) decreases food intake and reduces body weight in rats[38]. Further-more, OXM has been shown to increase energy expenditure[39]. The combination of decreasing energy intake and increasing energy consumption qualifies OXM to be a potential agent for bariatric treatment. Moreover, a plethora of other gut hormones and peptides are currently under intense investigation regarding weight loss. Interestingly, there is also evidence that various gut hormones are related to cancer growth and cancer development making their physiological understanding even more alluring[40].

Obesity and cancer

Morbid obesity is associated with various types of cancer: Epidemiological studies identified an association of morbid obesity and several types of cancer disease, such as colorectal cancer, endometrium carcinoma, postmenopausal breast cancer, kidney cancer, esophageal cancer, pancreatic cancer, gallbladder cancer, liver cancer, and hematological malignancies[41,42]. Obese patients have a tendency for worse prognosis and outcome after cancer treatment and an increased risk of cancer related morbidity[43]. Calle *et al*[5] conducted a prospective study to examine the association of obesity and cancer related mortality. They concluded that increased body weight is associated with increased death rates for all cancers combined.

The link between obesity and cancer is still poorly understood. Several adipokines, growth factors, signaling pathways, inflammatory processes as well as the general demodulation of energy-balance and the lack of calorie restriction are being intensively discussed.

Adipokines are involved in cancer development: Traditionally, the adipose tissue was considered to be an energy storage organ. In recent years, however, it became evident that it also functions as an endocrine organ. Besides estrogen, it produces and secretes various adipokines and cytokines. Leptin and adiponectin, two well characterized adipokines, are associated with cancer development[44].

Figure 3 Endocrine, inflammatory, and cancer promoting effects of adipose tissue. IL-8: Interleukin 8; PGE$_2$: Prostaglandin E$_2$; TNF-α: Tumor necrosis factor-α; MCP-1: Monocyte chemoattractant protein-1; STAT3: Signal transducer and activator of transcription 3; PI3K: Phosphatidylinositol-4,5-bisphosphate 3-kinase; MAPK: Mitogen-activated proteinkinase; NF-κB: Nuclear-factor-kappa-light-chain-enhancer activated B cells; IGF-1: Insulin-growth factor-1; AMPK: 5'AMP-activated protein kinase; mTOR: Mechanistic target of rapamycin.

Leptin concentration in serum correlates positively with the patients' adipose tissue reserves and their nutritional condition. Moreover, leptin has been identified to be a potential mediator of cancer development[45], which is able to activate various key players of different signaling cascades like phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), mitogen-activated proteinkinase (MAPK) and signal transducer and activator of transcription 3 (STAT3). More interestingly, leptin signaling promotes the progression of different cancers[46-48].

Adiponectin is mainly secreted by visceral fat cells and acts adversary to leptin. It is inversely associated with obesity, hyperinsulinemia, and inflammation and may have anti-cancer effects by decreasing insulin-growth factor-1 (IGF-1) and mechanistic target of rapamycin (mTOR) signaling by activation of 5'AMP-activated protein kinase (AMPK). Also anti-inflammatory actions of adiponectin are described through inhibition of nuclear-factor-kappa-light-chain-enhancer activated B cells signaling (NF-κB)[49].

Increased carcinogenesis in obese patients might be due to chronic inflammation

Recent studies suggest a causal link of obesity related diseases (Figure 3) and low-grade/chronic inflammation (Figure 3)[50-52].

In humans, the immune system is of major relevance, which in turn, is able to form a defence shield against bacteria, viruses, or injured cells. A hallmark of the immune system is its most powerful weapon, the "inflammatory response" which was already noticed by a German pathologist called Rudolf Virchow in 1863. Despite the fact that humans without a functional immune system are not able to survive, too much inflammation can have a great impact and may cause serious damage to the healthy individual. Well-known chronic inflammatory diseases occur in patients that suffer from psoriasis or rheumatoid arthritis. A possible link between infections and cancer already exists, since stomach cancer may result from *Helicobacter pylori* infections or liver cancer from hepatitis (B-, C-) virus infections. A unique feature of these infections is the chronic inflammation response, which is primarily mediated by specific immune cells, such as macrophages and granulocytes that infiltrate the tumor. The latter is known to be recruited by tumor-released attractants. Once leucocytes infiltrate the tumor, they start to secrete chemokines and thereby initiate blood vessel growth/angiogenesis to allocate oxygen and nutrients,

which are relevant for tumor growth.

Circulating immune cell recruitment is a crucial feature of immune response

Macrophages: In obese individuals, macrophages infiltrate and expand in adipose tissue. Quantitative and functional changes of these cells affect adipose tissue inflammation. Exposure of macrophages to cytokines promotes two different activation states inducing to divergent polarizations. M1 macrophages are activated by tumor necrosis factor-α (TNF-α), Interferon-γ (IFN-γ), and bacterial endotoxins such as lipopolysaccharides. They are characterized by high levels of interleukin (IL)-12 and IL-23, and low levels of IL-10 as well as inflammatory cytokines[53]. Contrarily, M2 macrophages are attracted by IL-4, IL-13, IL-10, and glucocorticoid hormones. Both types are part of innate immune response. M1 macrophages may induce chronic inflammation, whereas M2 macrophages tend to act anti-inflammatory[54]. It has been suggested, that a phenotypic switch from M2 to M1 occurs in fat tissue[55], however, this model is discussed controversially.

Eosinophiles: Eosinophiles levels are negatively correlated with obesity and adipose tissue in mice. Wu *et al*[54] could show, that eosinophiles promote an M2-polarization of macrophages by secreting IL-4 and IL-13 and a down regulation of M1 macrophages in adipose tissue.

Mast cells: Mast cell levels in adipose tissue are elevated in obese animals[56]. Mast cell ablation reduces body fat and benefits glucose homeostasis in mice. This effect is induced by IL-6 and IFN-γ. Also, pro-angiogenic factors such as Cathepsins may influence mast cell levels[56].

Myeloid-derived suppressor cells: In adipose tissue, Myeloid-derived suppressor cells (MDSCs) have an inhibitory effect on inflammation by suppressing CD8^{+}-T cells and promoting M1 to M2 macrophage switch in favour for M2 macrophages[57]. The state of chronic inflammation in adipose tissue leads to an accumulation of MDSCs[58]. Being part of immune autoregulation by MDSCs suppress overt inflammatory immune response in chronic inflammation[59].

CD4^{+}-T cells: CD4^{+}-T cell activation is mediated by class Ⅱ major histocompability complex (MHC Ⅱ) molecules presented by macrophages and dendritic cells. When activated, CD4^{+}-T cells secrete cytokines, which attract pro-inflammatory cells. Three groups of T cells can be distinguished, namely T_H1, T_H2, and T_H17.

The ratio of T_H1/T_H2 cells is significantly enhanced in high fat diet induced obesity, since T_H2 cells are undermined by IFN-γ producing T_H1 cells[60]. CD4^{+}-T cell substitution in immunodeficient mice eventuates in reduction of weight gain, adipocyte cell size, and improvement of glucose homeostasis[60]. The STAT6 pathway is essential for T_H2 differentiation, thus STAT6 deficient CD4^{+}-T cells do not show any effect of reconstitution on glucose

homeostasis and body weight gain[60].

Regulatory T cells: CD4^{+}-T cells can transdifferentiate into immunosuppressive CD4^{+}CD25^{+}-regulatory T cells (T_{reg})[61]. Obesity is associated with reduced levels of T_{reg} cells in visceral adipose tissue in mice and humans[62,63]. T_{reg} cell depletion enhances circulating insulin levels and levels of pro-inflammatory cytokines in adipose tissue of lean mice[62]. Up regulation of T_{reg} on the other hand improves insulin sensitivity and enhances anti-inflammatory cytokine IL-10 levels[62]. Also, T_{reg} function to suppress pro-inflammatory immune response and promote macrophage M1 to M2 switch by secreting IL-4, IL-10, and IL-13[64].

CD8^{+}-T cells: CD8^{+}-T cell activation is mediated by MHC I . Activated CD8^{+}-T cells induce lysis of target cells by producing various cytokines and chemokines.

Adipose tissue of obese animals[65] and humans[60] show a significant increase of CD8^{+}-T cell levels. CD8^{+}-T cells lead to elevation of macrophages in adipose tissue and promote polarization into M1 macrophages[63]. CD8^{+}-T cell deficient mice have fewer levels of macrophages in adipose tissue and less levels of TNF-α and IL-6[63].

Natural killer T cells: When activated by lipids, natural killer T (NKT) cells produce a significant amount of T_H1- and T_H2-responsive cytokines, such as IFN-γ and IL-4[66]. NKT cells can either promote or supress inflammatory response by promoting either T_H1 or T_H2 cell activation[67,68]. Interestingly, NKT cell levels are reduced in human omental adipose tissue[69]. The role of NKTs in obesity still remains unclear.

B cells: After high fat diet, accumulation of B cells can be detected in adipose tissue of mice. This accumulation is associated with high levels of pro-inflammatory immunoglobulin G2c[70]. B cells promote T cell modulation and macrophage polarization by producing pathogenic Ig-G antibodies. Ig-G, however, increases inflammatory response[70]. The specific role of B-cells and Ig-G in inflammatory response in obesity has yet to be further investigated.

Mediators of inflammatory response: Preadipocytes can transdifferentiate into macrophages[71]. Also they tend to enlarge due to oxygen diffusion resulting in hypoxia, inflammation and increased macrophage infiltration. Enlarged adipocytes produce a variety of inflammatory cytokines and show greater insulin resistance than normal sized ones. Levels of prostaglandin E2, TNF-α, IL-2, IL-8, IL-10, and monocyte chemoattractant protein-1 (MCP-1) are elevated in the microenvironment of enlarged adipocytes. The inflammatory environment attracts macrophages and induces production of additional pro-inflammatory mediators[71].

NF-κB is a central transcription factor that is activated upon bacterial and viral stimuli. It activates gene

expression associated with apoptosis, cell proliferation, inflammation, tumorigenesis, metastasis, and angiogenesis[72]. In addition, increased NF-κB expression and activation is associated with insulin resistance.

The frequent up-regulation of NF-κB in many cancers is already known[73]. The increased expres-sion and "uncontrolled" activation of NF-κB may induce cancerogenesis[74,75]. Interestingly, NF-κB gets activated upon leptin stimulation in preneoplastic and neoplastic human colonic epithelial cells *in vitro*[76,77].

Inflammasomes, by definition cytosolic multiprotein complexes, activate IL-1β and IL-18 during infection or tissue damage[78]. They can be sub-divided into different inflammasome sub-groups such as nucleotide-binding oligomerization domain-like receptors (NLR), NLR pyrin domain-containing 1 and 3 (NLRP1 and NLRP3), absent in melanoma 2, and caspase activation and recruitment domains domain containing 4 (NLRC4/IPAF)[78]. In-flammasomes secrete caspase 1, which cleaves cytokine preforms, such as IL-1β[79,80]. The activity of NLR is associated with autoimmune diseases, malignancies, in-flammation, infection, and metabolic disorders[59]. Inflam-masome components expression levels are elevated in ad-ipose tissue of obese mice[81-83]. Conversely, NLRP3 and IL-1β are decreased in low calorie dietary restriction[83]. It seems therefore, that NLRP3 integrates multiple signals, causing pathogenic inflammation in obese subjects[84]. Also NLPR6 has a critical role in gut homeostasis[85,86]. Mice with non-functional NLRP6 develop an altered commensal system, preventing normal glycaemic control on a high fat diet and promoting NASH[87].

In summary, there are at least two inflammasome types and substrates that can imbalance metabolism and inflammation in obesity[85].

Linking obesity to cancer-inflammation is a double-edged sword

The role of chronic inflammation as a precursor of tu-morigenesis can be observed in various cancers. A gas-tritis can give rise to gastric cancer, inflammatory bowel disease may promote colorectal cancer and patients suffering from a chronic pancreatitis may have a higher risk to develop pancreatic cancer[88]. The inflammatory effect of adipose fat tissue might therefore be a general precursor of cancerogenesis. Like adipose tissue, tumor microenvironment is composed of multiple cell types like fibroblasts, epithelial cells, mast cells, and cells of innate and adaptive immune system that favor a pro-inflamma-tory, pro-tumorigenic environment[89-91].

Contribution to the pro-inflammatory environment is the presence of macrophages that are attracted by MCP-1. Tumor tissue classically contains a high amount of M2 polarized macrophages[92]. Macrophages activated by obese states, infiltrate tumors and amplify the inflam-matory tumor environment through NF-κB dependent cytokine production and angiogenic factors[88]. Malignan-cies may be initiated or exacerbate by inflammation, and increased levels of inflammation may be a cause and/or a consequence of malignancy[88,93].

Steroid hormones

Production of steroid hormones in the adipose tis-sue are also relevant for various cancers: Steroid hor-mones such as progesterone, estrogen, androgens and adrenal steroids are associated with energy balance level and obesity associated development of several cancer types[94]. In women, the BMI correlates with the incidence of breast cancer, endometrium cancer and other cancer entities that are associated to sexual hormone levels. The relative contribution of adipose tissue steroid hormone production to the whole steroid metabolism is about 100% in postmenopausal women[44]. The risk of develop-ing breast cancer in post-menopausal women enhances with an increase of circulating levels of steroid hormones such as dehydroepiandrosterone, testosterone, estradiol and estrogen, and low levels of sex hormone binding globuline. There is evidence that estrogens are mitogenic, regulating the expression of insulin, and inducing DNA damage by free radicals, genetic instability and gene mu-tations in cells[95]. Increased estradiol levels can induce endometrial cell proliferation rates while inhibiting apop-tosis and activating the IGF-1 synthesis in endometrial tissue[5].

In men, testosterone has been the focus of most studies on sex hormones, obesity and metabolic compli-cations. Evidence indicates that most tissues, including adipose tissue, express steroid converting enzymes neces-sary for the local production of androgens and/or estro-gens[96]. Up to 40% of the active androgen production (dihydrotestosterone) is accounted for by tissue conver-sion of adrenal precursors[96]. In men, obesity has gener-ally been associated with reduction of testosterone levels in plasma and elevated estrogen concentrations[97-99]. It has also been reported, that men with visceral adiposity have decreased levels of testosterone[100,101]. A growing body of interest suggests, that obese men are more likely to be diagnosed with aggressive prostate cancer and high tumor volumes[102]. Furthermore, obese patients show a higher risk of cancer recurrence, as well as an increase in disease related deaths compared to lean patients[103,104].

Hyperinsulinemia and insulin growth factors

Increased insulin levels and insulin growth factor-1 sig-naling enhance cancer development[105]. Other observa-tional studies reported an increased mortality of obese cancer patients with T2DM due to hyperinsulinemia and elevated IGF-1 serum levels. In contrast, patients with lower insulin, IGF-1, and IGF-2 levels showed a lower risk to develop cancer[105-107].

Patients treated with insulin or drugs stimulating in-sulin secretion showed a significantly higher incidence of developing malignancies than those patients treated with anti-diabetic drugs like metformin. Therefore, metformin might be a potential anticancer agent[108].

Caloric restriction, which causes down-regulation of circulating insulin and IGF-1 levels is a potent suppressor in carcinogenesis[74]. Insulin and IGF-1 can trigger cell

growth and proliferation, while inhibiting cell survival *via* proteinkinase B (Akt)/PI3K/mTOR (Akt/PI3K/mTOR) pathway[73]. This signaling pathway is not only the most frequently mutated pathway in human cancers, it is also a signal mediator of leptin, adiponectin and pro-inflammatory cytokines[46,109,110].

Caloric restriction reduces cancer incidence by inhibiting the Akt/PI3K/mTOR pathway *via* AMPK activation[111-113]. In contrast, Kalaany *et al*[110] could show that tumors with PI3K activation do not respond to the anti-cancerous effects of caloric restriction.

Interestingly, mTOR activity is increased in obese patients. It plays a central role in obesity related inflammation. Multiple risk factors for cancer development in obesity have been identified, such as the insulin-IGF-1 axis, leptin/adiponectin, and pro-inflammatory cytokines like IL-6, IL-7 and TNF-α. These factors can activate multiple pathways including PI3K/Akt, MAPK and STAT3, resulting in increased mTOR activity. mTOR, however, inhibits the insulin-PI3K pathway by stimulating the STAT3 pathway[46].

IL-6 and TNF-α play a major role in obesity associated hepatocellular carcinoma by activating the STAT3 pathway[114]. The STAT3 pathway is involved in the regulation of various gene expressions including IL-17, IL-23, B-cell lymphoma 2, and vascular epithelial growth factor to promote cell survival, proliferation, invasion, angiogenesis, and metastasis[115]. Consistent activation of STAT3 increases tumor cell proliferation, survival and invasion in suppressing anti-tumor immunity. STAT3 activation also leads to activation of further pro-oncogenic pathways, such as NF-κB and the IL-6/Janus kinase pathways[115].

FUTURE OUTLOOKS

As time passes, advancement of technologies proceeds

In 2003, the Human Genome Project was accomplished. After 13 years and estimated costs of 2.7 billion USD, the first human genome was sequenced. In contrast, the human genome of an individual was sequenced over a 5 mo period of time at costs of 1.5 million United States-Dollars in 2008[116].

An overall trend in the public health sector is the tendency towards "individualized therapy" in order to tailor specific therapy options that are currently available for a given patient which is further supported by usage of sophisticated mouse models.

Without doubt, mouse models have helped to understand relevant pathways that are important in the regulation of human body fat on the molecular level[117-123]. Initial insights into molecules that are important in regulating body fat, resulted primarily from genetic mouse screenings[117,124-126]. The identification of specific inactivating gene mutations accompanied by an obese phenotype, have revealed that leptin, leptin receptor and melanocortin-4 receptor play central roles in the regulation of body fat[127-132]. Interestingly, these three obesity phenotypes as a result of inactivating mutations, are also

relevant in humans, suggesting that knockout mouse models are a powerful tool to gain new insights into obesity relevant human genes and proteins.

A clinical approach might further support the *in vivo* findings that resulted from former obesity mouse models. Extensive tissue banking combined with collected clinical data may open up new perspectives in translational medicine as well.

There already exist several methods for screening large patient cohorts such as next generation sequencing. Also, established methods (*e.g.*, Fluorescence *in situ* hybridization or immunohistochemistry) became powerful tools when featured with high-throughput methods such as tissue microarrays to gain knowledge in the distribution of potential obesity relevant proteins. Tools such as laser mass spectrometry combined with a large tissue database in a microarray format might enable the initiation of virtual protein expression profiling of cells in their natural tissue environment. Further development in this field and others will open up new possibilities to identify causal links between gene expression levels, RNA modification, protein expression levels, post translational modification of proteins, intrinsic enzyme activity, and initiation and progression of diseases on a molecular level.

Automated chip technologies for detection of structural variation discoveries on a DNA- and RNA-level may decrease sequencing time, streamline sample preparations and reduce costs in future studies.

Acquiring great amounts of patient cohorts' data in large databases combined with blood and tissue sampling will move clinical applicability of new gained knowledge into focus. New potential risk factors and/or therapy targets will be identified by high throughput tissue and blood screenings. Especially the combination of organ-tissue samples with respective blood samples, body fluids, and visceral/subcutaneous fat samples will help to understand complex causal connections between obesity and organ function failure and carcinogenesis on a molecular basis. The novel knowledge will be centralized and digitally organized, accompanied by its' access that will be provided to health care units and hospitals for data reconciliation.

Preclinical and clinical patient screening will provide the basis for individualized digital patient DNA-, RNA-, protein-, post translational modification-, and enzyme activity profiles that automatically may be compared to already identified risk factors or therapy targets in centralized data bases.

In the present, there already exist research projects that might serve as landmarks for individualized obesity research in the future.

Interestingly, the TG and HDL Working Group were able to identify rare mutations that disrupt apolipoprotein C3 function by sequencing the protein-coding regions of 18666 genes in each of 3734 participants. By correlating loss of function studies with clinical data, carriers of these mutations were found to have a reduced risk of

coronary heart disease[133].

In the future, these mutations might serve as clinical risk-markers for coronary heart disease in obese patients. Blood samples of obese patients could be easily tested for gene mutations and the presence of a mutation might then be interpreted as a protective factor in favor of the patients' health.

Another study conducted an association analysis of single nucleotide polymorphisms, identifying genetic variants that predispose to T2DM[134]. Testing blood or tissue samples right after birth for these genetic variants might probably change the way of clinical diagnostics entirely.

In the future, patients with genetic predisposition for *e.g.*, diabetes might be diagnosed before the onset of disease. This knowledge could then lead to an individualized treatment in terms of dietary intake, physical exercise, or to earlier elective surgical intervention in obese patients.

Also, gut hormone and adipokine serum levels could be screened on regular basis in obese individuals. When out of balance, pharmaceutical intervention with suitable drugs such as GOAT- inhibitors, GIP-Receptor antagonists, inhibitors of the mTOR-, STAT3- and MAPK-, PI3K-pathways or even Metformin might be applicable in the future to prevent relevant comorbidities such as cancer.

Morbid obesity is already a widespread problem not only in first-, but also in second world countries. It causes various major chronic diseases such as coronary heart disease, diabetes, hypertension, and cancer. As living standards in second and third world countries enhance, morbid obesity will proceed to be a huge challenge for health institutions and national health systems. Obesity is a potential human health threat and is likely to become even more present in the future. The relevance and possible long-terms effects of maternal obesity to the health of the offspring are not fully understood. Studies that deal with this issue are of high relevance to precisely understand the long-term adverse health outcomes for the upcoming new generations.

In conclusion, there is an urgent need for obesity research with a straightforward concentration on new studies that aim to identify and interpret the complex, multifactorial variables in order to develop new therapy approaches and prevention programs for patients suffering from this disease.

REFERENCES

1   **Swinburn BA**, Sacks G, Hall KD, McPherson K, Finegood DT, Moodie ML, Gortmaker SL. The global obesity pandemic: shaped by global drivers and local environments. *Lancet* 2011; **378**: 804-814 [PMID: 21872749 DOI: 10.1016/S0140-6736(11)60813-1]

2   **Ng M**, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, Mullany EC, Biryukov S, Abbafati C, Abera SF, Abraham JP, Abu-Rmeileh NM, Achoki T, AlBuhairan FS, Alemu ZA, Alfonso R, Ali MK, Ali R, Guzman NA, Ammar W, Anwari P, Banerjee A, Barquera S, Basu S, Bennett DA, Bhutta Z, Blore J, Cabral N, Nonato IC, Chang JC, Chowdhury R, Courville KJ, Criqui MH, Cundiff DK, Dabhadkar KC, Dandona L, Davis A, Dayama A, Dharmaratne SD, Ding EL, Durrani AM, Esteghamati A, Farzadfar F, Fay DF, Feigin VL, Flaxman A, Forouzanfar MH, Goto A, Green MA, Gupta R, Hafezi-Nejad N, Hankey GJ, Harewood HC, Havmoeller R, Hay S, Hernandez L, Husseini A, Idrisov BT, Ikeda N, Islami F, Jahangir E, Jassal SK, Jee SH, Jeffreys M, Jonas JB, Kabagambe EK, Khalifa SE, Kengne AP, Khader YS, Khang YH, Kim D, Kimokoti RW, Kinge JM, Kokubo Y, Kosen S, Kwan G, Lai T, Leinsalu M, Li Y, Liang X, Liu S, Logroscino G, Lotufo PA, Lu Y, Ma J, Mainoo NK, Mensah GA, Merriman TR, Mokdad AH, Moschandreas J, Naghavi M, Naheed A, Nand D, Narayan KM, Nelson EL, Neuhouser ML, Nisar MI, Ohkubo T, Oti SO, Pedroza A, Prabhakaran D, Roy N, Sampson U, Seo H, Sepanlou SG, Shibuya K, Shiri R, Shiue I, Singh GM, Singh JA, Skirbekk V, Stapelberg NJ, Sturua L, Sykes BL, Tobias M, Tran BX, Trasande L, Toyoshima H, van de Vijver S, Vasankari TJ, Veerman JL, Velasquez-Melendez G, Vlassov VV, Vollset SE, Vos T, Wang C, Wang X, Weiderpass E, Werdecker A, Wright JL, Yang YC, Yatsuya H, Yoon J, Yoon SJ, Zhao Y, Zhou M, Zhu S, Lopez AD, Murray CJ, Gakidou E. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2014; **384**: 766-781 [PMID: 24880830 DOI: 10.1016/S0140-6736(14)60460-8]

3   **Cawley J**, Meyerhoefer C. The medical care costs of obesity: an instrumental variables approach. *J Health Econ* 2012; **31**: 219-230 [PMID: 22094013 DOI: 10.1016/j.jhealeco.2011.10.003]

4   **Wong RJ**, Cheung R, Ahmed A. Nonalcoholic steatohepatitis is the most rapidly growing indication for liver transplantation in patients with hepatocellular carcinoma in the U.S. *Hepatology* 2014; **59**: 2188-2195 [PMID: 24375711 DOI: 10.1002/hep.26986]

5   **Calle EE**, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. *Nat Rev Cancer* 2004; **4**: 579-591 [PMID: 15286738 DOI: 10.1038/nrc1408]

6   **Ditillo M**, Pandit V, Rhee P, Aziz H, Hadeed S, Bhattacharya B, Friese RS, Davis K, Joseph B. Morbid obesity predisposes trauma patients to worse outcomes: a National Trauma Data Bank analysis. *J Trauma Acute Care Surg* 2014; **76**: 176-179 [PMID: 24368375 DOI: 10.1097/TA.0b013e3182ab0d7c]

7   **Wilson MZ**, Dillon PW, Hollenbeak CS, Stewart DB. How do risk factors for mortality and overall complication rates following laparoscopic and open colectomy differ between inpatient and post-discharge phases of care? A retrospective cohort study from NSQIP. *Surg Endosc* 2014; **28**: 3392-3400 [PMID: 24928234 DOI: 10.1007/s00464-014-3609-4]

8   **Wigfield CH**, Lindsey JD, Muñoz A, Chopra PS, Edwards NM, Love RB. Is extreme obesity a risk factor for cardiac surgery? An analysis of patients with a BMI > or = 40. *Eur J Cardiothorac Surg* 2006; **29**: 434-440 [PMID: 16504529 DOI: 10.1016/j.ejcts.2006.01.016]

9   **Buerba RA**, Fu MC, Gruskay JA, Long WD, Grauer JN. Obese Class III patients at significantly greater risk of multiple complications after lumbar surgery: an analysis of 10,387 patients in the ACS NSQIP database. *Spine J* 2014; **14**: 2008-2018 [PMID: 24316118 DOI: 10.1016/j.spinee.2013.11.047]

10 **Adams KF**, Schatzkin A, Harris TB, Kipnis V, Mouw T, Ballard-Barbash R, Hollenbeck A, Leitzmann MF. Overweight, obesity, and mortality in a large prospective cohort of persons 50 to 71 years old. *N Engl J Med* 2006; **355**: 763-778 [PMID: 16926275 DOI: 10.1056/NEJMoa055643]

11 **Katzmarzyk PT**, Janssen I, Ardern CI. Physical inactivity, excess adiposity and premature mortality. *Obes Rev* 2003; **4**: 257-290 [PMID: 14649376 DOI: 10.1046/j.1467-789X.2003.00120.x]

12 **Tsai AG**, Wadden TA. Systematic review: an evaluation of major commercial weight loss programs in the United States. *Ann Intern Med* 2005; **142**: 56-66 [PMID: 15630109 DOI: 10.7326/0003-4819-142-1-200501040-00012]

13   **Anderson JW**, Konz EC, Frederich RC, Wood CL. Long-term weight-loss maintenance: a meta-analysis of US studies. *Am J Clin Nutr* 2001; **74**: 579-584 [PMID: 11684524]

14   **Jeffery RW**, Wing RR, Sherwood NE, Tate DF. Physical activity and weight loss: does prescribing higher physical activity goals improve outcome? *Am J Clin Nutr* 2003; **78**: 684-689 [PMID: 14522725]

15   **Carlsson LM**, Peltonen M, Ahlin S, Anveden Å, Bouchard C, Carlsson B, Jacobson P, Lönroth H, Maglio C, Näslund I, Pirazzi C, Romeo S, Sjöholm K, Sjöström E, Wedel H, Svensson PA, Sjöström L. Bariatric surgery and prevention of type 2 diabetes in Swedish obese subjects. *N Engl J Med* 2012; **367**: 695-704 [PMID: 22913680 DOI: 10.1056/NEJMoa1112082]

16   **Schauer PR**, Bhatt DL, Kirwan JP, Wolski K, Brethauer SA, Navaneethan SD, Aminian A, Pothier CE, Kim ES, Nissen SE, Kashyap SR. Bariatric surgery versus intensive medical therapy for diabetes--3-year outcomes. *N Engl J Med* 2014; **370**: 2002-2013 [PMID: 24679060 DOI: 10.1056/NEJMoa1401329]

17   **Buchwald H**, Oien DM. Metabolic/bariatric surgery worldwide 2011. *Obes Surg* 2013; **23**: 427-436 [PMID: 23338049 DOI: 10.1007/s11695-012-0864-0]

18   **Mason EE**, Ito C. Gastric bypass in obesity. *Surg Clin North Am* 1967; **47**: 1345-1351 [PMID: 6073761]

19   **Griffen WO**, Young VL, Stevenson CC. A prospective comparison of gastric and jejunoileal bypass procedures for morbid obesity. *Ann Surg* 1977; **186**: 500-509 [PMID: 907395]

20   **Baltasar A**, Serra C, Pérez N, Bou R, Bengochea M, Ferri L. Laparoscopic sleeve gastrectomy: a multi-purpose bariatric operation. *Obes Surg* 2005; **15**: 1124-1128 [PMID: 16197783 DOI: 10.1381/0960892055002248]

21   **Aasheim ET**, Björkman S, Søvik TT, Engström M, Hanvold SE, Mala T, Olbers T, Bøhmer T. Vitamin status after bariatric surgery: a randomized study of gastric bypass and duodenal switch. *Am J Clin Nutr* 2009; **90**: 15-22 [PMID: 19439456 DOI: 10.3945/ajcn.2009.27583]

22   **Nguyen NT**, Slone JA, Nguyen XM, Hartman JS, Hoyt DB. A prospective randomized trial of laparoscopic gastric bypass versus laparoscopic adjustable gastric banding for the treatment of morbid obesity: outcomes, quality of life, and costs. *Ann Surg* 2009; **250**: 631-641 [PMID: 19730234 DOI: 10.1097/SLA.0b013e3181b92480]

23   **Padwal RS**, Rueda-Clausen CF, Sharma AM, Agborsangaya CB, Klarenbach S, Birch DW, Karmali S, McCargar L, Majumdar SR. Weight loss and outcomes in wait-listed, medically managed, and surgically treated patients enrolled in a population-based Bariatric program: prospective cohort study. *Med Care* 2014; **52**: 208-215 [PMID: 24374423 DOI: 10.1097/MLR.0000000000000070]

24   **Chang SH**, Stoll CR, Song J, Varela JE, Eagon CJ, Colditz GA. The effectiveness and risks of bariatric surgery: an updated systematic review and meta-analysis, 2003-2012. *JAMA Surg* 2014; **149**: 275-287 [PMID: 24352617 DOI: 10.1001/jamasurg.2013.3654]

25   **Ribaric G**, Buchwald JN, McGlennon TW. Diabetes and weight in comparative studies of bariatric surgery vs conventional medical therapy: a systematic review and meta-analysis. *Obes Surg* 2014; **24**: 437-455 [PMID: 24374842 DOI: 10.1007/s11695-013-1160-3]

26   **Sjöström L**. Review of the key results from the Swedish Obese Subjects (SOS) trial - a prospective controlled intervention study of bariatric surgery. *J Intern Med* 2013; **273**: 219-234 [PMID: 23163728 DOI: 10.1111/joim.12012]

27   **le Roux CW**, Aylwin SJ, Batterham RL, Borg CM, Coyle F, Prasad V, Shurey S, Ghatei MA, Patel AG, Bloom SR. Gut hormone profiles following bariatric surgery favor an anorectic state, facilitate weight loss, and improve metabolic parameters. *Ann Surg* 2006; **243**: 108-114 [PMID: 16371744 DOI: 10.1097/01.sla.0000183349.16877.84]

28   **Beckman LM**, Beckman TR, Sibley SD, Thomas W, Ikramud-din S, Kellogg TA, Ghatei MA, Bloom SR, le Roux CW, Earthman CP. Changes in gastrointestinal hormones and leptin after Roux-en-Y gastric bypass surgery. *JPEN J Parenter Enteral Nutr* 2011; **35**: 169-180 [PMID: 21378246 DOI: 10.1177/0148607110381403]

29   **Pories WJ**, Swanson MS, MacDonald KG, Long SB, Morris PG, Brown BM, Barakat HA, deRamon RA, Israel G, Dolezal JM. Who would have thought it? An operation proves to be the most effective therapy for adult-onset diabetes mellitus. *Ann Surg* 1995; **222**: 339-350; discussion 350-352 [PMID: 7677463]

30   **Schirra J**, Nicolaus M, Roggel R, Katschinski M, Storr M, Woerle HJ, Göke B. Endogenous glucagon-like peptide 1 controls endocrine pancreatic secretion and antro-pyloro-duodenal motility in humans. *Gut* 2006; **55**: 243-251 [PMID: 15985560 DOI: 10.1136/gut.2004.059741]

31   **Punjabi M**, Arnold M, Geary N, Langhans W, Pacheco-López G. Peripheral glucagon-like peptide-1 (GLP-1) and satiation. *Physiol Behav* 2011; **105**: 71-76 [PMID: 21371486 DOI: 10.1016/j.physbeh.2011.02.038]

32   **Ng SY**, Wilding JP. Liraglutide in the treatment of obesity. *Expert Opin Biol Ther* 2014; **14**: 1215-1224 [PMID: 24905058 DOI: 10.1517/14712598.2014.925870]

33   **Malin SK**, Samat A, Wolski K, Abood B, Pothier CE, Bhatt DL, Nissen S, Brethauer SA, Schauer PR, Kirwan JP, Kashyap SR. Improved acylated ghrelin suppression at 2 years in obese patients with type 2 diabetes: effects of bariatric surgery vs standard medical therapy. *Int J Obes* (Lond) 2014; **38**: 364-370 [PMID: 24166065 DOI: 10.1038/ijo.2013.196]

34   **Samat A**, Malin SK, Huang H, Schauer PR, Kirwan JP, Kashyap SR. Ghrelin suppression is associated with weight loss and insulin action following gastric bypass surgery at 12 months in obese adults with type 2 diabetes. *Diabetes Obes Metab* 2013; **15**: 963-966 [PMID: 23679188 DOI: 10.1111/dom.12118]

35   **Schellekens H**, Dinan TG, Cryan JF. Lean mean fat reducing "ghrelin" machine: hypothalamic ghrelin and ghrelin receptors as therapeutic targets in obesity. *Neuropharmacology* 2010; **58**: 2-16 [PMID: 19573543 DOI: 10.1016/j.neuropharm.2009.06.024]

36   **Gualillo O**, Lago F, Dieguez C. Introducing GOAT: a target for obesity and anti-diabetic drugs? *Trends Pharmacol Sci* 2008; **29**: 398-401 [PMID: 18606462 DOI: 10.1016/j.tips.2008.06.003]

37   **Andrade S**, Pinho F, Ribeiro AM, Carreira M, Casanueva FF, Roy P, Monteiro MP. Immunization against active ghrelin using virus-like particles for obesity treatment. *Curr Pharm Des* 2013; **19**: 6551-6558 [PMID: 23859551 DOI: 10.2174/13816128113199990506]

38   **Dakin CL**, Small CJ, Batterham RL, Neary NM, Cohen MA, Patterson M, Ghatei MA, Bloom SR. Peripheral oxyntomodulin reduces food intake and body weight gain in rats. *Endocrinology* 2004; **145**: 2687-2695 [PMID: 15001546 DOI: 10.1210/en.2003-1338]

39   **Wynne K**, Park AJ, Small CJ, Meeran K, Ghatei MA, Frost GS, Bloom SR. Oxyntomodulin increases energy expenditure in addition to decreasing energy intake in overweight and obese humans: a randomised controlled trial. *Int J Obes* (Lond) 2006; **30**: 1729-1736 [PMID: 16619056 DOI: 10.1038/sj.ijo.0803344]

40   **Ashrafian H**, Ahmed K, Rowland SP, Patel VM, Gooderham NJ, Holmes E, Darzi A, Athanasiou T. Metabolic surgery and cancer: protective effects of bariatric procedures. *Cancer* 2011; **117**: 1788-1799 [PMID: 21509756 DOI: 10.1002/cncr.25738]

41   **Wiseman M**. The second World Cancer Research Fund/American Institute for Cancer Research expert report. Food, nutrition, physical activity, and the prevention of cancer: a global perspective. *Proc Nutr Soc* 2008; **67**: 253-256 [PMID: 18452640 DOI: 10.1017/S002966510800712X]

42   **Lichtman MA**. Obesity and the risk for a hematological malignancy: leukemia, lymphoma, or myeloma. *Oncologist* 2010; **15**: 1083-1101 [PMID: 20930095 DOI: 10.1634/theoncolo-

gist.2010-0206]

43   **Kaidar-Person O**, Bar-Sela G, Person B. The two major epidemics of the twenty-first century: obesity and cancer. *Obes Surg* 2011; **21**: 1792-1797 [PMID: 21842287 DOI: 10.1007/s11695-011-0490-2]

44   **Kershaw EE**, Flier JS. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab* 2004; **89**: 2548-2556 [PMID: 15181022 DOI: 10.1210/jc.2004-0395]

45   **Drew JE**. Molecular mechanisms linking adipokines to obesity-related colon cancer: focus on leptin. *Proc Nutr Soc* 2012; **71**: 175-180 [PMID: 22014041 DOI: 10.1017/S0029665111003259]

46   **Chen J**. Multiple signal pathways in obesity-associated cancer. *Obes Rev* 2011; **12**: 1063-1070 [PMID: 22093240 DOI: 10.1111/j.1467-789X.2011.00917.x]

47   **Gao J**, Tian J, Lv Y, Shi F, Kong F, Shi H, Zhao L. Leptin induces functional activation of cyclooxygenase-2 through JAK2/STAT3, MAPK/ERK, and PI3K/AKT pathways in human endometrial cancer cells. *Cancer Sci* 2009; **100**: 389-395 [PMID: 19154413 DOI: 10.1111/j.1349-7006.2008.01053.x]

48   **Jaffe T**, Schwartz B. Leptin promotes motility and invasiveness in human colon cancer cells by activating multiple signal-transduction pathways. *Int J Cancer* 2008; **123**: 2543-2556 [PMID: 18767036 DOI: 10.1002/ijc.23821]

49   **Dalamaga M**, Diakopoulos KN, Mantzoros CS. The role of adiponectin in cancer: a review of current evidence. *Endocr Rev* 2012; **33**: 547-594 [PMID: 22547160 DOI: 10.1210/er.2011-1015]

50   **Hotamisligil GS**, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest* 1995; **95**: 2409-2415 [PMID: 7738205 DOI: 10.1172/JCI117936]

51   **Hotamisligil GS**, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* 1993; **259**: 87-91 [PMID: 7678183]

52   **Uysal KT**, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. *Nature* 1997; **389**: 610-614 [PMID: 9335502 DOI: 10.1038/39335]

53   **Gordon S**, Martinez FO. Alternative activation of macrophages: mechanism and functions. *Immunity* 2010; **32**: 593-604 [PMID: 20510870 DOI: 10.1016/j.immuni.2010.05.007]

54   **Wu D**, Molofsky AB, Liang HE, Ricardo-Gonzalez RR, Jouihan HA, Bando JK, Chawla A, Locksley RM. Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. *Science* 2011; **332**: 243-247 [PMID: 21436399 DOI: 10.1126/science.1201475]

55   **Lumeng CN**, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest* 2007; **117**: 175-184 [PMID: 17200717 DOI: 10.1172/JCI29881]

56   **Liu J**, Divoux A, Sun J, Zhang J, Clément K, Glickman JN, Sukhova GK, Wolters PJ, Du J, Gorgun CZ, Doria A, Libby P, Blumberg RS, Kahn BB, Hotamisligil GS, Shi GP. Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice. *Nat Med* 2009; **15**: 940-945 [PMID: 19633655 DOI: 10.1038/nm.1994]

57   **Xia S**, Sha H, Yang L, Ji Y, Ostrand-Rosenberg S, Qi L. Gr-1+ CD11b+ myeloid-derived suppressor cells suppress inflammation and promote insulin sensitivity in obesity. *J Biol Chem* 2011; **286**: 23591-23599 [PMID: 21592961 DOI: 10.1074/jbc.M111.237123]

58   **Ostrand-Rosenberg S**, Sinha P. Myeloid-derived suppressor cells: linking inflammation and cancer. *J Immunol* 2009; **182**: 4499-4506 [PMID: 19342621 DOI: 10.4049/jimmunol.0802740]

59   **Sun S**, Ji Y, Kersten S, Qi L. Mechanisms of inflammatory responses in obese adipose tissue. *Annu Rev Nutr* 2012; **32**: 261-286 [PMID: 22404118 DOI: 10.1146/annurev-nutr-071811-150623]

60   **Winer S**, Chan Y, Paltser G, Truong D, Tsui H, Bahrami J, Dorfman R, Wang Y, Zielenski J, Mastronardi F, Maezawa Y,

Drucker DJ, Engleman E, Winer D, Dosch HM. Normalization of obesity-associated insulin resistance through immunotherapy. *Nat Med* 2009; **15**: 921-929 [PMID: 19633657 DOI: 10.1038/nm.2001]

61   **Long SA**, Buckner JH. CD4+FOXP3+ T regulatory cells in human autoimmunity: more than a numbers game. *J Immunol* 2011; **187**: 2061-2066 [PMID: 21856944 DOI: 10.4049/jimmunol.1003224]

62   **Feuerer M**, Herrero L, Cipolletta D, Naaz A, Wong J, Nayer A, Lee J, Goldfine AB, Benoist C, Shoelson S, Mathis D. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. *Nat Med* 2009; **15**: 930-939 [PMID: 19633656 DOI: 10.1038/nm.2002]

63   **Nishimura S**, Manabe I, Nagasaki M, Eto K, Yamashita H, Ohsugi M, Otsu M, Hara K, Ueki K, Sugiura S, Yoshimura K, Kadowaki T, Nagai R. CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. *Nat Med* 2009; **15**: 914-920 [PMID: 19633658 DOI: 10.1038/nm.1964]

64   **Tiemessen MM**, Jagger AL, Evans HG, van Herwijnen MJ, John S, Taams LS. CD4+CD25+Foxp3+ regulatory T cells induce alternative activation of human monocytes/macrophages. *Proc Natl Acad Sci U S A* 2007; **104**: 19446-19451 [PMID: 18042719 DOI: 10.1073/pnas.0706832104]

65   **Rausch ME**, Weisberg S, Vardhana P, Tortoriello DV. Obesity in C57BL/6J mice is characterized by adipose tissue hypoxia and cytotoxic T-cell infiltration. *Int J Obes* (Lond) 2008; **32**: 451-463 [PMID: 17895881 DOI: 10.1038/sj.ijo.0803744]

66   **Yoshimoto T**, Bendelac A, Watson C, Hu-Li J, Paul WE. Role of NK1.1+ T cells in a TH2 response and in immunoglobulin E production. *Science* 1995; **270**: 1845-1847 [PMID: 8525383]

67   **Bendelac A**, Savage PB, Teyton L. The biology of NKT cells. *Annu Rev Immunol* 2007; **25**: 297-336 [PMID: 17150027 DOI: 10.1146/annurev.immunol.25.022106.141711]

68   **Kronenberg M**. Toward an understanding of NKT cell biology: progress and paradoxes. *Annu Rev Immunol* 2005; **23**: 877-900 [PMID: 15771592 DOI: 10.1146/annurev.immunol.23.021704.115742]

69   **Lynch L**, O'Shea D, Winter DC, Geoghegan J, Doherty DG, O'Farrelly C. Invariant NKT cells and CD1d(+) cells amass in human omentum and are depleted in patients with cancer and obesity. *Eur J Immunol* 2009; **39**: 1893-1901 [PMID: 19585513 DOI: 10.1002/eji.200939349]

70   **Winer DA**, Winer S, Shen L, Wadia PP, Yantha J, Paltser G, Tsui H, Wu P, Davidson MG, Alonso MN, Leong HX, Glassford A, Caimol M, Kenkel JA, Tedder TF, McLaughlin T, Miklos DB, Dosch HM, Engleman EG. B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. *Nat Med* 2011; **17**: 610-617 [PMID: 21499269 DOI: 10.1038/nm.2353]

71   **Charrière G**, Cousin B, Arnaud E, André M, Bacou F, Penicaud L, Casteilla L. Preadipocyte conversion to macrophage. Evidence of plasticity. *J Biol Chem* 2003; **278**: 9850-9855 [PMID: 12519759 DOI: 10.1074/jbc.M210811200]

72   **Dolcet X**, Llobet D, Pallares J, Matias-Guiu X. NF-kB in development and progression of human cancer. *Virchows Arch* 2005; **446**: 475-482 [PMID: 15856292 DOI: 10.1007/s00428-005-1264-9]

73   **Renehan AG**, Roberts DL, Dive C. Obesity and cancer: pathophysiological and biological mechanisms. *Arch Physiol Biochem* 2008; **114**: 71-83 [PMID: 18465361 DOI: 10.1080/13813450801954303]

74   **Hursting SD**, Smith SM, Lashinger LM, Harvey AE, Perkins SN. Calories and carcinogenesis: lessons learned from 30 years of calorie restriction research. *Carcinogenesis* 2010; **31**: 83-89 [PMID: 19969554 DOI: 10.1093/carcin/bgp280]

75   **Harvey AE**, Lashinger LM, Hursting SD. The growing challenge of obesity and cancer: an inflammatory issue. *Ann N Y Acad Sci* 2011; **1229**: 45-52 [PMID: 21793838 DOI: 10.1111/j.1749-6632.2011.06096.x]

76   **Fenton JI**, Hursting SD, Perkins SN, Hord NG. Interleukin-6 production induced by leptin treatment promotes cell proliferation in an Apc (Min/+) colon epithelial cell line. *Carcinogenesis* 2006; **27**: 1507-1515 [PMID: 16597643 DOI: 10.1093/carcin/bgl018]

77   **Rouet-Benzineb P**, Aparicio T, Guilmeau S, Pouzet C, Descatoire V, Buyse M, Bado A. Leptin counteracts sodium butyrate-induced apoptosis in human colon cancer HT-29 cells via NF-kappaB signaling. *J Biol Chem* 2004; **279**: 16495-16502 [PMID: 14752104 DOI: 10.1074/jbc.M312999200]

78   **Schroder K**, Tschopp J. The inflammasomes. *Cell* 2010; **140**: 821-832 [PMID: 20303873 DOI: 10.1016/j.cell.2010.01.040]

79   **Tschopp J**, Schroder K. NLRP3 inflammasome activation: The convergence of multiple signalling pathways on ROS production? *Nat Rev Immunol* 2010; **10**: 210-215 [PMID: 20168318 DOI: 10.1038/nri2725]

80   **Davis BK**, Wen H, Ting JP. The inflammasome NLRs in immunity, inflammation, and associated diseases. *Annu Rev Immunol* 2011; **29**: 707-735 [PMID: 21219188 DOI: 10.1146/annurev-immunol-031210-101405]

81   **Stienstra R**, Joosten LA, Koenen T, van Tits B, van Diepen JA, van den Berg SA, Rensen PC, Voshol PJ, Fantuzzi G, Hijmans A, Kersten S, Müller M, van den Berg WB, van Rooijen N, Wabitsch M, Kullberg BJ, van der Meer JW, Kanneganti T, Tack CJ, Netea MG. The inflammasome-mediated caspase-1 activation controls adipocyte differentiation and insulin sensitivity. *Cell Metab* 2010; **12**: 593-605 [PMID: 21109192 DOI: 10.1016/j.cmet.2010.11.011]

82   **Stienstra R**, van Diepen JA, Tack CJ, Zaki MH, van de Veerdonk FL, Perera D, Neale GA, Hooiveld GJ, Hijmans A, Vroegrijk I, van den Berg S, Romijn J, Rensen PC, Joosten LA, Netea MG, Kanneganti TD. Inflammasome is a central player in the induction of obesity and insulin resistance. *Proc Natl Acad Sci USA* 2011; **108**: 15324-15329 [PMID: 21876127 DOI: 10.1073/pnas.1100255108]

83   **Vandanmagsar B**, Youm YH, Ravussin A, Galgani JE, Stadler K, Mynatt RL, Ravussin E, Stephens JM, Dixit VD. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. *Nat Med* 2011; **17**: 179-188 [PMID: 21217695 DOI: 10.1038/nm.2279]

84   **Masters SL**. Specific inflammasomes in complex diseases. *Clin Immunol* 2013; **147**: 223-228 [PMID: 23294928 DOI: 10.1016/j.clim.2012.12.006]

85   **Normand S**, Delanoye-Crespin A, Bressenot A, Huot L, Grandjean T, Peyrin-Biroulet L, Lemoine Y, Hot D, Chamaillard M. Nod-like receptor pyrin domain-containing protein 6 (NLRP6) controls epithelial self-renewal and colorectal carcinogenesis upon injury. *Proc Natl Acad Sci USA* 2011; **108**: 9601-9606 [PMID: 21593405 DOI: 10.1073/pnas.1100981108]

86   **Elinav E**, Strowig T, Kau AL, Henao-Mejia J, Thaiss CA, Booth CJ, Peaper DR, Bertin J, Eisenbarth SC, Gordon JI, Flavell RA. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. *Cell* 2011; **145**: 745-757 [PMID: 21565393 DOI: 10.1016/j.cell.2011.04.022]

87   **Henao-Mejia J**, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, Thaiss CA, Kau AL, Eisenbarth SC, Jurczak MJ, Camporez JP, Shulman GI, Gordon JI, Hoffman HM, Flavell RA. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature* 2012; **482**: 179-185 [PMID: 22297845 DOI: 10.1038/nature10809]

88   **Coussens LM**, Werb Z. Inflammation and cancer. *Nature* 2002; **420**: 860-867 [PMID: 12490959 DOI: 10.1038/nature01322]

89   **Ishigami S**, Natsugoe S, Tokuda K, Nakajo A, Che X, Iwashige H, Aridome K, Hokita S, Aikou T. Prognostic value of intratumoral natural killer cells in gastric carcinoma. *Cancer* 2000; **88**: 577-583 [PMID: 10649250]

90   **Ribatti D**, Ennas MG, Vacca A, Ferreli F, Nico B, Orru S, Sirigu P. Tumor vascularity and tryptase-positive mast cells correlate with a poor prognosis in melanoma. *Eur J*

Clin Invest 2003; **33**: 420-425 [PMID: 12760367 DOI: 10.1046/j.1365-2362.2003.01152.x]

91   **Leek RD**, Landers RJ, Harris AL, Lewis CE. Necrosis correlates with high vascular density and focal macrophage infiltration in invasive carcinoma of the breast. *Br J Cancer* 1999; **79**: 991-995 [PMID: 10070902 DOI: 10.1038/sj.bjc.6690158]

92   **Allavena P**, Sica A, Garlanda C, Mantovani A. The Yin-Yang of tumor-associated macrophages in neoplastic progression and immune surveillance. *Immunol Rev* 2008; **222**: 155-161 [PMID: 18364000 DOI: 10.1111/j.1600-065X.2008.00607.x]

93   **Del Prete A**, Allavena P, Santoro G, Fumarulo R, Corsi MM, Mantovani A. Molecular pathways in cancer-related inflammation. *Biochem Med* (Zagreb) 2011; **21**: 264-275 [PMID: 22420240]

94   **Hursting SD**, Lashinger LM, Wheatley KW, Rogers CJ, Colbert LH, Nunez NP, Perkins SN. Reducing the weight of cancer: mechanistic targets for breaking the obesity-carcinogenesis link. *Best Pract Res Clin Endocrinol Metab* 2008; **22**: 659-669 [PMID: 18971125 DOI: 10.1016/j.beem.2008.08.009]

95   **De Pergola G**, Silvestris F. Obesity as a major risk factor for cancer. *J Obes* 2013; **2013**: 291546 [PMID: 24073332 DOI: 10.1155/2013/291546]

96   **Labrie F**, Bélanger A, Cusan L, Gomez JL, Candas B. Marked decline in serum concentrations of adrenal C19 sex steroid precursors and conjugated androgen metabolites during aging. *J Clin Endocrinol Metab* 1997; **82**: 2396-2402 [PMID: 9253307 DOI: 10.1210/jcem.82.8.4160]

97   **Strain GW**, Zumoff B, Kream J, Strain JJ, Deucher R, Rosenfeld RS, Levin J, Fukushima DK. Mild Hypogonadotropic hypogonadism in obese men. *Metabolism* 1982; **31**: 871-875 [PMID: 6811834 DOI: 10.1016/0026-0495(82)90175-5]

98   **Glass AR**, Swerdloff RS, Bray GA, Dahms WT, Atkinson RL. Low serum testosterone and sex-hormone-binding-globulin in massively obese men. *J Clin Endocrinol Metab* 1977; **45**: 1211-1219 [PMID: 338622 DOI: 10.1210/jcem-45-6-1211]

99   **Tchernof A**, Després JP, Bélanger A, Dupont A, Prud'homme D, Moorjani S, Lupien PJ, Labrie F. Reduced testosterone and adrenal C19 steroid levels in obese men. *Metabolism* 1995; **44**: 513-519 [PMID: 7723675 DOI: 10.1016/0026-0495(95)90060-8]

100   **Seidell JC**, Björntorp P, Sjöström L, Kvist H, Sannerstedt R. Visceral fat accumulation in men is positively associated with insulin, glucose, and C-peptide levels, but negatively with testosterone levels. *Metabolism* 1990; **39**: 897-901 [PMID: 2202881 DOI: 10.1016/0026-0495(90)90297-P]

101   **Pasquali R**, Casimirri F, Cantobelli S, Melchionda N, Morselli Labate AM, Fabbri R, Capelli M, Bortoluzzi L. Effect of obesity and body fat distribution on sex hormones and insulin in men. *Metabolism* 1991; **40**: 101-104 [PMID: 1984562]

102   **Jentzmik F**, Schnoeller TJ, Cronauer MV, Steinestel J, Steffens S, Zengerling F, Al Ghazal A, Schrader MG, Steinestel K, Schrader AJ. Corpulence is the crucial factor: association of testosterone and/or obesity with prostate cancer stage. *Int J Urol* 2014; **21**: 980-986 [PMID: 24865433 DOI: 10.1111/iju.12494]

103   **Cao Y**, Ma J. Body mass index, prostate cancer-specific mortality, and biochemical recurrence: a systematic review and meta-analysis. *Cancer Prev Res* (Phila) 2011; **4**: 486-501 [PMID: 21233290 DOI: 10.1158/1940-6207.CAPR-10-0229]

104   **Gong Z**, Agalliu I, Lin DW, Stanford JL, Kristal AR. Obesity is associated with increased risks of prostate cancer metastasis and death after initial cancer diagnosis in middle-aged men. *Cancer* 2007; **109**: 1192-1202 [PMID: 17311344 DOI: 10.1002/cncr.22534]

105   **Gallagher EJ**, LeRoith D. Minireview: IGF, Insulin, and Cancer. *Endocrinology* 2011; **152**: 2546-2551 [PMID: 21540285 DOI: 10.1210/en.2011-0231]

106   **Calle EE**, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of US adults. *N Engl J Med* 2003; **348**: 1625-1638 [PMID: 12711737 DOI: 10.1056/NEJMoa021423]

107 **Coughlin SS**, Calle EE, Teras LR, Petrelli J, Thun MJ. Diabetes mellitus as a predictor of cancer mortality in a large cohort of US adults. *Am J Epidemiol* 2004; **159**: 1160-1167 [PMID: 15191933 DOI: 10.1093/aje/kwh161]

108 **Dowling RJ**, Niraula S, Stambolic V, Goodwin PJ. Metformin in cancer: translational challenges. *J Mol Endocrinol* 2012; **48**: R31-R43 [PMID: 22355097 DOI: 10.1530/JME-12-0007]

109 **Liu P**, Cheng H, Roberts TM, Zhao JJ. Targeting the phosphoinositide 3-kinase pathway in cancer. *Nat Rev Drug Discov* 2009; **8**: 627-644 [PMID: 19644473 DOI: 10.1038/nrd2926]

110 **Kalaany NY**, Sabatini DM. Tumours with PI3K activation are resistant to dietary restriction. *Nature* 2009; **458**: 725-731 [PMID: 19279572 DOI: 10.1038/nature07782]

111 **Moore T**, Beltran L, Carbajal S, Strom S, Traag J, Hursting SD, DiGiovanni J. Dietary energy balance modulates signaling through the Akt/mammalian target of rapamycin pathways in multiple epithelial tissues. *Cancer Prev Res* (Phila) 2008; **1**: 65-76 [PMID: 19138937 DOI: 10.1158/1940-6207. CAPR-08-0022]

112 **Dann SG**, Selvaraj A, Thomas G. mTOR Complex1-S6K1 signaling: at the crossroads of obesity, diabetes and cancer. *Trends Mol Med* 2007; **13**: 252-259 [PMID: 17452018 DOI: 10.1016/j.molmed.2007.04.002]

113 **Jiang W**, Zhu Z, Thompson HJ. Dietary energy restriction modulates the activity of AMP-activated protein kinase, Akt, and mammalian target of rapamycin in mammary carcinomas, mammary gland, and liver. *Cancer Res* 2008; **68**: 5492-5499 [PMID: 18593953 DOI: 10.1158/0008-5472. CAN-07-6721]

114 **Park EJ**, Lee JH, Yu GY, He G, Ali SR, Holzer RG, Ostreicher CH, Takahashi H, Karin M. Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. *Cell* 2010; **140**: 197-208 [PMID: 20141834 DOI: 10.1016/j.cell.2009.12.052]

115 **Yu H**, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. *Nat Rev Cancer* 2009; **9**: 798-809 [PMID: 19851315 DOI: 10.1038/nrc2734]

116 **Wheeler DA**, Srinivasan M, Egholm M, Shen Y, Chen L, McGuire A, He W, Chen YJ, Makhijani V, Roth GT, Gomes X, Tartaro K, Niazi F, Turcotte CL, Irzyk GP, Lupski JR, Chinault C, Song XZ, Liu Y, Yuan Y, Nazareth L, Qin X, Muzny DM, Margulies M, Weinstock GM, Gibbs RA, Rothberg JM. The complete genome of an individual by massively parallel DNA sequencing. *Nature* 2008; **452**: 872-876 [DOI: 10.1038/nature06884]

117 **Bultman SJ**, Michaud EJ, Woychik RP. Molecular characterization of the mouse agouti locus. *Cell* 1992; **71**: 1195-1204 [PMID: 1473152 DOI: 10.1016/S0092-8674(05)80067-4]

118 **Allan MF**, Eisen EJ, Pomp D. The M16 mouse: an outbred animal model of early onset polygenic obesity and diabesity. *Obes Res* 2004; **12**: 1397-1407 [PMID: 15483204 DOI: 10.1038/oby.2004.176]

119 **Hummel KP**, Dickie MM, Coleman DL. Diabetes, a new mutation in the mouse. *Science* 1966; **153**: 1127-1128 [PMID: 5918576]

120 **Ingalls AM**, Dickie MM, Snell GD. Obese, a new mutation in the house mouse. *J Hered* 1950; **41**: 317-318 [PMID: 14824537]

121 **Jürgens HS**, Schürmann A, Kluge R, Ortmann S, Klaus S, Joost HG, Tschöp MH. Hyperphagia, lower body temperature, and reduced running wheel activity precede development of morbid obesity in New Zealand obese mice. *Physiol Genomics* 2006; **25**: 234-241 [PMID: 16614459 DOI: 10.1152/physiolgenomics.00252.2005]

122 **Nakamura M**, Yamada K. Studies on a diabetic (KK) strain of the mouse. *Diabetologia* 1967; **3**: 212-221 [PMID: 4907141]

123 **Suzuki W**, Iizuka S, Tabuchi M, Funo S, Yanagisawa T, Kimura M, Sato T, Endo T, Kawamura H. A new mouse model of spontaneous diabetes derived from ddY strain. *Exp Anim* 1999; **48**: 181-189 [PMID: 10480023]

124 **Zhang Y**, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994; **372**: 425-432 [PMID: 7984236 DOI: 10.1038/372425a0]

125 **Duhl DM**, Stevens ME, Vrieling H, Saxon PJ, Miller MW, Epstein CJ, Barsh GS. Pleiotropic effects of the mouse lethal yellow (Ay) mutation explained by deletion of a maternally expressed gene and the simultaneous production of agouti fusion RNAs. *Development* 1994; **120**: 1695-1708 [PMID: 8050375]

126 **Michaud EJ**, Bultman SJ, Klebig ML, van Vugt MJ, Stubbs LJ, Russell LB, Woychik RP. A molecular model for the genetic and phenotypic characteristics of the mouse lethal yellow (Ay) mutation. *Proc Natl Acad Sci USA* 1994; **91**: 2562-2566 [PMID: 8146154]

127 **Torres-Andrade R**, Moldenhauer R, Gutierrez-Bertín N, Soto-Covasich J, Mancilla-Medina C, Ehrenfeld C, Kerr B. The increase in body weight induced by lack of methyl CpG binding protein-2 is associated with altered leptin signalling in the hypothalamus. *Exp Physiol* 2014; **99**: 1229-1240 [PMID: 24996410 DOI: 10.1113/expphysiol.2014.079798]

128 **Halaas JL**, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM. Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 1995; **269**: 543-546 [PMID: 7624777]

129 **Clément K**, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, Gourmelen M, Dina C, Chambaz J, Lacorte JM, Basdevant A, Bougnères P, Lebouc Y, Froguel P, Guy-Grand B. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* 1998; **392**: 398-401 [PMID: 9537324 DOI: 10.1038/32911]

130 **van Swieten MM**, Pandit R, Adan RA, van der Plasse G. The neuroanatomical function of leptin in the hypothalamus. *J Chem Neuroanat* 2014; Epub ahead of print [PMID: 25007719 DOI: 10.1016/j.jchemneu.2014.05.004]

131 **Huszar D**, Lynch CA, Fairchild-Huntress V, Dunmore JH, Fang Q, Berkemeier LR, Gu W, Kesterson RA, Boston BA, Cone RD, Smith FJ, Campfield LA, Burn P, Lee F. Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell* 1997; **88**: 131-141 [PMID: 9019399 DOI: 10.1016/S0092-8674(00)81865-6]

132 **Woods SC**, Seeley RJ, Porte D, Schwartz MW. Signals that regulate food intake and energy homeostasis. *Science* 1998; **280**: 1378-1383 [PMID: 9603721 DOI: 10.1126/science.280.5368.1378]

133 **Crosby J**, Peloso GM, Auer PL, Crosslin DR, Stitziel NO, Lange LA, Lu Y, Tang ZZ, Zhang H, Hindy G, Masca N, Stirrups K, Kanoni S, Do R, Jun G, Hu Y, Kang HM, Xue C, Goel A, Farrall M, Duga S, Merlini PA, Asselta R, Girelli D, Olivieri O, Martinelli N, Yin W, Reilly D, Speliotes E, Fox CS, Hveem K, Holmen OL, Nikpay M, Farlow DN, Assimes TL, Franceschini N, Robinson J, North KE, Martin LW, DePristo M, Gupta N, Escher SA, Jansson JH, Van Zuydam N, Palmer CN, Wareham N, Koch W, Meitinger T, Peters A, Lieb W, Erbel R, Konig IR, Kruppa J, Degenhardt F, Gottesman O, Bottinger EP, O'Donnell CJ, Psaty BM, Ballantyne CM, Abecasis G, Ordovas JM, Melander O, Watkins H, Orho-Melander M, Ardissino D, Loos RJ, McPherson R, Willer CJ, Erdmann J, Hall AS, Samani NJ, Deloukas P, Schunkert H, Wilson JG, Kooperberg C, Rich SS, Tracy RP, Lin DY, Altshuler D, Gabriel S, Nickerson DA, Jarvik GP, Cupples LA, Reiner AP, Boerwinkle E, Kathiresan S. Loss-of-function mutations in APOC3, triglycerides, and coronary disease. *N Engl J Med* 2014; **371**: 22-31 [PMID: 24941081 DOI: 10.1056/NEJMoa1307095]

134 **Scott LJ**, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, Erdos MR, Stringham HM, Chines PS, Jackson AU, Prokunina-Olsson L, Ding CJ, Swift AJ, Narisu N, Hu T, Pruim R, Xiao R, Li XY, Conneely KN, Riebow NL, Sprau AG, Tong M, White PP, Hetrick KN, Barnhart MW, Bark CW, Goldstein JL, Watkins L, Xiang F, Saramies J, Buchanan

TA, Watanabe RM, Valle TT, Kinnunen L, Abecasis GR, Pugh EW, Doheny KF, Bergman RN, Tuomilehto J, Collins FS, Boehnke M. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* 2007; **316**: 1341-1345 [PMID: 17463248 DOI: 10.1126/science.1142382]

135 **Kim GW**, Lin JE, Blomain ES, Waldman SA. Antiobesity pharmacotherapy: new drugs and emerging targets. *Clin Pharmacol Ther* 2014; **95**: 53-66 [PMID: 24105257 DOI: 10.1038/clpt.2013.204]

REVIEW

Pathophysiological responses from human gut microbiome

Anindya Roy Chowdhury, Utpal Bakshi

Anindya Roy Chowdhury, Utpal Bakshi, Structural Biology and Bioinformatics Division, CSIR-Indian Institute of Chemical Biology, Kolkata 700032, India
Author contributions: Roy Chowdhury A designed the study, wrote the manuscript; Bakshi U contributed to the writing of the manuscript.
Supported by University Grants Commission (India) (Bakshi U)
Correspondence to: Dr. Anindya Roy Chowdhury, Structural Biology and Bioinformatics Division, CSIR-Indian Institute of Chemical Biology, 4, Raja S. C. Mullick Road, Kolkata 700032, India. ani.rc.29@gmail.com
Telephone: +91-33-24995875 Fax: +91-33-24735197
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Abstract

The human gastrointestinal tract harbors a vast collection of symbiotic microorganisms-collectively termed as "gut microbiome". This microbiota has important effect in immune system and other host activities. Recent studies have suggested that alterations of the normal gut microbiota are associated with various human diseases and psychological disorders. The underlying cause, once proven, may provide novel insights into the importance of gut flora in human health. In this review, we give an attempt to describe how the alteration in the microbial community causes the development of certain widespread pathophysiological disorders; focusing on inflammatory bowel disease, colorectal cancer, obesity and autism. Proper knowledge about the host-microbiota interaction and linkage could be essential for the development of future personalized strategies of therapeutics.

Key words: Human gut microbiome; Inflammatory bowel disease; Colorectal cancer; Obesity; Autism

Core tip: This review is an endeavor to provide an ac-

count about the human gut microbiome, their diversity, and disease causing capability. Till date, so many diseases have been associated with the alteration of gut microbiota. In this review we talk about four of the major diseases/disorders, *viz.*, inflammatory bowel disease, colorectal cancer, obesity and autism.

INTRODUCTION

The topic of human microbiome is pretty trendy in the present world of science. Human body is inhabited by more microbial cells than our own cell numbers. The term "microbiome" refers to the whole number of microorganisms residing in human body and their genetic material[1-3]. It is different from the term "microbiota", which describes the microbial population present in different niches in the body. Resident microbes contain ten times more cells than our own somatic and germ cells and hence more number of genes than present in a human body-as a consequence they represent a combined microbial genome with a size bigger than human genome itself[1,4]. Collectively, the flora has a metabolic action equal to a virtual organ within an organ[5]. Researchers at human microbiome project, NIH are sampling and exploring data from few specific sites of human body, *viz.*, airways, nasal passages, oral cavities, skin, blood, gastrointestinal tract, and urogenital tract[2]. The microbial density starts increasing in the distal small intestine, and in the large intestine it rises to an estimated of 10^{11}-10^{12} microbes per gram of colonic content, which contributes to 60% of the fecal mass. However, this is to bear in mind, since so many factors affect our body's ecosystem, the microbiota composition is different for every individual regardless of their age and sex.

Usually this microbiota is commensal and represents a healthy asset of our body, helping us to digest food and maintain immunity. Our typical understanding about a disease causing event states us that whenever a pathogenic organism enters our body, the disease takes shape. Introduction to the era of human microbiome enlightens us about a more susceptible way of causing disease-the imbalance of the microbiota within our body. Therefore human microbiome can be considered as a therapeutic drug target[6].

The organisms from this microbiome are hard to culture. Metagenomics, the study of the genetic material extracted directly from environmental samples in a given environment, has been applied to the studies of the human microbiome, since it can be used to investigate various microbes simultaneously, without cultivation. This approach gathers speed in studies of human microbiome and their medical relevance. Studies about diverse microbes from the human body site-specific microbiota, and the correlations between their composition and disease have rapidly increased our understanding towards the importance of the human microbiome and its roles in health and disease[3,7]. This bang of human microbiome data holds the promise of managing personal health, based on the genome and microbiome information of an individual.

HUMAN GUT MICROBIOME

A new chapter in medical science has emerged with the recognition of the crucial role of the gut microbiota in health and disease. At the time of birth human gut is completely sterile. However, immediately after birth the colonization of mammoth variety of microorganisms including bacteria, archaea, fungi and viruses starts within the body. The colonization of these microbial species within a body depends upon the mode of delivery, hygene level, infant diet, and medication[8].

Among all the niches, human GI tract contains the most number of microorganisms. The density of the microbiota increases from the proximal to the distal gut, reaching its maximum at the colon. In the different habitats of the gut, ecological sorting and competitive exclusion between microbes are the key factors influencing microbial diversity[9,10]. Stochastic factors during colonization and in situ evolution cause the diversity of gut microbiota between individuals[11]. The intestinal microbiota of infants lacks diversity and the major constituents are the phyla *Proteobacteria* and *Actinobacteria*. The microbiota attains diversity with age with the addition of *Fusobacteria*, *Cyanobacteria*, *Verrucomicrobia* and *Actinobacteria* amongst others and the dominance of *Firmicutes* and *Bacteroidetes* characterizes the adult microbiota[12-14]. The gut microbiota is mainly a collection of anaerobes, which outnumber facultative anaerobes and aerobic microbes by approximately 2-3 orders of magnitude[15]. In human, after the age of 2.5 years the gut microbiota remains almost the same throughout the adult age of that individual[16,17]. The

actual adult human gut microbiota composition is diverse and differs from person to person in a significant way. Therefore it has been suggested that it can be used as a substitute to fingerprinting[18]. In this regard three enterotypes have been found, *viz., Prevotella*, *Ruminococcus* and *Bacteroids* that are independent of age or sex. The normal human gut flora composition is subject to age, diet, medication and socioeconomic conditions. In a recent study of gut microbiota in elderly individuals, the associations with diet and age was documented[19].

It is a prominent fact that, although there is great variety in the composition of the gut microbiota among individuals, there still lays a conserved set shared between individuals, and this set of microbiota is called the core gut microbiome[20]. The functions and pathways encoded by the core gut microbiome offer the greatest benefit to the host and are essential for the correct functioning of the healthy gut. The gut microbiota helps the host in various ways, including protection against probable pathogens, production of essential vitamins, digestion of polysaccharides, regulation of fat storage and modulation of the host's immune system[21]. Latest studies have also revealed that the gut microbiota influences brain and the gut-brain axis configures the stress related symptoms such as anxiety and pain tolerance and few other psychological condition[22].

ROLE OF GUT MICROBIOTA IN HUMAN DISEASE/DISORDER

It has been well established that the human gut microbiota is essential for human health. However, an alteration of the normal composition of the gut microbiome leads to formation of various types of diseases. Therefore it is reasonable to conclude that modulation of the gut microbiota can be used as a therapeutic target in treating these chronic diseases. Before properly utilizing the gut microbiota as a therapeutic tool, it is necessary to understand the role of these microbes in shaping disease. Till date, a great number of physical and psychological disorders have been associated with the alteration of gut flora; addressing all can be quite unfeasible task for this review. Thus, in this review, brief overviews of the current understanding about the role of microbiota in four common disease and disorders have been discussed.

INFLAMMATORY BOWEL DISEASE

Inflammatory bowel disease (IBD) is chronic, relapsing, immunologically mediated disorder that affects the digestive tract, mainly colon and small intestine. IBD majorly includes ulcerative colitis (UC) and Crohn's disease (CD). There is considerable evidence suggesting the importance of gut microbiota in IBD[23-25]. Recent studies imply that an unbalanced microbial community composition is associated with a dysregulated immune response[26]. Although the exact mechanism of IBD is not yet being

fully elucidated, four broad mechanisms are proposed to explain the complex relationship between the commensal microbiota and IBD: (1) dysbiosis of conventional microbiota; (2) induction of intestinal inflammation by pathogens and functionally altered commensal bacteria; (3) host genetic defects in containing commensal microbiota; and (4) defective host immunoregulation[27]. The net result of these effects is continuous antigenic stimulation that activates pathogenic T cells, ultimately causing chronic intestinal inflammation. In case of patients suffering from CD, intestinal T lymphocytes have shown to be hyperactive against bacterial antigens as local tolerant mechanism is found to be abolished in them[28]. In addition, they have increased intestinal secretion of IgG antibodies against a broad spectrum of commensal bacteria that, unlike IgA, activate complement cascade and inflammatory mediators[29,30].

There are various hypothesis of how microbial composition in human gut plays vital role in the pathogenesis of IBD. Recent studies have shown that reduction of dominant commensal bacteria, such as *Firmicutes* and *Bacteroidetes*, and an increased number of *Proteobacteria* and *Actinobacteria*, may lead to this pathophysiological condition of human gut[31,32]. A wide variety of gut microbial species have directly being linked with IBD. Among Firmicutes, *Faecalibacterium prausnitzii* is shown to have anti inflammatory activity and is found to be significantly decreased in CD[33,34]. Furthermore, Joossens *et al*[35] have shown that reduction of *Faecalibacterium prausnitzii*, *Bifidobacterium adolescentis*, *Dialister invisus*, an unknown species of *Clostridium* clusters XIVA and increase of *Ruminococcus gnavus* are characteristic features in patients suffering from CD. A number of studies have shown that a wide variety of probiotic microorganisms, including *Escherichia coli*, *Saccharomyces boulardii*, and *Bifidobacterium* are involved in treating UC, although these are not always supported by high quality clinical trials[36-42]. Also the role of microbiota in fecal transplantation, efficiently utilized in severe *Clostridium difficile* infection, have shown to be effective in IBD[43,44].

COLORECTAL CANCER

Colorectal cancer (CRC) is one of the most common malignancies in the world, which accounts for about half million deaths annually[45]. Although the prevalence of CRC is higher in the western world but it is found to be increasing in the developing countries at an alarming pace. Two principle mechanisms are mainly involved for the development of CRC-molecular genetics mechanisms and environmental factors[46]. Dietary and genetic factors interact with each other *via* events taking place in the lumen of the large intestine[47].

The genetic mechanisms of CRC are well established[48]. A number of oncogenes and tumor-suppressor genes, such as *APC*, *KRAS*, *p53* and other regulatory genes are mutated in CRC patients. Together with DNA-methylation and chromatin-structure changes, the mutations

act to dysregulate conserved signaling networks that play vital role on cell phenotypes, including the regulation of cellular metabolism, proliferation, differentiation, and survival. Beside genes, environmental factors also influence the occurrence of CRC. Dietary carbohydrate and fat play critical roles in the development of colon tumorigenesis[49]. Studies suggested that dietary fat and high consumption of red meat are associated with high risk of CRC[50]. By contrast, a high intake of complex carbohydrate or dietary fibers, such as cellulose, lignin and pectin, that undergo bacterial fermentation in the colon, has been associated with reduced CRC risk[51-53].

The effect of diet on carcinogenic process is mediated by changes in metabolic activity and composition of the colonic microbiome that accounts for over 100 trillion bacteria grouped in about 1000 species in human gut[54,55]. Various studies have shown significant association between abundance of different bacterial species, particularly *Fusobacterium nucleatum*, with the prevalence of CRC[56,57]. CRC risk was found to be associated with decreased bacterial diversity of Gram-positive, fiber-fermenting Clostridia; and increased presence of Gram-negative, pro-inflammatory genera such as *Fusobacterium* and *Porphyromonas*[58]. The bacterial gut population can be shifted to a healthier composition by dietary fiber that provides substrates for bacterial fermentation[55]. On the other hand, diet rich in fat and meat but poor in vegetables increases the concentration of N-nitroso compounds, a group of genotoxic substances that are known initiators of colon cancer[59]. Another group of carcinogens are heterocyclic aromatic amines that are found in meat and some intestinal bacteria leads to DNA damage in colon cells due to the presence of such compounds[60]. A more descriptive human study highlighted that high risk of CRC is associated with the presence of *Bacteroides vulgatus* and *Bacteroides stercoris*, whereas presence of *Lactobacillus acidophilus*, *Lactobacillus S06* and *Eubacterium aerofaciens* are associated with low risk of CRC[61]. Although there is no conclusive evidence, gut microbiome seems to be a significant contributing factor that modulates risk of CRC in human beings.

OBESITY

Obesity is a medical disorder in which excess body fat accumulates over body. It is only recently that the problem of obesity has achieved global acknowledgment, in contrast to the problem of underweight and malnutrition-which have always conquered clinical attention. World Health Organization describes obesity as one of the major public health concern that threatens the modern world civilization and of late has become a global epidemic. A person is categorized as overweight when the body-mass index (BMI) is around 25 kg/m^2 or higher and people are classified as obese when the BMI is 30 kg/m^2 or more[62]. A plentiful of studies has demonstrated that obese individuals are lazy, lack self-discipline. There is a social disgrace and discrimination against obese people

in various fields of life, which in turn creates numerous consequences for their psychological and to some extent physical health[63]. However, obesity is not only a cosmetic concern. It has serious health concerns including increased risk for type 2 diabetes, cardiovascular diseases, non alcoholic fatty liver disease, pulmonary hypertension, asthma, sleep apnea, osteoarthritis, gall-bladder disease, a number of cancers, and most importantly an increased risk of mortality[64].

Numerous studies have suggested that the gut microbiota plays a crucial role in the development of fat mass and altered energy homeostasis[65]. Obese gut microbiota increases both the capacity to harvest energy from the diet and the accumulation of fat in adipose tissue and liver, by altering host metabolism. Studies in germ-free and conventionalized mice revealed that the microbiota helps in absorbing the monosaccharides from the gut lumen and adipocyte hypertrophy by suppressing fasting-induced adipocyte factor in the intestine, and this suggests that the gut microbiota is an important factor that affects energy harvest from the diet and energy storage in the host[66,67]. The gut microbial community is diverse; consisting of bacterial species, archaea and various microbial eukaryotes. Therefore competitive interactions among these species might also play crucial roles in promoting obesity. In this regard, methane producing archaea *Methanobrevibacter smithi* has been found to be present in greater abundance in obese mice and humans when compared with lean individuals[68,69]. Obesity and diet could be associated with altered gut microbiota characterized by a high *Firmicutes* to *Bacteroidetes* ratio and a dramatic fall in overall microbial diversity[70].

It has been proposed that the composition of the gut microbiota during childhood predicts the following development of obesity in humans. In this regard some studies were conducted to compare between the fecal samples from overweight/obese and normal weight children[68,71]. It shows that during infancy, a significantly higher number of *Bifidobacterial* species was observed in children who maintain a normal weight at age 7 years, while significantly greater numbers of *Staphylococcus aureus* were detected in children who became obese afterward. Therefore, it is hypothesized that an early modulation of gut microbiota can actually prevent obesity[72,73]. Interestingly, another study found that the microbiota composition is different in case of pregnant women also, with relatively higher numbers of *Bacteroides* and *Staphylococcus* found in overweight pregnant women[74]. Obese human twins also have different gut microbial composition as compared to their lean twin. The obese one has reduced levels of *Bacteroidetes* and also less bacterial diversity[69].

AUTISM

The brain is strongly coupled with the gut *via* 200-600 million neurons[75]. Currently, a growing number of clinical data and experimental observations suggest the presence of bidirectional gut-brain axis, implying that there

are probably many a type of neuro-atypical symptoms; including stress, depression, anxiety, associated with the alteration of the normal composition of gut microbial flora[76,77]. In this review we would like to restrict ourselves to one neuropsychiatric disorder-Autism. The Autism Spectrum Disorder (ASD) is an assemblage of neuro-developmental disorders characterized by obscurity in social interaction and communication in affected children. It is typically associated with limited, repetitive, and stereotypic behavior and is noticeable within the first 3 years of life[78,79]. Until 1990, Autism was treated as a rare psychological disorder. Today it is a major health concern, big emotional burden for families, and large financial burden for the government worldwide.

Though the principal cause of this disorder is yet to be known; gastrointestinal disorders have frequently been reported in the children with autism-suggesting the probable link between the atypical compositions of human gut microbiome with ASD[80]. The hypothesis regarding the gut microbiota and ASD linkage was first coined by Bolte[81]. Their study showed that interruption in the normal composition of native gut flora resulted colonization of some neurotoxin producing bacteria, contributing to the autistic symptom[82]. As the importance of gut microbiota in gut-brain function came emerging; probable role of diet, bacteria, and enzyme became a field of important study in autism research[83]. It has been proved that there is a significant difference between the stool sample from autistic and normal children in terms of frequency of occurence of four bacterial phyla specifically, *viz., Firmicutes, Bacteroids, Actinobacteria* and *Proteobacteria*. Further studies have shown higher count and diversity of *Clostridia* (mainly *Clostridium tetani, Clostridium perfringens* and *Clostridium bolteae*) and *Desulfovibrio* (mainly *D.desulfuricans, D.fairfieldensis, D.piger*) in fecal samples of children with autistic behavior as compared to the normal healthy children with same sex and age[81,84-91]. Evidence suggests that high occurrence of *Bifidobacterium* and *Lactobacillus* species is a biological indicator for healthy gut microbiota in breast-fed infants as they serve important probiotic function in the gut[92-94]. As expected, these organisms are frequently reported to be lower in patients with ASD. People are working with several animal models to investigate the expected link between gut microbiota and autism like disorders. One recent paper on maternal immune activation (MIA) mouse model has revealed gastrointestinal abnormalities and changes in the gut microbial community in offspring of MIA animals with autism-like symptoms[95].

Till date, several studies have demonstrated the presence of a perturbed intestinal microbiota composition in children with ASD compared to normal control children. However, caution should be applied while drawing conclusion from these results, as patients with ASD have probable history of using high antibiotic dosage and different diets compared with neuro-typical individuals, either of these can influence the normal composition of the gut microbiota[96,97]. Fortunately enough, a recent study demonstrated that the alteration in the concentra-

tions of short-chain fatty acids in the fecal sample of children with ASD[98]. This suggests that atypical production of such microbial metabolites may have a direct effect on our brain function and thus bacteria can modulate the brain function in a straight line.

CONCLUSION

It is well established fact that the gut microbiota influences host metabolism, immune function, and host homeostasis. Interruption in this balanced community may generate very serious health troubles for the host. Advancement of next-generation genomic technology will pave the way to the development of experimental models of representative examples from the human gut microbiome. This will consecutively accelerate the discovery, testing, and validation of novel drug targets. Future metagenomic research is also expected to focus on the complex relationships of the gut microbiome composition and host metabolism so that in time their actual importance to human health will also be understood better. More in depth understanding of the specific relationships between the gut microbiota and disease will enlighten us about the potential therapeutic targets. The issue of intelligent modulation of the intestinal community is a topic of great interest nowadays. The gut microbiome is expected to contribute immensely to the delivery of personalized healthcare strategies that are already being applied into the clinical environment for the benefit of patients. It can open new door to treating disease and potential modulation of human disease risk factors.

ACKNOWLEDGMENTS

REFERENCES

1 **Human Microbiome Project Consortium**. Structure, function and diversity of the healthy human microbiome. *Nature* 2012; **486**: 207-214 [PMID: 22699609 DOI: 10.1038/nature11234]

2 **Peterson J**, Garges S, Giovanni M, McInnes P, Wang L, Schloss JA, Bonazzi V, McEwen JE, Wetterstrand KA, Deal C, Baker CC, Di Francesco V, Howcroft TK, Karp RW, Lunsford RD, Wellington CR, Belachew T, Wright M, Giblin C, David H, Mills M, Salomon R, Mullins C, Akolkar B, Begg L, Davis C, Grandison L, Humble M, Khalsa J, Little AR, Peavy H, Pontzer C, Portnoy M, Sayre MH, Starke-Reed P, Zakhari S, Read J, Watson B, Guyer M. The NIH Human Microbiome Project. *Genome Res* 2009; **19**: 2317-2323 [PMID: 19819907 DOI: 10.1101/gr.096651.109]

3 **Turnbaugh PJ**, Ley RE, Hamady M, Fraser-Liggett MC, Knight R, Gordon JI. The human microbiome project: exploring the microbial part of ourselves in a changing world. *Nature* 2007; **449**: 804-810 [DOI: 10.1038/nature06244]

4 **Shanahan F**. The host-microbe interface within the gut. *Best Pract Res Clin Gastroenterol* 2002; **16**: 915-931 [PMID: 12473298]

5 **Bocci V**. The neglected organ: bacterial flora has a crucial immunostimulatory role. *Perspect Biol Med* 1992; **35**: 251-260 [PMID: 1557302]

6 **Wallace BD**, Redinbo MR. The human microbiome is a sou-

rce of therapeutic drug targets. *Curr Opin Chem Biol* 2013; **17**: 379-384 [PMID: 23680493 DOI: 10.1016/j.cbpa.2013.04.011]

7 **Costello EK**, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. Bacterial community variation in human body habitats across space and time. *Science* 2009; **326**: 1694-1697 [PMID: 19892944 DOI: 10.1126/science.1177486]

8 **Grönlund MM**, Lehtonen OP, Eerola E, Kero P. Fecal microflora in healthy infants born by different methods of delivery: permanent changes in intestinal flora after cesarean delivery. *J Pediatr Gastroenterol Nutr* 1999; **28**: 19-25 [PMID: 9890463]

9 **Benson AK**, Kelly SA, Legge R, Ma F, Low SJ, Kim J, Zhang M, Oh PL, Nehrenberg D, Hua K, Kachman SD, Moriyama EN, Walter J, Peterson DA, Pomp D. Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. *Proc Natl Acad Sci USA* 2010; **107**: 18933-18938 [PMID: 20937875 DOI: 10.1073/pnas.1007028107]

10 **Ley RE**, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell* 2006; **124**: 837-848 [PMID: 16497592]

11 **Walter J**, Ley R. The human gut microbiome: ecology and recent evolutionary changes. *Annu Rev Microbiol* 2011; **65**: 411-429 [PMID: 21682646 DOI: 10.1146/annurev-micro-090110-102830]

12 **Bäckhed F**. Programming of host metabolism by the gut microbiota. *Ann Nutr Metab* 2011; **58** Suppl 2: 44-52 [PMID: 21846980 DOI: 10.1159/000328042]

13 **Eckburg PB**, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA. Diversity of the human intestinal microbial flora. *Science* 2005; **308**: 1635-1638 [PMID: 15831718]

14 **Qin J**, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Doré J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, Bork P, Ehrlich SD, Wang J. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010; **464**: 59-65 [PMID: 20203603 DOI: 10.1038/nature08821]

15 **Clemente JC**, Ursell LK, Parfrey LW, Knight R. The impact of the gut microbiota on human health: an integrative view. *Cell* 2012; **148**: 1258-1270 [PMID: 22424233 DOI: 10.1016/j.cell.2012.01.035]

16 **Koenig JE**, Spor A, Scalfone N, Fricker AD, Stombaugh J, Knight R, Angenent LT, Ley RE. Succession of microbial consortia in the developing infant gut microbiome. *Proc Natl Acad Sci USA* 2011; **108** Suppl 1: 4578-4585 [PMID: 20668239 DOI: 10.1073/pnas.1000081107]

17 **Palmer C**, Bik EM, DiGiulio DB, Relman DA, Brown PO. Development of the human infant intestinal microbiota. *PLoS Biol* 2007; **5**: e177 [PMID: 17594176]

18 **Arumugam M**, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, Fernandes GR, Tap J, Bruls T, Batto JM, Bertalan M, Borruel N, Casellas F, Fernandez L, Gautier L, Hansen T, Hattori M, Hayashi T, Kleerebezem M, Kurokawa K, Leclerc M, Levenez F, Manichanh C, Nielsen HB, Nielsen T, Pons N, Poulain J, Qin J, Sicheritz-Ponten T, Tims S, Torrents D, Ugarte E, Zoetendal EG, Wang J, Guarner F, Pedersen O, de Vos WM, Brunak S, Doré J, Antolín M, Artiguenave F, Blottiere HM, Almeida M, Brechot C, Cara C, Chervaux C, Cultrone A, Delorme C, Denariaz G, Dervyn R, Foerstner KU, Friss C, van de Guchte M, Guedon E, Haimet F, Huber W, van Hylckama-Vlieg J, Jamet A, Juste C, Kaci G, Knol J, Lakhdari O, Layec S, Le Roux K, Maguin E, Mérieux A, Melo Minardi R, M'rini C, Muller J, Oozeer R, Parkhill J, Renault

P, Rescigno M, Sanchez N, Sunagawa S, Torrejon A, Turner K, Vandemeulebrouck G, Varela E, Winogradsky Y, Zeller G, Weissenbach J, Ehrlich SD, Bork P. Enterotypes of the human gut microbiome. *Nature* 2011; **473**: 174-180 [PMID: 21508958 DOI: 10.1038/nature09944]

19   **Claesson MJ**, Jeffery IB, Conde S, Power SE, O'Connor EM, Cusack S, Harris HM, Coakley M, Lakshminarayanan B, O'Sullivan O, Fitzgerald GF, Deane J, O'Connor M, Harnedy N, O'Connor K, O'Mahony D, van Sinderen D, Wallace M, Brennan L, Stanton C, Marchesi JR, Fitzgerald AP, Shanahan F, Hill C, Ross RP, O'Toole PW. Gut microbiota composition correlates with diet and health in the elderly. *Nature* 2012; **488**: 178-184 [PMID: 22797518 DOI: 10.1038/nature11319]

20   **Turnbaugh PJ**, Gordon JI. The core gut microbiome, energy balance and obesity. *J Physiol* 2009; **587**: 4153-4158 [PMID: 19491241 DOI: 10.1113/jphysiol.2009.174136]

21   **Sekirov I**, Russell SL, Antunes LC, Finlay BB. Gut microbiota in health and disease. *Physiol Rev* 2010; **90**: 859-904 [PMID: 20664075 DOI: 10.1152/physrev.00045.2009]

22   **Cryan JF**, O'Mahony SM. The microbiome-gut-brain axis: from bowel to behavior. *Neurogastroenterol Motil* 2011; **23**: 187-192 [PMID: 21303428 DOI: 10.1111/j.1365-2982.2010.01664.x]

23   **Sartor RB**, Muehlbauer M. Microbial host interactions in IBD: implications for pathogenesis and therapy. *Curr Gastroenterol Rep* 2007; **9**: 497-507 [PMID: 18377803]

24   **Shanahan F**. Inflammatory bowel disease: immunodiagnostics, immunotherapeutics, and ecotherapeutics. *Gastroenterology* 2001; **120**: 622-635 [PMID: 11179240]

25   **Shanahan F**. The microbiota in inflammatory bowel disease: friend, bystander, and sometime-villain. *Nutr Rev* 2012; **70** Suppl 1: S31-S37 [PMID: 22861805 DOI: 10.1111/j.1753-4887.2012.00502.x]

26   **Jostins L**, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY, Lee JC, Schumm LP, Sharma Y, Anderson CA, Essers J, Mitrovic M, Ning K, Cleynen I, Theatre E, Spain SL, Raychaudhuri S, Goyette P, Wei Z, Abraham C, Achkar JP, Ahmad T, Amininejad L, Ananthakrishnan AN, Andersen V, Andrews JM, Baidoo L, Balschun T, Bampton PA, Bitton A, Boucher G, Brand S, Büning C, Cohain A, Cichon S, D'Amato M, De Jong D, Devaney KL, Dubinsky M, Edwards C, Ellinghaus D, Ferguson LR, Franchimont D, Fransen K, Gearry R, Georges M, Gieger C, Glas J, Haritunians T, Hart A, Hawkey C, Hedl M, Hu X, Karlsen TH, Kupcinskas L, Kugathasan S, Latiano A, Laukens D, Lawrance IC, Lees CW, Louis E, Mahy G, Mansfield J, Morgan AR, Mowat C, Newman W, Palmieri O, Ponsioen CY, Potocnik U, Prescott NJ, Regueiro M, Rotter JI, Russell RK, Sanderson JD, Sans M, Satsangi J, Schreiber S, Simms LA, Sventoraityte J, Targan SR, Taylor KD, Tremelling M, Verspaget HW, De Vos M, Wijmenga C, Wilson DC, Winkelmann J, Xavier RJ, Zeissig S, Zhang B, Zhang CK, Zhao H, Silverberg MS, Annese V, Hakonarson H, Brant SR, Radford-Smith G, Mathew CG, Rioux JD, Schadt EE, Daly MJ, Franke A, Parkes M, Vermeire S, Barrett JC, Cho JH. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* 2012; **491**: 119-124 [PMID: 23128233 DOI: 10.1038/nature11582]

27   **Sartor RB**, Mazmanian SK. Intestinal microbes in inflammatory bowel diseases. *Am J Gastroenterol Suppl* 2012; **1**: 15-21 [DOI: 10.1038/ajgsup.2012.4]

28   **Pirzer U**, Schönhaar A, Fleischer B, Hermann E, Meyer zum Büschenfelde KH. Reactivity of infiltrating T lymphocytes with microbial antigens in Crohn's disease. *Lancet* 1991; **338**: 1238-1239 [PMID: 1682646]

29   **Brandtzaeg P**, Halstensen TS, Kett K, Krajci P, Kvale D, Rognum TO, Scott H, Sollid LM. Immunobiology and immunopathology of human gut mucosa: humoral immunity and intraepithelial lymphocytes. *Gastroenterology* 1989; **97**: 1562-1584 [PMID: 2684725]

30   **Macpherson A**, Khoo UY, Forgacs I, Philpott-Howard J,

Bjarnason I. Mucosal antibodies in inflammatory bowel disease are directed against intestinal bacteria. *Gut* 1996; **38**: 365-375 [PMID: 8675088]

31   **Fava F**, Danese S. Intestinal microbiota in inflammatory bowel disease: friend of foe? *World J Gastroenterol* 2011; **17**: 557-566 [PMID: 21350704 DOI: 10.3748/wjg.v17.i5.557]

32   **Manichanh C**, Borruel N, Casellas F, Guarner F. The gut microbiota in IBD. *Nat Rev Gastroenterol Hepatol* 2012; **9**: 599-608 [PMID: 22907164 DOI: 10.1038/nrgastro.2012.152]

33   **Sokol H**, Pigneur BD, Watterlot L, Lakhdari O, Bermudez-Humaran LG, Gratadoux JJ, Blugeon S, Bridonneau C, Furet JP, Corthier G, Grangette C, Vasquez N, Pochart P, Trugnan G, Thomas G, Blottiere HM, Dore J, Marteau P, Seksik P, Langella P. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *P Natl Acad Sci USA* 2008; **105**: 16731-16736 [DOI: 10.1073/pnas.0804812105]

34   **Sokol H**, Seksik P, Furet JP, Firmesse O, Nion-Larmurier I, Beaugerie L, Cosnes J, Corthier G, Marteau P, Doré J. Low counts of Faecalibacterium prausnitzii in colitis microbiota. *Inflamm Bowel Dis* 2009; **15**: 1183-1189 [PMID: 19235886 DOI: 10.1002/ibd.20903]

35   **Joossens M**, Huys G, Cnockaert M, De Preter V, Verbeke K, Rutgeerts P, Vandamme P, Vermeire S. Dysbiosis of the faecal microbiota in patients with Crohn's disease and their unaffected relatives. *Gut* 2011; **60**: 631-637 [PMID: 21209126 DOI: 10.1136/gut.2010.223263]

36   **Bibiloni R**, Fedorak RN, Tannock GW, Madsen KL, Gionchetti P, Campieri M, De Simone C, Sartor RB. VSL#3 probiotic-mixture induces remission in patients with active ulcerative colitis. *Am J Gastroenterol* 2005; **100**: 1539-1546 [PMID: 15984978]

37   **Guslandi M**, Giollo P, Testoni PA. A pilot trial of Saccharomyces boulardii in ulcerative colitis. *Eur J Gastroenterol Hepatol* 2003; **15**: 697-698 [PMID: 12840682]

38   **Ishikawa H**, Akedo I, Umesaki Y, Tanaka R, Imaoka A, Otani T. Randomized controlled trial of the effect of bifidobacteria-fermented milk on ulcerative colitis. *J Am Coll Nutr* 2003; **22**: 56-63 [PMID: 12569115]

39   **Kruis W**, Fric P, Pokrotnieks J, Lukás M, Fixa B, Kascák M, Kamm MA, Weismueller J, Beglinger C, Stolte M, Wolff C, Schulze J. Maintaining remission of ulcerative colitis with the probiotic Escherichia coli Nissle 1917 is as effective as with standard mesalazine. *Gut* 2004; **53**: 1617-1623 [PMID: 15479682]

40   **Kruis W**, Schütz E, Fric P, Fixa B, Judmaier G, Stolte M. Double-blind comparison of an oral Escherichia coli preparation and mesalazine in maintaining remission of ulcerative colitis. *Aliment Pharmacol Ther* 1997; **11**: 853-858 [PMID: 9354192]

41   **Rembacken BJ**, Snelling AM, Hawkey PM, Chalmers DM, Axon AT. Non-pathogenic Escherichia coli versus mesalazine for the treatment of ulcerative colitis: a randomised trial. *Lancet* 1999; **354**: 635-639 [PMID: 10466665]

42   **Zocco MA**, dal Verme LZ, Cremonini F, Piscaglia AC, Nista EC, Candelli M, Novi M, Rigante D, Cazzato IA, Ojetti V, Armuzzi A, Gasbarrini G, Gasbarrini A. Efficacy of Lactobacillus GG in maintaining remission of ulcerative colitis. *Aliment Pharmacol Ther* 2006; **23**: 1567-1574 [PMID: 16696804]

43   **Damman CJ**, Miller SI, Surawicz CM, Zisman TL. The microbiome and inflammatory bowel disease: is there a therapeutic role for fecal microbiota transplantation? *Am J Gastroenterol* 2012; **107**: 1452-1459 [PMID: 23034604 DOI: 10.1038/ajg.2012.93]

44   **Kahn SA**, Gorawara-Bhat R, Rubin DT. Fecal bacteriotherapy for ulcerative colitis: patients are ready, are we? *Inflamm Bowel Dis* 2012; **18**: 676-684 [PMID: 21618362]

45   **Parkin DM**, Pisani P, Ferlay J. Global cancer statistics. *CA Cancer J Clin* 1999; **49**: 33-64, 1 [PMID: 10200776]

46   **Chambers WM**, Warren BF, Jewell DP, Mortensen NJ. Cancer surveillance in ulcerative colitis. *Br J Surg* 2005; **92**: 928-936

[PMID: 16034807]

47   **Rafter J**, Glinghammar B. Interactions between the environ-ment and genes in the colon. *Eur J Cancer Prev* 1998; **7** Suppl 2: S69-S74 [PMID: 9696945]

48   **Fearon ER**. Molecular genetics of colorectal cancer. *Annu Rev Pathol* 2011; **6**: 479-507 [PMID: 21090969 DOI: 10.1146/annurev-pathol-011110-130235]

49   **Kushi LH**, Byers T, Doyle C, Bandera EV, McCullough M, McTiernan A, Gansler T, Andrews KS, Thun MJ. American Cancer Society Guidelines on Nutrition and Physical Activ-ity for cancer prevention: reducing the risk of cancer with healthy food choices and physical activity. *CA Cancer J Clin* 2006; **56**: 254-281; quiz 313-314 [PMID: 17005596]

50   **Bingham SA**. High-meat diets and cancer risk. *P Nutr Soc* 1999; **58**: 243-248 [DOI: 10.1017/S0029665199000336]

51   **Kaczmarczyk MM**, Miller MJ, Freund GG. The health ben-efits of dietary fiber: beyond the usual suspects of type 2 diabetes mellitus, cardiovascular disease and colon cancer. *Metabolism* 2012; **61**: 1058-1066 [DOI: 10.1016/j.metab-ol.2012.01.017]

52   **Nomura AM**, Hankin JH, Henderson BE, Wilkens LR, Mur-phy SP, Pike MC, Le Marchand L, Stram DO, Monroe KR, Kolonel LN. Dietary fiber and colorectal cancer risk: the mul-tiethnic cohort study. *Cancer Causes Control* 2007; **18**: 753-764 [PMID: 17557210]

53   **Peters U**, Sinha R, Chatterjee N, Subar AF, Ziegler RG, Kull-dorff M, Bresalier R, Weissfeld JL, Flood A, Schatzkin A, Hayes RB. Dietary fibre and colorectal adenoma in a colorec-tal cancer early detection programme. *Lancet* 2003; **361**: 1491-1495 [PMID: 12737857]

54   **Floch MH**. Intestinal microecology in health and wellness. *J Clin Gastroenterol* 2011; **45** Suppl: S108-S110 [PMID: 21992947 DOI: 10.1097/MCG.0b013e3182309276]

55   **Zhu Y**, Michelle Luo T, Jobin C, Young HA. Gut microbiota and probiotics in colon tumorigenesis. *Cancer Lett* 2011; **309**: 119-127 [PMID: 21741763 DOI: 10.1016/j.canlet.2011.06.004]

56   **Castellarin M**, Warren RL, Freeman JD, Dreolini L, Krzy-winski M, Strauss J, Barnes R, Watson P, Allen-Vercoe E, Moore RA, Holt RA. Fusobacterium nucleatum infection is prevalent in human colorectal carcinoma. *Genome Res* 2012; **22**: 299-306 [PMID: 22009989 DOI: 10.1101/gr.126516.111]

57   **Kostic AD**, Gevers D, Pedamallu CS, Michaud M, Duke F, Earl AM, Ojesina AI, Jung J, Bass AJ, Tabernero J, Baselga J, Liu C, Shivdasani RA, Ogino S, Birren BW, Huttenhower C, Garrett WS, Meyerson M. Genomic analysis identifies association of Fusobacterium with colorectal carcinoma. *Genome Res* 2012; **22**: 292-298 [PMID: 22009990 DOI: 10.1101/gr.126573.111]

58   **Ahn J**, Sinha R, Pei Z, Dominianni C, Wu J, Shi J, Goedert JJ, Hayes RB, Yang L. Human gut microbiome and risk for colorectal cancer. *J Natl Cancer Inst* 2013; **105**: 1907-1911 [PMID: 24316595 DOI: 10.1093/jnci/djt300]

59   **Hughes R**, Cross AJ, Pollock JR, Bingham S. Dose-dependent effect of dietary meat on endogenous colonic N-nitrosation. *Carcinogenesis* 2001; **22**: 199-202 [PMID: 11159760]

60   **Wollowski I**, Rechkemmer G, Pool-Zobel BL. Protective role of probiotics and prebiotics in colon cancer. *Am J Clin Nutr* 2001; **73**: 451S-455S [PMID: 11157356]

61   **Moore WE**, Moore LH. Intestinal floras of populations that have a high risk of colon cancer. *Appl Environ Microbiol* 1995; **61**: 3202-3207 [PMID: 7574628]

62   **James WPT**, Jackson-Leach R, Mhurchu CN, Kalamara E, Shayeghi M, Rigby NJ, Nishida C, Rodgers A. Overweight and obesity (high body mass index). Comparative quantifi-cation of health risks: global and regional burden of disease attribution to selected major risk factors volume 1. Geneva: World Health Organization, 2004: 497-596

63   **KD B**, Puhl RM. Weight bias; nature, consequences, and remedies. In: Fikkan J, Rothblum E. Weight bias in employ-ment. Guilford Press, 2005

64   **Ezzati M**, Lopez AD, Rodgers A, Vander Hoorn S, Murray CJ. Selected major risk factors and global and regional bur-den of disease. *Lancet* 2002; **360**: 1347-1360 [PMID: 12423980]

65   **Turnbaugh PJ**, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006; **444**: 1027-1031 [PMID: 17183312]

66   **Bäckhed F**, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. *Science* 2005; **307**: 1915-1920 [PMID: 15790844 DOI: 10.1126/sci-ence.1104816]

67   **Bäckhed F**, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obe-sity in germ-free mice. *Proc Natl Acad Sci USA* 2007; **104**: 979-984 [PMID: 17210919]

68   **Kalliomäki M**, Collado MC, Salminen S, Isolauri E. Early differences in fecal microbiota composition in children may predict overweight. *Am J Clin Nutr* 2008; **87**: 534-538 [PMID: 18326589]

69   **Turnbaugh PJ**, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B, Heath AC, Knight R, Gordon JI. A core gut microbiome in obese and lean twins. *Nature* 2009; **457**: 480-484 [PMID: 19043404 DOI: 10.1038/nature07540]

70   **Spencer MD**, Hamp TJ, Reid RW, Fischer LM, Zeisel SH, Fodor AA. Association between composition of the human gastrointestinal microbiome and development of fatty liver with choline deficiency. *Gastroenterology* 2011; **140**: 976-986 [PMID: 21129376 DOI: 10.1053/j.gastro.2010.11.049]

71   **Karlsson CL**, Onnerfält J, Xu J, Molin G, Ahrné S, Thorn-gren-Jerneck K. The microbiota of the gut in preschool chil-dren with normal and excessive body weight. *Obesity* (Silver Spring) 2012; **20**: 2257-2261 [PMID: 22546742 DOI: 10.1038/oby.2012.110]

72   **Luoto R**, Kalliomäki M, Laitinen K, Delzenne NM, Cani PD, Salminen S, Isolauri E. Initial dietary and microbiologi-cal environments deviate in normal-weight compared to overweight children at 10 years of age. *J Pediatr Gastro-enterol Nutr* 2011; **52**: 90-95 [PMID: 21150648 DOI: 10.1097/MPG.0b013e3181f3457f]

73   **Luoto R**, Kalliomäki M, Laitinen K, Isolauri E. The impact of perinatal probiotic intervention on the development of over-weight and obesity: follow-up study from birth to 10 years. *Int J Obes* (Lond) 2010; **34**: 1531-1537 [PMID: 20231842 DOI: 10.1038/ijo.2010.50]

74   **Collado MC**, Isolauri E, Laitinen K, Salminen S. Distinct composition of gut microbiota during pregnancy in over-weight and normal-weight women. *Am J Clin Nutr* 2008; **88**: 894-899 [PMID: 18842773]

75   **Furness JB**. Novel gut afferents: Intrinsic afferent neurons and intestinofugal neurons. *Auton Neurosci* 2006; **125**: 81-85 [PMID: 16476573]

76   **Collins SM**. Stress and the Gastrointestinal Tract IV. Modu-lation of intestinal inflammation by stress: basic mechanisms and clinical relevance. *Am J Physiol Gastrointest Liver Physiol* 2001; **280**: G315-G318 [PMID: 11171612]

77   **Wu JC**. Psychological Co-morbidity in Functional Gastro-intestinal Disorders: Epidemiology, Mechanisms and Man-agement. *J Neurogastroenterol Motil* 2012; **18**: 13-18 [PMID: 22323984 DOI: 10.5056/jnm.2012.18.1.13]

78   **Lyons V**, Fitzgerald M. Asperger (1906-1980) and Kanner (1894-1981), the two pioneers of autism. *J Autism Dev Disord* 2007; **37**: 2022-2023 [PMID: 17922179]

79   **Wolff S**. The history of autism. European Child and Adoles-cent *Psychiatry* 2004; **13**: 201 [DOI: 10.1007/s00787-004-0363-5]

80   **de Theije CG**, Wu J, da Silva SL, Kamphuis PJ, Garssen J, Korte SM, Kraneveld AD. Pathways underlying the gut-to-brain connection in autism spectrum disorders as future tar-gets for disease management. *Eur J Pharmacol* 2011; **668** Suppl 1: S70-S80 [PMID: 21810417 DOI: 10.1016/j.ejphar.2011.07.013]

81   **Bolte ER**. Autism and Clostridium tetani. *Med Hypotheses* 1998; **51**: 133-144 [PMID: 9881820]

82   **Sandler RH**, Finegold SM, Bolte ER, Buchanan CP, Maxwell AP, Väisänen ML, Nelson MN, Wexler HM. Short-term benefit from oral vancomycin treatment of regressive-onset autism. *J Child Neurol* 2000; **15**: 429-435 [PMID: 10921511]

83   **MacFabe DF**, Cain DP, Rodriguez-Capote K, Franklin AE, Hoffman JE, Boon F, Taylor AR, Kavaliers M, Ossenkopp KP. Neurobiological effects of intraventricular propionic acid in rats: possible role of short chain fatty acids on the pathogenesis and characteristics of autism spectrum disorders. *Behav Brain Res* 2007; **176**: 149-169 [PMID: 16950524]

84   **Adams JB**, Johansen LJ, Powell LD, Quig D, Rubin RA. Gastrointestinal flora and gastrointestinal status in children with autism--comparisons to typical children and correlation with autism severity. *BMC Gastroenterol* 2011; **11**: 22 [PMID: 21410934 DOI: 10.1186/1471-230X-11-22]

85   **De Angelis M**, Piccolo M, Vannini L, Siragusa S, De Giacomo A, Serrazzanetti DI, Cristofori F, Guerzoni ME, Gobbetti M, Francavilla R. Fecal microbiota and metabolome of children with autism and pervasive developmental disorder not otherwise specified. *PLoS One* 2013; **8**: e76993 [PMID: 24130822 DOI: 10.1371/journal.pone.0076993]

86   **Finegold SM**, Dowd SE, Gontcharova V, Liu C, Henley KE, Wolcott RD, Youn E, Summanen PH, Granpeesheh D, Dixon D, Liu M, Molitoris DR, Green JA. Pyrosequencing study of fecal microflora of autistic and control children. *Anaerobe* 2010; **16**: 444-453 [PMID: 20603222 DOI: 10.1016/j.anaerobe.2010.06.008]

87   **Finegold SM**, Downes J, Summanen PH. Microbiology of regressive autism. *Anaerobe* 2012; **18**: 260-262 [PMID: 22202440 DOI: 10.1016/j.anaerobe.2011.12.018]

88   **Finegold SM**, Molitoris D, Song Y, Liu C, Vaisanen ML, Bolte E, McTeague M, Sandler R, Wexler H, Marlowe EM, Collins MD, Lawson PA, Summanen P, Baysallar M, Tomzynski TJ, Read E, Johnson E, Rolfe R, Nasir P, Shah H, Haake DA, Manning P, Kaul A. Gastrointestinal microflora studies in late-onset autism. *Clin Infect Dis* 2002; **35**: S6-S16 [PMID: 12173102]

89   **Parracho HM**, Bingham MO, Gibson GR, McCartney AL. Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. *J Med Microbiol* 2005; **54**: 987-991 [PMID: 16157555]

90   **Song Y**, Liu C, Molitoris DR, Tomzynski TJ, Lawson PA, Collins MD, Finegold SM. Clostridium bolteae sp. nov., isolated from human sources. *Syst Appl Microbiol* 2003; **26**: 84-89 [PMID: 12747414]

91   **Williams BL**, Hornig M, Parekh T, Lipkin WI. Application of novel PCR-based methods for detection, quantitation, and phylogenetic characterization of Sutterella species in intestinal biopsy samples from children with autism and gastrointestinal disturbances. *MBio* 2012; **3**: pii: e00261-11 [PMID: 22233678 DOI: 10.1128/mBio.00261-11]

92   **Gueimonde M**, Laitinen K, Salminen S, Isolauri E. Breast milk: a source of bifidobacteria for infant gut development and maturation? *Neonatology* 2007; **92**: 64-66 [PMID: 17596738]

93   **Martín R**, Heilig GH, Zoetendal EG, Smidt H, Rodríguez JM. Diversity of the Lactobacillus group in breast milk and vagina of healthy women and potential role in the colonization of the infant gut. *J Appl Microbiol* 2007; **103**: 2638-2644 [PMID: 18045446]

94   **Sinkiewicz G,** Nordström EA. 353 occurrence of lactobacillus reuteri, lactobacilli and bifidobacteria in human breast milk. *Pediatr Res* 2005; **58**: 415 [DOI: 10.1203/00006450-200508000-00382]

95   **Hsiao EY**, McBride SW, Hsien S, Sharon G, Hyde ER, McCue T, Codelli JA, Chow J, Reisman SE, Petrosino JF, Patterson PH, Mazmanian SK. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell* 2013; **155**: 1451-1463 [PMID: 24315484 DOI: 10.1016/j.cell.2013.11.024]

96   **Forsythe P**, Sudo N, Dinan T, Taylor VH, Bienenstock J. Mood and gut feelings. *Brain Behav Immun* 2010; **24**: 9-16 [PMID: 19481599 DOI: 10.1016/j.bbi.2009.05.058]

97   **Dethlefsen L**, Huse S, Sogin ML, Relman DA. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biol* 2008; **6**: e280 [PMID: 19018661 DOI: 10.1371/journal.pbio.0060280]

98   **Wang L**, Christophersen CT, Sorich MJ, Gerber JP, Angley MT, Conlon MA. Elevated fecal short chain fatty acid and ammonia concentrations in children with autism spectrum disorder. *Dig Dis Sci* 2012; **57**: 2096-2102 [PMID: 22535281 DOI: 10.1007/s10620-012-2167-7]

MINIREVIEWS

Pharmacogenetics of type 2 diabetes mellitus: An example of success in clinical and translational medicine

Antonio Brunetti, Francesco S Brunetti, Eusebio Chiefari

Antonio Brunetti, Eusebio Chiefari, Department of Health Sciences, University "Magna Græcia" of Catanzaro, 88100 Catanzaro, Italy
Francesco S Brunetti, Department of Medical and Surgical Sciences, University "Magna Græcia" of Catanzaro, 88100 Catanzaro, Italy
Author contributions: Brunetti A wrote and edited the review; Brunetti FS contributed to editing of the final draft of the manuscript; Chiefari E contributed to writing the manuscript and drew the figures.
Correspondence to: Antonio Brunetti, Professor, Department of Health Sciences, University "Magna Græcia" of Catanzaro, V.le Europa (Loc. Germaneto), 88100 Catanzaro, Italy. brunetti@unicz.it
Telephone: +39-961-3694368   Fax: +39-961-996087
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Abstract

The pharmacological interventions currently available to control type 2 diabetes mellitus (T2DM) show a wide interindividual variability in drug response, emphasizing the importance of a personalized, more effective medical treatment for each individual patient. In this context, a growing interest has emerged in recent years and has focused on pharmacogenetics, a discipline aimed at understanding the variability in patients' drug response, making it possible to predict which drug is best for each patient and at what doses. Recent pharmacological and clinical evidences indicate that genetic polymorphisms (or genetic variations) of certain genes can adversely affect drug response and therapeutic efficacy of oral hypoglycemic agents in patients with T2DM, through pharmacokinetic- and/or pharmacodynamic-based mechanisms that may reduce the therapeutic effects or increase toxicity. For example, genetic variants in genes encoding enzymes of the cytochrome P-450 superfamily, or proteins of the ATP-sensitive potassium channel on the beta-cell of the pancreas, are responsible for the interindividual variability of drug response to sulfonylureas in patients with T2DM. Instead, genetic variants in the genes that encode for the organic cation transporters of metformin have been related to changes in both pharmacodynamic and pharmacokinetic responses to metformin in metformin-treated patients. Thus, based on the individual's genotype, the possibility, in these subjects, of a personalized therapy constitutes the main goal of pharmacogenetics, directly leading to the development of the right medicine for the right patient. Undoubtedly, this represents an integral part of the translational medicine network.

Key words: Type 2 diabetes; Anti-diabetic drugs; Personalized therapy; Genetic variants; Genome-wide association study

Core tip: Type 2 diabetes mellitus (T2DM) is a heterogeneous complex disorder, in which predisposing genetic variants (polymorphisms) and precipitating environmental factors interact synergistically in the development of the disease. Besides being useful in identifying individuals at risk for T2DM, knowledge of the polymorphisms associated with T2DM is also useful in pharmacogenetics for correlating individual variants with individual responses to anti-diabetic drugs. To date, a wide variety of genes that influence pharmacogenetics of anti-diabetic drugs have been identified. However, with few exceptions, drug therapy has not taken into account the individual genetic diversity of treated patients, representing, this, a substantial limitation of pharmacogenetics. This review focuses on clinically important polymorphisms affecting a patient's response to diabetic medications.

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INTRODUCTION

The common observation that patients with type 2 diabetes mellitus (T2DM) show a great variability in the individual response to the same drug treatment suggests the importance of a personalized care approach, in which the most appropriate treatment is indicated by the genetic peculiarities of each individual[1]. The introduction, in 2007, of genome-wide association study (GWAS) has greatly enhanced the number of genes that are known to be associated with common diseases. Applied to millions of people, this method has allowed the identification of several genetic variants which are associated with T2DM[2]. However, similarly to other complex diseases, none of the individual variants identified so far is in itself sufficient to cause the disease, but most of the genetic risk for T2DM is mediated by the combined influence of more genetic variants that individually have only a small degree of risk[3,4]. This combination (haplotype) defines the genetic profile of the individual. The fact that the pathogenesis of T2DM requires the involvement of multiple genes in different combination is in line with the assumption that T2DM, far from being a disease genetically identifiable in a few specific forms, actually consists of a large number of rather different disorders[3,4], each of which is associated with a specific disease phenotype only apparently identical to one another, and in which inter-individual variability in drug response can be identified both in terms of drug efficacy and undesired drug reactions.

Therefore, clarifying the molecular mechanisms by which genetic variations may cause differences in phenotypic traits and in individual drug response is essential not only to determine the etiological role of gene variants, but also to identify new personalized medical solutions. Personalized therapy, based on the genetic diversity of each individual, is one of the most fascinating challenges of modern medicine, representing an integral part of the translational medicine effort, whose ultimate goal is to translate advances in biomedical research into new medical treatments and improvements in patient care (Figure 1). Herein, we provide an overview of this area and its relevance to clinical practice in T2DM.

PHARMACOGENETICS AND GENE POLYMORPHISMS

Pharmacogenetics is defined as the influence of variations in DNA sequence on drug response (www.ema.europa.eu). Its relevance arises from the clinical observation that patients suffering from the same disease do not necessarily respond to the same drug treatment in terms of therapeutic efficacy as well as adverse effects. The principal aim of pharmacogenetics is to provide

Figure 1 From bench to bedside. Translational medicine is a discipline of biomedical research that attempts to connect basic research with clinical care.

personalized medicine, tailored to an individual's genetic makeup, in order to optimize the effectiveness and safety of drug treatment. Although elements of pharmacogenetics can be traced back to ancient Greece (510 years BC), when it was already known the risk of hemolytic anemia in certain individuals in response to the ingestion of uncooked fava beans[5], the term "pharmacogenetics" was first coined by Vogel[6] in 1959 to indicate the importance of genetic polymorphisms on the disposition and action of drugs. The first evidence on the role of genetic variants in drug response back to the '70s and refers to cytochrome P-450 2D6 (CYP2D6), an enzyme of the hepatic P-450 microsomal enzyme system, which is involved in the metabolism of numerous drugs. Studies of the genetic variations within the P-450 family of enzymes provided the first direct evidence for the genetic contributions to drug therapy and efficacy, and these studies continue to be an active part of the basic and clinical research performed today. In fact, numerous other genetic variations have been identified in subsequent years, within the P-450 family of enzymes, including the biotransformation enzymes CYP3A4/5 and the CYP2C9 enzyme. It has been shown that individuals carrying genetic variants of *CYP2D6* (and other P-450 isoforms resulting in poor enzymatic activity), who are concomitantly taking medications that are influenced by these enzymes, are at risk for increased or prolonged drug effect, influencing the speed and effectiveness of drug metabolism[7]. However, there is no doubt that the greatest contribution to pharmacogenetics has come from the sequencing of the entire human genome in 2003, showing that over 99% of DNA is identical in all humans and that, therefore, phenotypic differences among individuals, as well as differences in disease susceptibility and the inter-individual variability in drug response, are the result of sequence polymorphisms that

Figure 2 Single nucleotide polymorphism. As the most common type of variant, a single nucleotide polymorphism is characterized by a single DNA base pair substitution at a specific location in a gene. SNP: Single nucleotide polymorphism.

Figure 3 The ATP-sensitive K$^+$ channels regulate insulin release in beta-cells. Single nucleotide polymorphism in *SUR1* and/or *Kir6.2* genes may cause functional abnormalities of the ATP-sensitive K$^+$ channel on the pancreatic β-cell membrane, leading to abnormalities in insulin secretion.

affect less than 1% of 3 billion bases of human DNA. In most cases, these variants consist of the exchange of single nucleotides in both coding and noncoding DNA regions and are defined as single nucleotide polymorphisms (SNPs) (Figure 2). The ability of the SNP to influence drug response and therapeutic efficacy may rely on the capacity of the variant to induce changes in the expression of proteins that may influence either the pharmacokinetic and/or pharmacodynamic profile and hence the clinical efficacy of the drug. On the basis of these acquisitions, recent GWAS have identified several SNPs that can affect both the therapeutic efficacy and the occurrence of adverse reactions after drug intake[8-10].

PHARMACOGENETICS IN T2DM TREATMENT

Pharmacogenetics of sulfonylureas

In Caucasians, sulfonylureas are metabolized primarily in the liver by CYP2C9 to active metabolites, which are ultimately excreted by the kidney[11]. In previous work, it was demonstrated that polymorphisms of the *CYP2C9* gene significantly affect the pharmacological response of diabetic patients to sulfonylureas[12], due to the reduction of the catalytic activity in the metabolism of these drugs[13-16], with a consequent increase in drug bioavailability. In particular, in certain diabetic patients with the variants Ile359Leu (isoleucine changes to leucine in exon 7 position 359) and Arg144Cys (arginine changes to cysteine in exon 3 position 144) in the *CYP2C9* gene, the clearance of glibenclamide was reduced by 30%-80%, allowing the use of lower doses of this drug to limit the risk of hypoglycemia[12,17-20]. The risk of hypoglycemia in sulphonylurea treated patients was confirmed in a study with a larger population, in which the simultaneous presence (or the presence in homozygosity) of the variants Ile359Leu and Arg144Cys in the *CYP2C9* gene was associated with the improvement in markers of glycemic control, including glycated hemoglobin A1c (HbA1c)[21]. Therefore, genotyping of the *CYP2C9* gene may provide important additional information in predicting the adverse effects of these drugs and to assist physicians in prescribing oral hypoglycemic agents.

The ATP-sensitive potassium [ATP-sensitive K$^+$ (K-ATP)] channel plays a central role in mediating glucose-stimulated insulin release from pancreatic beta-cells (Figure 3). In physiological conditions, the rapid entry of glucose into the beta-cell results in an increase in the intracellular concentration of ATP, which promotes the closure of the K-ATP channel with consequent opening of the voltage-dependent calcium channel, elevation of intracellular calcium ion concentration and insulin secretion. The K-ATP channel is composed of two subunits: the sulphonylurea receptor (SUR1) and the pore-forming inward rectifier K$^+$ channel Kir6.2[22,23]. Genetic variants inactivating the *KCNJ11* (potassium inwardly-rectifying channel, subfamily J, member 11) gene, which encodes for the protein Kir6.2, and the ATP-binding cassette, subfamily C (CFTR/MRP), member 8 (*ABCC8*) gene, which encodes the SUR1 protein, are responsible for neonatal diabetes mellitus; conversely, activating mutations of

Figure 4 Organic cation transporter 1 plays a major role in drug uptake across the liver cell membrane. Single nucleotide polymorphism associated with organic cation transporter 1 may contribute to variation in response to metformin. AMPK: Adenosine 5'-monophosphate (AMP)-activated protein kinase; OCT1: Organic cation transporters 1.

these two genes lead to hyperinsulinism and neonatal hypoglycemia[24]. As an example of pharmacogenetics with important clinical implications, recent studies have found that diabetic patients carrying mutations in the *KCNJ11* gene respond better to treatment with sulfonylureas than to treatment with insulin[25-27].

Association of the polymorphism Ser1369Ala (serine 1369 to alanine substitution) in *ABCC8* with the antidiabetic efficacy of gliclazide was found in patients with T2DM, after two months of treatment[28]. In particular, patients with the genotype alanine/alanine had a greater reduction in either fasting plasma glucose or 2 h postload plasma glucose during oral glucose tolerance test, and a greater decrease in HbA1c levels compared to patients with the Serine/Serine genotype[28]. The variant Ser1369Ala in *ABCC8* is often associated in linkage disequilibrium with a variant, Glu23Lys (glutamine to lysine variant at position 23), in the *KCNJ11* gene, forming a haplotype that increases the risk of developing T2DM[29]. It has been observed that this haplotype displays large differences to the therapeutic effects of various sulfonylureas: greater to gliclazide, less apparent to tolbutamide, chlorpropamide and glimepiride, invariable in the glipizide and glibenclamide treatment group[30].

Interesting results, in this context, have been obtained from the study of the transcription factor 7-like 2 (*TCF7L2*) gene, which encodes a nuclear transcription factor that appears to play a role in beta-cell function. Genetic variants of *TCF7L2* are associated with increased risk of T2DM[3]. Recently, two variants of the *TCF7L2* gene, rs7903146 (G > T), and rs7903146 (C > T), have been shown to influence the therapeutic efficacy of sulfonylureas[31-33]. In particular, the reduction in both

HbA1c and fasting plasma glucose was higher in diabetic patients carrying either GG or CC genotypes[31-33]. In contrast, diabetic patients with the TT genotype in both the rs7903146 (G > T) and the rs7903146 (C > T) variants showed a lower response to sulfonylureas and appeared to be more prone to therapeutic failure[31-33].

Pharmacogenetics of metformin
Metformin, in use for control of diabetes since 1950s, is the first-line pharmacological therapy for T2DM. After oral administration, the drug is absorbed into the blood *via* the gastrointestinal tract, rapidly distributed in body tissues by travelling through specific transport proteins [including the organic cation transporters 1 (OCT1) and OCT2, the multidrug and toxin extrusion 1 (MATE1) transporters and MATE2-K, and the plasma membrane monoamine transporter (PMAT)] located on the cytoplasmic membrane of many cells, especially intestinal cells, liver cells and kidney cells[34], and excreted in the urine almost unchanged from the original drug. The individual's response to metformin is highly variable with less than 2/3 of treated patients achieving glycemic control[35]. Thus, identification of genetic variants that may influence the interindividual variability to metformin would be of major importance for the effective treatment of these patients. However, studies on the pharmacogenetics of metformin are relatively limited, mainly because its mechanism of action is still poorly defined. So far, most of the studies on this topic have involved the solute carrier family 22A1 (*SLC22A1*) gene, which by coding for the OCT1 transport protein, plays a key role in the cell absorption of the drug[36], and is essential for the anti-gluconeogenic effect of metformin into the liver[37] (Figure 4). It has been shown that polymorphisms of this gene (rs12208357; rs34130495; rs72552763; rs34059508), by reducing the functional capacity of OCT1, can alter the bioavailability of metformin and mitigate its hypoglycemic response in healthy people carrying these gene variants[37-39]. Recently, two polymorphisms of *SLC22A1* (rs628031 and rs36056065) have been associated with gastrointestinal side effects in diabetic patients treated with metformin[40]. At the same time, other authors[41,42] have also reported that the bioavailability of metformin was increased in healthy individuals carrying mutations of the *SLC22A2* gene, which encodes for the OCT2 transport protein. Variants of this gene, by adversely affecting OCT2 function, may decrease the renal clearance of metformin, and may contribute to increased plasma metformin levels with increased risk of hypoglycemic events.

Interindividual variation in metformin response has been recently reported in subjects with genetic variations in *SLC47A1* and *SLC47A2* genes coding for MATE1 and MATE2-K, respectively, which play important roles in the urine excretion of metformin. A better glycemic response to metformin, with lower HbA1c levels, has been reported in association with the *SLC47A1* gene variant rs2252281[43-46]. In contrast, the therapeutic response to metformin was reduced in diabetic patients

carriers of the variant rs12943590 in the *SLC47A2* gene[45,46]. Therefore, these observations imply that genetic variants of MATE1 and MATE2-K are important determinants of the therapeutic efficacy of metformin in patients treated with this drug. The first GWAS on the efficacy of metformin on glycemic control in diabetic patients resulted in the demonstration that a gene variant near ataxia telangiectasia mutated (*ATM*), rs11212617, is significantly associated with metformin treatment response in T2DM, more frequently with HbA1c levels < 7%[47]. The explanation of this phenomenon lies in the role ATM, the protein product of the *ATM* gene, plays in the context of insulin signaling and insulin action[48].

Thus, genetic variants of *SLC22A1* and *SLC22A2* may be determinant in the therapeutic efficacy of metformin. Furthermore, genotyping of *SLC22A1* and *SLC22A2* is useful in the management of diabetic patients under metformin theraphy.

Pharmacogenetics of thiazolidinediones

Genetic variants that can influence the pharmacogenetics of oral antidiabetic medications were also assessed in diabetic patients treated with pharmacogenetics of thiazolidinediones (TZDs) (pioglitazone and rosiglitazone). As agonists of peroxisome proliferator-activated receptor gamma (PPAR-γ), TZDs act as insulin-sensitizing, thus reducing the release of glucose from the liver and increasing glucose uptake in muscle[49]. The *PPAR-γ* gene has been extensively investigated in pharmacogenetic studies of TZDs, especially because genetic variants of this gene have been associated with an increased risk of T2DM[3]. However, pharmacogenetic studies with TZDs have shown conflicting results, probably due to insufficient sample size and low levels of statistical power[50]. Furthermore, it is worthy noting that the retrospective study design used in the majority of studies on pharmacogenetics has its own drawbacks, being able to expose to a variety of confounding and bias, including age, gender, ethnicity, lifestyle, concomitant use of other medications, *etc*. A similar discrepancy has emerged from studies on the genetic variants of the *CYP2C8* gene, which is responsible for metabolizing pioglitazone[50]. A reduction in the blood glucose-lowering effect of pioglitazone was recently observed in diabetic patients carriers of the truncation variant, Ser447X, of the lipoprotein lipase gene[51]. Another study has reported that the -420 C/G variant of the *resistin* gene promoter can also be used as an independent predictor of the reduction of fasting plasma glucose and insulin resistance by pioglitazone in T2DM[52]. As it is known, side effects of TZDs therapy include fluid retention and peripheral edema, worsening heart failure[53]. In this context, various genetic variations have been discovered in genes known to be involved in sodium and water reabsorption. Among these, the aquaporin 2 (*AQP2*) rs296766 variant and the *SLC12A1* rs12904216 variant, both of which have been associated with edema in T2DM patients treated with a TZD[54]. *AQP2* gene codes aquaporin-2, which function as a water channel in the

collecting duct of the kidney[55]. *SLC12A1* encodes the kidney-specific sodium-potassium-chloride cotransporter (NKCC2), which plays an important role in both urine concentration and NaCl reabsorption[54,56]. Therefore, it is quite evident that these variants may represent both a risk factor for the development of edema in diabetic patients during treatment with TZDs.

Pharmacogenetics of metiglinides

Metiglinides (repaglinide and nateglinide) are a class of rapid-acting, short duration insulin secretagogues that act in a manner similar to that of the sulfonylureas[57]. Nateglinide is also metabolized by the CYP2C9 enzyme of the cytochrome P-450 system, and gene variants of *CYP2C9* are associated with variability in glucose-lowering effect of nateglinide[58]. Repaglinide is metabolized by CYP2C8 and to a lesser degree by CYP3A4[59]. Also in this case, gene variants of *CYP2C8* have been associated with increased clearance of repaglinide, although with contradictory results[60]. The solute carrier organic anion transporter family member 1B1 (*SLCO1B1*) gene encodes for the organic anion transporting polypeptide, OATP1B1, which regulates cellular uptake of various drugs, including statins by the liver. Recent studies have reported the role of some variants of *SLCO1B1* in the pharmacokinetics of metiglinides[61-64]. For example, a more effective hypoglycemic effect of repaglinide was observed in diabetic patients carrying the Glu23Lys (E23K) polymorphism in the *KCNJ11* gene[65], and the rs13266634 variant in the *SLC30A8* gene[66]. Similarly, polymorphisms of neurogenic differentiation 1 (*NEUROD1*), also called beta2 (*NEUROD1/BETA2*), paired box gene 4[67] and uptake control 2[68] genes were also found to be associated with the hypoglycemic efficacy of repaglinide. An association of the variant G2677 T/A in the multidrug resistance gene, which encodes a multidrug efflux pump, with the variability in the pharmacokinetics of repaglinide was found recently in a Chinese study in healthy volunteers[69].

Pharmacogenetics of incretins

Glucagon-like peptide-1 (GLP-1) is part of the group of incretin hormones that are secreted from endocrine cells in the intestinal mucosa in response to meals. It mediates insulin secretion in a glucose-dependent manner and is easily inactivated after being secreted by the enzyme dipeptidyl peptidase-IV (DPP-IV). Recent pharmacological research has led to the development and synthesis of medications that are capable of acting at this level as both GLP-1 agonists (exenatide and liraglutide) and DPP-IV inhibitors (gliptins)[70]. Variants of the GLP-1 receptor gene have been shown to be associated with altered sensitivity to GLP-1[71]. Furthermore, whereas variants in the *TCF7L2* (rs7903146) and wolfram syndrome 1 (rs10010131) genes have been associated with a reduced response to exogenous GLP-1, variations in the *KCNQ1* (rs151290, rs2237892, and rs2237895) gene appear to alter the secretion of endogenous GLP-1[72]. The only significant study on the pharmacogenetics of gliptins

Figure 5 Pharmacogenetic testing. The pharmacogenetic test has the potential to provide personalized therapy based on individual genetic variability.

showed that three novel genetic loci (transmembrane protein 114, carbohydrate sulfotransferase 3 and Chymotrypsinogen B1/2) were identified, which affect GLP-1-induced insulin release during hyperglycemic clamp in nondiabetic Caucasian subjects[73].

CONCLUSION

Pharmacogenetics is an expanding area of research which seeks to understand how variations in the genome influence medication response. Pharmacogenetics has gained increasing attention in the context of translational medicine, providing an opportunity for personalized treatment strategies based on an individual's genetic makeup. The results obtained so far with the study of genetic variants in patients with T2DM (and other common diseases) may be used for the realization of a pharmacogenetic test, which can assist in making treatment decisions on the basis of each patient's genetic profile, thus improving the overall management of the disease and ensuring better results in terms of safety and therapeutic efficacy. The clinical use of pharmacogenetics, through the identification of individual genetic variants (genetic polymorphisms), can contribute to move to a more evidence-based and less empiric clinical management of patients, thereby avoiding treatment failures, while reducing the incidence of adverse drug reactions (Figure 5).

REFERENCES

1   **Hamburg MA**, Collins FS. The path to personalized medicine. *N Engl J Med* 2010; **363**: 301-304 [PMID: 20551152 DOI: 10.1056/NEJMP1006304]

2   **McCarthy MI**, Zeggini E. Genome-wide association studies in type 2 diabetes. *Curr Diab Rep* 2009; **9**: 164-171 [PMID: 19323962 DOI: 10.1007/s11892-009-0027-4]

3   **Brunetti A**, Chiefari E, Foti D. Recent advances in the molecular genetics of type 2 diabetes mellitus. *World J Diabetes* 2014; **5**: 128-140 [PMID: 24748926 DOI: 10.4239/WJD.V5.I2.128]

4   **Doria A**, Patti ME, Kahn CR. The emerging genetic architecture of type 2 diabetes. *Cell Metab* 2008; **8**: 186-200 [PMID: 18762020 DOI: 10.1016/J.Cmet.2008.08.006]

5   **Ingelman-Sundberg M**. Pharmacogenetics of cytochrome P450 and its applications in drug therapy: the past, present and future. *Trends Pharmacol Sci* 2004; **25**: 193-200 [PMID: 15063083 DOI: 10.1016/j.tips.2004.02.007]

6   **Vogel F**. Moderne Probleme der Humangenetik. *Ergeb Inn Med Kinderheilkd* 1959; **12**: 52-125 [DOI: 10.1007/978-3-642-94744-5_2]

7   **Mahgoub A**, Idle JR, Dring LG, Lancaster R, Smith RL. Polymorphic hydroxylation of Debrisoquine in man. *Lancet* 1977; **2**: 584-586 [PMID: 71400 DOI: 10.1016/S0140-6736(77)91430-1]

8   **Crowley JJ**, Sullivan PF, McLeod HL. Pharmacogenomic genome-wide association studies: lessons learned thus far. *Pharmacogenomics* 2009; **10**: 161-163 [PMID: 19207016 DOI: 10.2217/14622416.10.2.161]

9   **Daly AK**. Genome-wide association studies in pharmacogenomics. *Nat Rev Genet* 2010; **11**: 241-246 [PMID: 20300088 DOI: 10.1038/Nrg2751]

10   **Motsinger-Reif AA**, Jorgenson E, Relling MV, Kroetz DL, Weinshilboum R, Cox NJ, Roden DM. Genome-wide association studies in pharmacogenomics: successes and lessons. *Pharmacogenet Genomics* 2013; **23**: 383-394 [PMID: 20639796 DOI: 10.1097/FPC.0B013E32833D7B45]

11   **Kirchheiner J**, Bauer S, Meineke I, Rohde W, Prang V, Meisel C, Roots I, Brockmöller J. Impact of CYP2C9 and CYP2C19 polymorphisms on tolbutamide kinetics and the insulin and glucose response in healthy volunteers. *Pharmacogenetics* 2002; **12**: 101-109 [PMID: 11875364]

12   **Becker ML**, Visser LE, Trienekens PH, Hofman A, van Schaik RH, Stricker BH. Cytochrome P450 2C9 *2 and *3 polymorphisms and the dose and effect of sulfonylurea in type II diabetes mellitus. *Clin Pharmacol Ther* 2008; **83**: 288-292 [PMID: 17597710 DOI: 10.1038/sj.clpt.6100273]

13   **Kirchheiner J**, Brockmöller J, Meineke I, Bauer S, Rohde W, Meisel C, Roots I. Impact of CYP2C9 amino acid polymorphisms on glyburide kinetics and on the insulin and glucose response in healthy volunteers. *Clin Pharmacol Ther* 2002; **71**: 286-296 [PMID: 11956512 DOI: 10.1067/mcp.2002.122476]

14   **Elliot DJ**, Suharjono BC, Gillam EM, Birkett DJ, Gross AS, Miners JO. Identification of the human cytochromes P450 catalysing the rate-limiting pathways of gliclazide elimination. *Br J Clin Pharmacol* 2007; **64**: 450-457 [PMID: 17517049 DOI: 10.1111/j.1365-2125.2007.02943.x]

15   **Kidd RS**, Curry TB, Gallagher S, Edeki T, Blaisdell J, Goldstein JA. Identification of a null allele of CYP2C9 in an African-American exhibiting toxicity to phenytoin. *Pharmacogenetics* 2001; **11**: 803-808 [PMID: 11740344]

16   **Wang R**, Chen K, Wen SY, Li J, Wang SQ. Pharmacokinetics of glimepiride and cytochrome P450 2C9 genetic polymorphisms. *Clin Pharmacol Ther* 2005; **78**: 90-92 [PMID: 16003298 DOI: 10.1016/j.clpt.2005.03.008]

17   **Ragia G**, Petridis I, Tavridou A, Christakidis D, Manolopoulos VG. Presence of CYP2C9*3 allele increases risk for hypoglycemia in Type 2 diabetic patients treated with sulfonylureas. *Pharmacogenomics* 2009; **10**: 1781-1787 [PMID: 19891554 DOI: 10.2217/Pgs.09.96]

18   **Holstein A**, Plaschke A, Ptak M, Egberts EH, El-Din J, Brockmöller J, Kirchheiner J. Association between CYP2C9 slow metabolizer genotypes and severe hypoglycaemia on medication with sulphonylurea hypoglycaemic agents. *Br J Clin Pharmacol* 2005; **60**: 103-106 [PMID: 15963101 DOI: 10.1111/j.1365-2125.2005.02379.x]

19   **Bozkurt O**, de Boer A, Grobbee DE, Heerdink ER, Burger H, Klungel OH. Pharmacogenetics of glucose-lowering drug treatment: a systematic review. *Mol Diagn Ther* 2007; **11**: 291-302 [PMID: 17963417]

20   **Distefano JK**, Watanabe RM. Pharmacogenetics of Anti-Diabetes Drugs. *Pharmaceuticals* (Basel) 2010; **3**: 2610-2646 [PMID: 20936101 DOI: 10.3390/ph3082610]

21   **Zhou K**, Donnelly L, Burch L, Tavendale R, Doney AS, Leese G, Hattersley AT, McCarthy MI, Morris AD, Lang CC, Palmer CN, Pearson ER. Loss-of-function CYP2C9 variants improve therapeutic response to sulfonylureas in type 2 diabetes: a Go-DARTS study. *Clin Pharmacol Ther* 2010; **87**: 52-56 [PMID: 19794412 DOI: 10.1038/Clpt.2009.176]

22   **Shyng S**, Nichols CG. Octameric stoichiometry of the KATP channel complex. *J Gen Physiol* 1997; **110**: 655-664 [PMID: 9382894 DOI: 10.1085/Jgp.110.6.655]

23   **Winkler M**, Stephan D, Bieger S, Kühner P, Wolff F, Quast U. Testing the bipartite model of the sulfonylurea receptor binding site: binding of A-, B-, and A + B-site ligands. *J Pharmacol Exp Ther* 2007; **322**: 701-708 [PMID: 17495126 DOI: 10.1124/Jpet.107.123224]

24   **Flanagan SE**, Clauin S, Bellanné-Chantelot C, de Lonlay P, Harries LW, Gloyn AL, Ellard S. Update of mutations in the genes encoding the pancreatic beta-cell K(ATP) channel subunits Kir6.2 (KCNJ11) and sulfonylurea receptor 1 (ABCC8) in diabetes mellitus and hyperinsulinism. *Hum Mutat* 2009;

30: 170-180 [PMID: 18767144 DOI: 10.1002/Humu.20838]

25   **Pearson ER**, Flechtner I, Njølstad PR, Malecki MT, Flanagan SE, Larkin B, Ashcroft FM, Klimes I, Codner E, Iotova V, Slingerland AS, Shield J, Robert JJ, Holst JJ, Clark PM, Ellard S, Søvik O, Polak M, Hattersley AT. Switching from insulin to oral sulfonylureas in patients with diabetes due to Kir6.2 mutations. *N Engl J Med* 2006; **355**: 467-477 [PMID: 16885550 DOI: 10.1056/NEJMOA061759]

26   **Siklar Z**, Ellard S, Okulu E, Berberoğlu M, Young E, Savaş Erdeve S, Mungan IA, Hacihamdioğlu B, Erdeve O, Arsan S, Oçal G. Transient neonatal diabetes with two novel mutations in the KCNJ11 gene and response to sulfonylurea treatment in a preterm infant. *J Pediatr Endocrinol Metab* 2011; **24**: 1077-1080 [PMID: 22308870]

27   **Dupont J**, Pereira C, Medeira A, Duarte R, Ellard S, Sampaio L. Permanent neonatal diabetes mellitus due to KCNJ11 mutation in a Portuguese family: transition from insulin to oral sulfonylureas. *J Pediatr Endocrinol Metab* 2012; **25**: 367-370 [PMID: 22768671]

28   **Feng Y**, Mao G, Ren X, Xing H, Tang G, Li Q, Li X, Sun L, Yang J, Ma W, Wang X, Xu X. Ser1369Ala variant in sulfonylurea receptor gene ABCC8 is associated with antidiabetic efficacy of gliclazide in Chinese type 2 diabetic patients. *Diabetes Care* 2008; **31**: 1939-1944 [PMID: 18599530 DOI: 10.2337/Dc07-2248]

29   **Fatehi M**, Raja M, Carter C, Soliman D, Holt A, Light PE. The ATP-sensitive K(+) channel ABCC8 S1369A type 2 diabetes risk variant increases MgATPase activity. *Diabetes* 2012; **61**: 241-249 [PMID: 22187380 DOI: 10.2337/Db11-0371]

30   **Lang VY**, Fatehi M, Light PE. Pharmacogenomic analysis of ATP-sensitive potassium channels coexpressing the common type 2 diabetes risk variants E23K and S1369A. *Pharmacogenet Genomics* 2012; **22**: 206-214 [PMID: 22209866 DOI: 10.1097/FPC.0B013E32835001E7]

31   **Schroner Z**, Javorsky M, Tkacova R, Klimcakova L, Dobrikova M, Habalova V, Kozarova M, Zidzik J, Rudikova M, Tkac I. Effect of sulphonylurea treatment on glycaemic control is related to TCF7L2 genotype in patients with type 2 diabetes. *Diabetes Obes Metab* 2011; **13**: 89-91 [PMID: 21114608 DOI: 10.1111/J.1463-1326.2010.01324.X]

32   **Pearson ER**, Donnelly LA, Kimber C, Whitley A, Doney AS, McCarthy MI, Hattersley AT, Morris AD, Palmer CN. Variation in TCF7L2 influences therapeutic response to sulfonylureas: a GoDARTs study. *Diabetes* 2007; **56**: 2178-2182 [PMID: 17519421 DOI: 10.2337/Db07-0440]

33   **Holstein A**, Hahn M, Körner A, Stumvoll M, Kovacs P. TCF7L2 and therapeutic response to sulfonylureas in patients with type 2 diabetes. *BMC Med Genet* 2011; **12**: 30 [PMID: 21349175 DOI: 10.1186/1471-2350-12-30]

34   **Viollet B**, Guigas B, Sanz Garcia N, Leclerc J, Foretz M, Andreelli F. Cellular and molecular mechanisms of metformin: an overview. *Clin Sci* (Lond) 2012; **122**: 253-270 [PMID: 22117616 DOI: 10.1042/CS20110386]

35   **Kahn SE**, Haffner SM, Heise MA, Herman WH, Holman RR, Jones NP, Kravitz BG, Lachin JM, O'Neill MC, Zinman B, Viberti G; ADOPT Study Group. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N Engl J Med* 2006; **355**: 2427-2443 [PMID: 17145742 DOI: 10.1056/NEJMOA066224]

36   **Wang DS**, Jonker JW, Kato Y, Kusuhara H, Schinkel AH, Sugiyama Y. Involvement of organic cation transporter 1 in hepatic and intestinal distribution of metformin. *J Pharmacol Exp Ther* 2002; **302**: 510-515 [PMID: 12130709 DOI: 10.1124/Jpet.102.034140]

37   **Shu Y**, Sheardown SA, Brown C, Owen RP, Zhang S, Castro RA, Ianculescu AG, Yue L, Lo JC, Burchard EG, Brett CM, Giacomini KM. Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action. *J Clin Invest* 2007; **117**: 1422-1431 [PMID: 17476361 DOI: 10.1172/JCI30558]

38   **Shu Y**, Brown C, Castro RA, Shi RJ, Lin ET, Owen RP, Shear-

down SA, Yue L, Burchard EG, Brett CM, Giacomini KM. Effect of genetic variation in the organic cation transporter 1, OCT1, on metformin pharmacokinetics. *Clin Pharmacol Ther* 2008; **83**: 273-280 [PMID: 17609683 DOI: 10.1038/sj.clpt.6100275]

39   **Christensen MM**, Brasch-Andersen C, Green H, Nielsen F, Damkier P, Beck-Nielsen H, Brosen K. The pharmacogenetics of metformin and its impact on plasma metformin steady-state levels and glycosylated hemoglobin A1c. *Pharmacogenet Genomics* 2011; **21**: 837-850 [PMID: 21989078 DOI: 10.1097/FPC.0B013E32834C0010]

40   **Tarasova L**, Kalnina I, Geldnere K, Bumbure A, Ritenberga R, Nikitina-Zake L, Fridmanis D, Vaivade I, Pirags V, Klovins J. Association of genetic variation in the organic cation transporters OCT1, OCT2 and multidrug and toxin extrusion 1 transporter protein genes with the gastrointestinal side effects and lower BMI in metformin-treated type 2 diabetes patients. *Pharmacogenet Genomics* 2012; **22**: 659-666 [PMID: 22735389 DOI: 10.1097/FPC.0B013E3283561666]

41   **Song IS**, Shin HJ, Shim EJ, Jung IS, Kim WY, Shon JH, Shin JG. Genetic variants of the organic cation transporter 2 influence the disposition of metformin. *Clin Pharmacol Ther* 2008; **84**: 559-562 [PMID: 18401339 DOI: 10.1038/CLPT.2008.61]

42   **Wang ZJ**, Yin OQ, Tomlinson B, Chow MS. OCT2 polymorphisms and in-vivo renal functional consequence: studies with metformin and cimetidine. *Pharmacogenet Genomics* 2008; **18**: 637-645 [PMID: 18551044 DOI: 10.1097/FPC.0B013E328302CD41]

43   **Becker ML**, Visser LE, van Schaik RH, Hofman A, Uitterlinden AG, Stricker BH. Genetic variation in the multidrug and toxin extrusion 1 transporter protein influences the glucose-lowering effect of metformin in patients with diabetes: a preliminary study. *Diabetes* 2009; **58**: 745-749 [PMID: 19228809 DOI: 10.2337/DB08-1028]

44   **Jablonski KA**, McAteer JB, de Bakker PI, Franks PW, Pollin TI, Hanson RL, Saxena R, Fowler S, Shuldiner AR, Knowler WC, Altshuler D, Florez JC, Diabetes Prevention Program Research Group. Common variants in 40 genes assessed for diabetes incidence and response to metformin and lifestyle intervention in the diabetes prevention program. *Diabetes* 2010; **59**: 2672-2681 [PMID: 20682687 DOI: 10.2337/DB10-0543]

45   **Choi JH**, Yee SW, Ramirez AH, Morrissey KM, Jang GH, Joski PJ, Mefford JA, Hesselson SE, Schlessinger A, Jenkins G, Castro RA, Johns SJ, Stryke D, Sali A, Ferrin TE, Witte JS, Kwok PY, Roden DM, Wilke RA, McCarty CA, Davis RL, Giacomini KM. A common 5'-UTR variant in MATE2-K is associated with poor response to metformin. *Clin Pharmacol Ther* 2011; **90**: 674-684 [PMID: 21956618 DOI: 10.1038/CLPT.2011.165]

46   **Stocker SL**, Morrissey KM, Yee SW, Castro RA, Xu L, Dahlin A, Ramirez AH, Roden DM, Wilke RA, McCarty CA, Davis RL, Brett CM, Giacomini KM. The effect of novel promoter variants in MATE1 and MATE2 on the pharmacokinetics and pharmacodynamics of metformin. *Clin Pharmacol Ther* 2013; **93**: 186-194 [PMID: 23267855 DOI: 10.1038/CLPT.2012.210]

47   **Zhou K**, Bellenguez C, Spencer CC, Bennett AJ, Coleman RL, Tavendale R, Hawley SA, Donnelly LA, Schofield C, Groves CJ, Burch L, Carr F, Strange A, Freeman C, Blackwell JM, Bramon E, Brown MA, Casas JP, Corvin A, Craddock N, Deloukas P, Dronov S, Duncanson A, Edkins S, Gray E, Hunt S, Jankowski J, Langford C, Markus HS, Mathew CG, Plomin R, Rautanen A, Sawcer SJ, Samani NJ, Trembath R, Viswanathan AC, Wood NW, Harries LW, Hattersley AT, Doney AS, Colhoun H, Morris AD, Sutherland C, Hardie DG, Peltonen L, McCarthy MI, Holman RR, Palmer CN, Donnelly P, Pearson ER; The GoDARTS and UKPDS Diabetes Pharmacogenetics Study Group, The Wellcome Trust Case Control Consortium. Common variants near ATM are associated with glycemic response to metformin in type 2 diabetes. *Nat Genet*

2011; **43**: 117-120 [PMID: 21186350 DOI: 10.1038/NG.735]

48   **Yang DQ**, Kastan MB. Participation of ATM in insulin signalling through phosphorylation of eIF-4E-binding protein 1. *Nat Cell Biol* 2000; **2**: 893-898 [PMID: 11146653 DOI: 10.1038/35046542]

49   **Chen L**, Yang G. PPARs Integrate the Mammalian Clock and Energy Metabolism. *PPAR Res* 2014; **2014**: 653017 [PMID: 24693278 DOI: 10.1155/2014/653017]

50   **Becker ML**, Pearson ER, Tkáč I. Pharmacogenetics of oral antidiabetic drugs. *Int J Endocrinol* 2013; **2013**: 686315 [PMID: 24324494 DOI: 10.1155/2013/686315]

51   **Wang G**, Wang X, Zhang Q, Ma Z. Response to pioglitazone treatment is associated with the lipoprotein lipase S447X variant in subjects with type 2 diabetes mellitus. *Int J Clin Pract* 2007; **61**: 552-557 [PMID: 17394430 DOI: 10.1111/J.1742-1241.2006.01242.X]

52   **Makino H**, Shimizu I, Murao S, Kondo S, Tabara Y, Fujiyama M, Fujii Y, Takada Y, Nakai K, Izumi K, Ohashi J, Kawamura R, Yamauchi J, Takata Y, Nishida W, Hashiramoto M, Onuma H, Osawa H. A pilot study suggests that the G/G genotype of resistin single nucleotide polymorphism at -420 may be an independent predictor of a reduction in fasting plasma glucose and insulin resistance by pioglitazone in type 2 diabetes. *Endocr J* 2009; **56**: 1049-1058 [PMID: 19738363 DOI: 10.1507/ENDOCRJ.K08E-320]

53   **Karalliedde J**, Buckingham RE. Thiazolidinediones and their fluid-related adverse effects: facts, fiction and putative management strategies. *Drug Saf* 2007; **30**: 741-753 [PMID: 17722967]

54   **Chang TJ**, Liu PH, Liang YC, Chang YC, Jiang YD, Li HY, Lo MT, Chen HS, Chuang LM. Genetic predisposition and nongenetic risk factors of thiazolidinedione-related edema in patients with type 2 diabetes. *Pharmacogenet Genomics* 2011; **21**: 829-836 [PMID: 21934636 DOI: 10.1097/FPC.0B013E32834BFFF1]

55   **Knepper MA**, Wade JB, Terris J, Ecelbarger CA, Marples D, Mandon B, Chou CL, Kishore BK, Nielsen S. Renal aquaporins. *Kidney Int* 1996; **49**: 1712-1717 [PMID: 8743483]

56   **Ji W**, Foo JN, O'Roak BJ, Zhao H, Larson MG, Simon DB, Newton-Cheh C, State MW, Levy D, Lifton RP. Rare independent mutations in renal salt handling genes contribute to blood pressure variation. *Nat Genet* 2008; **40**: 592-599 [PMID: 18391953 DOI: 10.1038/NG.118]

57   **Yan FF**, Casey J, Shyng SL. Sulfonylureas correct trafficking defects of disease-causing ATP-sensitive potassium channels by binding to the channel complex. *J Biol Chem* 2006; **281**: 33403-33413 [PMID: 16956886 DOI: 10.1074/JBC.M605195200]

58   **Kirchheiner J**, Roots I, Goldammer M, Rosenkranz B, Brockmöller J. Effect of genetic polymorphisms in cytochrome p450 (CYP) 2C9 and CYP2C8 on the pharmacokinetics of oral antidiabetic drugs: clinical relevance. *Clin Pharmacokinet* 2005; **44**: 1209-1225 [PMID: 16372821 DOI: 10.2165/00003088-200544120-00002]

59   **Bidstrup TB**, Bjørnsdottir I, Sidelmann UG, Thomsen MS, Hansen KT. CYP2C8 and CYP3A4 are the principal enzymes involved in the human in vitro biotransformation of the insulin secretagogue repaglinide. *Br J Clin Pharmacol* 2003; **56**: 305-314 [PMID: 12919179 DOI: 10.1046/J.0306-5251.2003.01862.X]

60   **Tomalik-Scharte D**, Fuhr U, Hellmich M, Frank D, Doroshyenko O, Jetter A, Stingl JC. Effect of the CYP2C8 genotype on the pharmacokinetics and pharmacodynamics of repaglinide. *Drug Metab Dispos* 2011; **39**: 927-932 [PMID: 21270106 DOI: 10.1124/DMD.110.036921]

61   **Kalliokoski A**, Neuvonen M, Neuvonen PJ, Niemi M. The effect of SLCO1B1 polymorphism on repaglinide pharmacokinetics persists over a wide dose range. *Br J Clin Pharmacol* 2008; **66**: 818-825 [PMID: 18823304 DOI: 10.1111/J.1365-2125.2008.03287.X]

62 **Zhang W**, He YJ, Han CT, Liu ZQ, Li Q, Fan L, Tan ZR, Zhang WX, Yu BN, Wang D, Hu DL, Zhou HH. Effect of SLCO1B1 genetic polymorphism on the pharmacokinetics of nateglinide. *Br J Clin Pharmacol* 2006; **62**: 567-572 [PMID: 16796707 DOI: 10.1111/j.1365-2125.2006.02686.x]

63 **Kalliokoski A**, Neuvonen M, Neuvonen PJ, Niemi M. Different effects of SLCO1B1 polymorphism on the pharmacokinetics and pharmacodynamics of repaglinide and nateglinide. *J Clin Pharmacol* 2008; **48**: 311-321 [PMID: 18187595 DOI: 10.1177/0091270007311569]

64 **Kalliokoski A**, Backman JT, Neuvonen PJ, Niemi M. Effects of the SLCO1B1*1B haplotype on the pharmacokinetics and pharmacodynamics of repaglinide and nateglinide. *Pharmacogenet Genomics* 2008; **18**: 937-942 [PMID: 18854776 DOI: 10.1097/FPC.0B013E32830D733E]

65 **He YY**, Zhang R, Shao XY, Hu C, Wang CR, Lu JX, Bao YQ, Jia WP, Xiang KS. Association of KCNJ11 and ABCC8 genetic polymorphisms with response to repaglinide in Chinese diabetic patients. *Acta Pharmacol Sin* 2008; **29**: 983-989 [PMID: 18664331 DOI: 10.1111/J.1745-7254.2008.00840.X]

66 **Huang Q**, Yin JY, Dai XP, Wu J, Chen X, Deng CS, Yu M, Gong ZC, Zhou HH, Liu ZQ. Association analysis of SLC30A8 rs13266634 and rs16889462 polymorphisms with type 2 diabetes mellitus and repaglinide response in Chinese patients. *Eur J Clin Pharmacol* 2010; **66**: 1207-1215 [PMID: 20809084 DOI: 10.1007/S00228-010-0882-6]

67 **Gong ZC**, Huang Q, Dai XP, Lei GH, Lu HB, Yin JY, Xu XJ, Qu J, Pei Q, Dong M, Zhou BT, Shen J, Zhou G, Zhou HH, Liu ZQ. NeuroD1 A45T and PAX4 R121W polymorphisms are associated with plasma glucose level of repaglinide monotherapy in Chinese patients with type 2 diabetes. *Br J Clin Pharmacol* 2012; **74**: 501-509 [PMID: 22296034 DOI: 10.1111/J.1365-2125.2012.04202.X]

68 **Wang S**, Se YM, Liu ZQ, Lei MX, Hao-BoYang ZX, Nie SD, Zeng XM, Wu J. Effect of genetic polymorphism of UCP2-866 G/A on repaglinide response in Chinese patients with type 2 diabetes. *Pharmazie* 2012; **67**: 74-79 [PMID: 22393835]

69 **Xiang Q**, Cui YM, Zhao X, Yan L, Zhou Y. The Influence of MDR1 G2677T/a genetic polymorphisms on the pharmacokinetics of repaglinide in healthy Chinese volunteers. *Pharmacology* 2012; **89**: 105-110 [PMID: 22398664 DOI: 10.1159/000336345]

70 **Umpierrez GE**, Meneghini L. Reshaping diabetes care: the fundamental role of dipeptidyl peptidase-4 inhibitors and glucagon-like peptide-1 receptor agonists in clinical practice. *Endocr Pract* 2013; **19**: 718-728 [PMID: 23512382 DOI: 10.4158/EP12292.RA]

71 **Sathananthan A**, Man CD, Micheletto F, Zinsmeister AR, Camilleri M, Giesler PD, Laugen JM, Toffolo G, Rizza RA, Cobelli C, Vella A. Common genetic variation in GLP1R and insulin secretion in response to exogenous GLP-1 in nondiabetic subjects: a pilot study. *Diabetes Care* 2010; **33**: 2074-2076 [PMID: 20805279 DOI: 10.2337/DC10-0200]

72 **Smushkin G**, Sathananthan M, Sathananthan A, Dalla Man C, Micheletto F, Zinsmeister AR, Cobelli C, Vella A. Diabetes-associated common genetic variation and its association with GLP-1 concentrations and response to exogenous GLP-1. *Diabetes* 2012; **61**: 1082-1089 [PMID: 22461567 DOI: 10.2337/DB11-1732]

73 **'t Hart LM**, Fritsche A, Nijpels G, van Leeuwen N, Donnelly LA, Dekker JM, Alssema M, Fadista J, Carlotti F, Gjesing AP, Palmer CN, van Haeften TW, Herzberg-Schäfer SA, Simonis-Bik AM, Houwing-Duistermaat JJ, Helmer Q, Deelen J, Guigas B, Hansen T, Machicao F, Willemsen G, Heine RJ, Kramer MH, Holst JJ, de Koning EJ, Häring HU, Pedersen O, Groop L, de Geus EJ, Slagboom PE, Boomsma DI, Eekhoff EM, Pearson ER, Diamant M. The CTRB1/2 locus affects diabetes susceptibility and treatment via the incretin pathway. *Diabetes* 2013; **62**: 3275-3281 [PMID: 23674605 DOI: 10.2337/DB13-0227]

MINIREVIEWS

Psychotherapy in anorexia nervosa: What does the absence of evidence mean?

Emilio Gutiérrez, Olaia Carrera

Emilio Gutiérrez, Olaia Carrera, Unidad Venres Clínicos, Facultad de Psicología, Campus Vida, 15782 Santiago de Compostela, Spain
Emilio Gutiérrez, Departamento de Psicología Clínica y Psicobiología, Facultad de Psicología, Universidad de Santiago, Campus Vida, 15782 Santiago de Compostela, Spain
Author contributions: Both authors contributed to the manuscript.
Correspondence to: Emilio Gutiérrez, PhD, Departamento de Psicología Clínica y Psicobiología, Facultad de Psicología, Universidad de Santiago, Rua Xose María Suarez Nuñez, s/n, Campus Vida, 15782, Santiago de Compostela, Spain. emilio.gutierrez@usc.es
Telephone: +34-881-813730 Fax:. +34-881-813901

Abstract

Psychological treatment in anorexia nervosa (AN) is disheartening. Psychotherapy is the "treatment of choice" for adults though this recommendation is grounded on the absence of good quality clinical studies. This paper seeks to address the question of why improvements in the psychological treatment of AN have been thwarted, and why one of the best treatments available for adult patients is specialist supportive clinical management that has entered the stage through the backdoor of nonspecific supportive treatments originally serving as a placebo treatment assigned in randomized clinical trials to control for non-specific aspects of true psychosocial treatments. The possibility that most of the psychopathological features that characterise the AN symptoms profile could be best understood as the direct consequences of emaciation would enhance the utility of research with animal models for generating new hypothesis to improve AN treatment.

Core tip: This paper presents an alternative explanation conspicuously lacking in the literature as to the scarce evidence concerning the efficacy of psychotherapy in anorexia nervosa. The absence of data supporting a particular treatment undermines the basic tenets underlying the theory on which it is grounded, or is at least a defective translation of the theory into the "dos" and "don'ts" of manualized treatment. This assertion is elucidated by recent research on a placebo and nonspecific treatment that was found to be more effective than a number of specialized treatments.

INTRODUCTION

According to successive state-of-the-art reviews, contemporary treatment of anorexia nervosa (AN) is discouraging[1]. This pessimistic view is all the more disturbing when we consider the high rates of relapse after successful weight restoration in specialist inpatient settings[2]. With regard to pharmacological treatment, several reviews have reached a similar conclusion: No efficacy documented. "In general, studies have not consistently shown favourable results when pharmacotherapy is used for the treatment of anorexia" (p.114)[3]; and: "No pharmacological intervention for AN has a significant impact on weight gain or the psychological features of AN. Although mood may improve with tricyclic antidepressants, this outcome is not associated with improved weight

gain. Moreover, medication treatment for AN is associated with high dropout rates, suggesting that the currently available medications are not acceptable to individuals with AN"[4]. Furthermore, "In summary, at present, there is no convincing evidence of efficacy for any drug treatment for AN in either the acute or chronic phase of the illness; AN is one of the few psychiatric disorders of which this may be said"[5].

For example, in the case of antipsychotics, one of the drugs most extensively administered to AN patients, chlorpromazine, has not made much progress since it was deemed to be ineffective and toxic when administered to AN patients five decades ago[6]. In combination with supportive psychotherapy and bed rest (to avoid possible fractures due to episodes of hypotension), chlorpromazine was no better in the follow-up with respect to control patients not receiving the drug, although it caused severe extrapyramidal effects in up to 50% of cases[7]. With the advent of the so-called atypical antipsychotics, patients are free of these serious side effects, but still with no substantial positive effects, as concluded in a recent meta-analysis: "Compared with placebo, atypical antipsychotics were associated with a nonsignificant increase in body mass index (BMI), and a nonsignificant effect on the drive for thinness and body dissatisfaction. Compared with placebo or active control, these medications led to an increase in anxiety and overall eating disorder symptoms" (p.1)[8]. In spite of the poor response to the core symptoms of AN, pharmacotherapy continues to be frequently employed as part of a comprehensive treatment plan in an attempt to alleviate negative emotions and obsessive ruminations[9].

According to current clinical practice guidelines on psychosocial treatments[10,11], family therapy is recommended for medically stable adolescents [a practice that is supported by the findings of several randomized clinical trials (RCTs) excellently reviewed elsewhere[12,13]], whereas generic psychotherapy is the recommended "treatment of choice" for adults. However, this later recommendation is based on poor scientific evidence, that is, an expert's clinical confidence, consensus or opinion (Grade C), which indicates the absence of good quality clinical studies. Thus, the panoply of recommended therapeutic approaches spans the entire theoretical spectrum of psychotherapy, namely cognitive analytic therapy, cognitive behaviour therapy (CBT), Interpersonal Therapy (IPT), Focal Psychodynamic Therapy, and Family Therapy. However, although the philosophy inherent in these Guidelines is that not "everything-is-worth-the-same", the fact is that these recommendations lack any specific indications or specificity for selecting among them. This gloomy panorama was described some years ago with a succinct "Barely" to the question "Is evidence-based treatment of anorexia nervosa possible?" with the author concluding that: "New forms of treatment are needed for adults with anorexia nervosa, and the true value of family-based treatment for adolescents has yet to be established"[14].

Attempts at explaining the limited evidence on AN treatment efficacy have enumerated several factors responsible either for the lack of research, or the difficulties in performing more randomized controlled treatment trials. For instance, it is claimed that AN is an uncommon disorder with a relatively low prevalence in the general population. This hinders efforts to collect data from large patient samples, which consequently limits the methodological strength both in terms of the internal validity of most RCTs performed up to date (*e.g.*, randomization procedures, adequate control groups), and in terms of external validity as the lack of replication is the overriding norm. Furthermore, the ego-syntonic nature of AN symptomatology and the ambivalence of AN patients about recovery hinder their enrolment, participation, and the acceptability of treatments in clinical studies, which paves the way to the high dropout rates characteristic of AN clinical trials.

Though there may be sound reasons for understanding the lack of evidence regarding effective AN treatments, there is an alternative explanation conspicuously lacking in the literature, namely that the absence of documented efficacy is related to the current conceptualization of AN. In contrast to pharmacological treatments for AN, where the expected action of drugs are loosely connected to any aetiological or maintenance theory of a disorder, psychological treatments are supposed to be more committed with a particular conceptualization of AN. According to this stronger theoretical link, the absence of data supporting a particular AN treatment would be indicative of a failure in the basic tenets underlying the theory on which it is founded, or at least a defective translating of the theory into the "dos" and "don'ts" of manualized treatment.

A plausible hypothesis is that the lack of documented efficacy is related to the current conceptualization of AN, a view which has been reinforced by the outcomes of a new treatment originally termed as "Nonspecific Supportive Clinical Management"[15], which later, in the light of its unexpected efficacy, has lost its disconcerting "Nonspecific" qualification which has been euphemistically rebranded with the more reassuring label of "Specialist Supportive Clinical Management"[16]. However, the truth behind this relabeling reinforced by its abbreviation (SSCM) is that a placebo and non-specific treatment was found to be more effective than two specialized treatments, *i.e.*, CBT or IPT, and was as effective as these treatments at 5-year follow-up[17]. Moreover, in a further randomized controlled trial, SSCM was found to be as efficacious as the Maudsley Model of Anorexia Nervosa Treatment for Adults (MANTRA)[18,19], which was specifically devised to address the disorder according to a rather complex rationale in comparison to SSCM as illustrated in Table 1.

Furthermore, in a third RCT with a good retention rate (85%), SSCM was again similar to CBT at the end of the study[20]. Similar to differences with MANTRA shown in Table 1, differences between CBT and SSCM treatment manuals modified for treating a group of chronic

Table 1 Descriptive characteristics of two "novel" treatments for Anorexia Nervosa	
SSCM (previously, nonspecific supportive clinical management[16])	**MANTRA[19]**
"Nonspecific supportive clinical management was developed for the present study, and its aim was to mimic outpatient treatment that could be offered to individuals with anorexia nervosa in usual clinical practice. It combined features of clinical management and supportive psychotherapy. Clinical management includes education, care, and support and fostering a therapeutic relationship that promotes adherence to treatment. Supportive psychotherapy aims to assist the patient through use of praise, reassurance, and advice. The abnormal nutritional status and dietary patterns typical of anorexia nervosa were central to nonspecific supportive clinical management, which emphasized the resumption of normal eating and the restoration of weight and provided information on weight maintenance strategies, energy requirements, and relearning to eat normally. Information was provided verbally and as written handouts" (p. 742)	"MANTRA, aims to tackle maintaining factors related to rigid thinking styles (*e.g.*, perfectionism and obsessive-compulsive personality traits), avoidance of strong emotion, pro-anorectic beliefs and responses of close others. The modularised treatment that has resulted from this model maintains a focus on specific changes required in eating and weight within a motivational interviewing and cognitive behavioural therapy frame-work, including individualised case conceptualisation, in addition to summary letters from the therapist to the patient. Due to its modularized nature, it results in a targeted treatment of AN that is matched to the clinical symptoms, personality traits and neuropsychological profile of participants" (pp. 2-3)

SSCM: Specialist supportive clinical management; MANTRA: Maudsley model of anorexia nervosa treatment for adults; AN: Anorexia nervosa.

anorexia nervosa patients were significant. Thus, while CBT sessions were highly structured and included motivational enhancement strategies to improve motivation and willingness for change with the therapist adopting a directive stance, SSCM treatment was less structured and mostly circumscribed to what the patient brought to the session. In CBT psychoeducational material was discussed with the patients to increase their motivation, and their eating behaviours were confronted through the use of cognitive strategies and behavioural experiments, while patients receiving SSCM were encouraged to change their eating behaviours using advice and education about nutrition but they were not taught specific strategies. Finally, homework assignments and reviewing of the content of each session was systematically employed with patients receiving CBT, but no homework was employed in the SSCM, and when patients were provided with some educational material it was not necessarily reviewed in the next session.

Although the authors reported significant effects for CBT in the Weissman Social Adjustment Scale at the 6-mo follow-up, the better Eating Disorder Examination global scores and higher readiness for recovery at 12-mo follow-up were comparable to SSCM, these differences were not confirmed by sensitivity analysis using complete case data. In short, as the authors report: "both groups experienced significant changes on all primary and secondary measures of outcome at EOT, 6- and 12-mo follow-ups… The magnitude of improvements for health-related quality of life, depression and social adjustment were somewhat larger for SSCM, whereas those for ED symptoms and readiness for change were generally larger for CBT" (p.7)[20].

Thus, for the fourth time consecutively, SSCM has yielded a better or similar outcome in comparison to sophisticated treatments that presumably have sound theoretical foundations. Bearing in mind the constitutive non-specific nature of SSCM, it is rather paradoxical that SSCM should be the first AN treatment for adults to attain the distinction of a "well established psychosocial intervention" according to the criteria of the American Psychological Association Task Force for the Promotion

and Dissemination of Psychological Procedures[21].

The aim of treatment such as SSCM was to control for nonspecific therapeutic influences inherent in CBT, IPT, and MANTRA, as illustrated in their original conception in Table 1. In contrast to these highly structured, directive and modular treatments, SSCM is nondirective, *i.e.*, the patient meets the therapist in an unstructured setting with an emphasis on patient self-exploration and understanding. According to conventional practice in the psychotherapy research literature, SSCM is a non-psychotherapy control placebo condition[22]. Its two components, clinical management with a strong component in education and supportive psychotherapy only retain the contextual and relationship elements of any therapeutic encounter and it is devoid of any further specific ingredient[23]. Thus, clinical management and supportive therapy are the pragmatic baseline elements of SSCM that stand in contrast with the efficacy of theory driven specific treatments built according to an explicit focus on specific cognitive, behavioural and interpersonal domains which are highly structured, and introduced by the therapist in a directive way according to a manualized protocol. As in previous RCTs[24], SSCM was intended to be a routine type of outpatient treatment.

The unexpected good outcome of patients receiving SSCM has not led to any critical reappraisal of presumably "genuine" treatments, but instead the fundamental nature of SSCM as a non-specific placebo treatment has been called into questioned. Thus, some have argued the possibility of hidden specific active ingredients in SSCM[12], or even more astonishing is the recommendation that "it would have been desirable to have included a third treatment arm, such as 'treatment as usual' (TAU). Such a group would have controlled for non-specific therapy factors (of SSCM)" (p. 9)[20]. Hence, the burden of proof is placed on attesting that a non-specific treatment is truly non-specific instead of questioning the theory underlying MANTRA, CBT or IPT.

As for the employment of "TAU" as a control condition, the authors of the recent Anorexia Nervosa Treatment of OutPatients (ANTOP) study[25] in Germany should be congratulated as they have undertaken an am-

bitious multicentre randomized clinical trial in AN adults, whose methodological quality will be quite difficult to match in the coming years. However, as the authors themselves have acknowledged, once again the results were not as expected, *i.e.*, two manual-based specialized treatments- focal psychodynamic therapy (FPT), and enhanced CBT (CBT-E)-were not superior to an optimized treatment as usual.

In short, the list of specialized brand name treatments (CBT, IPT, MANTRA, FPT, CBT-E) with a "non-superiority" score over non-specific treatments initially conceived as a control condition continues to grow. Remarkably, the unfulfilled expectations regarding the efficacy of these specialized AN treatments have not prompted any reappraisal of theoretical assumptions underpinning these treatments, but rather has led to the euphemistic renaming of Nonspecific Supportive Clinical Management, as SSCM. However, as long as the aims of treatment are a logical corollary of the basic understanding of the disorder, the reluctance to critically reappraise the current conceptualization of AN is quite disturbing, bearing in mind that the parity with nonspecific treatments has been the norm since the first RCT in AN twenty six years ago[26]. In that study, a nonspecific form of individual therapy was already found to be more beneficial than family therapy in older patients, and the authors' proposed improving individual supportive therapy by incorporating: "more specific therapeutic components in the individual therapy" (p. 1056)[26]. Notwithstanding, the evidence-base for AN treatments gathered since the recommendation was proposed would suggest this goal is far from being accomplished.

This recurrent pattern of failed attempts at developing a successful treatment for AN challenges established beliefs underlying failed treatments and their specific components. In other words, the parity between nonspecific treatments (not based on any singular trait of AN), and specialized brand type treatments that have been the primary focus of research in recent decades cannot continue to be overlooked, which compels one to consider the possibility that conceptualizations of the disorder may be misleading, and research on AN treatment developed over the last four decades may have been on a misguided path[27].

Furthermore, with the publication of the ANTOP study the full spectrum of theoretical assumptions underlying treatments (cognitive, interpersonal, psychodynamic) have been encompassed, yet the outcomes of these treatments remain similar or marginally better than SSCM nonspecific treatment, or optimized treatment as usual. Strikingly, it makes no difference whatever the theoretical foundations of treatments are when compared with nonspecific treatments mimicking treatment as usual. This applies regardless of whether treatments are founded on theories aligned with weight and shape concerns prevalent in current AN diagnosis, as is the case of different cognitive behaviour treatments[28-30], or if they depart significantly from this mainstream thinking[31], or still if

they are derived from treatments developed for other disorders such as depression, as in the case of IPT[32].

Several options are open for overcoming the virtual impasse in current treatments for AN. To date the most common strategy has been to try to enhance[29] and/or refine existing treatments[33]. However this "more-of-the-same" solution fails to take into account that the similar efficacy of SSCM derives from what SSCM "lacks" in comparison to specialized treatment. For example, despite SSCM lacking specific techniques for addressing complex problems and psychological needs in AN patients, which frustrated more the therapists than the patients themselves[34], SSCM instilled hope in these patients[35]. Likewise, the process evaluation of the Maudsley Outpatient Study of Treatments for Anorexia Nervosa and Related conditions (MOSAIC) has revealed the relative unstructured agenda of SSCM, except from its focus on weight restoration and target symptoms, which was addressed in a supportive therapeutic atmosphere, and its slower pace and time to listen the patients were helpful characteristics in developing a positive therapeutic relationship. In the same line, the authors involved in the comparison of SSCM and CBT reported that: "there were no significant differences in patient ratings of therapeutic alliance of the two treatments. Although CBT-AN and SSCM use unique intervention strategies to achieve therapy aims, both were able to promote moderate therapeutic alliance in early treatment, increasing to strong therapeutic alliance in late treatment, to relatively the same degree" (p. 787)[36]. However, this common therapeutic alliance factor across the two treatments was affected by the absence of an emphasis on weight gain owing to these treatments administered to "severe and enduring anorexia nervosa"[20] patients. Under different circumstances, where the pressure to gain weight arouses anxiety, early therapeutic alliance seems not to be associated "with either the likelihood of completing treatment or subsequent weight gain. In contrast, both early and later weight gain were associated with the strength of subsequent alliance. These findings indicate that it might be advisable to focus on techniques to drive weight gain rather than rely on the therapeutic alliance to bring about therapeutic change" (p. 216)[37], which highlight the golden rule in AN treatment, *i.e.*, psychotherapy only works after the starvation process has been properly managed.

Moreover, as the authors involved in the MOSAIC project recognized: "The overlaps between MANTRA and SSCM remind us of the significance of the most basic features of any psychological treatment, such as regularity and predictability of appointments, being given time to talk, and above all the importance of a solid therapeutic relationship.., (Furthermore) two thirds of the patients interviewed about their experience in the process evaluation, embedded in the MOSAIC study, reported external factors that had influenced therapy outcome positively or negatively" (p. 137)[35], which underscores that being involved in an RCT does not exclude external interferences that may outweigh any involvement in an RCT.

Thus, the lack of any greater efficacy as compared to SSCM, profoundly undermines the conceptualization of AN underlying CBT, IPT, MANTRA, FPT and CBT-E. Either these fundamentals do not represent the essence of AN itself or there are flaws in the way the theory translates into treatment. And, by the same token, these elements purportedly reflecting the essence of the AN disorder might be epiphenomena with respect to its true essence and its maintenance factors, and are therefore irrelevant for the purposes of making them targets for treatment development. Should the latter assertion be correct, it would follow that treatments based on flawed assumptions may be not only ineffective, which is currently the norm, but worse still by being counterproductive and iatrogenic in preventing spontaneous remission: *i.e.*, by requiring patients to work on motives that are not so much in the patients' minds as in the minds of clinicians.

This assertion, which will undoubtedly prove unsettling in some quarters, and runs along the lines of a previous warning against firmly grounded beliefs governing routine treatment of AN patients: "an over-emphasis on weight/BMI and targets is inappropriate, misleading and potentially harmful. Although this view is not always greeted with enormous enthusiasm by some, others are relieved that this particular 'holy cow' is at last being challenged. It is important that we should all have an open mind to the possibility that one of the main tenets of our practice may actually be unhelpful"[38].

To contend that AN is an elusive, multifactorial disorder refractory to treatment should not preclude fresh avenues of research that may eventually generate alternative AN conceptualizations and treatment, *e.g.*, assessing concurring circumstances in spontaneous remissions, considering psychopathology as an epiphenomenon of malnutrition, and researching the signs of the AN disorder such as hyperactivity and its link to starvation. Anorexia nervosa is extreme in many ways, low incidence, high mortality, and a detrimental impact on health and quality of life, but it is the mental disorder with more objective signs that may serve to guide diagnosis. However, the diagnosis of AN has been primarily based on psychopathology (symptoms), and in the DSM-5[39] two of the three criteria for AN diagnosis involve symptom complexes of unobservable aspects, *e.g.*, body image disturbance or fear of fatness, whilst the only sign referring to the low bodyweight criterion lacks any clear standard of reference[40]. The DSM-5 not only discards a previously included sign such as amenorrhea, but also continues to ignore hyperactivity as a relevant sign[41]. However, as the developers of MANTRA have judiciously pointed out, the unexpected efficacy of SSCM has underscored that treatment: "that does not focus specifically on weight and shape concerns may just (if not more) effective treatments that do" (p. 357)[31]. It follows that the next step should be to further simplify AN treatment by removing the unnecessary and by incorporating new facets to it.

Much remains to be improved concerning existing AN treatments, as "contemporary etiological hypotheses have not produced informative research for predictably effective treatments" (p. 163)[42]. Nevertheless, animal research with analogous models of the human disorder, as is the case of Activity-based anorexia (ABA)[43], and semi-starvation induced hyperactivity[44] may be helpful in circumventing the assumption of an internal agency organized around a core motive (weight and shape concerns) underlying restrictive eating and excessive exercising in AN, an assumption which unfortunately has not advanced the treatment of this serious disorder[45].

The utility of these animal models in generating new hypothesis or for improving AN treatment is further enhanced by the possibility that most of the psychopathological features that characterise AN patients are best understood as the direct consequences of emaciation. The semi-starvation study of Minnesota[46] has shown that typical symptoms in AN patients (elation and sense of liveliness, irritability, obsessive thinking, depression, anxiety, decreased libido, decreased sociability, and a feeling of personal inefficiency) were associated to a state of starvation. The young men volunteers who lost 25% of body weight suffered insomnia, complained of cold hands and feet and showed an increased tolerance to heat. As their weight loss progressed bizarre food rituals began to show up including cutting food into small pieces, increased gum chewing, food hoarding and an inordinate interest in cooking, and the collection of food recipes[47,48].

Likewise, the recommendations by members of the Keys' research team may prove to be instructive with respect to the attitude and behaviour patterns of those who have experienced starvation: "One of the more profound changes which took place was the decreased sociability of the men (p. 30)... You are working with people who are living in a narrow world of their own interests and concerns, who must be patiently dealt with as individuals. They are similar to normal people, but have most of the peculiarities and sensitivities of normal people in a greatly exaggerated form" (p. 71)[49]. Further recommendations have been proposed for one of the signs of AN, *i.e.*, hypothermia: "The lowering of body temperature is more serious than it sounds, for it makes the starving very sensitive to cold weather. This means it is necessary to provide warm clothing, warm blankets, and some warm place where people can spend their daytime hours"(p. 62)[49]. Due to this hypothermia, the influence of ambient temperature and protection from the cold should be taken into consideration: "the fact that the starving are emotionally affected by the weather (p. 66)[49], and bad weather is a sufficient cause to explain" the frequent irritability and mood swings: "Such cyclic tendencies were markedly influenced by the weather; warm, sunny days brightened the spirits immeasurably, while cold, damp, cloudy days lowered the men further in their abyss of dejection"[49]. Notwithstanding, the role of ambient temperature (AT) and climate on the course of AN has been unduly overlooked in research[50].

Surprisingly most of these recommendations have gone unnoticed so far in spite of being explicitly men-

tioned in Gull's seminal paper, in which he coined the term "anorexia nervosa", and stated: "I have observed that in the extreme emaciation, when the pulse and respiration are slow, the temperature is below the normal standard. This fact together with the observation made by Chossat on the effect of starvation on animals, and their inability to digest food in the state of inanition, without the aid of external heat, has direct clinical bearings- it being often necessary to supply external heat as well as food to patients" (p.24) [51].

It is worth noting that the first recommendation for the treatment for AN was translational from findings on animal studies. Since Gull's time, however, successive "too human" conceptualizations of AN treatment have evolved that have, with some notable exceptions[52], relegated animal research as unworthy.

Twelve years ago a paper entitled "Ambient temperature: A neglected factor in Activity-based Anorexia"[53] brought to the forefront the deficient control and even the absence of reports of AT in research performed with the ABA animal models analogous to anorexia nervosa. This oversight of ambient temperature in studies with the ABA procedure -in which rats on a restricted feeding schedule can exercise freely in a running wheel- violated a well-established recommendation that "one cannot study food intake without specifying or controlling the conditions of temperature regulation"[54]. Accordingly, AT mishandling has been widespread in ABA research as self-starvation was acknowledged to be the core element in the conceptualization of ABA.

However, research performed in recent years has established the paramount importance of AT on the development[45,55], and more importantly on the reversal of exhausting running activity, severe weight loss and self-starvation of rats exposed to the ABA experimental procedure[56-58]. The manipulation of AT in animals exposed to ABA was an attempt to prevent hypothermia resulting from weight loss due to constraints in adequate energy replenishment exerted by the restricted feeding schedule. Together, these studies demonstrated that under the ABA experimental conditions the increase in AT over the thermoneutral range reversed excessive running and favoured weight gain.

The effect of ambient temperature on body weight gain was illustrated in a study where food restricted (1.5 h/d) sedentary rats were housed at either 21 ℃ or 32 ℃[59]. Under this arrangement cumulative food ingestion of rats housed at 21 ℃ for a 2-wk period was a 21.5% higher than that of rats maintained at 32 ℃, but rats housed at 21 ℃ gained even less weight than the rats housed at 32 ℃. This study also included two additional pair-fed groups of rats housed either at 21 ℃ or 32 ℃ that were fed according to the amount of food ingested the previous day by the animals housed at a different AT. Thus, under food restricted conditions, a warmer environment was more influential for body weight gain than food availability, and that under a given fixed food intake only increased AT enhanced body weight gain.

The absence of evidence-based efficacy in AN treat-

ment, and the parity of efficacy compared with a placebo nonspecific treatment such as SSCM are a clear red flag that something has gone awry in the development of treatments for a disorder mostly prevalent in young women that has remained unchanged for centuries. A promising alternative may be to look towards clues provided by animal research, but in the words of John Maynard Keynes: "The difficulty lies not so much in developing new ideas as in escaping from old ones".

REFERENCES

1 **Bulik CM**, Berkman ND, Brownley KA, Sedway JA, Lohr KN. Anorexia nervosa treatment: a systematic review of randomized controlled trials. *Int J Eat Disord* 2007; **40**: 310-320 [PMID: 17370290 DOI: 10.1002/eat.20367]
2 **Carter JC**, Blackmore E, Sutandar-Pinnock K, Woodside DB. Relapse in anorexia nervosa: a survival analysis. *Psychol Med* 2004; **34**: 671-679 [PMID: 15099421 DOI: 10.1017/S0033291703001168]
3 **Attia E**, Walsh BT. Behavioral management for anorexia nervosa. *N Engl J Med* 2009; **360**: 500-506 [PMID: 19179317 DOI: 10.1056/NEJMct0805569]
4 **Dunican KC**, DelDotto D. The role of olanzapine in the treatment of anorexia nervosa. *Ann Pharmacother* 2007; **41**: 111-115 [PMID: 17190846]
5 **Crow SJ**, Mitchell JE, Roerig JD, Steffen K. What potential role is there for medication treatment in anorexia nervosa? *Int J Eat Disord* 2009; **42**: 1-8 [PMID: 18683884 DOI: 10.1002/eat.20576]
6 **Dally PJ**, Oppenheim GB, Sargant W. Anorexia nervosa. *Brit Med J* 1958; **2**: 633-634
7 **Dally PJ**, Sargant W. Treatment and outcome of anorexia nervosa. *Brit Med J* 1966; **2**: 793-795
8 **Lebow J**, Sim LA, Erwin PJ, Murad MH. The effect of atypical antipsychotic medications in individuals with anorexia nervosa: a systematic review and meta-analysis. *Int J Eat Disord* 2013; **46**: 332-339 [PMID: 23001863 DOI: 10.1002/eat.22059]
9 **Casper RC**. How useful are pharmacological treatments in eating disorders? *Psychopharmacol Bull* 2002; **36**: 88-104 [PMID: 12397843]
10 **National Institute for Clinical Excellence**. Eating Disorders. Core Interventions in the Treatment and Management of Anorexia Nervosa. Bulimia Nervosa and Related Eating Disorders. London: NHS, 2004
11 **American Psychiatric Association**. Practice Guideline for the Treatment of Patients with Eating Disorders Third Edition. Washington, DC: American Psychiatric Association, 2006
12 **Watson HJ**, Bulik CM. Update on the treatment of anorexia nervosa: review of clinical trials, practice guidelines and emerging interventions. *Psychol Med* 2013; **43**: 2477-2500 [PMID: 23217606 DOI: 10.1017/S0033291712002620]
13 **Keel PK**, Haedt A. Evidence-based psychosocial treatments for eating problems and eating disorders. *J Clin Child Adolesc Psychol* 2008; **37**: 39-61 [PMID: 18444053 DOI: 10.1080/15374410701817832]
14 **Fairburn CG**. Evidence-based treatment of anorexia nervosa. *Int J Eat Disord* 2005; **37** Suppl: S26-S30; discussion S41-S42 [PMID: 15852315]
15 **McIntosh VV**, Jordan J, Carter FA, Luty SE, McKenzie JM, Bulik CM, Frampton CM, Joyce PR. Three psychotherapies for anorexia nervosa: a randomized, controlled trial. *Am J Psychiatry* 2005; **162**: 741-747 [PMID: 15800147 DOI: 10.1176/appi.ajp.162.4.741]
16 **McIntosh VV**, Jordan J, Luty SE, Carter FA, McKenzie JM, Bulik CM, Joyce PR. Specialist supportive clinical manage-

ment for anorexia nervosa. *Int J Eat Disord* 2006; **39**: 625-632 [PMID: 16937382 DOI: 10.1002/eat.20297]

17　**Carter FA**, Jordan J, McIntosh VV, Luty SE, McKenzie JM, Frampton CM, Bulik CM, Joyce PR. The long-term efficacy of three psychotherapies for anorexia nervosa: a randomized, controlled trial. *Int J Eat Disord* 2011; **44**: 647-654 [PMID: 21997429 DOI: 10.1002/eat.20879]

18　**Schmidt U**, Oldershaw A, Jichi F, Sternheim L, Startup H, McIntosh V, Jordan J, Tchanturia K, Wolff G, Rooney M, Landau S, Treasure J. Out-patient psychological therapies for adults with anorexia nervosa: randomised controlled trial. *Br J Psychiatry* 2012; **201**: 392-399 [PMID: 22995632 DOI: 10.1192/bjp.bp.112.112078]

19　**Wade TD**, Treasure J, Schmidt U. A case series evaluation of the Maudsley Model for treatment of adults with anorexia nervosa. *Eur Eat Disord Rev* 2011; **19**: 382-389 [PMID: 21280166 DOI: 10.1002/erv.1078]

20　**Touyz S**, Le Grange D, Lacey H, Hay P, Smith R, Maguire S, Bamford B, Pike KM, Crosby RD. Treating severe and enduring anorexia nervosa: a randomized controlled trial. *Psychol Med* 2013; **43**: 2501-2511 [PMID: 23642330]

21　**Chambless DL**, Hollon SD. Defining empirically supported therapies. *J Consult Clin Psychol* 1998; **66**: 7-18 [PMID: 9489259 DOI: 10.1037/0022-006X.66.1.7]

22　**Luborsky L**. The Dodo Bird Verdict Is Alive and Well-Mostly. *Clin Psychol Sci Prac* 2002; **9**: 2-12

23　**Fawcett J**, Epstein P, Fiester SJ, Elkin I, Autry JH. Clinical management--imipramine/placebo administration manual. NIMH Treatment of Depression Collaborative Research Program. *Psychopharmacol Bull* 1987; **23**: 309-324 [PMID: 3303100]

24　**Walsh BT**, Wilson GT, Loeb KL, Devlin MJ, Pike KM, Roose SP, Fleiss J, Waternaux C. Medication and psychotherapy in the treatment of bulimia nervosa. *Am J Psychiatry* 1997; **154**: 523-531 [PMID: 9090340]

25　**Zipfel S**, Wild B, Groß G, Friederich HC, Teufel M, Schellberg D, Giel KE, de Zwaan M, Dinkel A, Herpertz S, Burgmer M, Löwe B, Tagay S, von Wietersheim J, Zeeck A, Schade-Brittinger C, Schauenburg H, Herzog W. Focal psychodynamic therapy, cognitive behaviour therapy, and optimised treatment as usual in outpatients with anorexia nervosa (ANTOP study): randomised controlled trial. *Lancet* 2014; **383**: 127-137 [PMID: 24131861 DOI: 10.1016/S0140-6736(13)61746-8]

26　**Russell GF**, Szmukler GI, Dare C, Eisler I. An evaluation of family therapy in anorexia nervosa and bulimia nervosa. *Arch Gen Psychlatry* 1987; **44**: 1047-1056

27　**Gutierrez E**, Carrera O. Psychological therapies in anorexia nervosa: on the wrong track? *Br J Psychiatry* 2013; **202**: 384 [PMID: 23637113 DOI: 10.1192/bjp.202.5.384]

28　**Fairburn CG**, Shafran R, Cooper Z. A cognitive behavioural theory of anorexia nervosa. *Behav Res Ther* 1999; **37**: 1-13 [PMID: 9922553 DOI: 10.1016/S0005-7967(98)00102-8]

29　**Fairburn CG**, Cooper Z, Shafran R. Cognitive behaviour therapy for eating disorders: a "transdiagnostic" theory and treatment. *Behav Res Ther* 2003; **41**: 509-528 [PMID: 12711261 DOI: 10.1016/S0005-7967(02)00088-8]

30　**Pike KM**, Walsh BT, Vitousek K, Wilson GT, Bauer J. Cognitive behavior therapy in the posthospitalization treatment of anorexia nervosa. *Am J Psychiatry* 2003; **160**: 2046-2049 [PMID: 14594754 DOI: 10.1176/appi.ajp.160.11.2046]

31　**Schmidt U**, Treasure J. Anorexia nervosa: valued and visible. A cognitive-interpersonal maintenance model and its implications for research and practice. *Br J Clin Psychol* 2006; **45**: 343-366 [PMID: 17147101]

32　**Klerman GL**, Weissman MM, Rounsaville BJ, Chevron ES. Interpersonal Psychotherapy of Depression. New York: Basic Books, 1984

33　**Treasure J**, Schmidt U. The cognitive-interpersonal maintenance model of anorexia nervosa revisited: a summary of the evidence for cognitive, socio-emotional and interpersonal predisposing and perpetuating factors. *J Eat Disord* 2013; **1**:

13 [DOI: 10.1186/2050-2974-1-13]

34　**Waterman-Collins D**, Renwick B, Lose A, Kenyon M, Serpell L, Richards L, Boughton N, Treasure J, Schmidt U. Process evaluation of the MOSAIC Trial, Part I: Therapist experiences of delivering two psychological therapies for treatment of anorexia nervosa. *Eur Eat Disord Rev* 2014; **22**: 122-130 [PMID: 24446244 DOI: 10.1002/erv2278]

35　**Lose A**, Davies C, Renwick B, Kenyon M, Treasure J, Schmidt U. Process evaluation of the maudsley model for treatment of adults with anorexia nervosa trial. Part II: Patient experiences of two psychological therapies for treatment of anorexia nervosa. *Eur Eat Disord Rev* 2014; **22**: 131-139 [PMID: 24590563 DOI: 10.1002/erv.2279]

36　**Stiles-Shields C**, Touyz S, Hay P, Lacey H, Crosby RD, Rieger E, Bamford B, Le Grange D. Therapeutic alliance in two treatments for adults with severe and enduring anorexia nervosa. *Int J Eat Disord* 2013; **46**: 783-789 [PMID: 24014042 DOI: 10.1002/eat.22187]

37　**Brown A**, Mountford V, Waller G. Therapeutic alliance and weight gain during cognitive behavioural therapy for anorexia nervosa. *Behav Res Ther* 2013; **51**: 216-220 [PMID: 23435122 DOI: 10.1016/j.brat.2013.01.008]

38　**Lask B**, Frampton I. Anorexia nervosa--irony, misnomer and paradox. *Eur Eat Disord Rev* 2009; **17**: 165-168 [PMID: 19382127 DOI: 10.1002/erv.933]

39　**American Psychiatric Association**. Diagnostic and statistical manual of mental disorders. 5th ed. Arlington, VA: American Psychiatric Publishing, 2013

40　**Föcker M**, Knoll S, Hebebrand J. Anorexia nervosa. *Eur Child Adolesc Psychiatry* 2013; **22** Suppl 1: S29-S35 [PMID: 23224275 DOI: 10.1007/s00787-012-0358-6]

41　**Casper RC**. The 'drive for activity' and "restlessness" in anorexia nervosa: potential pathways. *J Affect Disord* 2006; **92**: 99-107 [PMID: 16448703]

42　**Halmi KA**. Perplexities and provocations of eating disorders. *J Child Psychol Psychiatry* 2009; **50**: 163-169 [PMID: 19220599 DOI: 10.1111/j.1469-7610.2008.01983.x]

43　**Epling WF**, Pierce WD, Stefan L. A theory of activity-based anorexia. *Int J Eat Disord* 1983; **3**: 27-46

44　**Broocks A**, Liu J, Pirke KM. Semistarvation-induced hyperactivity compensates for decreased norepinephrine and dopamine turnover in the mediobasal hypothalamus of the rat. *J Neural Transm Gen Sect* 1990; **79**: 113-124 [PMID: 2297396]

45　**Gutierrez E**. A rat in the labyrinth of anorexia nervosa: contributions of the activity-based anorexia rodent model to the understanding of anorexia nervosa. *Int J Eat Disord* 2013; **46**: 289-301 [PMID: 23354987 DOI: 10.1002/eat.22095]

46　**Keys A**, Brozek J, Henschel A, Mickelsen O, Taylor HL. The biology of human starvation (2 vols). Minneapolis: University of Minnesota Press, 1950

47　**Schiele BC**, Brozek J. Experimental neurosis resulting from semistarvation in man. *Psychosom Med* 1948; **10**: 31-50 [PMID: 18905659]

48　**Franklin JC**, Scheile BC. Observations on human behavior in experimental semi-starvation and rehabilitation. *J Clin Psychol* 1948; **4**: 28-45 [PMID: 18903450]

49　**Guetzkow HG**, Bowman PH. Men and Hunger: A Psychological Manual for Relief Workers. Elgin, IL: Brethren Publishing House, 1946

50　**Gutierrez E**, Carrera O, Vazquez R, Birmingham CL. Climate might be considered as a risk factor for anorexia nervosa? A hypothesis worth another look. *Eat Behav* 2013; **14**: 278-280 [PMID: 23910766 DOI: 10.1016/j.eatbeh.2013.05.006]

51　**Gull WW**. Anorexia nervosa (apepsia hysterica, anorexia hysterica). 1868. *Obes Res* 1997; **5**: 498-502 [PMID: 9385628]

52　**Tassignon MJ**, Merckaert I, De Coninck A, Cornelis MT. [Treatment of choroidal neovascular membranes in a case of pseudoxanthoma elasticum]. *Bull Soc Belge Ophtalmol* 1985; **214**: 107-112 [PMID: 2434171 DOI: 10.1037/a0034921]

53　**Gutiérrez E**, Vázquez R, Boakes RA. Activity-based anorex-

ia: ambient temperature has been a neglected factor. *Psychon Bull Rev* 2002; **9**: 239-249 [PMID: 12120785]

54   **Brobeck JR**. Food and temperature. In Pincus G. ed. Recent progress in hormone research. New York: Academic Press, 1960: 439-459

55   **Hillebrand JJ**, de Rijke CE, Brakkee JH, Kas MJ, Adan RA. Voluntary access to a warm plate reduces hyperactivity in activity-based anorexia. *Physiol Behav* 2005; **85**: 151-157 [PMID: 15924912 DOI: 10.1016/j.physbeh.2005.03.017]

56   **Gutiérrez E**, Baysari MT, Carrera O, Whitford TJ, Boakes RA. High ambient temperature reduces rate of body-weight loss produced by wheel running. *Q J Exp Psychol* (Hove) 2006; **59**: 1196-1211 [PMID: 16769620 DOI: 10.1080/17470210500417688]

57   **Gutierrez E**, Cerrato M, Carrera O, Vazquez R. Heat reversal of activity-based anorexia: implications for the treatment of anorexia nervosa. *Int J Eat Disord* 2008; **41**: 594-601 [PMID: 18446833 DOI: 10.1002/eat.20535]

58   **Farraway L**, Huizinga JD. Potassium channel activation by cromakalim affects the slow wave type action potential of colonic smooth muscle. *J Pharmacol Exp Ther* 1991; **257**: 35-41 [PMID: 1902258 DOI: 10.1016/j.psyneuen.2008.10.003]

59   **Cerrato M**, Carrera O, Vazquez R, Echevarría E, Gutierrez E. Heat makes a difference in activity-based anorexia: a translational approach to treatment development in anorexia nervosa. *Int J Eat Disord* 2012; **45**: 26-35 [PMID: 22170019 DOI: 10.1002/eat.20884]

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Ruggero Ridolfi, MD, Director, Immunotherapy and Somatic Cell Therapy Unit, Romagna Cancer Institute, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori, Via Piero Maroncelli, 40 - 47014 Meldola, Italy

Editorial office
Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
World Journal of Translational Medicine
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
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In press

3   **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA*

2006; In press

Organization as author

4   **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377   DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5   **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764   DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6   21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303   DOI:10.1136/bmj.325.7357.184]

Volume with supplement

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Issue with no volume

8   **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900   DOI:10.1097/00003086-200208000-00026]

No volume or issue

9   Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books
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10   **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

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Author(s) and editor(s)

12   **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13   **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14   **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15   Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16   **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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