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PHARMACOGENETIC STUDY ON METABOLIC DISORDERS INDUCED BY PSYCHOTROPIC AND IMMUNOSUPPRESSANT DRUGS

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Département de Psychiatrie (DP-CHUV)

PHARMACOGENETIC STUDY ON METABOLIC DISORDERS INDUCED BY PSYCHOTROPIC AND IMMUNOSUPPRESSANT DRUGS

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par

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Pharmacogenetic Study on Metabolic Disorders Induced by Psychotropic and Immunosuppressant Drugs

Lausanne, le 4 juillet 2016

pour Le Doyen
de la Faculté de Biologie et de Médecine

Prof. Pierre-Alexandre Bart

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"Twenty years from now you will be more disappointed by the things that you didn't do than by the ones you did do, so throw off the bowlines, sail away from safe harbor, catch the trade winds in your sails. Explore, Dream, Discover." – Mark Twain

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List of Acronyms and Abbreviations

5HT(R)	Serotonin (Receptor)
AgRP	Agouti-Related Peptide
ARC	Arcuate Nucleus
BBB	Blood Brain Barrier
BMI	Body Mass Index
CART	Cocaine and Amphetamine Related Transcript
CG	Candidate Gene
CNI	Calcineurin Inhibitor
CNS	Central Nervous System
CNV	Copy Number Variation
CoLaus	*Cohorte Lausannoise*
CRTC1	CREB-regulated transcription coactivator 1
CVD	Cardiovascular Disease
DNA	Desoxirribonucleic Acid
DP-CHUV	Department of Psychiatry – *Centre Hospitalier Universitaire Vaudois*
FGA	First Generation Antipsychotics
FKBP5	FK506 Binding Protein 5
FTO	Fat mass and Obesity-associated gene
GABA	Gamma Aminobutyric Acid
GIANT	Genetic Investigation of Anthropometric Traits consortium
GWAS	Genome Wide Association Studies
HDL-C	High Density Lipoprotein Cholesterol
HLA	Human leukocyte antigen
LD	Linkage Disequilibrium
LDL-C	Low Density Lipoprotein Cholesterol
LEP (R)	Leptine (Receptor)
MCH	Melanin Concentrating Hormone
MCR	Melanocortin Receptor
NODAT	New Onset Diabetes After Transplant
NPY	Neuropeptide Y
PCK1	Phosphoenol Piruvate Carboxikinase 1
POMC	Pro-Opiomelanocortin
SEP	Socio Economic Position
SES	Socioeconomic factors
SGA	Second Generation Antipsychotics
SNP	Single Nucleotide Polymorphism
SOT	Solid Organ Transplant
STCS	Swiss Transplant Cohort Study
T2DM	Type 2 Diabetes Mellitus
TDM	Therapeutic Drug Monitoring
TRH	Thyrotropin Releasing Hormone
UCP	Uncoupling Protein
w-GRS	Weighted Genetic Risk Scores
α-MSH	Alpha Melanocyte Stimulating Hormone

Summary

Obesity has become a worldwide epidemic with negative impact on health leading to serious consequences such as metabolic and cardiovascular disease, type 2 diabetes, musculoskeletal disorders and some cancers. Psychiatric and transplanted populations are at risk of metabolic disorders with a significant reduction in life expectancy when compared to general populations. Several factors such as the pharmacological treatment, the illness itself or socioeconomic (SES) factors as well as genetic factors influence the development of common obesity. Weight gain is the first step leading to obesity and metabolic disorders. The aim of the present thesis work is to study and describe the factors (notably genetic and SES factors) related to weight gain and metabolic outcomes in two high risk collectives (psychiatric and transplanted populations). To do so, psychiatric and transplanted populations under psychotropic and immunosuppressant treatments, respectively, were followed over time.

Several genetic factors were associated with metabolic outcomes in both studied populations. More specifically, in psychiatric populations, carriers of the *PCK1 AA* genotype had lower BMI units when compared to non-carriers. Additionally, this polymorphism was significantly associated with BMI, waist circumference and lipid levels, particularly among women younger than 45 years. An analysis combining both polymorphisms of *PCK1* and *CRTC1* (an upstream gene of the *PCK1* previously associated with weight gain in our psychiatric populations), showed that carriers of the *PCK1 AA* genotype and *CRTC1 G-allele* had lower BMI units during psychotropic treatment when compared to non carriers. The combined analysis of several variants into genetic risk scores (GRS) showed an association with BMI in three psychiatric samples. Extremes of the GRS (i.e. p5 vs p95) showed 1.89 kg/m^2 of BMI difference when combining all studied psychiatric samples. When stratifying by gender, stronger associations were found in men, whereas no association was found in women. In the transplanted samples, GRS showed associations with BMI and with New Onset Diabetes After Transplant (NODAT). Moreover, some of the variants predicted an increase of 10% or more of weight and NODAT one year after transplantation.

Regarding SES factors, an exploratory analysis showed that living alone and occupational status (including people with disability pension) were associated with BMI and weight change in psychiatric populations. Additionally, a Swiss socioeconomic position index (integrating income, education, occupation and housing conditions) was inversely correlated with BMI.

The results found in this project explore further the risk factors for metabolic disorders within at risk populations, improving the understanding of interindividual variability in weight gain and metabolic syndrome, and contributing towards personalized medicine.

Résumé

L'obésité est devenue une épidémie mondiale avec un impact négatif sur la santé, menant à des troubles métaboliques et cardiovasculaires, à des diabètes du type 2, à des troubles musculo-squelettiques et/ou à certains cancers. Les populations psychiatriques et transplantées sont à risque de développer ces troubles métaboliques avec une réduction de l'espérance de vie. Plusieurs facteurs dont le traitement pharmacologique, la maladie ou le niveau socio-économique (NSE) ainsi que des facteurs génétiques ont été décrits comme impliqués dans le développement de l'obésité. Le but de ce travail de thèse est d'étudier et de décrire les facteurs (notamment génétiques et NSE) liés à la prise de poids et à d'autres symptômes métaboliques dans deux populations (psychiatrique et transplantée). C'est pour cela que nous avons instauré un suivi de populations psychiatriques prenant des psychotropes, ainsi qu'un suivi de populations transplantées sous traitement immunosuppresseur.

Plusieurs facteurs génétiques ont été associés à des paramètres métaboliques dans les deux populations. Plus spécifiquement, dans la population psychiatrique, les porteurs du génotype *AA* du *PCK1* ont montré un Indice de Masse Corporelle (IMC) plus faible. En outre, ce polymorphisme a montré des associations significatives avec l'IMC, le tour de taille et les niveaux lipidiques, en particulier chez les femmes de moins de 45 ans. Une analyse combinant deux polymorphismes du *PCK1* et du *CRTC1* (gène en amont de *PCK1* déjà associé à la prise de poids dans notre population psychiatrique) montre que les porteurs du génotype *AA* du *PCK1* et de l'allèle *G* du *CRTC1*, ont un IMC plus faible au cours du traitement par rapport aux non-porteurs. La combinaison de différents polymorphismes dans des scores de risque génétique (GRS) a montré une association significative avec l'IMC dans plusieurs cohortes psychiatriques. La différence d'IMC entre les extrêmes (p5 vs p95) du GRS est de 1.89 kg/m^2 dans les trois cohortes psychiatriques étudiées. Des effets plus forts ont été trouvés chez les hommes tandis qu'aucune association n'est constatée chez les femmes. Dans la population transplantée, plusieurs GRS ont montré des associations avec l'IMC et avec le développement du diabète post-transplantation (NODAT). Par ailleurs, ces facteurs génétiques intégrés dans un modèle clinique, améliorent la prédiction d'une prise de poids de 10% ou plus et de la survenue d'un diabète une année après transplantation.

Concernant les facteurs NSE, une analyse exploratoire montre que le fait de vivre seul ainsi que la catégorie professionnelle sont associés à l'IMC et à la prise pondérale dans la population psychiatrique. En outre, un indice socio-économique Suisse (intégrant le revenu, la formation, le métier et les conditions de logement) a été inversement corrélé avec l'IMC et d'autres paramètres métaboliques.

Les résultats présentés dans ce projet permettent d'expliquer et de comprendre, en partie, la variabilité interindividuelle dans la prise de poids et l'apparition de symptômes métaboliques dans deux populations à risque, pouvant ainsi contribuer à une médecine personnalisée.

Résumé large publique

L'obésité est devenue une épidémie mondiale avec un impact négatif sur la santé, menant à des maladies cardiovasculaires et/ou à la survenue d'un diabète. Les populations psychiatriques et transplantées sont à risque de développer ce type de problèmes avec une réduction de l'espérance de vie. Plusieurs facteurs dont le traitement pharmacologique, la maladie ou le niveau socioéconomique (NSE) ainsi que des facteurs génétiques ont été décrits dans le développement de l'obésité. Le but de ce travail de thèse est d'identifier les facteurs (notamment génétiques et NSE) liés à la prise de poids, à la survenue d'un diabète ou à l'altération du cholestérol sanguin dans deux populations (psychiatrique et transplantée) à risque élevé de développer ces maladies. C'est pour cela qu'un suivi des populations psychiatriques et transplantées sous médication pouvant induire une forte prise de poids a été instauré dans la routine clinique. Dans le cadre de cette thèse, nous avons montré que plusieurs facteurs génétiques sont associés à la prise de poids. Plus spécifiquement, dans la population psychiatrique, nous avons observé que les individus ayant une variation sur un gène régulant la production de glucose ont montré un Indice de Masse Corporelle (IMC) plus faible. En outre, cette variation génétique est significativement associée au tour de taille et aux niveaux de lipides sanguins, en particulier chez les femmes de moins de 45 ans. Dans une deuxième partie, nous avons étudié la combinaison de différentes variations génétiques intégrées dans un score de risque génétique. Ce score a montré une association significative avec l'IMC dans plusieurs cohortes psychiatriques. Dans les trois cohortes étudiées, nous avons calculé la différence d'IMC entre les individus situés aux extrêmes du score (les 5% plus bas ; risque faible et les 5% plus haut ; risque élevé), avec une différence de 1.89 kg/m^2. Des effets plus importants ont été trouvés chez les hommes tandis qu'aucune association n'est constatée chez les femmes. Dans la population transplantée, plusieurs scores génétiques testés ont montré des associations avec l'IMC et avec le développement du diabète après transplantation. Par ailleurs, ces facteurs génétiques intégrés dans un modèle clinique, améliorent la prédiction pour une prise de poids égale ou supérieure à 10% et pour la survenue d'un diabète une année après transplantation.

Concernant les facteurs socio-économiques, une analyse exploratoire montre que le fait de vivre seul ainsi que la catégorie professionnelle sont associés à l'IMC et à la prise pondérale dans une population psychiatrique. En outre, nous avons constaté que les individus ayant un indice de position socio-économique élevé (indice intégrant le revenu, le niveau de formation, le métier et les conditions de logement) ont des valeurs plus faibles d'IMC que ceux ayant un index faible.

Les résultats présentés dans cette thèse permettent d'expliquer et de comprendre, en partie, la variabilité de la sensibilité de chaque patient envers les problèmes métaboliques, ce qui peut contribuer à une médecine personnalisée.

INTRODUCTION

1. Generalities of obesity

1.1 Epidemiological factors

Overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health (1). The Body Mass Index (BMI) remains among the most commonly used assessment of weight in obesity studies and has been correlated to body fat content. Obesity can lead to a number of chronic diseases, including type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD), musculoskeletal disorders and some cancers being the fifth most common risk factor for death in the general population (2, 3). According to World Health Organization (WHO), over the last decades, obesity has more than doubled all over the world. Thirty nine percent of adults aged 18 years and over were overweight (25 kg/m^2≤ BMI <30 kg/m^2) in 2014, and 13% were obese (BMI ≥30 kg/m^2) (1). The epidemic of obesity is not limited to developed countries but has been rising in most countries, becoming a global phenomenon. Between 1980 and 2008, the number of overweight and obese adults in developing countries has more than tripled to reach 900 million of adult cases (4). The economic impact of obesity should not be underestimated including direct costs (i.e. medical, preventive, diagnostic and treatment services directly related to obesity) and indirect ones (i.e. obesity related co-morbidities as well as premature mortality and disability) (5). A recent published review concluded that obesity absorbs a huge amount of health-care resources, however it is difficult to estimate the total costs, especially the indirect ones (6).

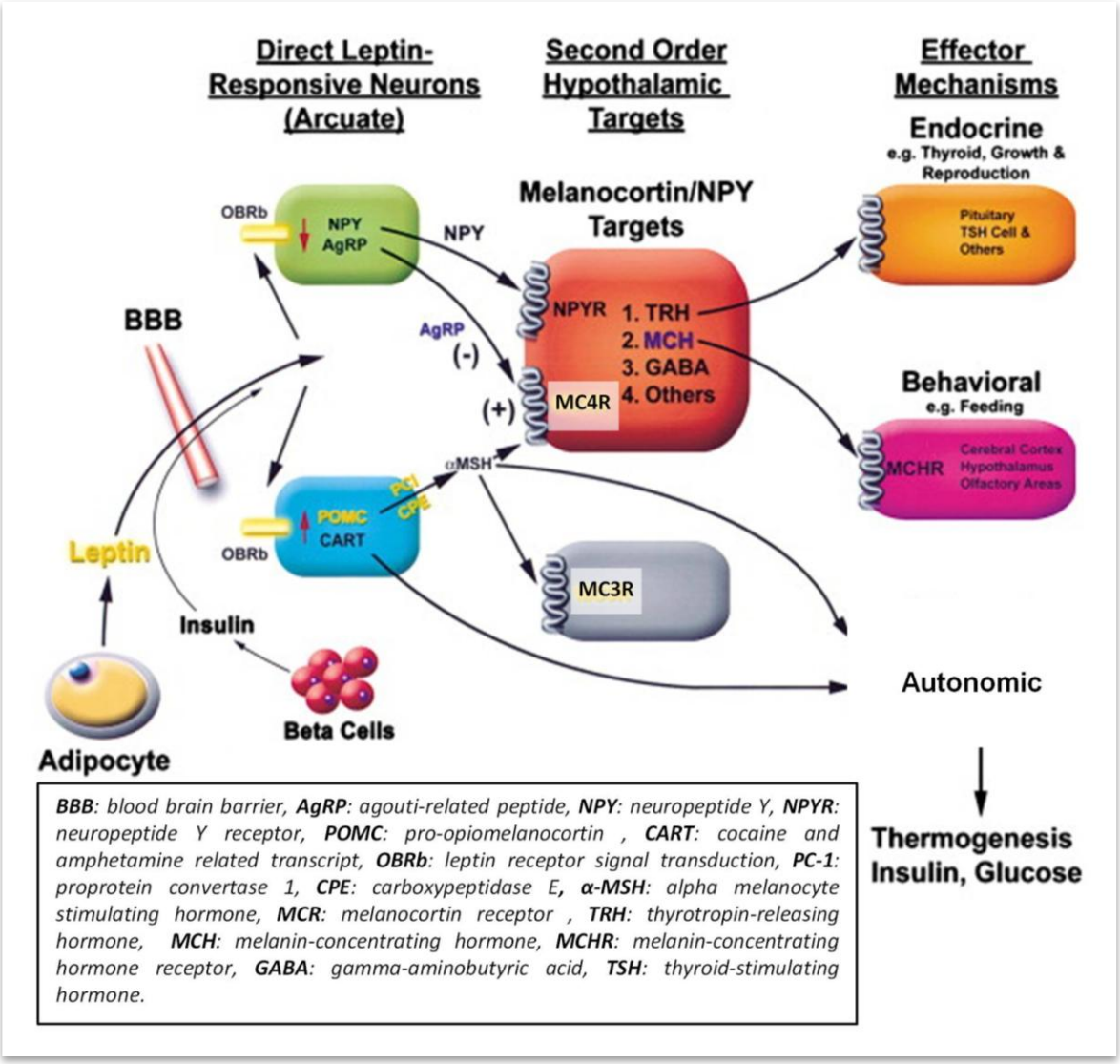
1.2 Obesity and the regulation of energy balance

Since obesity is preventable (1) a better understanding of the disease is crucial. The capacity of storing fat is a trait that has been selected during many years of human evolution. The *thrifty gene* hypothesis supports the idea that humans evolved efficiently to store excess of energy in order to deal with famine periods (7). Part of this energy is stored in the adipose cells which were thought to be a passive storage organ. However, over the past decades, the metabolic role of adipocytes on endocrine regulation and energy balance (i.e. the balance

between energy intake and expenditure) has been well described, as well as its connection with the Central Nervous System (CNS). Energy balance is controlled centrally by the CNS through neuroendocrine pathways regulation. From a molecular point of view, the regulation of energy intake is divided in short- and long- term regulation systems. A short-term regulation refers to appetite and satiety signals such as Glucagon-like peptide-1, which can induce reduction in food intake or Ghrelin, which stimulates food intake by acting in the hypothalamus (8). The long term-regulation mechanisms are related to adiposity signals (leptin and insulin among others) reflecting the status of energy storages. Not surprisingly, the short- and long- term systems are interrelated (9).

The best characterized and clinically relevant pathway in energy balance regulation is the leptin-melanocortin pathway (Figure 1), where seven proteins of this pathway have been related to weight regulation and obesity. Energy balance is regulated through brain factors mainly located in the hypothalamic arcuate nucleus (ARC). This includes agouti-related peptide (AgRP) and neuropeptide Y (NPY), which are produced by neurons with orexigenic (feeding-inducing) properties. Near to the orexigenic neurons, there are pro-opiomelanocortin (POMC) and cocaine and amphetamine related transcript (CART), expressed by neurons with anorexigenic properties (10). The hormone leptin (LEP) secreted in the adipocytes crosses the Blood Brain Barrier (BBB) and through OBRb receptors acts on the orexigenic and anorexigenic neurons. LEP stimulates (+) the alpha melanocyte stimulating hormone (α-MSH) production, an agonist of the melanocortin-4 receptor (MC4R) and inhibits (-) the AgRP, an antagonist of MC4R. Activation of MC4R by α-MSH reduces food intake whereas suppression of MC4R by AgRP increases feeding and diminishes the hypophagic response to LEP (11). MC4R expressing neurons receive the leptin-regulated signals and other signals such as NPY. MC4R neurons include the thyrotropin-releasing hormone (TRH, thyroid regulation), the melanin-concentrating hormone (MCH, feeding regulation) and gamma-aminobutyric acid (GABAergic) neurons acting on other neurons also implicated in energy balance. As a result, these mechanisms have implications at endocrine, behavioral and autonomic levels related to growth, feeding and energy expenditure regulation (12) .

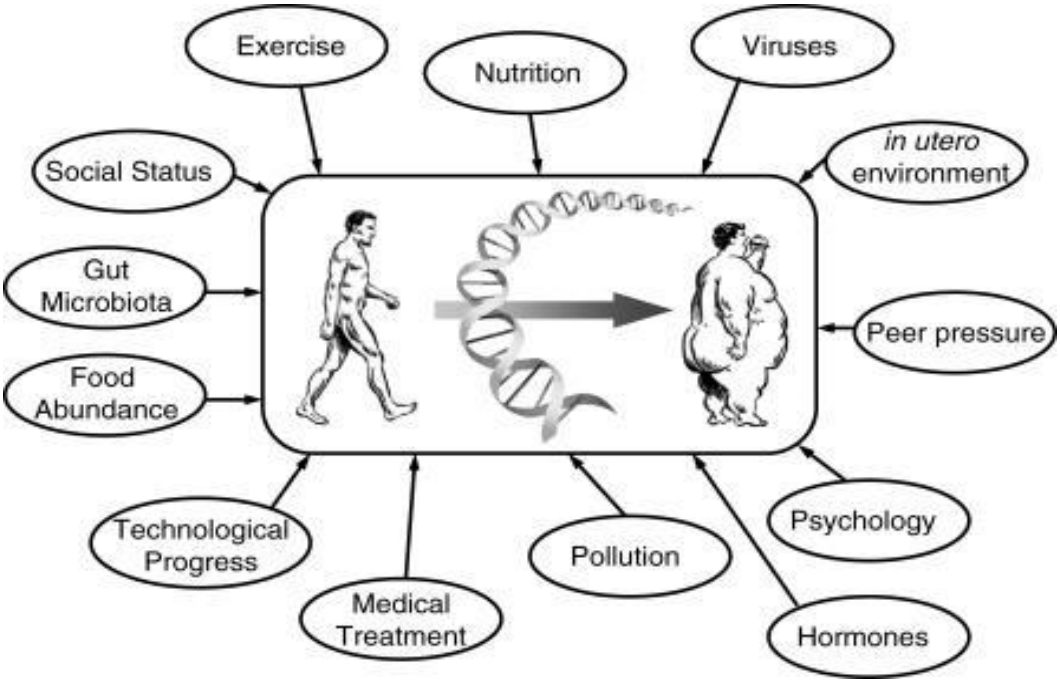
Figure 1. The leptin-melanocortin pathway regulating energy homeostasis. Adapted from (12)



2. Factors influencing obesity

The onset of obesity and metabolic disorders is the result of the combination of several factors, including environmental and genetic factors as well as gene-environment interactions (Figure 2).

Figure 2: Gene, environment and gene-environment interactions influencing obesity (13)



2.1 Environmental factors

Part of the environmental factors influencing energy balance refers to energy expenditure (i.e. physical activity) and energy intake (i.e. metabolic rate and food intake). These factors may be highly variable between individuals (14). In addition, measuring these covariates might be quite difficult in an experimental setting. Body mass composition differs as well between genders with women having more body fat and men more central distribution of fat. In both genders, weight gain has been described until 60-65 years, being the largest increase between the ages of 20 to 40 years (14-16). Also, a critical role of environmental factors in the development of obesity is highlighted in several societies (17). In general population, socioeconomic factors (SES) have been described as moderators of obesity. A pattern of inverse relationship between SES and

obesity has been reported in women from developed societies, whereas among men this pattern is less clear (18, 19). In contrast, in developing societies higher SES has been associated with higher obesity prevalence (20). Finally, studies conducted in children found a higher prevalence of obese children among parents with lower SES and educational level status (21).

In addition, recent research has revealed a new insight into obesity risk factor, suggesting that gut microbiota is implicated in weight gain, regulating fat metabolism, energy harvest, storage, and expenditure (22, 23). The gut microbiota composition differs between lean and obese individuals (24) and the dietary variation and caloric intake may induce changes in gut microbiome composition (25, 26). Therefore, how the microbiota influences health and disease is a new research area that needs to be further explored. This promising field of research may lead to new therapeutic ideas for prevention of obesity and its complications (23).

2.2 Genetic factors

The present work focuses on common obesity described as a polygenic disease (27). Environmental factors may certainly contribute to gain weight, however, genetic factors should also be considered. Genetic predisposition to obesity (BMI) is estimated to be around 50% to 80% (28). Genetic factors are known to influence obesity for some decades. For instance, a study examining more than 500 adopted Danish subjects found a strong relationship between the weight of adopted children and the BMI of their biological parents. Interestingly, no association was observed between the weight of adopted children and the BMI of adoptive parents (29).

Several gene association studies are currently used to identify disease-causing variants. Candidate Gene (CG) approaches are based on known biological, physiological or functional pathways relevant to the disease, being hypothesis-driven. Some of the most studied and replicated genes are related to food intake and energy homeostasis especially from the previously described leptin-melanocortin pathways (i.e. *LEP, leptine receptor (LEPR), MC4R, POMC*). Others refer to peripheral regulation of energy expenditure (i.e. *uncoupling proteins 2*

(UCP2) and *3 (UCP3)*). In general these genes are implicated in a wide variety of biological functions such as regulation of food intake, energy expenditure, lipid and glucose metabolism or adipose tissue development. Further details are reviewed elsewhere (30).

With the increasing use of Genome Wide Association Studies (GWAS) hundreds of thousands of individuals are being tested, reporting many other variants associated with BMI and other metabolic phenotypes. Unlike CG approaches, GWAS use hypothesis-free methodologies and are based on genotyped Single Nucleotide Polymorphisms (SNPs) showing an association with the trait (i.e. BMI) that will be in Linkage Disequilibrium (LD) with the causal variant. However, because they identify common variants with small size effect, large cohorts are needed in order to have enough power to detect the effect. Therefore, replication of the results is one of the major challenges. To date, at least 97 loci associated with BMI have been elucidated in European populations (31). These loci integrated in genetic risk scores (GRS) explained altogether only 2.7% of BMI variability. The best replicated SNPs lie in *MC4R* and *fat mass and obesity associated (FTO)* gene regions which have been involved in food intake, energy homeostasis and energy expenditure (32). These results have been replicated in adults and children from European and Asian populations (33-35). However, the low BMI variability explained by all these polymorphisms leads to the question of the missing heritability of obesity, which could be attributed to factors such as the complex, polygenic nature of obesity, as well as to other mechanisms and variants (e.g. copy number variation (CNV), rare mutations, epigenetics) (36).

2.3 Gene-environment interactions

Neither genetic factors nor environmental factors alone appear to be the only regulating components of obesity. Complex traits are highly dependent on gene-environment interactions, thus the complexity in humans lies in the fact that alleles influence common diseases in different genetic backgrounds eventually influenced by different environmental factors. A number of studies examined whether specific gene-environment interactions influenced weight gain. Studies focusing on genetic variants and physical activity

showed an interaction between the *FTO rs9939609* SNP and physical activity in Danish participants. Those physically inactive subjects had significant differences in BMI according to the *AA* or *TT* genotypic groups (37). Another study reported that the *LEPR* genetic variants were associated with weight loss in women who were prescribed a low-calorie diet (38). A gene-diet interaction was found between *Melanocortin-3 receptor (MC3R) C17A and G241A* variants and low-calorie diet on weight loss in childhood obesity (39). However, quite often, the importance of gene-environment interactions is not properly appreciated leading to non-replicated results (40).

3. Obesity and metabolic disorders in Psychiatric Populations

It is well described that psychiatric populations have an increased risk of obesity and cardio metabolic disorders compared to the general population. The illness itself, lifestyle (e.g. unhealthy diet, physical inactivity, smoking habits) and also the psychotropic drug treatment, with an independent dose effect, contribute to that risk (41). Evidence shows a 2-3 fold increased mortality rate attributed to natural causes such as cardiovascular diseases compared to healthy populations which corresponds to a reduction of 10-25 years in life expectancy (42). Some of the modifiable risk factors contributing to cardiovascular diseases include obesity, smoking, diabetes, hypertension, dyslipidaemia and metabolic syndrome. Relative risks for these factors are twice or three times higher compared to the general populations in both schizophrenic and bipolar populations (43), as shown in Table 1. Finally, besides of these physical comorbidities, these populations have a poorer access to a good health system which efficiently takes care of the metabolic diseases.

Table 1. Relative risks of metabolic side effects in schizophrenia and bipolar disorders compared to general population (43)

	Estimated prevalence, % (RR)	
Modifiable risk factors	Schizophrenia	Bipolar disorder
Obesity	45-55 (1.5-2)	21-49 (1-2)
Smoking	50-80 (2-3)	54-68 (2-3)
Diabetes	10-15 (2)	8-17 (1.5-2)
Hypertension	19-58 (2-3)	35-61 (2-3)
Dyslipidaemia	25-69 (≤5)	23-38 (≤3)
Metabolic syndrome	37-63 (2-3)	30-49 (1.5-2)

3.1 Psychotropic drugs and metabolic disorders

In the present research work, we focus particularly on psychotropic drugs including antipsychotic and mood stabilizers which are mainly used in the treatment of schizophrenia and bipolar disorders (44). Schizophrenia is characterized by a combination of positive (e.g. delusions, hallucinations, disorganized behavior, agitation) (44) and negative symptoms (e.g. social withdrawal, lack of empathy and self-care, anhedonia), affecting also cognitive and affective functions (45). The negative symptoms of the disease respond poorly to drug treatment in many patients as do cognitive symptoms, which are among the major factors in determining the opportunity to return to society for these individuals. Bipolar disorders refer to individuals experiencing at different time or simultaneously maniac phases (euphoria, abnormally elevated or irritable mood) and depression combined with euthymia (normal mood) periods (44).

3.1.1 Antipsychotic drugs

The key pharmacological mechanism of antipsychotic drugs, both for typical and atypical, is the blockage of dopamine-2 receptors which confers the antipsychotic properties (46). This action, however, is also responsible for most of their undesirable side-effects, such as metabolic ones. Concerning first generation antipsychotics (FGA) they have been mostly related to extrapyramidal symptoms, tardive dyskinesia, hyperprolactinemia and exacerbation of negative symptoms. Second generation antipsychotics (SGA) or atypical antipsychotics have replaced FGA in the treatment of schizophrenia and bipolar disorders. SGA can be defined as serotonin (5HT) and dopamine antagonists, dopamine antagonists with rapid dissociation, partial dopamine agonists or serotonin partial agonists at 5HT1A receptors (44). Due to their affinity to serotonin receptors (5HTR), they have less extrapyramidal symptoms and they are somehow better for treating negative symptoms compared to FGA. SGA may interact with a large variety of receptors such as serotonine, dopamine, histamine, muscarinic and adrenergic receptors which confer the pharmacological properties of these molecules as well as their side effects (47). Table 2 summarizes different side effects depending on the receptor interaction.

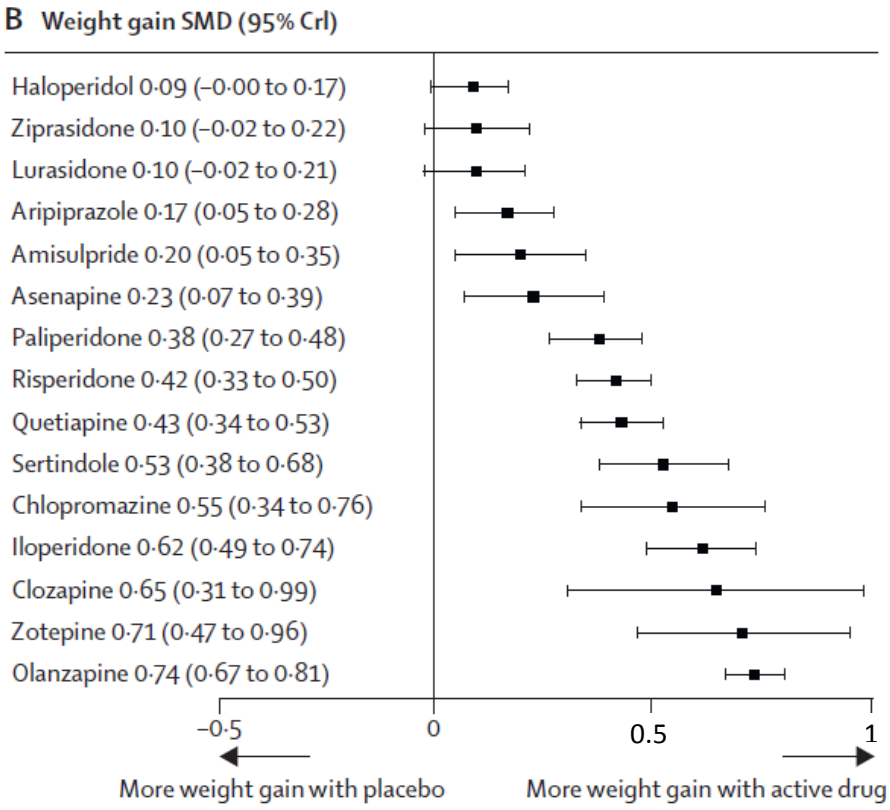
Table 2. Target receptors of antipsychotic drugs and its metabolic side effects (48).

Receptor	Side effect	Mechanism
Serotonin 5-HT 2C	Diabetes	Antagonists disrupt sympathetic regulation of peripheral glucose metabolism; also inhibit skeletal muscle and hepatic glucose uptake
	Weight gain	Antagonists disinhibit hypothalamic NPY neurons (resulting in elevated NPY) and inhibit POMC neurons (resulting in decreased α-MSH); may also play a role in leptin resistance
Serotonin 5-HT 1A	Diabetes	Antagonists inhibit skeletal muscle and hepatic glucose uptake and downregulate pancreatic β-cell sensitivity to glucose
	Weight gain	Agonists increase food intake; partial agonists may mitigate 5-HT2C antagonism; partial agonists may also decrease carbohydrate craving
Histamine H1	Weight gain	Antagonists cause increased hypothalamic AMPK activity, mimicking depletion of cellular energy stores and causing increased appetite
	Diabetes	Antagonists disrupt sympathetic regulation of adipose tissue
	Sedation	Antagonists inhibit cholinergic neurons of basal forebrain and serotonergic neurons of dorsal raphe
Dopamine D2	Weight gain	Antagonists cause overall decrease in limbic dopaminergic activity, possibly leading to increased engagement reward-seeking behaviors such as food intake; agonists (psychostimulants, cocaine) are appetite suppressants
	Extrapyramidal side effects	Antagonists disinhibit indirect descending motor pathway in basal ganglia
	Endocrine effects	Antagonists disinhibit prolactin release from posterior hypothalamus, also contributing to weight gain
Muscarinic M1	Anticholinergic effects	Antagonists cause dry mouth, urinary retention, cognitive dysfunction, urinary retention, and constipation
Muscarinic M3	Diabetes	Antagonists cause impaired glucose tolerance and reduced insulin secretion from pancreatic β cells

α-MSH, α-melanocyte-stimulating hormone; AMPK, AMP-related kinase; NPY, neuropeptide Y; POMC, pro-opiomelanocortin.

In addition, the type of antipsychotic drug determines the incidence of metabolic side effects due to different mechanisms of action and binding profiles. Thus, olanzapine and clozapine are associated with the highest weight gain whereas amisulpride or aripiprazole are at low or moderate risk (49, 50). A meta-analysis conducted in schizophrenic patients integrated the available evidence in order to create hierarchies of efficacy, non-compliance and major side effects (e.g. weight gain) of typical and atypical antipsychotics compared to placebo (50). Figure 3 shows the risk of weight gain according to different typical and atypical antipsychotics.

Figure 3: Forest plot for weight gain of antipsychotic drugs compared with placebo. (adapted from (50))



SMD=standardised mean difference. CrI=credible interval.

3.1.2 Mood stabilisers

Lithium and valproate are mood stabilizers that can treat both phases of bipolar disorders, reducing symptoms of mania and of bipolar depression, and also preventing relapse. Lithium is the classic mood stabilizer and it has been used for more than 50 years. Its mechanism of action might involve signal transduction (e.g. via inositol monophosphatase), modulation of G-proteins or interaction at various sites of downstream signal transduction cascades (44). For valproate, an anticonvulsivant used as mood stabilizer, several hypotheses are proposed for its mechanism of action. Some of them include inhibition of voltage-sensitive sodium channels and/or boosting the actions of GABA neurotransmitter, among others (44).

Although the mechanisms of these molecules are not completely understood, they certainly contribute to weight gain. A meta-analysis showed that almost 40% of bipolar patients developed metabolic syndrome.

This prevalence is twice than in general population (51). Studies focusing on treatment showed that patients under lithium and valproate treatment gain more weight than those receiving placebo (52, 53). Therefore a good monitoring of side effects is strongly recommended when starting any of these weight gain inducing treatments in order to mitigate the risk of developing CVD, T2DM or other metabolic side-effects (49).

3.2 Genetic, psychiatric disorders and metabolic disturbances.

The interaction of antipsychotics with leptin-melanocortin pathway correlates with an increase of appetite. Over time, a high food intake leads to obesity and lipid alteration profile such as hypertriglyceridemia. This dyslipidemia is followed by insulin resistance to finally lead to T2DM associated with a high risk of cardiovascular events, thus increasing mortality rate among this population (44). Also, it has been shown that psychiatric disorders such as major depressive disorder, schizophrenia or bipolar disorder share common pathways with obesity. Studies in the past decades have demonstrated that obesity is related to increased inflammation, oxidative stress, hypothalamus-pituitary-adrenocortical axis dysregulation, upregulation of kynurenine pathway which affects neurotransmitter production (e.g. serotonin) (54). Interestingly, high levels of inflammatory adiponectin and other cytokines are found among first-episode drug naïve schizophrenia patients (55) and upregulation of kynurenine pathway is observed in major depressive disorder, schizophrenia and bipolar disorder (56). In addition, schizophrenia and bipolar disorders have been related to neurotransmitters dysregulation (e.g. glutamate, dopamine and/or serotonin) (54, 57). Also, mental disorders such as schizophrenia, bipolar disorder and major depressive disorder are associated with impairment in cognitive functioning (executive functioning and attention memory) (58). This deficiency appears over time and worsens with psychiatric illness duration. This might be explained in part, by the combination of clinical and treatment variables: Although treatment used may improve some aspects of cognition, the anticholinergic effect of some of the psychotropic drugs and comedications prescribed may increase the risk of cognitive impairment (59, 60). Furthermore, weight gain associated with the use of some psychotropic drugs (notably

atypical antipsychotics) may have negative consequences on cognition (59). Further studies on how interaction occurs between medication use, weight gain and cognition in this population, are warranted.

Obesity may impair cognitive function in neuropsychiatric disorders by inflammatory processes (61), but the implicated pathways are only partially elucidated. The neuronal insulin signaling pathway (affecting the hippocampus) is implicated in cognitive functions and is involved in glucose homeostasis. Hippocampus is a neuronal-insulin sensitive organ and neuronal-insulin mediates metabolic biological actions and modulates neurotransmitter concentrations in the central nervous system which are implicated in the physiopathology of mood disorders, schizophrenia and alzheimer disease (62). Disturbances in this pathway might lead to cognition worsening and appearance of psychiatric disorders.

From the classical candidate gene approach, the best replicated variants concerning psychotropic-induced weight gain concern the *-759C/T 5HTR2C* polymorphism, showing that carriers of the *T-allele* had less weight gain than *C-allele* carriers (63). These results have been found in different ethnicities (48, 64). Additionally, the *C-allele* has been related with obesity in general population (48, 65). The *LEP -2548G/A* showed that *GG* genotype was associated with weight gain in Caucasian with multiple antipsychotics (66). Evidence of antipsychotic drugs and receptors and genes implicated in metabolic disturbances has been described in detail elsewhere (66, 67). In addition, some polymorphisms have been related to both psychiatric disease and obesity, such as the *A-allele* from the *FTO rs9939609* variant which has been associated with lower risk of depression and an increased BMI (68). Recently, the *CREB-regulated transcription coactivator 1 (CRTC1)* gene has been also associated with BMI and fat mass but only in lifetime depressed individuals. The mechanism of this gene in depression and psychiatric diseases needs to be further investigated (69). Other genes, such as *FKBP5* have shown a positive correlation with insulin levels in adults having suffered from early life stress (70). Further studies are needed to disentangle the shared pathways between obesity and psychiatric diseases. An extensive genetic analysis in psychiatric cohorts is presented in a later section of this thesis (see **Results** chapter). Recently, a study examining genetic influences on BMI and cognition found that some genetic

factors overlapped between both traits. This study took into account two large GWAS meta-analyses assessing cognition and BMI (31, 71). Low BMI was correlated with high cognitive function; thus genes variants associated with increased cognitive function were associated with lower BMI. Additionally, some polymorphisms associated with BMI, explained a significant –although small- proportion of cognition variance and vice-versa. When looking at SNPs individually, seven hits were significant for both traits and were found in genes of insulin-related functions, among others, thus supporting the link of type 2 diabetes and/or disturbances in insulin pathway and impaired cognitive function (61).

4. Obesity and Diabetes in Solid Organ Transplant populations

4.1 Generalities

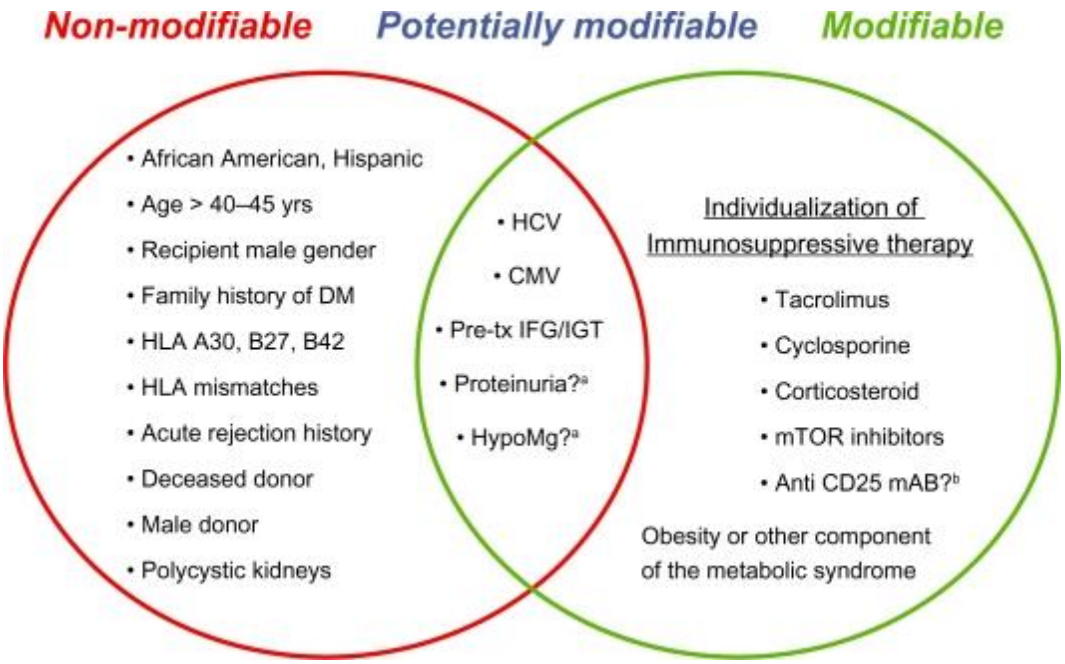
In 2003, international experts in the field of transplant and diabetes defined the diagnosis and management of new onset diabetes after transplant (NODAT) based on the World Health Organization and the American Diabetes Association recommendations (72). The incidence of NODAT ranges widely, depending on the transplanted organ and/or the study design among other factors. More specifically, it has been described that NODAT occurs in 4-25% in renal transplant recipients, 2.5-25% in liver transplantation, 4-40% in heart and 30-35% in lung transplant recipients (73-76). Pretransplantation diabetes and NODAT contribute to the risk of CVD, which remains the most common cause of death after kidney transplantation worldwide (77). Weight gain leading to obesity has been associated with insulin resistance and post-transplant weight gain exacerbates this condition leading to NODAT development (78, 79). Post-transplant weight-gain has been described in patients regardless of their weight prior to transplant. Excessive weight gain in transplanted individuals can lead to severe consequences such as delayed graft function related to surgical and post-operative complications (80) and decreased graft survival (81, 82).

4.2 Risk factors contributing to metabolic disorders

Because obesity and other components of the metabolic syndrome are modifiable factors, early intervention to prevent these conditions is of major importance. In liver transplanted recipients, factors such as older age (83), family history of overweight, high BMI prior to the transplantation and/or high donor BMI (83, 84), hypo-metabolism and physical inactivity (83, 85) are some of the risk factors associated with weight gain, overweight and obesity. In kidney-transplant recipients, obesity increases NODAT with a relative risk of 1.73 and this risk increases linearly above 45 kg (86). Additionally, NODAT has been associated with low levels of adiponectin (a marker inversely correlated with adiposity) and higher levels of C-reactive protein; potentially suggesting an inflammatory process. Regarding NODAT, some of the risk factors (grouped into non-modifiable, potentially modifiable and modifiable, Figure 4) are family history of diabetes, where the risk is

increased by 50% (87) or older age (72). Other factors specifically related to the transplanted populations concern HLA group mismatches, donor-recipient mismatch, acute rejection, graft from a deceased donor, virus load (i.e. hepatitis C virus –HCV- and cytomegalovirus –CMV-) or immunosuppressant treatment (72). NODAT incidence depends on HCV status being 25.6% in HCV positive and 14.4% in HCV negative groups (78). Patients may acquire the virus through blood contamination in hemodialysis units. Although the mechanism is poorly understood, HCV causes glomerular disease leading to end stage renal disease (88). Many HCV infected patients will therefore undergo kidney transplantation. CMV has been associated with impaired insulin release through proinflammatory cytokines that cause functional disturbances in the beta-pancreatic cells. CMV is, however, treated easily in post-transplant recipients compared to HCV. Also, the risk of NODAT increases with pre-transplant impaired fasting glucose and glucose tolerance. Finally, proteinuria and hypomagnesaemia and its association with NODAT development need to be further investigated. Proteinuria might reflect the normal condition just after renal transplantation due to residual native kidney proteinuria or the high dose of corticosteroids used at the beginning and might resolve spontaneously several weeks after transplantation. Studies have reported an inverse correlation between serum magnesium levels and glycemic regulation (72, 88). Additionally, some of the factors contributing to NODAT and metabolic disorders (e.g. increased weight gain) are shared. Such is the case for a high BMI and high fasting glucose levels prior to transplant (89).

F**igure 4.** Factors influencing NODAT classified by modifiable, potentially modifiable and non-modifiable (72)



4.3 Immunosuppressive treatment and metabolic risk

Organ rejection is reduced by immunosuppressant treatments and although new immunosuppressive drugs have improved short-time patient survival, they are not devoid of side effects. CVD together with malignancies and infections are the main causes of mortality in transplanted patients (90). Focusing on cardiovascular and metabolic-related events, immunosuppressant treatments are of particular interest. Immunosuppressive drugs have different impacts on metabolic risk factors. Glucocorticoids, a major component of immunosuppressant therapy, are associated with body fat accumulation and liquid retention. They damage the insulin-secreting beta cells of the pancreas (91) and long-term glucocorticoid therapy may result in clinically significant weight gain through leptin modulation and appetite increase (92). Besides, corticosteroids would have a dose-dependent diabetogenic effect (93). Therefore, standardized steroid withdrawal protocols are suggested in order to reduce the risk of NODAT (94). Cyclosporine was the first calcineurin inhibitor (CNI) approved in 1983 for Solid Organ Transplant (SOT) with the idea of reducing corticosteroids dose, thus decreasing the incidence of NODAT (95-97). Despite the steroid-dose lowering effect, cyclosporine later showed a strong negative impact on weight gain, blood pressure and lipids (98, 99). The CNI second-generation tacrolimus has been associated with an increased risk of NODAT, especially when combined with glucocorticoids (100, 101). mTOR inhibitors (i.e. Sirolimus and Everolimus) are one of the major causes of hyperlipidaemia after transplantation and also diabetogenic, especially when combined with CNI (102, 103). Although induction therapy (i.e. polyclonal and monoclonal antibody preparations) is not directly associated with weight gain or metabolic alterations, it has been associated with malignancies which lead to mortality (104). Thus, the optimal immunosuppressive therapy combines different drugs improving the immunosuppressive potential and decreasing the toxic effects by lowering each single dose.

4.4 Genetics of metabolic disorders in Solid Organ Transplant populations

Some of the genetic factors influencing obesity have already been described in **Chapter 2.2**. Focusing on transplant populations, some specific variants have shown an association with obesity and weight gain within

this population. Candidate gene approaches found two single nucleotide polymorphisms (SNPs) and one insertion/deletion associated with BMI-related phenotypes. In liver transplant recipients, carriers of the D allele of the angiotensin-converting enzyme had and carriers of the G-allele of the PNPLA had an increased risk of weight gain and higher obesity prevalence dependent of diabetes, respectively, compared to non-carriers. Finally, in kidney transplanted recipients, carriers of the CC genotype in an ATF6 polymorphism had higher BMI compared to non-carriers. The ATF6 gene is involved in lipogenesis and gluconeogenesis and would be activated via Tacrolimus during endoplasmatic reticulum stress (105-107). Weight change in kidney transplant has been associated with the expression of genes involved in T2DM, obesity and neurological concepts such as dopamine, nicotine, and cognition (108). To date, no weight gain-related GWAS in SOT have yet been conducted. Since NODAT and T2DM share some mechanisms, some variants associated with T2DM have also been associated with NODAT. Such is the case of *TCF7L2* or *KCNQ1* (109). Recently, a GWAS of NODAT in kidney transplanted recipients revealed eight significant SNPs (110). In **Projects III and IV** an extensive analysis of the genetic variants influencing obesity and NODAT in SOT is conducted.

5. The challenge of personalized medicine

Pharmacogenomics, the study of how genes affect a persons' response to drugs, is part of personalized medicine that customizes health care, tailoring treatments to each individual (111). Personalized medicine concerns treatment response and/or side effects. To date, the FDA approved more than 100 drugs that include label information on pharmacogenomic biomarkers (111, 112). CYP2D6-related dose recommendations obtained from pharmacokinetic studies were one of the first steps in developing guidelines for therapeutic use of antidepressant treatments (113). Another example is the Steven-Johnson side effect of carbamazepine for the treatment of bipolar disorders or epilepsy. In particular, carriers of the *HLA allele B*1502* have an increased risk of developing Steven-Johnson syndrome in Asian populations. Therefore, the Food and Drug Administration (FDA) recommended genotyping all Asians for the allele before starting the treatment (114). However, the number of pharmacogenetic tests that are used nowadays in the clinic remain small. This could be explained by several facts: First, there is a need to better understand the mechanism of action of a drug. Furthermore, many studies are hypothesis-driven and focus on gene candidate approaches, missing other gene contributions or gene-gene interactions. The polygenic nature of many phenotypes, such as obesity or diabetes, should be taken into account. On the same line, information is missing on how the gene-environment interactions affect drug response and side effects. All these factors may reduce the predictive value of pharmacogenetic testing (115). Finally, the use of pharmacogenetic tests has to overcome important scientific, economic, commercial, political and educational barriers (116). To date, there are no reliable biomarkers that can accurately predict the risk of weight gain, making difficult to translate risk prediction into clinical practice.

Finally, if pharmacogenetic testing is combined with therapeutic drug monitoring (TDM), both tools can optimally individualize drug therapy in order to maximize the efficacy and the safety of the drug. For instance, in tacrolimus treated individuals, carriers of *CYP3A*1 allele* have a greater clearance needing a dose readjustment. TDM may be helpful at the beginning of tacrolimus treatment in order to determine the appropriate starting dosage and to avoid serious adverse effects (117). In psychotropic and antidepressant

drugs, for instance, the combination of TDM and *CYP2D6/CYP2C19* genotyping is particularly useful in verifying concentration-dependent adverse drug reactions or in interpreting psychotropic drug response in polypharmacy (116, 118).

AIMS

Obesity has reached epidemic proportions globally, with at least 2.8 million people dying each year as a result of being overweight or obese (1). Obesity is preventable and is often associated with other diseases leading to metabolic complications and reducing indirectly life expectancy. The present thesis work focuses on two particular populations at high risk of metabolic disorders: The psychiatric and the transplanted populations.

The first aim of this work is to study, from a genetic point of view, the association of several polymorphisms with metabolic disturbances in these two populations (**Projects I, II, III and IV**). This will improve the understanding of the molecular pathways associated with the pathogenesis and the onset of metabolic disorders which worsen health quality and increase mortality rate. In a second step (**Project V**) we aimed to explore how SES factors influence weight related parameters (i.e. BMI and weight change) over time in a psychiatric sample under psychotropic treatment. This work will contribute to better understand interindividual variability in order to better adapt treatments.

A short summary of the secondary aims is included below.

Project I: Association of *PCK1* with Body Mass Index and Other Metabolic Features in Patients with Psychotropic Treatments

The aim of this project was to study the influence of *Phosphoenolpyruvate carboxykinase 1 (PCK1)* on BMI change over time and other metabolic features in a psychiatric population under psychotropic treatment. This gene codes for an enzyme which is a key regulator of the gluconeogenesis and it has been described as a downstream gene of *CRTC1* (implicated in weight gain in psychiatric populations).

Project II: Association of Genetic Risk Scores (GRS) with Body Mass Index in Swiss Psychiatric Cohorts

Since common obesity is influenced by many risk variants, in this project we wanted to study how weighted-GRS (w-GRS) built from previously BMI-related polymorphisms influence BMI in psychiatric cohorts under psychotropic treatment. Additionally, w-GRS from previously identified polymorphisms influencing antipsychotics-induced weight gain in psychiatric populations were tested for association.

Project III: Clinical and Genetic factors influencing Body Mass Index and risk prediction of weight gain in Solid Organ Transplant populations

Similarly to what it has been done with polygenic risk scores in psychiatric populations, in the present project we wanted to see how previously BMI related polymorphisms in GWAS based in general population, influenced BMI in two SOT populations. Additionally, we wanted to assess the ability of these variants to predict 10% weight gain one year after transplantation.

Project IV: Genetic and clinic predictors of New Onset Diabetes Mellitus after Transplantation

The present study focuses on multiple variants at risk for T2DM and NODAT, integrated in w-GRS. We wanted to determine whether these genetic factors influence NODAT and how they contribute in predicting NODAT one year after transplantation.

Project V: Socioeconomic and metabolic risk factors in Psychiatric patients

In the present work we wanted to explore how the sociodemographic factors (i.e. living status and professional condition) would influence weight gain and metabolic outcomes over time in psychiatric patients treated with psychotropic drugs. In a second step, we wanted to study whether a Swiss socioeconomic position index (SEP) based on income, education, occupation and housing conditions is associated with BMI and weight change over time.

METHODS

1. The psychiatric cohorts

1.1 The *Suivi Metabolique* cohort

This ongoing study started in the psychiatric department of the Lausanne University Hospital (DP-CHUV) in 2007. The aim of the *Suivi Metabolique* is to follow patients starting a psychotropic treatment (i.e. aripiprazole, amisulpride, clozapine, olanzapine, quetiapine, risperidone, mirtazapine, lithium and/or valproate) which can potentially lead to metabolic disturbances. Currently, this follow-up is integrated as a routine basis practice in the adult DP-CHUV. The follow-up consists of several regular medical checkups where anthropometric parameters (i.e. weight, height, waist circumference) and blood samples are collected at baseline, 1, 2, 3, 6, 12 months after initiating psychotropic treatment according to guidelines (119). Metabolic parameters, drug plasma concentration and genetic analysis are obtained for those patients who signed a written informed consent. As of March 2016, 934 patients who gave a written informed consent to participate in the study have been recruited.

1.2 The *Ambulatoire* cohort

Similarly to the *Suivi Metabolique* cohort, the aim of this cohort is to follow-up individuals who have been taking a psychotropic treatment (i.e. aripiprazole, amisulpride, clozapine, olanzapine, quetiapine, risperidone, mirtazapine, lithium and/or valproate) for more than one year in an outpatient setting. Started in 2010, through yearly medical checkups, data on anthropometric parameters and blood tests are collected. To date (ongoing study), we have obtained the informed consent for 250 patients.

Recently, for both *Suivi Metabolique* and *Ambulatoire* cohorts, patients treated with tricyclic antidepressants, FGA and/or newly commercialized SGA (i.e. paliperidone, asenapine, lurasidone) have been also recruited, but not taken into account for the present analyses.

1.3 The Geneva cohort

Geneva cohort concerns individuals who have been taking olanzapine, clozapine, quetiapine, risperidone, lithium, and/or valproate for at least 3 months between June 2006 and May 2008. This is a retrospective study that was conducted in an outpatient setting from Psychiatric Department of Geneva University Hospital. Questionnaires were filled and blood samples were collected during one of the patient routine follow-ups. Weight before starting psychotropic treatment was self-reported or extracted from medical files. Informed consent and blood samples were obtained for 198 patients.

In all samples, patients with previous treatments were included after having switched medication. The latest introduced psychotropic medication was considered as the main psychotropic treatment. A detailed description of the three cohorts can be found in a previous publication (120).

2. The Transplanted Populations

2.1 The Swiss Transplant Cohort Study (STCS)

The STCS is an ongoing prospective multicenter study (Basel, Bern, Geneva, Lausanne, St. Gallen and Zurich) started in May 2008 which enrolls SOT recipients with no particular eligibility or exclusion criteria. The sample used for the present work (May 2008 - May 2011) included individuals with a functional graft for at least 12 months after transplantation. A total of 1294 patients were followed up in their respective transplant centers at baseline and at 6, 12, 24, 36 and 48 months after transplantation. Informed consents and genetic analysis were obtained for this sample. Further details can be found elsewhere (121, 122).

2.2 The Swiss Immunosuppressant Study Cohort

This transplanted cohort concerned a total of 197 patients who were enrolled between 2003 and 2005 from the outpatient clinic of the transplant center of the University Hospital of Lausanne, Switzerland. Only

patients with a functional graft for more than 12 months were eligible to participate in the study. Several clinical data and blood samples were collected at baseline and during the regular medical check-ups up to five years after transplant. Informed consents were obtained and genetic analyses were conducted for this sample. Further details can be found elsewhere (122-124).

3. The General Population

Participants in the *Cohorte Lausannoise* (CoLaus, n = 5338) were recruited between June 2003 and May 2006 in the Lausanne area as described previously (125). The CoLaus was used to test for replication the positive results found in the psychiatric samples. Data related to general population lipid traits (n = 100,184), integrating the Global Lipids Genetics Consortium, was downloaded from Genome Wide Associations Scans for Total Cholesterol, HDL-C, LDL-C and triglycerides website (126). This data base aimed at testing for replication the positive findings in the psychiatric cohorts. Finally, the Genetic Investigation of Anthropometric Traits Consortium (GIANT) performed a meta-analysis of genomewide association study data with a discovery set of 123,865 individuals of European ancestry from 46 studies for several anthropometric traits (i.e. height, waist-to-hip ratio, BMI) (34, 127, 128). The GIANT data set was used to test for replication results from psychiatric samples and to calculate effect sizes (beta-estimates) of the w-GRS analyses.

4. Genotyping method

The cardiometabochip is the array used for genotyping our samples (i.e. the three Psychiatric cohorts and The Swiss Immunosuppressant study cohort). This customized chip is designed to test more than 200'000 DNA variation of SNPs from regions identified by large scale meta-analyses of GWAS for anthropometric, metabolic and cardiovascular traits. Around 7000 customized SNPs were additionally added in the chip. This method allows for processing thousands of samples quickly and cost-effectively, with loci-defined content. The genotyping was conducted at the iGE3 genomics platform of the University of Geneva (http://www.ige3.unige.ch/genomics-platform.php).

RESULTS

1. Project I

Association of *PCK1* with Body Mass Index and other metabolic features in patients with psychotropic treatments.

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Summary

The present work illustrates the association of the *PCK1* polymorphisms with BMI in a psychiatric population under psychotropic treatment. The *PCK1* is a downstream gene of *CRTC1* which is implicated in hypothalamic control of food intake. The main results of the present gene-candidate study showed that, after examining several tagging SNPs of the *PCK1* gene, a polymorphism was significantly associated with BMI (with stronger results in women younger than 45 years) and with other metabolic parameters. The association with BMI was replicated in two other samples. In addition, the same polymorphism was associated with waist-to-hip ratio in general population cohorts. Finally, when combining *CRTC1* and *PCK1* genes, carriers of protective alleles had lower BMI when compared to non-carriers. The present work contributes to the understanding of metabolic risk pathways, bringing special attention to high risk psychiatric populations.

ORIGINAL CONTRIBUTION

Association of *PCK1* with Body Mass Index and Other Metabolic Features in Patients With Psychotropic Treatments

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Abstract: Weight gain is a major health problem among psychiatric populations. It implicates several receptors and hormones involved in energy balance and metabolism. Phosphoenolpyruvate carboxykinase 1 is a rate-controlling enzyme involved in gluconeogenesis, glyceroneogenesis and cataplerosis and has been related to obesity and diabetes phenotypes in animals and humans. The aim of this study was to investigate the association of *phosphoenolpyruvate carboxykinase 1* polymorphisms with metabolic traits in psychiatric patients treated with psychotropic drugs inducing weight gain and in general population samples. One polymorphism (*rs11552145G > A*) significantly associated with body mass index in the psychiatric discovery sample (n = 478) was replicated in 2 other psychiatric samples (n_1 = 168, n_2 = 188), with *AA*-genotype carriers having lower body mass index as compared to *G-allele* carriers. Stronger associations were found among women younger than 45 years carrying *AA*-genotype as compared to *G-allele* carriers (−2.25 kg/m^2, n = 151, P = 0.009) and in the discovery sample (−2.20 kg/m^2, n = 423, P = 0.0004). In the discovery sample for which metabolic parameters were available, *AA*-genotype showed lower waist circumference (−6.86 cm, P = 0.008) and triglycerides levels (−5.58 mg/100 mL, P < 0.002) when compared to *G-allele* carriers. Finally, waist-to-hip ratio was associated with *rs6070157* (proxy of *rs11552145*, r^2 = 0.99) in a population-based sample (N = 123,865, P = 0.022). Our results suggest an association of *rs11552145G > A* polymorphism with metabolic-related traits, especially in psychiatric populations and in women younger than 45 years.

Key Words: metabolic syndrome, body mass index, psychotropic drugs, pharmacogenetics

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Weight gain is a known side effect of psychotropic drugs, such as antipsychotics, mood stabilizers, and antidepressants.[1] Psychotropic-induced weight gain can lead to many metabolic complications (eg, increase in triglycerides [TG], cholesterol [CHOL], waist circumference [WC]) and is related to comorbidities, such as diabetes, hypertension, and other cardiovascular diseases.[2] Psychiatric populations have a 10- to 25-year reduction in life expectancy due to comorbidities and to the psychiatric illness itself, corresponding to a 2- to 3-fold increased mortality rate when compared to healthy populations.[3] Obesity is attributed to the psychiatric illness, to behavioral and environmental factors (ie, diet, exercise, smoking), as well as genetic factors.[4] Besides, an interaction between genetic factors and psychotropic drug-inducing weight gain has been described implicating several receptors (eg, serotonin and dopamine receptors) and hormones (eg, leptin) involved in energy balance or metabolism pathways.[5,6]

The *Phosphoenolpyruvate carboxykinase* (*PCK*) gene codes for an enzyme involved in the gluconeogenesis[7] and is found in 2 forms, PCK1 (cytosolic) and PCK2 (mitochondrial). Both enzymes are expressed equally in the liver but their expression may vary depending on the tissue.[7,8] The PCK catalyzes the conversion from oxalacetate into phosphoenolpyruvate (a rate-controlling step of gluconeogenesis) and is also involved in glyceroneogenesis and cataplerosis.[7] Of note, *PCK* is a downstream gene of the *CREB-regulated transcription coactivator 1* (*CRTC1*) which is implicated in hypothalamic control of food intake,[9,10] and we recently found in general and psychiatric populations that carriers of a variant allele of a *CRTC1* polymorphism appear to be protected against weight gain especially in women younger than 45 years.[11]

Rodents who overexpress *PCK1* and *PCK2* were obese, hyperglycemic, and insulin-resistant,[12,13] whereas mice that underexpress *PCK1* and *PCK2* developed a lipodistrophy type of metabolic syndrome.[14] This is in line with the positive correlation found between PCK1 mRNA expression levels and body mass index (BMI), body fat percentage, TG, and CHOL levels in subcutaneous adipose tissue of nonmenopausal women.[15] In humans, regions near *PCK1* locus have been related to obesity or fat mass,[16,17] and several positive associations have been reported between *PCK1* polymorphisms and type 2 diabetes mellitus (T2DM)[18–20] although these results could not always be replicated.[21] Other studies conducted in the general population showed no significant association between *PCK1* polymorphisms and

BMI, WC or physical activity.[22] A case-control study in a diabetic versus non diabetic population also found that nondiabetic homozygous for the minor allele of a *PCK1* polymorphism (*+4824 T > C*) had increased levels of high-density lipoproteins (HDL) and lower TG levels when compared to wild type.[23] Thus, growing evidence supports that *PCK* contributes to obesity and metabolic syndrome in the general population but, to our knowledge, no studies have yet been conducted in psychiatric populations which are at high risk for developing obesity and metabolic syndrome. The aim of the present study was to analyze whether *PCK1* polymorphisms were associated with BMI and other metabolic traits (ie, WC, blood glucose levels [BGL], low-density lipoprotein [LDL], HDL, CHOL, and TG in 3 independent psychiatric populations treated with drugs inducing weight gain and in 3 large general population cohorts. As a secondary aim, we wanted to explore how *PCK1* and *CRTC1* polymorphisms are associated with BMI in a combined analysis.

MATERIALS AND METHODS

Psychiatric Sample Description

The first psychiatric sample (discovery sample) was recruited during a longitudinal follow-up study on metabolic syndrome at the Lausanne Psychiatric University Hospital (started in 2007, ongoing). Four hundred seventy-eight white patients switching or starting a treatment with drugs known to potentially induce weight gain (aripiprazole, amisulpride, clozapine, olanzapine, quetiapine, risperidone, mirtazapine, lithium, and/or valproate) were included. Weight, height, and other clinical variables were reported at baseline and at 1, 2, 3, 6, 9, and 12 months after starting the treatment according to published monitoring guidelines of weight and metabolic syndrome parameters.[24] Most patients had already received other psychotropic treatment before the current treatment. Fasting BGL and lipid levels (ie, CHOL, TG, LDL, HDL) were analyzed on a routine basis on blood samples using a Modular P apparatus (Roche Diagnostics, Switzerland). For patients for whom drug plasma determinations were available, we conducted preliminary analysis on the influence of compliance on the observed associations. For this purpose, we defined an arbitrary threshold at 10% of the minimal therapeutic drug plasma concentration[25] (ie, 2, 35, 10, 2, 15, 10, 2 ng/mL, 0.05 mmol/L, 5 mg/L for olanzapine, clozapine, quetiapine, risperidone + hydroxy-risperidone, aripiprazole, amisulpride, paliperidone, lithium, and valproate) to ensure psychotropic drug intake. Similar results to those described in the present article were obtained (data not shown). Thus, to increase the power of the study, the whole cohort was used for statistical analysis. Two other psychiatric samples were used as replication samples. A retrospective study (replication sample 1) was conducted in an outpatient setting in Geneva University Hospital in 2007. One hundred sixty-eight white patients treated for at least 3 months with olanzapine, clozapine, quetiapine, risperidone, lithium, and/or valproate were recruited. Another retrospective outpatient study in Lausanne, replication sample 2 (started in 2010, ongoing) included 188 white patients mostly treated for more than 1 year with aripiprazole, amisulpride, clozapine, olanzapine, quetiapine, risperidone, mirtazapine, lithium, and/or valproate. For both replication samples, questionnaires were filled during one of the patient routine follow-ups and weight, height, WC, and treatment duration were reported among other clinical variables. Weight before starting psychotropic treatment was self-reported or extracted from medical files. As shown previously,[11] self-reported weight was found to be a reliable estimate of the measured weight extracted from medical files.

In all samples, patients with previous treatments were included after having switched medication. The latest introduced psychotropic medication was considered as the main psychotropic treatment. Weight (patients with light clothes and without shoes) was measured in kilograms to the nearest kg. Height was measured using a height gauge to the nearest cm. The WC was measured to the nearest centimeter. The BMI for all individuals was obtained by dividing weight (in kg) by squared height (in m^2).

Written informed consent was provided by all individuals or by their legal representatives, and the studies were approved by the ethics committee of the corresponding centers. Further details of the 3 psychiatric cohorts have already been described elsewhere.[11,26] Of note, the present study refers to the same 3 psychiatric populations than in our previous article,[11] but with a larger number of patients included in the discovery cohort and in the replication sample 2 (inclusions ongoing).

Population-Based Samples

Significant results were tested for replication in 3 population based samples: participants in CoLaus (n = 5338) were recruited between June 2003 and May 2006 in the Lausanne area as described previously.[27] The Genetic Investigation of Anthropometric Traits Consortium (GIANT) performed a meta-analysis of genomewide association study data with a discovery set of 123,865 individuals of European ancestry from 46 studies for height,[28] BMI,[4] and waist-to-hip ratio (WHR).[29] Finally, the second set of association summary statistics for general populations (Global Lipids Genetics Consortium) was downloaded from "Genome Wide Associations Scans for Total Cholesterol, HDL-C, LDL-C and triglycerides" website[30] and contains data related to lipid traits (n = 100,184). Of note, CoLaus is part of both GIANT and Global Lipids Genetics Consortium.

SNP Selection and Genotyping

In a first step, the best replicated and studied *PCK1* polymorphism in the literature (ie, *rs2071023*) was manually genotyped using TaqMan allelic discrimination assay (ABI PRISM 7000 Sequence Detection System; Applied Biosystems, Rotkreuz, Switzerland, TaqMan SNP genotyping assays ID: C 2508731 1). Additionally, 3 SNPS which were available in the CardioMetaboChip were also considered for analysis (ie, *rs11552145, rs707555*, and *rs8123020*). The CardioMetaboChip is a custom Illumina iSelect genotyping array designed to test DNA variation of 200,000 SNPs from regions identified by large scale meta-analyses of genomewide association studies (GWAS) for metabolic and cardiovascular traits. Quality control excluded samples from the analysis if sex was inconsistent with genetic data from X-linked markers, genotype call rate less than 0.96, Gene Call score less than 0.15. GenomeStudio Data Analysis Software was used to export results generated by Illumina CardioMetaboChip. In total, 4 SNPs were considered for analyses with minor allele frequency (MAF) higher than 0.10 (Supplementary Table S-1, Supplemental Digital Content 1, http://links.lww.com/JCP/A313). All of them were in Hardy Weinberg Equilibrium (Supplementary Table S-2, Supplemental Digital Content 2, http://links.lww.com/JCP/A314). Finally, looking at HapMap Genome Browser (release 27, MAF > 0.10, cutoff of r^2 set at 0.8),[31] we found that several *PCK1* tagging SNPs were in linkage disequilibrium with our four selected SNPs (see details in Supplementary Figure S-1, Supplemental Digital Content 3, http://links.lww.com/JCP/A315).

DNA was extracted from blood samples as described by the manufacturer's protocol using Flexigene DNA kit and QIAamp DNA Blood Mini QIAcube Kit (Qiagen AG, Switzerland) for 834 Caucasian patients from the three psychiatric cohorts. Genotyping

of the *rs3746266A > G* SNP from *CRTC1* was performed using TaqMan allelic discrimination assay (ABI PRISM 7000 Sequence Detection System; Applied Biosystems) and according to the manufacturer's protocol as described elsewhere.[11] Genotyping of the CoLaus subjects was performed using the Affymetrix GeneChip Human Mapping 500 K array set as previously described.[27]

Variables of the Study

The main outcome analyzed in the three psychiatric samples was the BMI (kg/m^2) used as a continuous variable. Other outcomes studied were WC (cm), LDL, HDL, TG, CHOL and BGL (mg/100 mL). *PCK1* genotypes were grouped and analyzed in recessive (for *rs11552145, rs707555 and rs8123020*) and dominant (for *rs2071023*) models according to their association with BMI showed in preliminary analyses. Other covariates were extracted from medical files or during the interview and included demographic data (ie, sex, age, and ethnicity) as well as history of treatment (type of psychotropic drug and treatment duration). To preserve homogeneity of the samples, only patients treated up to 24 months were taken into account in combined (ie, discovery plus replication) psychiatric sample analyses.

Statistical Analysis

Psychiatric Samples

Hardy Weinberg Equilibrium was determined for each polymorphism by a χ^2 test. Statistical analyses were done using STATA 12.1 (StataCorp, College Station TX) and R version 2.11.1 software.[32] *P* values less than 0.05 were considered as statistically significant, and when necessary, Bonferroni correction for multiple tests was applied. Eventually, differences in sample size might be due to missing genotypes and/or covariates. First, exploratory analyses were conducted to explore differences in BMI between genetic groups in the 3 psychiatric samples using Mann-Whitney *U* nonparametric test. To fit a longitudinal model on the BMI trend, due to complex and nonlinear BMI evolution in time and presence of multiple observations per individual which introduces interdependence among observations, a Generalized Additive Mixed Model was used to assess the association of genetic polymorphism with BMI adjusted by sex, age, treatment and treatment duration. This allowed a smooth trend for the response in time based on multiple observations for each patient (using a thin plate regression spline basis). A random effect at the subject level was also introduced to take the dependence structure of observed data into account.[33] The Generalized Additive Mixed Models were fitted using the mgcv package of R (settings were fixed at package defaults). To be more conservative, the uncertainty of estimated parameters was assessed by 1000 bootstraps on individuals. For those *P* values lower than 0.001, 10,000 bootstraps were performed whenever possible. Multivariate analysis used the same methodology as previously described for the upstream *CRTC1* gene:[11] It was first conducted in the discovery sample, and the significant results were tested for replication in the 2 replication samples. In fitted longitudinal models, stratification by sex, and in some cases by age, was applied when analyzing all samples together. Also, analyses on WC and on other metabolic traits (ie, BGL and lipid levels) were conducted in the discovery sample (data available only in this sample) and only for *rs11552145* and *rs2071023* polymorphisms. Because of some missing data and the relatively low number of variant alleles of *rs707555* and *rs8123020*, analysis could not be conducted for these polymorphisms. Finally, it should be mentioned that preliminary analysis on *PCK1* haplotypes and BMI for the 3 SNPs that formed a haplotype block (ie, *rs11552145, rs707555* and *rs8123020*) showed no significant results (results not shown).

Population-Based Samples

Significant results from *PCK1* polymorphisms in the discovery sample (ie, *rs6070157*, proxy of *rs11552145*; $r^2 = 0.99$ and *rs2071023*) were further tested for replication in the three population samples (CoLaus, GIANT, and Global Lipids Genetics Consortium).

The associations of *PCK1* polymorphisms with adiposity markers, such as BMI, WC, fat mass, and lipid factors, were analyzed using multiple linear regression with additive model in which potential confounding factors, such as age, sex, and smoking status, were added as covariates in the CoLaus study. For anthropometric traits (BMI, WHR), we performed lookups from the summary statistics of the GIANT consortium. For lipid traits (ie, TG, HDL, CHOL), we looked up association results from the Global Lipid Consortium.[30]

RESULTS

Supplementary Table S-3, Supplemental Digital Content 4, http://links.lww.com/JCP/A316 shows the characteristics of the 3 psychiatric samples. The discovery sample included patients with the shortest treatment duration (median of 6 months versus 27.4 and 36 months in the replications 1 and 2, respectively, $P = 0.0001$), as well as the lowest BMI (current median BMI of 25 versus 28 and 27 kg/m^2 for replications 1 and 2, respectively, $P = 0.0001$) and the lowest prevalence of obesity (BMI ≥ 30 kg/m^2) (18% versus 40% and 27%, respectively, $P < 0.001$).

Association of *PCK1* Polymorphisms With BMI in Psychiatric Populations

Supplementary Table S-2, Supplemental Digital Content 2, http://links.lww.com/JCP/A314 shows *PCK1* genotype distribution among the 3 psychiatric samples. No significant associations were found between *PCK1* polymorphisms and baseline BMI when exploratory analyses were conducted (Supplementary Table S-4, Supplemental Digital Content 5, http://links.lww.com/JCP/A317). However, a trend and a significant association were found between *rs11552145* and *rs2071023* and current BMI (BMI at the last follow-up assessment) in the discovery (*P*-corrected 0.08 and 0.018, respectively) and in the combined sample (*P*-corrected 0.01 and 0.003, respectively). Figure 1 shows the association of PCK1 *rs11552145* polymorphism with BMI.

Multivariate analyses were first conducted in the discovery sample for the 4 SNPs (Table 1). Carriers of *rs11552145-AA* genotype had, on average, 2.20 lower BMI units when compared to carriers of *G-allele* ($P = 0.0004$). Similar results were found for *rs2071023-CC* genotype which had 1.27 lower BMI units when compared to *G-allele* carriers ($P = 0.004$). Significant results were replicated for *rs11552145* and BMI when combining the 2 replication samples. *AA* carriers had 1.42 lower BMI units when compared to *G-allele* carriers ($P = 0.009$). When combining the 3 samples, similar results were found for both *rs11552145* and *rs2071023* (estimates, −1.89 and −1.11 kg/m^2; and $P < 0.001$ and $P < 0.001$, respectively). Explained variances in the combined sample for *rs11552145* and *rs2071023* were 0.65% and 0.85%, respectively. For both *rs11552145* and *rs2071023*, further analyses stratified by sex and age were conducted in the 3 samples combined. *rs2071023* was associated with BMI only in women, whereas for *rs11552145* an association was found in both sexes, but a stronger association was found among women younger than 45 years, where *rs11552145 AA*-carriers had 2.25 lower BMI units when compared to *G-allele* carriers (*P* value of 0.009, explained variance 0.77%). No significant results were found for the other 2 SNPs *rs8123020* and *rs707555*.

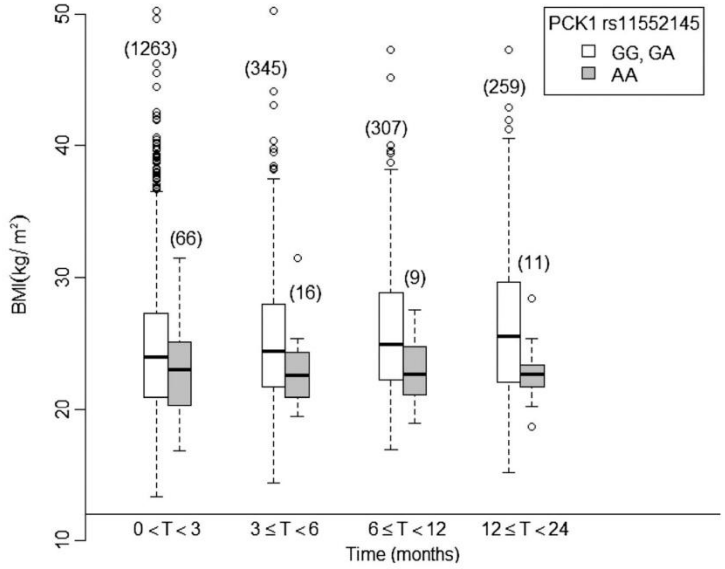

FIGURE 1. BMI in relation to *rs11552145 G > A* genotypes in the combined sample presented at different time periods of the current psychotropic treatment. Boxplots show median values of BMI for each time of the treatment duration (solid horizontal line), 25th and 75th percentile values (box outline), the lowest and upper value within 1.5 interquartile range (whiskers) and outlier values (open circles).

PCK1 Polymorphisms and Metabolic Parameters in Psychiatric Populations

The association of *rs11552145* and *rs2071023* with other metabolic parameters (ie, WC, BGL, CHOL, HDL, LDL, and TG) was analyzed in the discovery sample (Table 2). In agreement with results on BMI, both carriers of *rs11552145-AA* genotype and *rs2071023-CC* genotype had significantly lower WC (−6.86 and −3.45 cm, *P* values of 0.008 and 0.004, respectively). In addition, *rs11552145-AA* genotype carriers had lower TG levels when compared to *G-allele* carriers (−27.59 mg/100 mL, $P < 0.002$).

Association of *CRTC1* and *PCK1* with BMI

Since *PCK1* is a downstream gene of *CRTC1*, we wanted to further analyze the association of both *CRTC1 rs3746266A > G* previously associated with BMI[11] and *PCK1 rs11552145G > A* with BMI over treatment duration (Fig. 2). In the combined analysis, *CRTC1 G-allele* and *PCK1 AA* genotype were pooled together since carriers of these alleles showed lower BMI units when compared to others when analyzed individually. Thus, in the multivariate analysis adjusted by age, sex, treatment, and treatment duration (n = 610), those carriers of *AA* genotype for *CRTC1* and *PCK1* or carriers of *G-allele* of *CRTC1* and *PCK1* had 0.79 less units of BMI when compared to the reference group ($P = 0.009$). Similarly, carriers of *PCK1 AA* genotype and *CRTC1 G-allele* had 2.43 less units of BMI compared to the reference group ($P < 0.001$).

Functional Relevance of *PCK1* Polymorphisms

We explored further the functional relevance of *PCK1* polymorphisms. For *rs11552145* and *rs707555*, the 2 variants in coding regions, PolyPhen-2[34] predicted both mutations to be benign. Further analysis on gene expression platform (GTEX portal[35]) showed significant differences in *rs11552145* expression in subcutaneous adipose tissue with homozygous carriers of the variant allele having lower expression ($P = 0.03$). No differences were found for *rs707555*, *rs8123020* or *rs2071023*.

PCK1 Polymorphisms in Population-Based Samples

The association of *rs6070157* (proxy of *rs11552145*, $r^2 = 0.97$) and *rs2071023* with BMI and other metabolic features was further analyzed for replication in 3 population-based samples (GIANT, CoLaus, and Global Lipids Genetics Consortium). Significant associations were found between the 2 *PCK1* polymorphisms and the WHR in the GIANT cohort (N = 123,865) for women and for both sexes combined. In addition, significant associations were found for *rs2071023* with HDL and TGL in the Global Lipids Genetics Consortium (N = 100,184; *P* values of 0.003 and 0.03, respectively) (Table 3).

DISCUSSION

Growing evidence supports that *PCK* can contribute to obesity and metabolic syndrome both in animal models and in the general population.[12–14,16,17] The main results from this study suggest that carriers of *PCK1 rs11552145-AA* genotype have lower BMI when compared to *G-allele* carriers in psychiatric patients treated with weight gain inducing drugs, this association being found in the discovery sample and in the replication samples analyzed together. Moreover, low WC and TG levels were associated with *rs11552145-AA* in the discovery sample, and low BMI and WC were found as well for *rs2071023-CC* genotype. To our knowledge, this is the first study carried out in psychiatric patients and the first one to find a positive association between *PCK1* polymorphisms and BMI.

In addition, as a proof of concept, a positive association was found in the general population (GIANT cohort) with WHR and *rs6070157* (proxy of *rs11552145*, $r^2 = 0.99$) and *rs2071023*, again suggesting an association of the polymorphisms with obesity traits, although the value was much weaker than in

TABLE 1. Multivariate Analysis of *PCK1* Polymorphisms and BMI

		rs11552145			*rs2071023*			*rs707555*			*rs8123020*		
Sample	**n**	**BMI Difference (kg/m^2) Between AA and G-allele Carriers (95% CI)**	***P***	**E var, %**	**BMI Difference (kg/m^2) Between CC and G-allele Carriers (95% CI)**	***P***	**E var, %**	**BMI Difference (kg/m^2) Between GG and C-allele Carriers (95% CI)**	***P***	**E var, %**	**BMI Difference (kg/m^2) Between TT and C-allele Carriers (95% CI)**	***P***	**E var, %**
Discovery Sample*	423	−2.20 (−3.35 to −1.12)	**0.0004**†	0.84	−1.27 (−2.09 to −0.49)	**0.004**†	1.24	−0.38 (−3.26 to 2.21)	1.00†		−0.83 (−2.46 to 0.82)	0.5†	
Replication 1	168	−1.82 (−4.24 to 0.45)	0.07		−0.73 (−1.97 to 0.61)	0.1							
Replication 2	183	−0.64 (−2.72 to 1.22)	0.2		−0.18 (−1.40 to 1.04)	0.4							
Replication 1 and Replication 2‡	337	−1.42 (−2.69 to (−)0.25)	**0.009**	0.49	−0.53 (−1.40 to 0.41)	0.1							
Combined sample‡	760	−1.89 (−2.67 to (−)1.09)	**<0.001**	0.65	−1.11 (−1.71 to −0.52)	**<0.001**	0.85						
Combined sample men‡	377	−1.98 (−3.18 to (−)0.85)	**0.001**	1.01	−0.63 (−1.49 to 0.23)	*0.08*							
Combined sample women‡	383	−1.70 (−2.79 to (−)0.62)	**0.002**	0.35	−1.58 (−2.41 to −0.72)	**0.0001**	1.55						
Combined sample women <45 y‡	151	−2.25 (−4.18 to (−)0.45)	**0.009**	0.77	−1.48 (−2.74 to −0.11)	**0.01**	0.57						
Combined sample women ≥ 45 y‡	235	−1.54 (−3.59 to 0.86)	*0.06*		−1.68 (−2.74 to −0.60)	**0.002**	1.63						

*bootstrap at 10 000. Only significant results in discovery sample were further tested for replication.

†*P*-corrected value for discovery sample.

‡Patients treated for up to 24 months.

E var (%): explained variance by the polymorphism, only calculated for significant tests.

Adjusted by: age, sex, treatment (antipsychotic or mood stabilizer) and treatment duration. Bootstrap at 1000.

95% CI indicates 95% confidence interval. Values in bold are significant.

TABLE 2. Association of *PCK1* Polymorphisms With Other Metabolic Phenotypes in the Discovery Sample

rs11552145	n	Difference Between AA and G-allele Carriers (95% CI)	*P**	E.var (%)
WC, cm	408	−6.86 (−11.07 to (−)2.59)	**0.008**	1.04
HDL,† mg/100 mL	305	5.85 (−1.95 to 14.04)	0.13	
TG,† mg/100 mL	305	−27.59 (−39.16 to (−)14.24)	**<0.002**	0.90
LDL,† mg/100 mL	299	−10.14 (−19.89 to 2.34)	0.12	
CHOL,† mg/100 mL	307	−10.53 (−28.08 to 8.19)	0.28	
BGL,† mg/100 mL	289	−3.6 (−8.28 to 0.36)	0.09	
rs2071023	**n**	**Difference between CC and G-allele carriers (95% CI)**	***P****	**E.var (%)**
WC, cm	409	−3.45 (−5.74 to −1.18)	**0.004**	1.14
HDL,† mg/100 mL	305	1.95 (−0.39 to 4.29)	0.12	
TG,† mg/100 mL	305	−8.01 (−19.58 to 3.56)	0.64	
LDL,† mg/100 mL	299	−2.34 (−10.14 to 5.07)	0.54	
CHOL,† mg/100 mL	307	−3.12 (−11.7 to 5.07)	0.32	
BGL,† mg/100 mL	289	2.52 (−2.16 to 5.94)	0.42	

*P-corrected value for discovery sample.

†Fasting patients

E. var (%): explained variance by the polymorphism (%) calculated only for significant tests.

Adjusted by: BMI, age, sex, treatment (antipsychotic or mood stabilizer) and treatment duration. Bootstrap at 1000. Values in bold are significant.

psychiatric samples and being of no clinical significance in the general population. This goes in the same line of what we found in previous results,[11] because psychiatric populations are at high risk of obesity and/or metabolic syndrome. PCK1 function has been previously associated in animal models with glucose and lipid homeostasis and also with weight gain.[36] In humans, the main investigated polymorphism is the *-232C/G* (*rs2071023*) which is located in the promoter region of *PCK1.* This polymorphism has been previously associated with T2DM and gestational diabetes mellitus but with conflicting results in different

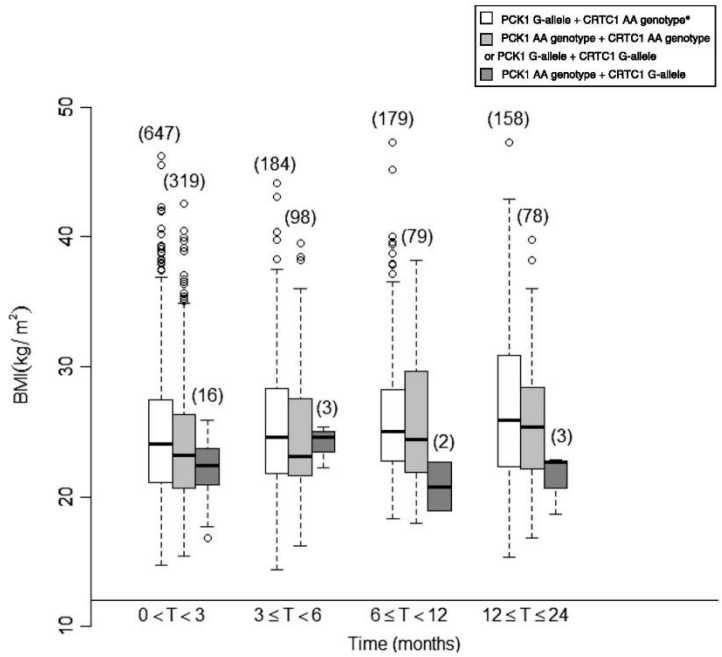

FIGURE 2. Association of *PCK1 rs11552145* and *CRTC1 rs3746266* genotypes with BMI over the time in all samples. * Reference group. Boxplots show median values of BMI for each time of the treatment duration (solid horizontal line), 25th and 75th percentile values (box outline), the lowest and upper value within 1.5 interquartile range (whiskers) and outlier values (open circles).

TABLE 3. Association of *PCK1* Polymorphisms With Metabolic Traits in Population-Based Samples

	CoLaus (n = 5338)		GIANT (n = 123,865)		Global Lipids Genetics Consortium (n = 100,184)	
***rs6070157* (proxy of *rs11552145*, $r^2 = 0.99$)**	**β (SE)**	***P***	**β (SE)**	***P***	**β (SE)**	***P***
Anthropometric traits						
BMI, kg/m^2	−0.0016 (0.0258)	0.95	0.0025 (0.0053)	0.63	N.A.	N.A.
WC, cm	−0.0026 (0.0258)	0.92	N.A.	N.A.	N.A.	N.A.
WHR	−0.0123 (0.0258)	0.63	−0.0163 (0.0071)	**0.02**	N.A.	N.A.
Men	0.0086 (0.038)	0.82	0.0151 (0.0096)	0.11	N.A.	N.A.
Women	−0.0308 (0.035)	0.39	−0.0202 (0.0089)	**0.02**	N.A.	N.A.
Lipids						
HDL, mg/100 mL	0.38 (0.37)	0.30	N.A.	N.A.	0.16 (0.12)	0.20
CHOL, mg/100 mL	−0.14 (1.02)	0.89	N.A.	N.A.	0.05 (0.12)	0.69
TG, mg/100 mL	−3.25 (2.57)	0.21	N.A.	N.A.	−0.10 (−0.28)	0.73
LDL, mg/100 mL	−0.41 (0.90)	0.65	N.A.	N.A.	N.A.	N.A.
BGL, mg/100 mL	0.85 (0.55)	0.12	N.A.	N.A.	−0.06 (−0.08)	0.50
rs2071023	**β (SE)**	***P***	**β (SE)**	***P***	**β (SE)**	***P***
Anthropometric traits						
BMI, kg/m^2	−0.0196 (0.0198)	0.32	−0.0028 (0.0043)	0.2	vN.A.	N.A.
WC, cm	−0.0087 (0.0198)	0.66	N.A.	N.A.	N.A.	N.A.
WHR	0.0026 (0.0198)	0.90	−0.0195 (0.0057)	**0.001**	N.A.	N.A.
Men	−0.0145 (0.029)	0.61	−0.0013 (0.0077)	0.87	N.A.	N.A.
Women	0.0184 (0.028)	0.50	−0.0154 (0.0071)	**0.03**	N.A.	N.A.
Lipids						
HDL, mg/100 mL	−0.54 (0.28)	0.06	N.A.	N.A.	0.28 (0.12)	**0.003**
CHOL, mg/100 mL	−0.99 (0.78)	0.20	N.A.	N.A.	0.078 (0.12)	0.54
TG, mg/100 mL	1.11 (1.98)	0.57	N.A.	N.A.	−0.61 (−0.28)	**0.03**
LDL, mg/100 mL	−0.58 (0.69)	0.41	N.A.	N.A.	N.A.	N.A.
BGL, mg/100 mL	−0.35 (0.42)	0.41	N.A.	N.A.	−0.09 (−0.07)	0.16

N.A. indicates data not available. Values in bold are significant.

ethnicities. Positive associations were found among South Asian and Japanese populations[20,37] concluding that carriers of the minor allele (*GG*) were at risk of developing T2DM, whereas no significant findings were found in German or Danish Caucasian populations.[18,21] Finally, a case series study conducted in 3 Maltese women found that those who developed gestational diabetes mellitus carried the homozygous variant allele, but these results must be replicated in larger cohorts.[38] In the present study, no association was found between *rs2071023* and BGL, although the diabetes phenotype was not assessed. Additionally, and consistent with our results, another *PCK1* polymorphism (*rs707555*) showed no significant association with anthropometric traits such as WC, weight, and fat mass or BMI.[22,39]

Analyses were conducted in the combined discovery and replication samples for treatment duration up to 24 months. Different effect sizes, detected in the discovery versus the replication samples, could be explained by lower prevalence of obesity at baseline and shorter treatment durations in the discovery sample (Supplementary Table S-3, Supplemental Digital Content 4, http://links.lww.com/JCP/A316), because both baseline BMI and treatment duration are moderators of weight gain.[40] However, to exclude a winner's curse event, these results need to be replicated in other short treatment duration samples.

Of note, in the present study as in previous genetic studies, genetically explained variances of BMI are quite low, suggesting that BMI and metabolic features are influenced by multiple genetic factors as previously described in the literature.[4] However, in the present study, *rs11552145* was strongly associated with BMI in the subgroup of women younger than 45 years, and the observed difference in BMI between genotypes is of clinical significance. This result is in agreement with our previous study showing that the association between a polymorphism of *CRTC1* (an upstream gene of *PCK1*) and BMI was higher in women younger than 45 years as compared to nongender stratified sample.[11] In addition, a positive correlation was found between PCK1 mRNA expression levels and BMI in a study conducted with nonmenopausal women.[15] Other pharmacogenetic studies also highlighted the importance of stratifying by sex.[41,42] This finding could be tentatively explained by the influence of estrogen circulating levels on energy balance.[43] Thus, a lack of estrogen in mice was related to obesity, decreasing fasting BGL, activating adenosine monophosphate protein kinase (AMPK), and reducing the expression of gluconeogenic genes, such as *PCK* in the liver.[44,45] However, this hypothesis could not be tested in our samples because estrogen circulating levels were not measured.

To assess the contribution of *PCK1* and *CRTC1* polymorphisms on BMI, analyses combining both SNPs were conducted. An additive association with BMI was observed over treatment duration among carriers of *CRTC1 rs3746266 G-allele* and *PCK1 rs11552145 AA* genotype which had lower BMI when compared to the reference group. As described elsewhere,[46] *PCK* family genes contain in their promoter region a CREB-regulated element binding site where CRTC1 binds, enhancing *PCK* expression. In the present study, the strongest associations

were found among psychiatric population under psychotropic treatment which could be explained by the additive effect between *PCK1* and *CRTC1* genes and psychotropic drugs. In particular, *CRTC1* is modulated, among other mechanisms, by AMPK which is increased by antipsychotics.[47] Besides, several polymorphisms on the *AMPK* gene, showed an association with weight gain induced by antipsychotics.[48] *AMPK* has also been related to gluconeogenesis modulation.[49] Another study conducted in rats showed that olanzapine increased the mRNA levels of glucose-6-phosphatase in the liver.[47] Although little is known about *PCK* family genes and psychotropic drugs, *PCK* expression is inhibited by lithium in isolated hepatocytes from fasted rats.[50] In addition, chronic clozapine administration upregulates *PCK* expression in rat liver.[51] Therefore, several genes coding for enzymes implicated in the gluconeogenic pathway have been associated with antipsychotics.

Finally, in our sample, higher associations were found among psychiatric patients rather than in general population possibly explained by the high prevalence of overweight or obesity in psychiatric patients induced by the illness, the lifestyle (diet, physical activity), in addition to the direct effect of drug inducing weight gain.

Some limitations of the present study must be mentioned. First, patients were not drug naive, therefore, we could not assess whether the association between the polymorphisms and BMI or other phenotypes was influenced by the psychiatric illness itself and/or by the psychotropic treatment. Second, although the main inclusion criteria for patients in the present study was that they were receiving psychotropic drugs known to induce weight gain (ie, aripiprazole, amisulpride, clozapine, olanzapine, quetiapine, risperidone, mirtazapine, lithium, and/or valproate), other drugs possibly inducing weight (psychotropic and/or somatic drugs) were prescribed, the influence of which could not be evaluated. This study was conducted in whites, thus results cannot be extrapolated to other ethnicities. Not all tagging SNPs could be tested due to limited availability of the genotypes. In addition, no significant associations with BMI were found for the 2 other tested SNPs *(rs707555* and *rs8123020*), either because of a lack of effect or a lack of power due to the low MAF. Further replications of this study should increase sample size to test low MAF polymorphisms and to increase the coverage of *PCK1* gene by including other tagging SNPs. Finally, variants obtained through GWAS should be also considered in further analysis, in particular those on gluconeogenic pathway. It has thus been recently shown that *PCK1* expression is regulated by *CAMK1D*,[52] a gene previously related to diabetes in GWAS.[53]

In conclusion, this is the first study investigating the association of *PCK1* polymorphisms with BMI and other metabolic traits in psychiatric populations. Higher associations were found in psychiatric patients treated with psychotropic drugs over short periods, and in women younger than 45 years. In addition, the present study supports research on pathway related genes, such as *CRTC1* and *PCK1*, which may have an additive association with BMI. Further studies on the same and other pathways are therefore warranted, to increase our knowledge on the multiple genetic risk factors influencing obesity, lipid disturbances or metabolic syndrome in psychiatric population. This could ultimately help, by the determination and the combination of multiple genetic and clinical risk factors, to better adapt pharmacological treatments among particular populations at risk.

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REFERENCES

1. Nihalani N, Schwartz TL, Siddiqui UA, et al. Obesity and psychotropics. *CNS Neurosci Ther.* 2012;18:57–63.
2. De Hert M, Dekker JM, Wood D, et al. Cardiovascular disease and diabetes in people with severe mental illness position statement from the European Psychiatric Association (EPA), supported by the European Association for the Study of Diabetes (EASD) and the European Society of Cardiology (ESC). *Eur Psychiatry.* 2009;24:412–424.
3. Laursen TM, Munk-Olsen T, Vestergaard M. Life expectancy and cardiovascular mortality in persons with schizophrenia. *Curr Opin Psychiatry.* 2012;25:83–88.
4. Speliotes EK, Willer CJ, Berndt SI, et al. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nat Genet.* 2010;42:937–948.
5. Balt SL, Galloway GP, Baggott MJ, et al. Mechanisms and genetics of antipsychotic-associated weight gain. *Clin Pharmacol Ther.* 2011;90: 179–183.
6. Kao AC, Muller DJ. Genetics of antipsychotic-induced weight gain: update and current perspectives. *Pharmacogenomics.* 2013;14:2067–2083.
7. Beale EG, Harvey BJ, Forest C. PCK1 and PCK2 as candidate diabetes and obesity genes. *Cell Biochem Biophys.* 2007;48:89–95.
8. Yang J, Kalhan SC, Hanson RW. What is the metabolic role of phosphoenolpyruvate carboxykinase? *J Biol Chem.* 2009;284: 27025–27029.
9. Kotelevtsev Y, Holmes MC, Burchell A, et al. 11beta-hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid-inducible responses and resist hyperglycemia on obesity or stress. *Proc Natl Acad Sci U S A.* 1997;94:14924–14929.
10. Oh KJ, Han HS, Kim MJ, et al. CREB and FoxO1: two transcription factors for the regulation of hepatic gluconeogenesis. *BMB Rep.* 2013;46: 567–574.
11. Choong E, Quteineh L, Cardinaux JR, et al. Influence of CRTC1 polymorphisms on body mass index and fat mass in psychiatric patients and in the general adult population. *JAMA Psychiatry.* 2013;70:1011–1019.
12. Franckhauser S, Munoz S, Elias I, et al. Adipose overexpression of phosphoenolpyruvate carboxykinase leads to high susceptibility to diet-induced insulin resistance and obesity. *Diabetes.* 2006;55:273–280.
13. Valera A, Pujol A, Pelegrin M, et al. Transgenic mice overexpressing phosphoenolpyruvate carboxykinase develop non-insulin-dependent diabetes mellitus. *Proc Natl Acad Sci U S A.* 1994;91:9151–9154.
14. Olswang Y, Cohen H, Papo O, et al. A mutation in the peroxisome proliferator-activated receptor gamma-binding site in the gene for the cytosolic form of phosphoenolpyruvate carboxykinase reduces adipose tissue size and fat content in mice. *Proc Natl Acad Sci U S A.* 2002;99: 625–630.
15. Chang TJ, Lee WJ, Chang HM, et al. Expression of subcutaneous adipose tissue phosphoenolpyruvate carboxykinase correlates with body mass index in nondiabetic women. *Metabolism.* 2008;57:367–372.

16. Zhao J, Xiao P, Guo Y, et al. Bivariate genome linkage analysis suggests pleiotropic effects on chromosomes 20p and 3p for body fat mass and lean mass. *Genet Res (Camb)*. 2008;90:259–268.
17. Lee JH, Reed DR, Li WD, et al. Genome scan for human obesity and linkage to markers in 20q13. *Am J Hum Genet*. 1999;64:196–209.
18. Wegner L, Andersen G, Albrechtsen A, et al. Large-scale study of the -232C > G polymorphism of PCK1 in Type 2 diabetes. *Diabet Med*. 2006; 23:1140–1144.
19. Dong Y, Zhang H, Wang X, et al. A Leu184Val polymorphism in PCK1 gene is associated with type 2 diabetes in Eastern Chinese population with BMI < 23 kg/m2. *Diabetes Res Clin Pract*. 2009;83:227–232.
20. Rees SD, Britten AC, Bellary S, et al. The promoter polymorphism -232C/G of the PCK1 gene is associated with type 2 diabetes in a UK-resident South Asian population. *BMC Med Genet*. 2009;10:83.
21. Gouni-Berthold I, Giannakidou E, Faust M, et al. Association of the promoter polymorphism -232C/G of the phosphoenolpyruvate carboxykinase gene (PCK1) with type 2 diabetes mellitus. *Diabet Med*. 2006;23:419–425.
22. Larsen LH, Angquist L, Vimaleswaran KS, et al. Analyses of single nucleotide polymorphisms in selected nutrient-sensitive genes in weight-regain prevention: the DIOGENES study. *Am J Clin Nutr*. 2012;95: 1254–1260.
23. Shin HD, Park BL, Kim LH, et al. Association of a polymorphism in the gene encoding phosphoenolpyruvate carboxykinase 1 with high-density lipoprotein and triglyceride levels. *Diabetologia*. 2005;48:2025–2032.
24. Choong E, Solida A, Lechaire C, et al. Suivi du syndrome métabolique induit par les antipsychotiques atypiques: recomendations et perspectives pharmacogénétiques. *Rev Med Suisse*. 2008;4:1994–1999.
25. Hiemke C, Baumann P, Bergemann N, et al. AGNP consensus guidelines for therapeutic drug monitoring in psychiatry: update 2011. *Pharmacopsychiatry*. 2011;44:195–235.
26. Choong E, Bondolfi G, Etter M, et al. Psychotropic drug induced weight gain and other metabolic complications in a Swiss Psychiatric population. *J Psychiatr Res*. 2012;46:540–548.
27. Firmann M, Mayor V, Vidal PM, et al. The CoLaus study: a population-based study to investigate the epidemiology and genetic determinants of cardiovascular risk factors and metabolic syndrome. *BMC Cardiovasc Disord*. 2008;8:6.
28. Lango Allen H, Estrada K, Lettre G, et al. Hundreds of variants clustered in genomic loci and biological pathways affect human height. *Nature*. 2010; 467:832–838.
29. Heid IM, Jackson AU, Randall JC, et al. Meta-analysis identifies 13 new loci associated with waist-hip ratio and reveals sexual dimorphism in the genetic basis of fat distribution. *Nat Genet*. 2010;42:949–960.
30. Teslovich TM, Musunuru K, Smith AV, et al. Biological, clinical and population relevance of 95 loci for blood lipids. *Nature*. 2010;466: 707–713.
31. International HapMap Consortium. The International HapMap Project. *Nature*. 2003;426:789–796.
32. R Core Team. R: A language and environment for statistical computing, R Fondation for Statistical Computing,Vienna, Austria. In: *R Foundation for Statistical Computing*. Vienna, Austria: R Development Core Team; 2013.
33. Lin X, Zhang D. Inference in generalized additive mixed models by using smoothing splines. *J R Statist Soc B*. 1999;61:381–400.
34. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods*. 2010;7:248–249.
35. Keen JC, Moore HM. The Genotype-Tissue Expression (GTEx) Project: linking clinical data with molecular analysis to advance personalized medicine. *J Pers Med*. 2015;5:22–29.
36. Rosella G, Zajac JD, Baker L, et al. Impaired glucose tolerance and increased weight gain in transgenic rats overexpressing a non-insulin-responsive phosphoenolpyruvate carboxykinase gene. *Mol Endocrinol*. 1995;9:1396–1404.
37. Horikawa Y, Yamasaki T, Nakajima H, et al. Identification of a novel variant in the phosphoenolpyruvate carboxykinase gene promoter in Japanese patients with type 2 diabetes. *Horm Metab Res*. 2003;35: 308–312.
38. Abou-Hussein S, Savona-Ventura C, Grima S, et al. Genetic factors in risk assessment for the development of type 2 diabetes mellitus in a small case series. *Int J Risk Saf Med*. 2011;23:119–123.
39. Vimaleswaran KS, Franks PW, Brage S, et al. Lack of association between PCK1 polymorphisms and obesity, physical activity, and fitness in European Youth Heart Study (EYHS). *Obesity*. 2010;18:1975–1980.
40. De Hert M, Detraux J, van Winkel R, et al. Metabolic and cardiovascular adverse effects associated with antipsychotic drugs. *Nat Rev Endocrinol*. 2012;8:114–126.
41. Vehof J, Al Hadithy AF, Burger H, et al. Association between the ROBO1 gene and body mass index in patients using antipsychotics. *Psychiatr Genet*. 2011;21:202–207.
42. Gregoor JG, van der Weide J, Loovers HM, et al. Polymorphisms of the LEP, LEPR and HTR2C gene: obesity and BMI change in patients using antipsychotic medication in a naturalistic setting. *Pharmacogenomics*. 2011;12:919–923.
43. Clegg DJ, Brown LM, Woods SC, et al. Gonadal hormones determine sensitivity to central leptin and insulin. *Diabetes*. 2006;55:978–987.
44. Mandour T, Kissebah AH, Wynn V. Mechanism of oestrogen and progesterone effects on lipid and carbohydrate metabolism: alteration in the insulin: glucagon molar ratio and hepatic enzyme activity. *Eur J Clin Invest*. 1977;7:181–187.
45. Kim JY, Jo KJ, Kim OS, et al. Parenteral 17beta-estradiol decreases fasting blood glucose levels in non-obese mice with short-term ovariectomy. *Life Sci*. 2010;87:358–366.
46. Hanson RW, Reshef L. Regulation of phosphoenolpyruvate carboxykinase (GTP) gene expression. *Annu Rev Biochem*. 1997;66:581–611.
47. Ikegami M, Ikeda H, Ohashi T, et al. Olanzapine increases hepatic glucose production through the activation of hypothalamic adenosine 5′-monophosphate-activated protein kinase. *Diabetes Obes Metab*. 2013;15: 1128–1135.
48. Souza RP, Tiwari AK, Chowdhury NI, et al. Association study between variants of AMP-activated protein kinase catalytic and regulatory subunit genes with antipsychotic-induced weight gain. *J Psychiatr Res*. 2012;46: 462–468.
49. Kahn BB, Alquier T, Carling D, et al. AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. *Cell Metab*. 2005;1:15–25.
50. Bosch F, Rodriguez-Gil JE, Hatzoglou M, et al. Lithium inhibits hepatic gluconeogenesis and phosphoenolpyruvate carboxykinase gene expression. *J Biol Chem*. 1992;267:2888–2893.
51. Tulipano G, Rizzetti C, Bianchi I, et al. Clozapine-induced alteration of glucose homeostasis in the rat: the contribution of hypothalamic-pituitary-adrenal axis activation. *Neuroendocrinology*. 2007; 85:61–70.
52. Haney S, Zhao J, Tiwari S, et al. RNAi screening in primary human hepatocytes of genes implicated in genome-wide association studies for roles in type 2 diabetes identifies roles for CAMK1D and CDKAL1, among others, in hepatic glucose regulation. *PLoS One*. 2013;8:e64946.
53. Zeggini E, Scott LJ, Saxena R, et al. Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. *Nat Genet*. 2008;40:638–645.

2. Project II

Association of Genetic Risk Scores (GRS) with Body Mass Index in Swiss Psychiatric Cohorts

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Summary

Common obesity is a polygenic disease; therefore studies focusing on polygenic scores are warranted. In this second project conducted in psychiatric populations, a polygenic risk score or genetic risk scores (GRS) approach was used to test the association of combined genetic factors with BMI. Several GRS were built from Candidate Gene (CG) or Genome Wide Association (GWA) studies from psychiatric and general populations, respectively. When stratifying analysis by sex, stronger associations were found between GRS and BMI among men while no association was found among women. The present work replicates in different psychiatric cohorts treated with weight gain inducing drugs the results found in large general populations.

Original article 1

Association of genetic risk scores with body mass index in Swiss psychiatric cohorts

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Objective **Weight gain is associated with psychiatric disorders and/or with psychotropic drug treatments. We analyzed in three psychiatric cohorts under psychotropic treatment the association of weighted genetic risk scores (w-GRSs) with BMI by integrating BMI-related polymorphisms from the candidate-gene approach and Genome-Wide Association Studies (GWAS).**

Materials and methods **w-GRS of 32 polymorphisms associated previously with BMI in general population GWAS and 20 polymorphisms associated with antipsychotics-induced weight gain were investigated in three independent psychiatric samples.**

Results **w-GRS of 32 polymorphisms were significantly associated with BMI in the psychiatric sample 1 ($n = 425$) and were replicated in another sample ($n = 177$). Those at the percentile 95 (p95) of the score had 2.26 and 2.99 kg/m^2 higher predicted BMI compared with individuals at the percentile 5 (p5) in sample 1 and in sample 3 ($P = 0.009$ and 0.04, respectively). When combining all samples together ($n = 750$), a significant difference of 1.89 kg/m^2 predicted BMI was found between p95 and p5 individuals at 12 months of treatment. Stronger associations were found among men (difference: 2.91 kg/m^2 of predicted BMI between p95 and p5, $P = 0.0002$), whereas no association was found among women. w-GRS of 20 polymorphisms was not associated with BMI. The w-GRS of 52 polymorphisms and the clinical variables (age, sex, treatment) explained 1.99 and 3.15%, respectively, of BMI variability.**

Conclusion **The present study replicated in psychiatric cohorts previously identified BMI risk variants obtained in GWAS analyses from population-based samples. Sex-specific analysis should be considered in further analysis. *Pharmacogenetics and Genomics* 00:000–000**

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Keywords: body mass index, genetic risk score, psychiatry, psychotropic drugs

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Introduction

Obesity has become a major public health concern, its prevalence increasing considerably over the last decades. Obesity is a complex disease that results from imbalance of energy intake and energy expenditure, being highly influenced by an individual's lifestyle or environment (i.e. diet, physical activity) and also by genetic predisposition [1]. Twin and family studies reported 40–80% of heritability in obesity [2,3]. Several forms of monogenic obesity have been described, especially those related to leptin–melanocortin pathways [4,5]. The most prevalent form of obesity, however, is the polygenic or common obesity, which results from the combined effect of common genetic variants as well as additional rare variants, copy number variants, and epigenetic changes [6]. Among psychiatric populations, the risk of developing obesity and related problems is increased compared with the general population [7]. Several factors have been attributed to this increased risk of obesity, such as the illness, the lifestyle, and/or the medication [8,9].

Since their introduction onto the market, second-generation antipsychotics have been used widely over first-generation antipsychotics as they clearly show an advantage in terms of reduced risks of extrapyramidal side-effects as well as some advantages for the treatment of negative symptoms. However, most second-generation antipsychotics can induce strong metabolic disturbances in particular as a consequence of the dual antagonism on serotonin and dopamine receptors and its effect on food

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intake regulation [10]. Over the last decade, pharmacogenetics of psychotropic-induced weight gain has been studied widely through hypothesis-driven candidate-gene (CG) approaches. The most studied and best-replicated polymorphisms focused on dopamine and serotonin receptors [11,12]. In addition, other genes implicated in leptin–melanocortin pathways (e.g. *LEP*, *LEPR*, *MC4R*, *NPY*), endocannabinoids (*CNR1*), or genes involved in fatty acids and cholesterol production (*SCARB1*, *INSIG2*) showed an association with weight gain among psychiatric cohorts treated with antipsychotics (see review in Lett *et al.* [13]). Recently, research carried out in our laboratory showed other CGs that could potentially induce weight gain among psychiatric populations under psychotropic treatment. These genes code for enzymes involved in metabolic pathways (PCK1, 11βHSD1) [14–16] for receptors (MCHR2, IRS2, and PPARGC1A) and for transcriptional coactivators (CRTC1, CRTC2) involved in energy balance, appetite regulation, and glucose homeostasis [17–21].

With the emergence of Genome-Wide Association Studies (GWAS), thousands of new polymorphisms associated with obesity and metabolic phenotypes have been elucidated. In particular, the associations with BMI and/or obesity in the *FTO* [22–25], *MC4R* [26–28], and *TMEM18* [23,24,28,29] genes have been replicated widely in general populations. The largest BMI meta-analysis of GWAS carried out to date in general populations reported 97 polymorphisms [30]. These variants also included 32 previously reported loci [31] that have been replicated in other cohorts and different ethnicities [32–34]. Individually, these variants have shown little effect on the BMI [31]. As an alternative way of testing individual single-nucleotide polymorphism (SNP) effects, genetic risk scores (GRSs) summarize risk-associated variations across the genome by aggregating information from multiple-risk SNPs [35], with small effects increasing the consistency and power to determine genetic risk in polygenic diseases (i.e. obesity) [36]. To date, GRS methods have been used in diabetes [37], schizophrenia [38], and obesity [31], among other diseases. Studies on obesity have been carried out in adults [36,39] or children from the general population [40,41], and recently, two studies were carried out among depressed patients [42,43]. The aim of the present study was to determine whether GRS constructed from previous BMI and/or weight gain-related variants from GWAS and CGs were associated with BMI in three independent psychiatric samples. In addition, we aimed to analyze whether previous variants related to diabetes (21 SNPs) and psychiatric disorders (nine SNPs) also showed an association with BMI as these diseases seem to share some genetic components with obesity [8,44,45].

Materials and methods

Samples description

Psychiatric samples

Sample 1 ($n = 425$) consisted of an on-going follow-up study that started in 2007 in the psychiatric ward from the Lausanne University Hospital already described elsewhere [46]. Briefly, 425 White patients starting psychotropic treatment including atypical antipsychotics, mood stabilizers, and/or mirtazapine were recruited. Anthropometric parameters such as weight, height, and waist circumference were measured. Other demographic covariates (i.e. sex, age, and ethnicity) as well as history of treatment (treatment duration, psychotropic treatment) were obtained from medical files or during the interview. Medical questionnaires were filled in and blood samples were collected at baseline and at 1, 2, 3, 6, and 12 months after initiating psychotropic treatment according to guidelines [47,48]. Patients switching to one of the studied treatments were also included. BMI (kg/m^2), the outcome in the present study, was used as a continuous variable and whenever required, stratified into three categories as normal ($BMI < 25\ kg/m^2$), overweight ($25 \geq BMI < 30\ kg/m^2$), and obese ($BMI \geq 30\ kg/m^2$). Twenty-one percent of patients had the first psychotic episode and/or were diagnosed within the first year of study inclusion [first episode and newly diagnosed (FEND) patients].

Two other psychiatric samples were used as replication. They consisted of two retrospective studies from outpatient settings in Geneva and in Lausanne (sample $2 = 148$, sample $3 = 177$, respectively). Both samples included patients receiving atypical antipsychotics and/or mood stabilizers (i.e. aripiprazole, amisulpride, clozapine, olanzapine, quetiapine, risperidone, lithium, and/or valproate). The Geneva study was conducted in 2007 in an outpatient Geneva setting and patients recruited had been under psychotropic treatment for at least 3 months. In the Lausanne study (sample 3 started in 2010, inclusions ongoing), most of the patients had been treated for more than 1 year in a Lausanne outpatient setting. For both studies, blood samples were collected and questionnaires were filled out during one of the routine checkups. Weight, height, waist circumference, serum lipids, and/or glucose were measured and several clinical variables (e.g. treatment, treatment duration) were recorded. Baseline weight (before the current psychotropic treatment) was extracted from medical files or self-reported. Further description of these samples has been published elsewhere [46].

Psychiatric diagnoses for the three samples were made according to ICD-10 classification criteria. The main diagnostic groups were F20.0–F24.9 and F28–F29: psychotic disorders; F25.0–F25.9: schizoaffective disorders; F30.0–F31.9: bipolar disorders; F32.00–F33.9: depression. The latest introduced psychotropic medication was considered the main psychotropic treatment. Written

informed consent was provided by all individuals or by their legal representatives and the studies were approved by the ethics committee of the corresponding centers.

General population-based sample

The Genetic Investigation of Anthropometric Traits Consortium (GIANT) carried out a meta-analysis of GWAS data with a discovery set of 123 865 individuals of European ancestry from 46 studies for height [49], BMI [31], and waist-to-hip ratio [50]. This general population-based sample was used to obtain β-coefficients (allele effect) that assigned weights to each variant when constructing the GRSs.

Single-nucleotide polymorphism selection, genotyping, and construction of genetic risk scores

The initial 32 polymorphisms selected for the present study had been associated with BMI in a GWAS meta-analysis carried out in an adult general population [31]. All selected variants reached GWAS significance ($P < 5 \times 10^{-8}$) (Supplementary Table S1, Supplemental digital content 1, *http://links.lww.com/FPC/A990*). Another 20 SNPs that had been previously related to antipsychotic-induced weight gain through the CG approach were also selected [13]. From the reviewed variants, only SNPs or proxies of SNPs genotyped in our sample and in GIANT, and only those in the literature yielding significant results in both sexes were retained for the analysis. A detailed description of the SNPs considered can be found in Supplementary Table S2 (Supplemental digital content 1, *http://links.lww.com/FPC/A990*).

Finally, we considered two meta-analyses of GWAS on the basis of 21 SNPs associated with type 2 diabetes (8130 cases and 38 987 controls; Supplementary Table S3, Supplemental digital content 1, *http://links.lww.com/FPC/A990*) and another one on the basis of nine SNPs associated with five major psychiatric disorders (final dataset: 33 332 cases and 27 888 controls; Supplementary Table S4, Supplemental digital content 1, *http://links.lww.com/FPC/A990*) including schizophrenia, bipolar disorder, major depressive disorder, autism, and attention deficit-hyperactivity disorder [51,52]. To avoid an indirect correlation between variants [i.e. in high linkage disequilibrium (LD) correlation], which is one of the problems when constructing GRS [53], and to avoid overrepresentation of a particular gene, only one SNP per gene was considered. Selection was made by selecting the SNP with the lowest *P*-value. We verified that the resulting SNPs were not in LD. Note that this approach is analogous to an LD-based pruning, but we typically select less SNPs by ignoring secondary (independent) SNP contributions from the same gene (allelic heterogeneity). The study protocol was approved by the ethics committees of the recruiting centers and all patients provided written informed consent for the genetic analysis. DNA was extracted from blood samples as described by the manufacturer's protocols using the Flexigene DNA Kit (Qiagen AG, Hombrechtikon, Switzerland) and the QIAamp DNA Blood Mini QIAcube Kit (Qiagen AG).

Genotyping of 895 White patients was performed using the Illumina 200K CardiometaboChip (Illumina, San Diego, California, USA). Briefly, the CardiometaboChip is a custom Illumina iSelect genotyping array designed to test DNA variation of 200 000 SNPs from regions identified by large-scale meta-analyses of GWAS for metabolic and cardiovascular traits [54]. A quality control was performed for the genotyped SNPs. Polymorphisms or proxies were chosen on the basis of genotype availability in the CardiometaboChip and GIANT cohort. In addition, samples were excluded from the analysis if sex was inconsistent with genetic data from X-linked markers and when genotype call rate was less than 0.96, gene call score less than 0.15, and minor allele frequency less than 0.05. GenomeStudio Data Analysis Software (San Diego, California, USA) was used to export results generated by Illumina CardiometaboChip. In addition, the rs7799039 from the *LEP* gene largely associated with antipsychotic-induced weight gain [55] and that was not available in CardiometaboChip was genotyped by the KBioscience Institute in UK using the novel fluorescence-based competitive allele-specific PCR technology (KASP; details of this technology are available at: *http://www.lgcgenomics.com/genotyping/kasp-genotyping-chemistry/*). Out of the 895 White genotyped individuals, 750 were finally analyzed (145 patients excluded because of missing data).

Among the several existing methods to build a GRS, it has been shown in disease risk modeling that weighted genetic risk score (w-GRS) methods are preferred to the simple count method when relative risks vary among SNPs [56]. Supplementary Fig. 1 (Supplemental digital content 2, *http://links.lww.com/FPC/A991*) represents the distribution of the weighted genetic score by the number of risk alleles (unweighted score) calculated for each individual in the entire cohort showing that weighted and unweighted scores are not perfectly correlated, thus highlighting the importance of weighting each risk allele using w-GRS methods. The w-GRS for selected SNPs was calculated as described previously [31]. In summary, genotypes from each SNP were coded as 0, 1, or 2 according to the number of BMI risk alleles. Then, each polymorphism was weighted by its β-coefficient (allele effect) on the basis of the assumption that all SNP of interest have independent effects and contribute in an additive manner toward BMI. Allele effect on BMI was assessed by performing lookups from the summary statistics of an independent population sample (GIANT, $n = 123\,865$), thus preserving the homogeneity of β-coefficient calculations (Supplementary Table S5, Supplemental digital content 1, *http://links.lww.com/FPC/A990*) for all SNPs included in the genetic score.

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Statistical analyses

Principal components of ancestry was used to assess ethnicity and only Whites were considered in the analysis. Hardy–Weinberg equilibrium (HWE) was determined for each polymorphism using a χ^2-test. HWE and genotype frequencies are shown in Supplementary Tables S1 and S2 (Supplemental digital content 1, *http://links.lww.com/FPC/A990*). *P*-values equal to or less than 0.05 were considered statistically significant and Bonferroni's correction for multiple tests was applied when necessary. Initially, individual SNP effects on BMI were calculated for sample 1. Genotypes were analyzed in an additive model of inheritance, except for one SNP (*HSD11β1* rs3753519C > T), which had too few homozygous for the variant allele ($n = 7$) and a dominant model was used. Second, a GRS was built and tested in sample 1 and it was further tested for replication in two other psychiatric samples (samples 2 and 3). Finally, to determine the general effect of the GRS on BMI, we combined all samples as they were similar overall in terms of the individual's origin (Lausanne and Geneva regions), type of treatment, age, and diagnostic. Because of interdependence between observations (i.e. BMI) made on the same individual over time, a generalized linear mixed model (GLMM) was fitted using the MASS library of R language [57,58] to assess the influence of genetic parameters on BMI in a model adjusted by age, sex, main psychotropic treatment, and treatment duration. The appropriate link function that we chose for the BMI variable is the inverse function, which is the canonical link function for the gamma family. GLMMs combine both linear mixed models (which incorporate random effects) and generalized linear models (which deal with non-normal data using link functions and exponential family) [59]. The glmmPQL function of the MASS library uses the penalized quasi-likelihood to estimate model parameters [60]. Finally, predicted BMI differences were calculated at baseline, 12, and 24 months of treatment between the percentile 95 (p95) (the upper extreme of an unfavorable genetic background) and percentile 5 (p5) (the lower extreme of an unfavorable genetic background) of the GRS. To preserve homogeneity within samples and to deal with treatment durations when combining all samples together (i.e. shorter treatment duration up to 12 months in sample 1), predicted BMI was obtained at baseline and at 12 months of treatment. The corresponding 95% confidence intervals (95% CIs) were calculated. Some exploratory analyses were also carried out to obtain the explained variance of BMI by genetic and nongenetic covariates in the psychiatric sample 1 for a subgroup of individuals aged between 18 and 65 years. A generalized additive mixed model was used to deal with complex and nonlinear BMI evolution in time and the presence of multiple observations per individual introducing interdependence among observations. A random effect at the patient level was also introduced to take the dependence structure of observed data into account. The generalized additive mixed models were fitted using the mgcv package of R (settings were fixed at package defaults). To be more conservative, the uncertainty of estimated parameters was assessed by 10 000 bootstraps on individuals [57,61,62]. Individuals with missing data or genotypes were excluded from the analysis (see Supplementary Methods for further details, Supplemental digital content 3, *http://links.lww.com/FPC/A992*).

Results

Population description

Table 1 presents the characteristics of sample 1 ($n = 425$) and replication samples 2 and 3 ($n_1 = 148$, $n_2 = 177$). All samples together included 750 White individuals, with 50% men and a median age of 45 years (range: 13–97 years). Sample 2 had the highest prevalence of obesity (BMI ≥ 30 kg/m^2) (35% compared with 18% in samples 1 and 3, $P = 0.006$). Sample 1 had the lowest olanzapine and clozapine prescription (11 and 8%, respectively, compared with 16 and 14% in sample 2, respectively, and 12 and 9% in sample 3, respectively, $P = 0.001$) as well as the shortest treatment duration (6 months) compared with samples 2 and 3 (27 and 36 months, respectively). Supplementary Tables S6 (Supplemental digital content 1, *http://links.lww.com/FPC/A990*) and S7 (Supplemental digital content 1, *http://links.lww.com/FPC/A990*) show the characteristics of the combined cohort stratified by sex and by FEND patients, respectively. Men were younger than women (median: 40 vs. 49 years, respectively, $P = 0.0001$) and had higher BMI at baseline (24.6 vs. 24.1 kg/m^2 in men and women, respectively, $P = 0.004$). Besides, treatment duration was longer for men than women (9 months compared with 6 months, respectively, $P = 0.05$) (Supplementary Table S6, Supplemental digital content 1, *http://links.lww.com/FPC/A990*).

Genetic analysis

Genotype analysis

Supplementary Tables S1 and S2 (Supplemental digital content 1, *http://links.lww.com/FPC/A990*) list the 32 and 20 SNPs from GWAS and CG studies, respectively, analyzed in the psychiatric samples. All of them were in HWE after multiple test correction ($P_{\text{corrected}} < 0.001$). Thirty-two previously reported SNPs associated with BMI in the general population [31] were analyzed in sample 1. One SNP located in *CADM2* gene showed a nominal association with BMI over time ($P = 0.01$) (Table 2). At 12 months of treatment, the rs13078807 polymorphism showed a 1.04 BMI units increase per additional risk allele. Twenty other SNPs were selected from CG studies associated with psychotropic-induced weight gain and two of them (i.e. *HSD11β1* rs3753519 and *CRTC2* rs8450) showed an association with BMI (difference of predicted BMI of −2.35 units for

Table 1 Description of demographic and clinical psychiatric White samples

Characteristics	Sample 1 ($n=425$)	Sample 2 ($n=148$)	Sample 3 ($n=177$)	Combined sample ($n=750$)
Male (%)	43	55	62	50
Age [median (range)] (years)	51 (13–97)	42 (19–64)	42 (18–69)	45 (13–97)
Diagnosis (%)				
Psychotic disorders	28.6	24.5	9.0	31.4
Schizoaffective disorders	7.3	17.0	12.1	10.3
Bipolar disorders	18.8	34.7	16.8	21.5
Depression disorders	16.4	17.0	12.7	15.7
Other diagnosis	28.9	6.8	14.5	21.2
Initial BMI status (kg/m^2)[a]				
BMI [median (range)]	23 (13–44)	25 (15–46)	24 (16–46)	24 (13–46)
25 ≥ initial BMI < 30 (%)	22	37	31	28
Initial BMI ≥ 30 (%)	13	16	15	14
Current BMI status (kg/m^2)[b]				
BMI [median (range)]	25 (15–50)	28 (16–40)	25 (17–43)	26 (15–50)
25 ≥ current BMI < 30 (%)	25	38	29	27
Current BMI ≥ 30 (%)	18	35	18	21
Initial WC (cm)[a]				
WC [median (range)]	87 (54–138)	–	–	87 (54–138)
High WC ≥ 94 (male), ≥ 88 (female) (%)	41	–	–	41
Current WC (cm)[b]				
WC [median (range)]	93 (57–162)	–	92 (73–136)	90 (57–162)
High WC ≥ 94 (male), ≥ 88 (female) (%)	51	–	53	51
Initial lipid status (%)[a]				
High LDL cholesterol[c]	9	–	–	9
High triglycerides[d]	18	–	–	18
Low HDL cholesterol[e]	23	–	–	23
Current lipid status (%)[b]				
High LDL cholesterol[c]	15	–	–	15
High triglycerides[d]	28	–	–	28
Low HDL cholesterol[e]	26	26	17	26
Smoker (%)	46	59	76	56
Prescribed psychotropic drug (%)[f]				
Amisulpride	8	–	11	7
Aripiprazole	8	–	7	6
Clozapine	8	14	9	9
Olanzapine	11	16	12	12
Quetiapine	35	20	24	29
Risperidone	15	17	17	16
Lithium	8	20	12	11
Valproate	5	14	8	8
Treatment duration [median (range)] (months)	6 (1–12)	27 (3–333)	36 (1–390)	12 (1–390)

WC, waist circumference; –, missing clinical values or obtained in nonfasting conditions.
[a]Before the current psychotropic treatment.
[b]For samples 2 and 3: current observation; for sample 1: last observed data.
[c]High LDL cholesterol: ≥ 4.1 mmol/l.
[d]High triglycerides: ≥ 2.2 mmol/l.
[e]Low HDL cholesterol: < 1 mmol/l.
[f]2% of the sample 1 was under paliperidone treatment.

rs3753519 at 12 months of treatment between patients homozygous for the variant allele and wild types and 0.69 units of BMI increase per additional risk allele for rs8450 ($P=0.00001$, 0.04, respectively; Table 2).

Genetic risk score analysis

On combining all 32 GWAS SNPs in a w-GRS (w-GRS 32), the score was significantly associated with BMI in sample 1 ($P=0.009$), in sample 3 ($P=0.04$), and also in the three combined samples ($P=0.002$) (see Table 3). In sample 1, those at the p95 of the GRS (i.e. a high GRS) had 2.26 units more of predicted BMI when compared with those individuals at the p5 of the GRS (low GRS) at 12 months of treatment. Results were similar in sample 3 and when all samples were combined together at 24 and 12 months of treatment (difference of predicted BMI between p95 and p5 of the GRS: 2.99 and 1.89 units, respectively). A higher effect on BMI was found among men when analyses were stratified by sex in the combined sample (interaction sex × GRS, $P<0.10$): individuals at the p95 score had 2.91 units more of predicted BMI compared with individuals at the p5 score at 24 months of treatment ($P=0.0002$). For the subgroup of FEND patients, a difference of predicted BMI of 3.79 units was observed between individuals at the p95 and p5 of the GRS ($P=0.008$) (Table 3). Figure 1 shows the evolution of BMI (nonadjusted) over time between extreme percentiles [low genetic risk (p5) vs. high genetic risk (p95)]. In addition, predicted BMI differences between p10 and p90 extremes are presented in Supplementary Table S8 (Supplemental digital content 1, *http://links.lww.com/FPC/A990*) and Supplementary Fig. S2 (Supplemental digital content 2, *http://links.lww.com/FPC/A991*).

When pooling all samples together, 1 unit increase of the risk allele at 24 months of treatment in the GRS was associated with an increase in BMI of 0.19 units ($P=0.011$). Among men, this increase in BMI was of 0.30 units ($P=0.0001$), whereas in women it was of 0.08 ($P=0.38$).

In contrast to what we found with GWAS SNPs, when the 20 CG SNPs were combined in a w-GRS (w-GRS 20), no association with BMI was observed in the entire sample ($P=0.46$) (Supplementary Table S9, Supplemental digital content 1, *http://links.lww.com/FPC/A990*).

Finally, the 20 CG SNPs were combined with the 32 GWAS SNPs in another w-GRS (w-GRS 52) (Supplementary Table S10, Supplemental digital content 1, *http://links.lww.com/FPC/A990*). w-GRS 52 was significantly associated with BMI in sample 1 ($P=0.01$), sample 3 ($P=0.04$), and when combining all samples ($P=0.001$). Only a trend was observed in samples 2 and 3 when pooled together ($P=0.06$). Thus, an individual in the p95 score had 2.08, 2.79, and 1.94 more predicted units of BMI in sample 1 (12 months of treatment), in sample 3 (24 months of treatment), and in all samples combined together (12 months of treatment) compared with individuals at the p5 of the score, respectively. When analyses were stratified by sex, a significant effect

Table 2 Significant results obtained from individual single-nucleotide polymorphism association with body mass index in the psychiatric sample 1 at baseline and at 12 months of follow-up treatment

			Difference of predicted BMI per risk allele increase (95% CI)		
Nearest genes	SNP	Major/minor allele	At baseline	At 12 months of treatment	*P*-value
CADM2	rs13078807	A>G	0.93 (0.89–1.97)	1.04 (−0.14–2.22)	**0.01***
HSD11β1	rs3753519[a]	C>T	−2.11 (−3.22 to −1.00)	−2.35 (−3.60 to −1.10)	**0.00001**
CRTC2	rs8450	G>A	0.62 (0.28–1.62)	0.69 (−0.44–1.83)	**0.04***

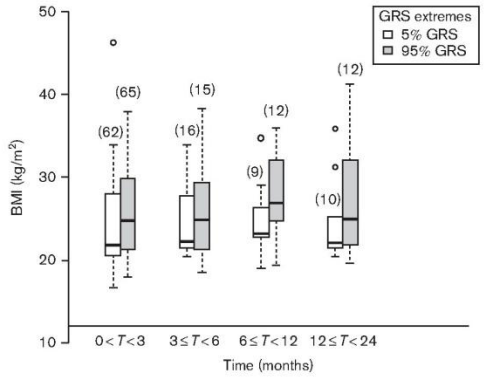
Bold values: significant at $P \leq 0.05$.
Predicted differences of BMI were calculated for polymorphisms that showed significant results ($P < 0.05$).
CI, confidence interval; SNP, single-nucleotide polymorphism.
[a]A dominant model was used for this SNP (carriers of the variant allele were compared with the wild type).
*Not significant after Bonferroni's correction.

Table 3 Weighted genetic risk score association with body mass index obtained from 32 Genome-Wide Association Studies single-nucleotide polymorphisms

		BMI difference between GRS p95 and GRS p5 (95% CI)			
	n	At baseline	At 12 months	At 24 months	*P*-value
Sample 1[a]	425	2.01 (0.52–3.51)	2.26 (0.48–4.04)	–	**0.009**
Sample 2[b]	148	−0.51 (−3.02–2.00)	−0.61 (−3.61–2.40)	−0.73 (−4.67–3.22)	0.7
Sample 3[b]	177	2.54 (0.26–4.81)	2.75 (0.23–5.27)	2.99 (−0.01–6.00)	**0.04**
Samples 2 and 3[b]	325	1.43 (−0.27–3.13)	1.61 (−0.33–3.56)	1.82 (−0.59–4.24)	0.1
All samples combined	750	1.68 (0.65–2.72)	1.89 (0.71–3.06)	–	**0.002**
FEND patients[a]	116	3.29 (0.79–5.78)	3.79 (0.88–6.71)	–	**0.008**
Men	375	2.59 (1.45–3.74)	2.91 (1.06–4.22)	–	**0.0002**
Women	375	0.76 (−0.55–2.06)	0.84 (−0.63–2.32)	–	0.3

Bold values: significant at $P \leq 0.05$.
CI, confidence interval; FEND, first episode and newly diagnosed; GRS, genetic risk score; p5, percentile 5 of GRS; p95, percentile 95 of GRS.
[a]Follow-up to 12 months of treatment.
[b]Follow-up to 24 months of treatment.

Fig. 1



Evolution of BMI over time between genetic risk score (GRS) extreme percentiles. Boxplots show the median values of BMI for each time of the treatment duration (solid horizontal line), 25th and 75th percentile values (box outline), the lowest and upper value within 1.5 interquartile range (whiskers), and outlier values (open circles). Values given in parentheses correspond to number of individuals.

was found among men at the p95 of the score, who showed 3.09 more units of predicted BMI compared with men at the p5 ($P = 0.0001$). FEND patients who were at the top percentile (p95) also had 3.66 more units of predicted BMI compared with patients at the p5 of the GRS ($P = 0.01$).

GLMM according to different quartiles showed significant differences between individuals within the third and fourth quartile of the GRS compared with the first quartile. At 24 months of treatment, those at the third and fourth quartiles had 1.84 (0.40–3.29) and 1.91 (0.51–3.32) more units of predicted BMI compared with the first quartile, respectively (results not shown). Table 4 shows the characteristics for the four groups stratified by GRS quartiles. Those at the fourth quartile had higher BMI before starting and during the current psychotropic treatment (baseline and current median BMI: 25.1 and 25.9 kg/m^2, respectively) compared with the first quartile (baseline and current median BMI: 23.2 and 24.3 kg/m^2, respectively), which could possibly be explained by the interaction between genetics, previous psychiatric episodes, and/or psychotropic treatments. The prevalence of baseline overweight and obesity increased in higher quartiles (i.e. 48% in the fourth quartile vs. 30% in the first quartile, $P = 0.007$). No differences in age, treatment, treatment duration, high waist circumference prevalence, diagnostic, and FEND individuals distribution were observed between the different quartile groups (Table 4).

Finally, on comparing the distribution of genetic scores without adjusting by other covariates, no differences

Table 4 Description of four quartiles of genetic risk score for 32 single-nucleotide polymorphism in the combined sample

GRS (n)	1st quartile (192)	2nd quartile (170)	3rd quartile (186)	4th quartile (202)	P-value
Score [mean (SD)]	0.87 (0.06)	0.97 (0.02)	1.05 (0.02)	1.16 (0.07)	**0.0001**
Men (%)	47	55	44	53	0.1
Age [median (range)] (years)	47 (17–96)	47 (13–90)	48 (14–97)	48 (15–93)	0.9
Initial BMI [median (range)] (kg/m^2)[a]	23.2 (13–46)	24.6 (15–39)	25.1 (16–46)	25.1 (14–39)	**0.0005**
Current BMI [median (range)] (kg/m^2)[b]	24.3 (16–40)	25.2 (15–40)	25.9 (16–50)	25.9 (17–41)	**0.04**
First episode and newly diagnosed patients (%)	13	15	16	17	0.6
Treatment prescription					
Ami, Ari, Li, Quet, Risp	74	70	71	67	0.5
Clo, Olan, Valp	26	30	29	33	
Treatment duration [median (range)] (months)	6 (1–23)	3 (1–21)	3 (1–24)	3 (1–24)	0.9
High WC (≥94 cm men, 88 cm women) (%)	40	47	49	53	0.2
Diagnostic (%)					
Psychotic disorders	42	42	38	46	0.6
Bipolar disorders	21	22	21	21	
Depression disorders	17	15	17	14	

Bold values: significant at $P \leq 0.05$.
Ami, amisulpride; Ari, aripiprazole; Clo, clozapine; GRS, genetic risk score; Li, lithium; Olan, olanzapine; Quet, quetiapine; Risp, risperidone; Valp, valproate; WC, waist circumference.
[a]Before the current psychotropic treatment.
[b]Last observed data.

were found between men and women (Supplementary Table S6, Supplemental digital content 1, *http://links.lww.com/FPC/A990*) or FEND patients (Supplementary Table S7, Supplemental digital content 1, *http://links.lww.com/FPC/A990*).

Genetic risk scores and Genome-Wide Association Studies genes for psychiatric diseases and diabetes

The SNPs selected from GWAS associated with psychiatric diseases (i.e. schizophrenia, bipolar disorder, major depressive disorder, autism, and hyperattention deficit) and diabetes were combined in two different w-GRS and tested for association with BMI. No significant results were found (results not shown).

Explained variability

We calculated the BMI variability explained by the clinical and genetic covariates in sample 1 for individuals ranging in age from 18 to 65 years ($n = 263$). Thus, in our model, the genetic component considering the w-GRS 32 explained 1.97% of BMI variability, whereas nongenetic components such as age, sex, and treatment explained 2.23, 0.42, and 0.6%, respectively, out of the total 7.01% BMI variability explained by the model. Finally, the BMI-explained variance of the 52 SNPs (32 SNPs added to the 20 SNPs) was 1.99%, whereas the important clinical variables known to influence weight (age, sex, treatment) represented altogether 3.15% of the BMI variability.

Discussion

In the present study, we found that w-GRS built from 32 polymorphisms previously associated with BMI in the general population GWAS were also significantly associated with BMI in our sample 1, being replicated in another sample. The stronger effects were found among men and FEND patients. Some studies have replicated the association of the 32-SNPs GRS with BMI and obesity-related genotypes in different cohorts and ethnicities [32–34]. Two cross-sectional studies using a Mendelian randomization approach [42] and a case–control design [43] replicated the association of w-GRS in depressed patients. However, type of treatment, treatment duration, or BMI variation over time were not taken into account, whereas BMI at baseline and treatment duration are known moderators of weight gain in populations under psychotropic treatment [9]. Moreover, the number of patients treated was not described in the previous studies. The present study, in contrast, includes longitudinal data considering long treatment duration (i.e. analysis has been carried out up to 24 months), type of treatment, and other diagnostics in addition to depression. Explained BMI variability by GRS when including 32-SNPs GWAS GRS in our model was slightly higher than that reported initially in general population cohorts in the literature (1.45%) [31] or that found in French and Chinese general populations (1 and 0.90%, respectively). It is noteworthy that addition of the 20 CG in the model did not improve the explained BMI variability (1.97 vs. 1.99%). The effect on BMI per risk allele increase of the 32-SNPs GWAS GRS was similar to those reported previously (0.11 [32] and 0.13 [34]) when considering both sexes together. However, higher BMI increase per risk allele was found among men.

Individual SNP analyses showed few significant effects in sample 1. Only one GWAS SNP (rs13078807) located in the *CADM2* gene region was nominally associated with BMI. *CADM2* has been associated previously with obesity in Whites and other ethnicities [31,63,64]. Among the CG polymorphisms, two SNPs (*HSD11β1* rs3753519 and *CRTC2* rs8450) were associated with BMI in sample 1; however, rs8450 did not survive Bonferroni's correction. In addition to weight gain association in psychiatric samples [16], *HSD11β1* has been associated with metabolic syndrome in a general population [14] and *CRTC2*

has been associated with type 2 diabetes in Asian populations [65]. CRTC2 is a coactivator that binds to CREB and stimulates the expression of PEPCK and G6Pase and this increases hepatic gluconeogenesis through dephosphorylation [17,66]. In addition, a deletion of CRTC2 impairs the expression of the gluconeogenic genes and the ability of glucagon to stimulate glucose production in hepatocytes [67]. The *HSD11β1* gene codes for a microsomal enzyme-catalyzing tissue regeneration of active cortisol from the inactive form cortisone [68]. It is highly expressed in metabolic tissues such as the liver and adipose tissue. Increased plasma cortisol levels have been associated with visceral obesity and metabolic syndrome. An overexpression of this gene has been associated with hyperphagia and obesity in mice [69,70].

The stronger effects observed when combining all SNPs in a w-GRS could be explained by the fact that common variants individually have little effect on BMI and very large sample sizes are needed to detect small effects. Thus, when integrating many small variant effects in a w-GRS, the consistency and the power to detect these effects increase, even in smaller sample sizes [35]. In addition, the BMI explained variability in the entire model was 7.01%, with 1.97% of it corresponding to the w-GRS. It is noteworthy that although this is not a high percentage, it represents 28% of the total BMI variability explained by the model. The present study is in the same line as a very recently published study on GWAS meta-analysis of large population data-sets (>300 000 individuals) where the genetic component (i.e. w-GRS) explained up to 2.7% of BMI variability [30].

The w-GRS 32 SNPs could not be replicated in sample 2. This might be tentatively explained by the fact that BMI and overweight prevalence at baseline were the highest among the three samples. Low BMI at baseline has been described as a risk factor for gaining weight [71]. In the same line, when analyzing the 20 CG variants previously associated with antipsychotic-induced weight gain in a w-GRS, no significant association was observed between the w-GRS and BMI. SNPs from CG studies that were selected included very heterogeneous studies, with small sample sizes and with different ethnicities, treatment, and treatment durations (see Supplementary Table S2, Supplemental digital content 1, *http://links.lww.com/FPC/A990*), which could explain the nonsignificant results in our psychiatric samples. In addition, some very promising variants (i.e. *5HT$_{2C}$ receptor*) could not be included in our w-GRS model as the allele effect (β-coefficient) calculation was not available, but on calculating unweighted GRS (in which this variant was included), the results did not change significantly ($P = 0.22$). Finally, an a-priori use of an additive model for the effect of all variants could have contributed toward the negative findings.

We also found significant effects for the w-GRS 32 among FEND patients who had lower BMI and obesity prevalence at baseline and shorter treatment duration compared with others. This is in agreement with previous studies showing that low baseline BMI and first-episode patients are known risk factors for important weight gain during psychotropic drug treatment [9]. To our knowledge, this is the first study reporting a stronger effect in men when analyzing the influence of genetic scores on BMI despite the fact that sex differences in fat storage and metabolism have already been described [72]. This emphasizes the need to consider sex when studying obesity-related phenotypes such as BMI. In the present study, men were, on average, younger and had longer treatment duration compared with women, which could contribute toward the observed sex effect as both young age and treatment duration are known risk factors for important weight gain [9]. It is noteworthy that when calculating GRS and sex interaction, a trend was observed when all three samples were combined ($P = 0.09$, $n = 750$). Because of the exploratory nature of these findings, further analysis including sex stratification should be carried out in larger psychiatric cohorts.

Finally, no association was found with BMI of GRS built from SNPs obtained from psychiatric disorders and diabetes GWAS. Although obesity, type 2 diabetes, and psychiatric disorders are known to share common etiological pathways [8], these results could be considered as negative controls as we only obtained significant BMI–GRS association results when we combined previously BMI-related SNPs.

This study has some limitations that should be mentioned: weighted scores were calculated from β-coefficients obtained from general population samples and the relative influence of these genes might differ in psychiatric patients. Other factors influencing weight gain, such as previous treatment history, were not reported. This study has been carried out in Whites; therefore, these results cannot be extrapolated to other ethnicities. Variants included in the genetic score model should be consistent with their effects (i.e. tested in large sample sizes and replicated effects). Finally, the 95% CI suggests that the genetic effect is variable within the groups and sample size should increase to narrow 95% CI and improve outcome precision.

In conclusion, the present study replicated in psychiatric cohorts previously identified BMI risk variants obtained in GWAS analyses from population-based samples. GRS can be a useful tool to integrate multiple variants with low impact, which, when tested individually, do not show any significant effect. This approach can contribute toward a better understanding of the genetic variability of polygenic obesity in psychiatric patients and our results suggest that particular care should be taken in sex-specific analyses when working with GRS. Thus, the

clinical utility of the w-GRS in obesity-related traits needs to be explored further in prospective studies, especially among populations at high risk of developing metabolic disorders.

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Conflicts of interest

C.B.E. has received research support from Takeda and from the Roche Organ Transplantation Research Foundation in the past 3 years. He has received honoraria for conferences or teaching CME courses from Advisis, Astra Zeneca, Janssen-Cilag, Lundbeck, Merck Sharp & Dohme, Otsuka, Sandoz, Servier, and Vifor-Pharma in the past 3 years. A.v.G. has received honoraria for a conference and a workshop participation unrelated to the topic of this study from Vifor and Bayer Sheringer in the past 3 years. For the remaining authors there are no conflicts of interest.

References

1 Yang W, Kelly T, He J. Genetic epidemiology of obesity. *Epidemiol Rev* 2007; **29**:49–61.
2 Stunkard AJ, Sørensen TI, Hanis C, Teasdale TW, Chakraborty R, Schull WJ, Schulsinger F. An adoption study of human obesity. *N Engl J Med* 1986; **314**:193–198.
3 Katzmarzyk PT, Malina RM, Pérusse L, Rice T, Province MA, Rao DC, Bouchard C. Familial resemblance in fatness and fat distribution. *Am J Hum Biol* 2000; **12**:395–404.
4 Farooqi IS, O'Rahilly S. Monogenic obesity in humans. *Annu Rev Med* 2005; **56**:443–458.
5 Strobel A, Issad T, Camoin L, Ozata M, Strosberg AD. A leptin missense mutation associated with hypogonadism and morbid obesity. *Nat Genet* 1998; **18**:213–215.
6 Waalen J. The genetics of human obesity. *Transl Res* 2014; **164**:293–301.
7 De Hert M, Dekker JM, Wood D, Kahl KG, Holt RI, Möller HJ. Cardiovascular disease and diabetes in people with severe mental illness position statement from the European Psychiatric Association (EPA), supported by the European Association for the Study of Diabetes (EASD) and the European Society of Cardiology (ESC). *Eur Psychiatry* 2009; **24**:412–424.
8 Lopresti AL, Drummond PD. Obesity and psychiatric disorders: commonalities in dysregulated biological pathways and their implications for treatment. *Prog Neuropsychopharmacol Biol Psychiatry* 2013; **45**:92–99.
9 De Hert M, Detraux J, van Winkel R, Yu W, Correll CU. Metabolic and cardiovascular adverse effects associated with antipsychotic drugs. *Nat Rev Endocrinol* 2011; **8**:114–126.
10 Stahl S. *Stahl's essential psychopharmacology neuroscientific basis and practical applications*, 3rd ed. New York: Cambridge University Press; 2008.
11 Ryu S, Cho EY, Park T, Oh S, Jang WS, Kim SK, *et al.* –759 C/T polymorphism of 5-HT2C receptor gene and early phase weight gain associated with antipsychotic drug treatment. *Prog Neuropsychopharmacol Biol Psychiatry* 2007; **31**:673–677.
12 Balt SL, Galloway GP, Baggott MJ, Schwartz Z, Mendelson J. Mechanisms and genetics of antipsychotic-associated weight gain. *Clin Pharmacol Ther* 2011; **90**:179–183.
13 Lett TA, Wallace TJ, Chowdhury NI, Tiwari AK, Kennedy JL, Müller DJ. Pharmacogenetics of antipsychotic-induced weight gain: review and clinical implications. *Mol Psychiatry* 2012; **17**:242–266.
14 Wamil M, Seckl JR. Inhibition of 11beta-hydroxysteroid dehydrogenase type 1 as a promising therapeutic target. *Drug Discov Today* 2007; **12**:504–520.
15 Beale EG, Harvey BJ, Forest C. PCK1 and PCK2 as candidate diabetes and obesity genes. *Cell Biochem Biophys* 2007; **48**:89–95.
16 Quteineh L, Vandenberghe F, Saigi Morgui N, Delacrétaz A, Choong E, Gholam-Rezaee M, *et al.* Impact of HSD11B1 polymorphisms on BMI and components of the metabolic syndrome in patients receiving psychotropic treatments. *Pharmacogenet Genomics* 2015; **25**:246–258.
17 Koo SH, Flechner L, Qi L, Zhang X, Screaton RA, Jeffries S, *et al.* The CREB coactivator TORC2 is a key regulator of fasting glucose metabolism. *Nature* 2005; **437**:1109–1111.
18 Gibson WT, Pissios P, Trombly DJ, Luan J, Keogh J, Wareham NJ, *et al.* Melanin-concentrating hormone receptor mutations and human obesity: functional analysis. *Obes Res* 2004; **12**:743–749.
19 Sadagurski M, Leshan RL, Patterson C, Rozzo A, Kuznetsova A, Skorupski J, *et al.* IRS2 signaling in LepR-b neurons suppresses FoxO1 to control energy balance independently of leptin action. *Cell Metab* 2012; **15**:703–712.
20 Esterbauer H, Oberkofler H, Krempler F, Patsch W. Human peroxisome proliferator activated receptor gamma coactivator 1 (PPARGC1) gene: cDNA sequence, genomic organization, chromosomal localization, and tissue expression. *Genomics* 1999; **62**:98–102.
21 Choong E, Quteineh L, Cardinaux JR, Gholam-Rezaee M, Vandenberghe F, Dobrinas M, *et al.* Influence of CRTC1 polymorphisms on body mass index and fat mass in psychiatric patients and the general adult population. *JAMA Psychiatry* 2013; **70**:1011–1019.
22 Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, *et al.* A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* 2007; **316**:889–894.
23 Willer CJ, Speliotes EK, Loos RJ, Li S, Lindgren CM, Heid IM, *et al.* Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. *Nat Genet* 2009; **41**:25–34.
24 Thorleifsson G, Walters GB, Gudbjartsson DF, Steinthorsdottir V, Sulem P, Helgadottir A, *et al.* Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. *Nat Genet* 2009; **41**:18–24.
25 Okada Y, Kubo M, Ohmiya H, Takahashi A, Kumasaka N, Hosono N, *et al.* Common variants at CDKAL1 and KLF9 are associated with body mass index in east Asian populations. *Nat Genet* 2012; **44**:302–306.
26 Loos RJ, Lindgren CM, Li S, Wheeler E, Zhao JH, Prokopenko I, *et al.* Common variants near MC4R are associated with fat mass, weight and risk of obesity. *Nat Genet* 2008; **40**:768–775.
27 Pei YF, Zhang L, Liu Y, Li J, Shen H, Liu YZ, *et al.* Meta-analysis of genome-wide association data identifies novel susceptibility loci for obesity. *Hum Mol Genet* 2014; **23**:820–830.
28 Berndt SI, Gustafsson S, Mägi R, Ganna A, Wheeler E, Feitosa MF, *et al.* Genome-wide meta-analysis identifies 11 new loci for anthropometric traits and provides insights into genetic architecture. *Nat Genet* 2013; **45**:501–512.
29 Wheeler E, Huang N, Bochukova EG, Keogh JM, Lindsay S, Garg S, *et al.* Genome-wide SNP and CNV analysis identifies common and low-frequency variants associated with severe early-onset obesity. *Nat Genet* 2013; **45**:513–517.
30 Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, *et al.* Genetic studies of body mass index yield new insights for obesity biology. *Nature* 2015; **518**:197–206.
31 Speliotes EK, Willer CJ, Berndt SI, Monda KL, Thorleifsson G, Jackson AU, *et al.* Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nat Genet* 2010; **42**:937–948.

32 Zhu J, Loos RJ, Lu L, Zong G, Gan W, Ye X, *et al.* Associations of genetic risk score with obesity and related traits and the modifying effect of physical activity in a Chinese Han population. *PLoS One* 2014; **9**:e91442.

33 Peterson RE, Maes HH, Lin P, Kramer JR, Hesselbrock VM, Bauer LO, *et al.* On the association of common and rare genetic variation influencing body mass index: a combined SNP and CNV analysis. *BMC Genomics* 2014; **15**:368.

34 Goumidi L, Cottel D, Dallongeville J, Amouyel P, Meirhaeghe A. Effects of established BMI-associated loci on obesity-related traits in a French representative population sample. *BMC Genet* 2014; **15**:62.

35 Horne BD, Anderson JL, Carlquist JF, Muhlestein JB, Renlund DG, Bair TL, *et al.* Generating genetic risk scores from intermediate phenotypes for use in association studies of clinically significant endpoints. *Ann Hum Genet* 2005; **69** (Pt 2):176–186.

36 Belsky DW, Moffitt TE, Sugden K, Williams B, Houts R, McCarthy J, Caspi A. Development and evaluation of a genetic risk score for obesity. *Biodemography Soc Biol* 2013; **59**:85–100.

37 Lin X, Song K, Lim N, Yuan X, Johnson T, Abderrahmani A, *et al.* Risk prediction of prevalent diabetes in a Swiss population using a weighted genetic score – the CoLaus Study. *Diabetologia* 2009; **52**:600–608.

38 Derks EM, Vorstman JA, Ripke S, Kahn RS, Ophoff RA. Schizophrenia Psychiatric Genomic Consortium. Investigation of the genetic association between quantitative measures of psychosis and schizophrenia: a polygenic risk score analysis. *PLoS One* 2012; **7**:e37852.

39 Rouskas K, Kouvatsi A, Paletas K, Papazoglou D, Tsapas A, Lobbens S, *et al.* Common variants in FTO, MC4R, TMEM18, PRL, AIF1, and PCSK1 show evidence of association with adult obesity in the Greek population. *Obesity (Silver Spring)* 2012; **20**:389–395.

40 Ntalla I, Panoutsopoulou K, Vlachou P, Southam L, William Rayner N, Zeggini E, Dedoussis GV. Replication of established common genetic variants for adult BMI and childhood obesity in Greek adolescents: the TEENAGE study. *Ann Hum Genet* 2013; **77**:268–274.

41 Warrington NM, Howe LD, Wu YY, Timpson NJ, Tilling K, Pennell CE, *et al.* Association of a body mass index genetic risk score with growth throughout childhood and adolescence. *PLoS One* 2013; **8**:e79547.

42 Hung CF, Rivera M, Craddock N, Owen MJ, Gill M, Korszun A, *et al.* Relationship between obesity and the risk of clinically significant depression: Mendelian randomisation study. *Br J Psychiatry* 2014; **205**:24–28.

43 Hung CF, Breen G, Czamara D, Corre T, Wolf C, Kloiber S, *et al.* A genetic risk score combining 32 SNPs is associated with body mass index and improves obesity prediction in people with major depressive disorder. *BMC Med* 2015; **13**:86.

44 Wali JA, Thomas HE, Sutherland AP. Linking obesity with type 2 diabetes: the role of T-bet. *Diabetes Metab Syndr Obes* 2014; **7**:331–340.

45 Rui L. SH2B1 regulation of energy balance, body weight, and glucose metabolism. *World J Diabetes* 2014; **5**:511–526.

46 Choong E, Quteineh L, Cardinaux JR, Gholam-Rezaee M, Vandenberghe F, Dobrinas M, *et al.* Influence of CRTC1 polymorphisms on body mass index and fat mass in psychiatric patients and the general adult population. *JAMA Psychiatry* 2013; **70**:1011–1019.

47 [No authors listed]. Comparative study of the efficacy of dipyrone, diclofenac sodium and pethidine in acute renal colic. Collaborative Group of the Spanish Society of Clinical Pharmacology. *Eur J Clin Pharmacol* 1991; **40**:543–546.

48 American Diabetes A, American Psychiatric A, American Association of Clinical E, North American Association for the Study of O. Consensus development conference on antipsychotic drugs and obesity and diabetes. *Diabetes Care* 2004; **27**:596–601.

49 Lango Allen H, Estrada K, Lettre G, Berndt SI, Weedon MN, Rivadeneira F, *et al.* Hundreds of variants clustered in genomic loci and biological pathways affect human height. *Nature* 2010; **467**:832–838.

50 Heid IM, Jackson AU, Randall JC, Winkler TW, Qi L, Steinthorsdottir V, *et al.* Meta-analysis identifies 13 new loci associated with waist–hip ratio and reveals sexual dimorphism in the genetic basis of fat distribution. *Nat Genet* 2010; **42**:949–960.

51 Voight BF, Scott LJ, Steinthorsdottir V, Morris AP, Dina C, Welch RP, *et al.* Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. *Nat Genet* 2010; **42**:579–589.

52 Cross-Disorder Group of the Psychiatric Genomics Consortium, Smoller JW, Craddock N, Kendler K, Lee PH, Neale BM, *et al.* Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. *Lancet* 2013; **381**:1371–1379.

53 Winham SJ, Colby CL, Freimuth RR, Wang X, de Andrade M, Huebner M, Biernacka JM. SNP interaction detection with Random Forests in high-dimensional genetic data. *BMC Bioinformatics* 2012; **13**:164.

54 Voight BF, Kang HM, Ding J, Palmer CD, Sidore C, Chines PS, *et al.* The metabochip, a custom genotyping array for genetic studies of metabolic, cardiovascular, and anthropometric traits. *PLoS Genet* 2012; **8**:e1002793.

55 Lett TA, Wallace TJ, Chowdhury NI, Tiwari AK, Kennedy JL, Müller DJ. Pharmacogenetics of antipsychotic-induced weight gain: review and clinical implications. *Mol Psychiatry* 2012; **17**:242–266.

56 Che R, Motsinger-Reif AA. A new explained-variance based genetic risk score for predictive modeling of disease risk. *Stat Appl Genet Mol Biol* 2012; **11**:Article 15.

57 R Core Team. *R: A language and environment for statistical computing, R Fondation for Statistical Computing*. Vienna, Austria: R Development Core Team; 2013. Available at: *http://www.R-project.org*. [Accessed 11 June 2014].

58 Venables WN, Ripley BD. *Modern applied statistics with S*. New York: Springer; 2002.

59 Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MH, White JS. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol Evol* 2009; **24**:127–135.

60 Wolfinger R, O'connell M. Generalized linear mixed models: a pseudo-likelihood approach. *J Statist Comput Simulation* 1993; **48**:233–243.

61 Lin X, Zhang D. Inference in generalized additive mixed models by using smoothing splines. *J R Statist Soc B* 1999; **61**:381–400.

62 Wood SN. GAMMs with R. In: Carlin BP, Chatfield C, Tanner M, Zidek J, editors. *Generalized additive models – an introduction with R*. Broken: Chapman & Hall/CRC; 2006. pp. 319–324.

63 Dorajoo R, Blakemore AI, Sim X, Ong RT, Ng DP, Seielstad M, *et al.* Replication of 13 obesity loci among Singaporean Chinese, Malay and Asian-Indian populations. *Int J Obes (Lond)* 2012; **36**:159–163.

64 Wen W, Cho YS, Zheng W, Dorajoo R, Kato N, Qi L, *et al.* Meta-analysis identifies common variants associated with body mass index in east Asians. *Nat Genet* 2012; **44**:307–311.

65 Keshavarz P, Inoue H, Nakamura N, Yoshikawa T, Tanahashi T, Itakura M. Single nucleotide polymorphisms in genes encoding LKB1 (STK11), TORC2 (CRTC2) and AMPK alpha2-subunit (PRKAA2) and risk of type 2 diabetes. *Mol Genet Metab* 2008; **93**:200–209.

66 Zhou XY, Shibusawa N, Naik K, Porras D, Temple K, Ou H, *et al.* Insulin regulation of hepatic gluconeogenesis through phosphorylation of CREB-binding protein. *Nat Med* 2004; **10**:633–637.

67 Wang Y, Inoue H, Ravnskjaer K, Viste K, Miller N, Liu Y, *et al.* Targeted disruption of the CREB coactivator Crtc2 increases insulin sensitivity. *Proc Natl Acad Sci USA* 2010; **107**:3087–3092.

68 Tomlinson JW, Walker EA, Bujalska IJ, Draper N, Lavery GG, Cooper MS, *et al.* 11Beta-hydroxysteroid dehydrogenase type 1: a tissue-specific regulator of glucocorticoid response. *Endocr Rev* 2004; **25**:831–866.

69 Masuzaki H, Paterson J, Shinyama H, Morton NM, Mullins JJ, Seckl JR, Flier JS. A transgenic model of visceral obesity and the metabolic syndrome. *Science* 2001; **294**:2166–2170.

70 Paterson JM, Morton NM, Fievet C, Kenyon CJ, Holmes MC, Staels B, *et al.* Metabolic syndrome without obesity: hepatic overexpression of 11beta-hydroxysteroid dehydrogenase type 1 in transgenic mice. *Proc Natl Acad Sci USA* 2004; **101**:7088–7093.

71 De Hert M, Dekker JM, Wood D, Kahl KG, Holt RI, Möller HJ. Cardiovascular disease and diabetes in people with severe mental illness position statement from the European Psychiatric Association (EPA), supported by the European Association for the Study of Diabetes (EASD) and the European Society of Cardiology (ESC). *Eur Psychiatry* 2009; **24**:412–424.

72 Power ML, Schulkin J. Sex differences in fat storage, fat metabolism, and the health risks from obesity: possible evolutionary origins. *Br J Nutr* 2008; **99**:931–940.

3. Project III

Weighted genetic risk scores and prediction of weight gain in Solid Organ Transplant populations

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Summary

Weight gain is often described in Solid Organ Transplant (SOT) populations. However, genetic studies on weight gain in transplanted populations are scarce. The present project was conducted in two SOT cohorts (n_A=995, n_B=156). The aim of the study was to determine whether genetic variants previously associated with BMI in general population were also associated with BMI in SOT individuals when combined in Genetic Risk Scores (GRS). Two GRS were significantly associated with BMI in the first sample, however, only one GRS showed significant results in the second cohort. In addition, an exploratory analysis of a third GRS was conducted with polymorphisms coming from genes whose expression is associated with weight change in renal transplant recipients. Significant results were found only in the second cohort. Finally, we assessed whether adding these variants in a clinical model improved prediction of 10% weight gain or more 12 months after transplantation. In general, models containing clinical and genetic predictors performed better than clinical models alone, highlighting the importance of taking into account genetic effects.

Weighted genetic risk scores and prediction of weight gain in Solid Organ Transplant populations

ABSTRACT

BACKGROUND: Polygenic obesity in Solid Organ Transplant (SOT) populations is considered a risk factor for the development of metabolic abnormalities and graft survival. Few studies to date have studied the genetics of weight gain in SOT recipients.

AIM: To determine whether weighted genetic risk scores (w-GRS) integrating genetic polymorphisms from GWAS studies (SNP group#1 and SNP group#2) and from Candidate Gene studies (SNP group#3) influence BMI in SOT populations and if they predict ≥10% weight gain (WG) one year after transplantation.

METHODS: Two samples (n_A=995, n_B=156) were obtained from naturalistic studies and three w-GRS were constructed and tested for association with BMI. Prediction of 10% WG at one year after transplantation was assessed with models containing genetic and clinical factors.

RESULTS: w-GRS were associated with BMI in sample A and B combined (BMI increased by 0.14 and 0.12 units per additional risk allele in SNP group#1 and #2, respectively, p-values<0.008). w-GRS of SNP group#3 showed an effect of 0.01 kg/m^2 per additional risk allele when combining sample A and B (p-value 0.04). Models with genetic factors performed better than models without in predicting 10% WG at one year after transplantation.

CONCLUSIONS: This is the first study in SOT evaluating extensively the association of w-GRS with BMI and the influence of clinical and genetic factors on 10% of WG one year after transplantation, showing the importance of integrating genetic factors in the final model. Genetics of obesity among SOT recipients remains an important issue and can contribute to treatment personalization and prediction of WG after transplantation.

INTRODUCTION

Obesity has become a worldwide major concern since it has more than doubled in the last decades. In 2014, 39% of adults were overweight (25 kg/m^2≤ Body Mass Index (BMI)< 30 kg/m^2) and 13% were obese (BMI ≥ 30 kg/m^2) (1). Obesity is a risk factor leading to other co-morbidities such as diabetes, cardiovascular diseases and certain type of cancers (1). Among solid organ transplant (SOT) recipients, the rate of overweight and obesity has increased over the past years. By 2011, 34% of liver transplant candidates were obese, compared to 29% in 2001 (2). Similar results have been found for kidney, heart and lung transplant recipients (3-5). Although overweight and obesity prevalence are similar to those measured in general population studies, in SOT recipients the consequences are more serious. Indeed, obesity in SOT is an important risk factor for the development of New Onset Diabetes after Transplant (NODAT) (6) which has a deleterious effect on graft survival (7, 8). Moreover, it can be often associated with delayed graft function related to surgical and post-operative complications (9).

Few longitudinal studies examining weight gain (WG) among transplant recipients have been conducted to date, most of them focusing on WG during the first year post transplantation. A WG ranging from 3.5 to 10 Kg has been reported in heart, liver and kidney transplant recipients (10-14) and a mean of 10% WG during the first year after transplantation was described in kidney transplant recipients (15). A threshold of 10% increase of ideal body weight, defined as the metropolitan relative weight criteria (16), has been related to a risk of developing cardiovascular disease in general populations followed for more than 25 years (16, 17). Ethnicity, sex, age in addition to specific factors such as transplanted organ, glucocorticoids and immunosuppressive treatments are some of the described factors influencing WG following SOT and NODAT (18), as well as genetic factors. Most studies on transplant populations focused mainly on the NODAT rather than WG (19, 20). Regarding BMI-related phenotypes, a protocol for the first systematic literature review has been published. The aim is to condense and compare the

current state of evidence on WG, overweight and obesity in SOT individuals including genetic and non-genetic factors (21). Regarding candidate gene approach, two single nucleotide polymorphisms (SNPs) and one insertion/deletion have previously been associated with BMI-related phenotypes (22-24). These studies were conducted in three heterogeneous populations with small or moderate sample sizes (n<270), with different obesity-related outcomes and type of transplanted organ. Furthermore, different polymorphisms were analyzed. To our knowledge, no Genome Wide Association Studies (GWAS) investigating BMI variants within SOT recipients have yet been published. Recently, a microarray study examining gene expression in subcutaneous adipose tissue in kidney transplant recipients found that the expression of obesity-related genes was correlated with weight change (25). The top 41 ranked genes were further associated with obesity through a text mining approach (26), including genes related to diabetes, obesity and neurological concepts such as dopamine, nicotine, and cognition (25). Interestingly, two of these genes (i.e. *MTCH2* and *TFAP2B*) were also found in the largest BMI GWAS meta-analysis conducted to date in the general population (27). This meta-analysis was conducted in more than 300 000 individuals and reported 97 SNPs associated with BMI, also including 32 previously replicated BMI SNPs (28-31). All 97 polymorphisms explained up to 2.7% of BMI variability within these individuals (27). Since polygenic or common obesity is influenced by many genetic polymorphisms, genetic risk scores (GRS) provide a useful tool summarizing risk-associated variations across the genome by aggregating information from multiple-risk SNPs, and they may improve the consistency and the power to determine genetic risk in polygenic diseases (32, 33).

In the present study, we aimed to study the association of three weighted GRS, integrating previously published SNPs, with BMI in two cohorts of Swiss transplanted individuals. In addition, we assessed whether these genetic polymorphisms could predict a ≥10% WG during the first year post transplant.

METHODS

Sample A

The Swiss transplant cohort study (STCS) is an ongoing prospective multicenter study (Basel, Bern, Geneva, Lausanne, St. Gallen and Zurich) started in May 2008 which enrolls SOT recipients with no particular eligibility or exclusion criteria. The present study (May 2008- May 2011) included SOT recipients (i.e. kidney, liver, lung, heart, or multi-organ) with a functional graft for at least 12 months after transplantation. A total of 1294 patients were followed up in their respective transplant centers at baseline and at 6, 12, 24, 36 and 48 months after transplantation. Lipid profile, BMI, blood pressure and patient characteristics were collected at the different time-points of the follow up. Further details have been published elsewhere (34, 35). Only Caucasians and Recipients of 18 years or older were retained. If an individual was subjected to more than one transplant, only the first SOT was considered. A total of 995 patients were considered for analysis.

Sample B

A total of 197 SOT recipients (i.e. lung, liver and kidney) were enrolled between 2003 and 2005 from the outpatient clinic of the transplant center of the University Hospital of Lausanne, Switzerland. Only patients with a functional graft for more than 12 months were eligible to participate in the study. Further details can be found elsewhere (35-37). Briefly, data regarding patients' age, gender, BMI, ethnicity, immunosuppressive treatments among others were collected retrospectively from the medical files. Additionally, data concerning weight, at baseline, at 1, 3, 6, 9, 12 and at the yearly follow-up during the 5 years after transplantation were collected retrospectively from the medical files between October 2011 and April 2012. Blood samples were collected for further genotyping analysis.

156 individuals of 18 years or older for whom Caucasian ethnicity was reported and had clinical data available, were included in the analysis.

All patients gave their written informed consent and the studies were approved by the ethics committee of the corresponding centers.

Genotype selection and genotyping

SNP selection was done according to large Meta analyses of GWAS published on BMI. SNP group#1 included 32 BMI associated polymorphisms in general adult populations (28). A second group consisted of 97 SNPs (SNP group#2) recently associated with BMI in general populations and which included the previous 32 SNPs (or its proxies) (27). Only SNPs significant at GWAS levels (i.e. p-value < $5x10^{-8}$) were retained for the analysis. Tables S1 and S2 show a detailed description of the selected SNPs.

Additionally, 41 genes whose expression in subcutaneous adipose tissue has been previously associated with weight change in kidney transplant recipients (25) were included. A selection of tagging SNPs of these genes was obtained using HapMap Genome Browser (release 28). In order to avoid over representation of a particular gene, one tagging SNP per gene was selected based on the number of SNPs tagged and on the genotype availability in our samples. Six genes were excluded since no tagging SNPs were found in HapMap. Of note, two genes (i.e. *MTCH2*, *TFAP2B*) were also present in the GWAS mentioned previously (SNP group#1). Finally 19 SNPs, for which genotype was available in both samples A and B, were retained in the SNP group#3. A detailed description of these genes and polymorphisms can be found in Table S3.

For the sample A, genotypes were analyzed with the Human OmniExpress-24 BeadChip Kit as described by the manufacturer's protocol (Illumina, San Diego, CA). For the sample B, genotyping was performed using the Illumina 200K Cardiometabochip (Illumina, San Diego, CA). Briefly, the CardioMetabochip is a custom Illumina iSelect genotyping array designed to test DNA variation of 200'000 SNPs from regions identified by large scale meta-analyses of genome wide association studies (GWAS) for metabolic and cardiovascular traits (38). Polymorphisms or proxies were chosen based on genotype availability. A Quality Control was done for the genotyped SNPs. Samples were excluded from the analysis if sex was inconsistent with genetic data from X-linked markers, and when genotype call rate was <0.96 and gene call score <0.15. GenomeStudio Data Analysis Software was used to export results generated by Illumina CardiometaboChip.

Construction of Genetic Risk Scores

Three GRS were built following a weighted GRS (w-GRS) method as previously described (28) with 32 SNPs (SNP group#1) and 97 SNPs (SNP group#2) both from GWAS, and 19 SNPs (SNP group#3) from candidate genes. Briefly, genotypes from each SNP were coded as 0, 1 or 2 according to the number of BMI risk alleles and each polymorphism was then weighted by its β-coefficient (allele effect) based on the assumption that all SNP of interest have independent effects and contribute in an additive manner on BMI. Each unit increase in the GRS corresponded approximately to one additional risk allele. Allele effects on BMI were obtained from those published in the literature for the SNPs group#1 and #2 (27, 28). For the SNP group#3 allele effects were calculated from a large population based sample, GIANT, which consisted in a meta-analysis of GWAS with a discovery set of 123,865 individuals of European ancestry from 46 studies for height (39), BMI (28) and waist-to-hip ratio (40).

Statistical analysis

Descriptive analysis of quantitative data is presented as median and range unless otherwise specified whereas qualitative data is expressed as percentages. Chi-squared test or rank sum test were used for association studies within categorical data or non-parametric continuous variables, respectively. Hardy-Weinberg Equilibrium (HWE) was determined for each polymorphism by a chi-square test. P-value threshold was set at <0.05 and Bonferroni multiple test correction (0.05/6) was applied when necessary.

For multivariate analysis, a Generalized Additive Mixed Model (GAMM) was used to deal with complex and non-linear BMI evolution in time and presence of multiple observations per individual introducing interdependence among observations. A random effect at the subject level was also introduced to take the dependence structure of observed data into account. The GAMMs were fitted using the mgcv package of R (settings were fixed at package defaults). To be more conservative, the uncertainty of estimated parameters was assessed by 1'000 bootstraps on individuals (41). Because sex and age have been described as factors influencing WG (15), further analyses were conducted stratifying by gender and the median age when the interactions with w-GRS were significant.

Prediction of ≥10 % WG one year after transplantation in the sample A and B

A binary logistic regression model at 12 months after transplant was used to determine whether clinical and genetic factors influence a ≥10% WG one year after transplantation for those cases where genetic components were significantly associated with BMI. The ability to discriminate between gainers of 10% weight versus those who did not gain 10% one year after transplantation was assessed with the Area Under the Receiver Operating Characteristic Curve (AUROC) for a model containing only clinical covariates (i.e. age, sex, transplanted organ, BMI at baseline, immunosuppressant treatment) and

another model integrating clinical and genetic factors. In addition, Sensitivity (percentage of correctly predicted individuals with ≥10% WG among all individuals with ≥10% WG), Specificity (percentage of correctly predicted individuals with <10% WG among all truly individuals with <10% WG) and Accuracy (percentage of correctly classified gainers of ≥10% weight among all subjects) were obtained for each model using "pROC" R package (42). An AUROC lower than 0.70 indicates low discriminative accuracy (43). As previously described, (44, 45) in order to assess the added value of selected SNPs in predicting a ≥10% WG one year after transplantation (i.e. comparison of genetic and non-genetic models), likelihood ratio tests (LRT) and Integrated Discrimination Improvement (IDI) estimates with their respective p-values were calculated. Finally, the number needed to genotype (NNG) (i.e. the average number of patients who need to be genotyped to detect one case of 10% WG one year after transplantation) was calculated based on the inverse of the difference between the accuracy of clinical and genetic models (46).

RESULTS

Population description

The characteristics of sample A are presented Table 1. Sixty-six percent were men, 17.0% were obese one year after transplantation and 27.1% were diagnosed of NODAT. Similar patterns ($p>0.05$) were observed in sample B (60.9%, 18.5% and 28.8%, respectively, Table 2). Twenty three percent of individuals in sample A gained ≥10% of weight the first year after transplantation and 35% of individuals in sample B ($p<0.001$). The mean of WG one year after transplantation was 3.5% and 6.3% for samples A and B, respectively. Sample A included also heart and multi-organ transplant, individuals were older than in sample B (median age: 54 years compared to 48, $p<0.001$) and there was a high prevalence of living donors (27.1% and 11.5%, respectively, $p<0.001$). Tacrolimus (TAC) was more frequently

prescribed in sample A, whereas cyclosporine (CSA) was more used in sample B (45.1% versus 34.6%, respectively for TAC and 19.6% versus 65.4%, respectively for CSA; p<0.05). For sample A, individuals with at least 3 immunosuppressive treatments (i.e. cyclosporine, tacrolimus, glucocorticoids, azatioprine and/or mycophenolate) gained significantly more weight at one year after transplantation compared to the others (p=0.01, Figure 1). Of note, 99% of those with at least 3 immunosuppressants had a glucocorticoid treatment prescribed, possibly explaining this weight gain. No significant results were found in sample B.

10% WG one year after transplantation

In both samples A and B, those gaining ≥10% of weight had lower BMI at baseline and higher BMI 12 months after transplantation compared to those gaining <10% (Table 1 and 2). The prevalence of overweight and obese was lower at baseline and higher at one year after transplantation for ≥10% when compared to <10% WG. The transplanted organ differed between ≥10% and <10% for both A and B samples. The kidney was the most prevalent transplanted organ in both groups. The second most prevalent transplanted organ in the ≥10% WG group was the lung while the heart and the liver were the third most frequently transplanted organs. In the <10% WG group, the liver and the lung were among the second and the third most frequently transplanted organs. Additionally, in sample A donors were younger and individuals had higher cholesterol levels at 12 months in the ≥10% WG group (median: 50 years and 5.0 cholesterol mmol/L, p 0.04 and p 0.01, respectively). In sample B, significant differences were found in the prescribed immunosuppressive treatments; CSA was highly prescribed in the ≥10% WG when compared to the <10% WG group (73.8% versus 52.7%, respectively; p 0.02).

Genetic Risk Score analysis

Weighted genetic risk score with GWAS polymorphisms

In samples A and B, w-GRS ranged from 16 to 40 (SNP group#1) and from 63 to 107 (SNP group#2), respectively. Figure S1 shows the w-GRS distribution percentage in each sample. The association between w-GRS and BMI over time for sample A is shown on Table 3. w-GRS built from the SNP group#1 was significantly associated with BMI, showing a 0.16 BMI units increase per additional risk allele and an explained variability of 1.46%. When stratified by the median of age (w-GRS*age p=0.001 and p=0.02 for SNP group#1 and #2, respectively) individuals older than 54 years old had 0.23 BMI unit increase per additional risk allele and an explained BMI variability of 2.74% whereas those at 54 years or younger showed a trend of 0.10 units increase and 0.56% of explained BMI variability after multiple test correction (p=0.08). For SNP group#2, the effect was slightly lower (0.11 units of BMI per risk allele increase, explained variability of 2.08%). These results could be partially replicated in sample B (Table 4) for SNP group#1 with an effect of 0.20 BMI units per risk allele increase and explained variability of 2.40%. Analysis stratified by sex (w-GRS*sex, p=0.03 for SNP group#1) showed no significant associations after multiple test correction (Table 4). Additionally, a significant interaction between w-GRS and organ (i.e. kidney/non kidney) was found for sample B and SNP group#1 (n=83, p-value 0.04) showing a slightly higher effect (0.30 units of BMI per risk allele increase) in kidney transplanted individuals when compared to the overall 0.20 units (results not shown). When combining samples A and B, BMI increased by 0.14 and 0.12 units per additional risk allele in SNP group#1 and #2, respectively (p-values<0.001).

Weighted genetic risk score in Candidate Gene polymorphisms (SNP group#3)

No association of w-GRS from SNP group#3 and BMI was found in sample A whereas an increase of 0.05 units of BMI per additional risk allele was found in sample B (p-value 0.048) with an explained BMI variability of 1.72% (Table S4). In addition, in sample B, when SNPs group#3 and #1 were combined (49 SNPs excluding repeated SNPs) a significant association with BMI was found with an increase of 0.16 BMI units per additional risk allele and an explained BMI variability of 4.1% (p-value: 0.001) (Table S5). The w-GRS in the combined A and B sample showed an effect of 0.01 kg/m^2 per additional risk allele (p-value 0.04).

Prediction of 10% WG one year after transplantation

For the models in which the w-GRS was significantly associated with BMI, we evaluated the ability of the model to discriminate between gainers of ≥10% of weight and those who gained <10% the first year after transplantation. In sample A, a model adjusted by clinical covariates (i.e. age, sex, immunosuppressant treatment (tacrolimus and/or cyclosporine), baseline BMI and transplanted organ) as well as genetic factors (i.e. SNP group#1) performed better than a model adjusted only by clinical covariates (LRT-p: 0.0004). The predictive value for gaining 10% or more weight when including SNP group#1 in the model resulted in an AUROC of 0.74, a specificity of 0.61, a sensitivity of 0.77 and an accuracy of 0.65, whereas the model without genetic components had 0.66, 0.59, 0.66 and 0.61 of AUROC, specificity, sensitivity and accuracy, respectively (Table 5). Similarly, the genetic model including SNP group#2, performed better (LRT-p: 0.008) and had higher AUROC (0.80) than the non genetic model (AUROC non genetic: 0.66). Similarly, for sample B, the genetic model including clinical covariates and SNP group#1 was significantly different from the clinical model, (LRT-p: 0.04) had an AUROC of 0.89 and a specificity, sensitivity and accuracy of 0.78, 0.88 and 0.81, respectively (Table 5). The prediction

performance of the genetic model compared to the non-genetic one was significantly improved as shown by the IDI score. An IDI of 0.17 (Sample 1, SNP group#2, Table 5) indicates that the difference in predicted risks between those who gain at least 10% of weight and those who did not, increased by 17% in the genetic model.

The lowest NNG in order to detect one misclassified case of 10% weight increase one year after transplantation (Table 5) was 6 (obtained for sample B, SNP group#1). In sample A, the NNG was 13 for SNP group#2 and 24 for SNP group#1.

DISCUSSION

To our knowledge, this is the first study examining the association of clinical and genetic risk scores with WG in SOT patients. Our results showed that, in transplanted populations, previously GWAS-BMI related SNPs in general populations, were associated with BMI when combined in w-GRS. These results could be partly replicated in a second sample (i.e. sample B).

The influence of weighted score including SNP group#1 on BMI has been extensively replicated in several general populations from different ethnicities (29-32). This is the first study evaluating the effect of these polymorphisms on BMI in SOT recipients (kidney, liver, lung, heart, or multi-organ) and WG, with positive results being found in both samples. SNPs group#2 was recently published (27) and contained a higher number of SNPs (including those from SNP group# 1 except of 2 SNPs). However, significant results were found only in sample A. The non replication using SNPs group#2 in sample B could be attributed either to no effect at all or to the low number of patients in the latter sample and the large number of polymorphisms in group#2, each one of small effect size, thus necessitating large sample sizes in order to observe an effect (47).

In addition, an exploratory analysis of 19 polymorphisms combined in a w-GRS (SNP group#3) showed an association with BMI in sample B. These variants were selected from a microarray study examining subcutaneous gene expression which was correlated with weight change in kidney transplant recipients (25). These findings should be considered as preliminary as they were not further replicated. In sample B individuals were younger, had lower percentage of living donors and gained more weight after the first year of transplant compared to sample A. Young age, low BMI at baseline and deceased donors increase the risk of gaining weight, as previously described in the literature (15, 48). Adding SNP group#3 to SNP group#1 resulted in an increased explained BMI variability of 4.1%. However, when all SNPs were combined (i.e. SNP group#2 and SNP group#3), no significant results were found, probably due to the low effect and sample size.

In a second step, we showed that a combination of extensive genetic factors and clinical data predicts better a 10% WG after the first year of treatment than considering the model with clinical data alone, increasing AUROC and accuracy. When examining genetic factors in sample A, several polymorphisms were significantly associated with 10% WG one year post-transplant. Interestingly, when looking at SNPs individually, only *MC4R* and *SEC16B* remained significant in both SNP group#1 and #2 analyses. *MC4R* is one of the most common genetic causes of obesity and this gene participates in appetite regulation and energy balance (49). *SEC16B* has been associated with obesity-related phenotypes but the mechanism behind remains unknown. In sample B, 4 SNPs in or near *MTIF3*, *ETV5*, *GNPDA2* and *FAIM2* gene regions were associated with 10% WG one year post-transplant. Most of these gene functions are not clear yet. *ETV5* modulates circulating glucocorticoids levels (50) and *GNPDA2* regulates metabolic pathways leading to insulin resistance (51). Interestingly, the best group of polymorphisms predicting 10% WG at 12 months post-transplant was SNP group#2 (n=97 SNPs) for sample A and SNP group#1 (n=32 SNPs) for sample B. This could be tentatively explained by the fact that a higher sample size (i.e. sample A) is

necessary to demonstrate the association with larger set of SNPs (i.e. SNP group#2). Finally, only the SNP group#1 was associated with BMI at different time points in both samples A and B.

In samples A and B, the mean of WG after one year post-transplant is 3.5% and 6.3%, respectively, i.e. much lower than the 10% mean value described in the literature (15). It should be noted that a solid consensus does not exist yet regarding WG after the first year post-transplantation; a mean of 10% has been described but a range from 3.5 to 10 kg as well. A WG of 10 kg over the first year following kidney (12, 13, 52) liver (14) and cardiac (10) transplantation as described in some studies would correspond to an increase of 14% of weight in our samples (considering a mean baseline weight in sample A and B of 71 kg and 69.5 kg, respectively) which would be much higher than the WG mean in our samples.

Some limitations of the present study should be acknowledged. These results can only be extrapolated to Caucasians. We could not obtain all genotypes, in particular those from the SNP group#3 and possible co-medications influencing weight in addition to the immunosuppressant treatment were not reported and/or considered. Finally, sample B size was small and other replication in larger cohorts should be tested. However, both Samples were obtained from naturalistic setting studies, which should represent the real cases in clinical practice.

To conclude, this is the first study evaluating extensively the association of w-GRS with BMI and the influence of clinical and genetic components on ≥10% WG over the first year post transplant. The results obtained in the present study, showed the importance of integrating genetic factors in the final model, since they contain predictive information on ≥10% WG. Genetics of obesity among SOT recipients remains an important issue and will definitely contribute towards treatment personalizing and prediction improvement of WG in these populations by identifying at risk-individuals.

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References

1. WHO, obesity and overweight (Fact sheet N° 311): World Health Organization; 2015 [updated January 2015; cited 2015 July 2015]. World Health Organization]. Available from: http://www.who.int/mediacentre/factsheets/fs311/en/.
2. Kim WR, Stock PG, Smith JM, Heimbach JK, Skeans MA, Edwards EB, et al. OPTN/SRTR 2011 Annual Data Report: liver. Am J Transplant. 2013;13 Suppl 1:73-102.
3. Friedman AN, Miskulin DC, Rosenberg IH, Levey AS. Demographics and trends in overweight and obesity in patients at time of kidney transplantation. Am J Kidney Dis. 2003;41(2):480-7.
4. Weiss ES, Allen JG, Russell SD, Shah AS, Conte JV. Impact of recipient body mass index on organ allocation and mortality in orthotopic heart transplantation. J Heart Lung Transplant. 2009;28(11):1150-7.
5. Allen JG, Arnaoutakis GJ, Weiss ES, Merlo CA, Conte JV, Shah AS. The impact of recipient body mass index on survival after lung transplantation. J Heart Lung Transplant. 2010;29(9):1026-33.
6. Reisaeter AV, Hartmann A. Risk factors and incidence of posttransplant diabetes mellitus. Transplant Proc. 2001;33(5A Suppl):8s-18s.
7. Halme L, Eklund B, Kyllonen L, Salmela K. Is obesity still a risk factor in renal transplantation? Transpl Int. 1997;10(4):284-8.
8. Pfeiffer T, Albrecht KH, Wagner K, Herget S, Eigler FW. [The effect of obesity on outcome of kidney transplantation]. Med Klin (Munich). 1994;89(12):635-9.
9. Pischon T, Sharma AM. Obesity as a risk factor in renal transplant patients. Nephrol Dial Transplant. 2001;16(1):14-7.
10. Williams JJ, Lund LH, LaManca J, Kunavarapu C, Cohen DJ, Heshka S, et al. Excessive weight gain in cardiac transplant recipients. J Heart Lung Transplant. 2006;25(1):36-41.
11. Cashion AK, Sanchez ZV, Cowan PA, Hathaway DK, Lo Costello A, Gaber AO. Changes in weight during the first year after kidney transplantation. Prog Transplant. 2007;17(1):40-7.
12. Patel MG. The effect of dietary intervention on weight gains after renal transplantation. J Ren Nutr. 1998;8(3):137-41.
13. Clunk JM, Lin CY, Curtis JJ. Variables affecting weight gain in renal transplant recipients. Am J Kidney Dis. 2001;38(2):349-53.
14. Wawrzynowicz-Syczewska M, Karpinska E, Jurczyk K, Laurans L, Boron-Kaczmarska A. Risk factors and dynamics of weight gain in patients after liver transplantation. Ann Transplant. 2009;14(3):45-50.
15. Johnson CP, Gallagher-Lepak S, Zhu YR, Porth C, Kelber S, Roza AM, et al. Factors influencing weight gain after renal transplantation. Transplantation. 1993;56(4):822-7.
16. Hubert HB, Feinleib M, McNamara PM, Castelli WP. Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. Circulation. 1983;67(5):968-77.
17. Simopoulos AP. Characteristics of obesity: an overview. Ann N Y Acad Sci. 1987;499:4-13.
18. Pham PT, Pham PM, Pham SV, Pham PA, Pham PC. New onset diabetes after transplantation (NODAT): an overview. Diabetes Metab Syndr Obes. 2011;4:175-86.
19. McCaughan JA, McKnight AJ, Maxwell AP. Genetics of new-onset diabetes after transplantation. J Am Soc Nephrol. 2014;25(5):1037-49.
20. Balla A, Chobanian M. New-onset diabetes after transplantation: a review of recent literature. Curr Opin Organ Transplant. 2009;14(4):375-9.
21. Beckmann S, Ivanovic N, Drent G, Ruppar T, De Geest S. Weight gain, overweight and obesity in solid organ transplantation--a study protocol for a systematic literature review. Syst Rev. 2015;4:2.

22. Toniutto P, Fabris C, Minisini R, Apollonio L, Fumo E, Caldato M, et al. Weight gain after liver transplantation and the insertion/deletion polymorphism of the angiotensin-converting enzyme gene. Transplantation. 2005;79(10):1338-43.
23. Watt KD, Dierkhising R, Fan C, Heimbach JK, Tillman H, Goldstein D, et al. Investigation of PNPLA3 and IL28B genotypes on diabetes and obesity after liver transplantation: insight into mechanisms of disease. Am J Transplant. 2013;13(9):2450-7.
24. Fougeray S, Loriot MA, Nicaud V, Legendre C, Thervet E, Pallet N. Increased body mass index after kidney transplantation in activating transcription factor 6 single polymorphism gene carriers. Transplant Proc. 2011;43(9):3418-22.
25. Cashion A, Stanfill A, Thomas F, Xu L, Sutter T, Eason J, et al. Expression levels of obesity-related genes are associated with weight change in kidney transplant recipients. PLoS ONE. 2013;8(3):e59962.
26. Homayouni R, Heinrich K, Wei L, Berry MW. Gene clustering by latent semantic indexing of MEDLINE abstracts. Bioinformatics. 2005;21(1):104-15.
27. Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, et al. Genetic studies of body mass index yield new insights for obesity biology. Nature. 2015;518(7538):197-206.
28. Speliotes EK, Willer CJ, Berndt SI, Monda KL, Thorleifsson G, Jackson AU, et al. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. Nat Genet. 2010;42(11):937-48.
29. Zhu J, Loos RJ, Lu L, Zong G, Gan W, Ye X, et al. Associations of genetic risk score with obesity and related traits and the modifying effect of physical activity in a Chinese Han population. PLoS ONE. 2014;9(3):e91442.
30. Peterson RE, Maes HH, Lin P, Kramer JR, Hesselbrock VM, Bauer LO, et al. On the association of common and rare genetic variation influencing body mass index: a combined SNP and CNV analysis. BMC Genomics. 2014;15:368.
31. Goumidi L, Cottel D, Dallongeville J, Amouyel P, Meirhaeghe A. Effects of established BMI-associated loci on obesity-related traits in a French representative population sample. BMC Genet. 2014;15:62.
32. Horne BD, Anderson JL, Carlquist JF, Muhlestein JB, Renlund DG, Bair TL, et al. Generating genetic risk scores from intermediate phenotypes for use in association studies of clinically significant endpoints. Ann Hum Genet. 2005;69(Pt 2):176-86.
33. Belsky DW, Moffitt TE, Sugden K, Williams B, Houts R, McCarthy J, et al. Development and evaluation of a genetic risk score for obesity. Biodemography Soc Biol. 2013;59(1):85-100.
34. Koller MT, van Delden C, Muller NJ, Baumann P, Lovis C, Marti HP, et al. Design and methodology of the Swiss Transplant Cohort Study (STCS): a comprehensive prospective nationwide long-term follow-up cohort. Eur J Epidemiol. 2013;28(4):347-55.
35. Quteineh L, Bochud PY, Golshayan D, Crettol S, Venetz JP, Manuel O, et al. CRTC2 polymorphism as a riks factor for the incidence of metabolic syndrome traits in patients with solid organ transplantation. The pharmacogenomics Journal. 2015.
36. Crettol S, Venetz JP, Fontana M, Aubert JD, Ansermot N, Fathi M, et al. Influence of ABCB1 genetic polymorphism on cyclosporine intracellular concentrations in transplant recipients. Pharmacogen Genomics. 2008;18:307-15.
37. Crettol S, Venetz JP, Fontana M, Aubert JD, Pascual M, Eap CB. CYP3A7, CYP3A5, CYP3A4, and ABCB1 genetic polymorphisms, cyclosporine concentration, and dose requirement in transplant recipients. Ther Drug Monit. 2008;30(6):689-99.
38. Voight BF, Kang HM, Ding J, Palmer CD, Sidore C, Chines PS, et al. The metabochip, a custom genotyping array for genetic studies of metabolic, cardiovascular, and anthropometric traits. PLoS Genet. 2012;8(8):e1002793.

39. Lango Allen H, Estrada K, Lettre G, Berndt SI, Weedon MN, Rivadeneira F, et al. Hundreds of variants clustered in genomic loci and biological pathways affect human height. Nature. 2010;467(7317):832-8.
40. Heid IM, Jackson AU, Randall JC, Winkler TW, Qi L, Steinthorsdottir V, et al. Meta-analysis identifies 13 new loci associated with waist-hip ratio and reveals sexual dimorphism in the genetic basis of fat distribution. Nat Genet. 2010;42(11):949-60.
41. R Core Team. R: A language and environment for statistical computing, R Fondation for Statistical Computing,Vienna, Austria Vienna, Austria: R Development Core Team; 2013 [June 11, 2014]. Available from: http://www.R-project.org.
42. Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez JC, et al. pROC: display and analyze ROC curves. 1.7.3 ed2014.
43. Janssens AC, Moonesinghe R, Yang Q, Steyerberg EW, van Duijn CM, Khoury MJ. The impact of genotype frequencies on the clinical validity of genomic profiling for predicting common chronic diseases. Genet Med. 2007;9(8):528-35.
44. Pencina MJ, D'Agostino RB, Sr., D'Agostino RB, Jr., Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med. 2008;27(2):157-72; discussion 207-12.
45. Pencina MJ, D'Agostino RB, Pencina KM, Janssens AC, Greenland P. Interpreting incremental value of markers added to risk prediction models. Am J Epidemiol. 2012;176(6):473-81.
46. Borobia AM, Lubomirov R, Ramirez E, Lorenzo A, Campos A, Muñoz-Romo R, et al. An acenocoumarol dosing algorithm using clinical and pharmacogenetic data in Spanish patients with thromboembolic disease. PLoS ONE. 2012;7(7):e41360.
47. Dudbridge F. Power and predictive accuracy of polygenic risk scores. PLoS Genet. 2013;9(3):e1003348.
48. Hoehn RS, Wilson GC, Wima K, Hohmann SF, Midura EF, Woodle ES, et al. Comparing living donor and deceased donor liver transplantation: A matched national analysis from 2007 to 2012. Liver Transpl. 2014;20(11):1347-55.
49. Adan RA, Tiesjema B, Hillebrand JJ, la Fleur SE, Kas MJ, de Krom M. The MC4 receptor and control of appetite. Br J Pharmacol. 2006;149(7):815-27.
50. Gutierrez-Aguilar R, Thompson A, Marchand N, Dumont P, Woods SC, de Launoit Y, et al. The obesity-associated transcription factor ETV5 modulates circulating glucocorticoids. Physiol Behav. 2015;150:38-42.
51. Gutierrez-Aguilar R, Kim DH, Woods SC, Seeley RJ. Expression of new loci associated with obesity in diet-induced obese rats: from genetics to physiology. Obesity (Silver Spring). 2012;20(2):306-12.
52. Chakkera HA, Weil EJ, Pham PT, Pomeroy J, Knowler WC. Can new-onset diabetes after kidney transplant be prevented? Diabetes Care. 2013;36(5):1406-12.

Table 1: Characteristics of Sample A (all and by 10% weight gain one year after transplantation)

Characteristic	**All** **n=995**	**wg≥10%*** **n=204**	**wg<10%*** **n=673**	**p-value #**
Recipient age at transplantation (years), median (range)	54 (18 - 79)	51 (18-73)	55 (18-79)	**0.0001**
Recipient men (%)	66.0	56.8	68.9	**0.001**
Period of follow up (months), median (range)	12 (0-48)	12 (0-48)	12 (0-48)	0.55
Living donor (%)	27.1	27.9	29.6	0.6
Donor age (years), median (range)	53 (1 - 86)	50 (1-80)	53 (1-86)	**0.04**
Transplanted organ (%)				
Kidney	62.4	61.3	67.2	
Liver	15.9	10.3	14.7	
Lung	9.5	14.7	7.8	**<0.001**
Heart	6.5	11.3	4.6	
Multi-organ transplantation	4.1	2.5	4.5	
Before transplant				
BMI (kg/m^2), median (range)	24.6 (13.7 - 41.2)	23.1 (14.9 - 37.4)	24.9 (14.3 - 41.2)	**0.0001**
Overweight (25 kg/m^2 ≤ BMI >30 kg/m^2), %	30.7	23.0	32.5	**<0.001**
Obese (BMI ≥ 30 kg/m^2), %	15.3	9.3	16.8	
HDL (mmol/L), median (range)	1.2 (0.01-8)	1.2 (0.1 - 4.1)	1.2 (0.09 - 8)	0.7
LDL (mmol/L), median (range)	2.2 (0.06-10.02)	2.2 (0.1 - 7.1)	2.2 (0.08 - 10.0)	0.3
Cholesterol (mmol/L), median (range)	4.2 (0.3-11.7)	4.0 (0.3 - 9.9)	4.2 (0.8 - 11.7)	0.2
At 12 months after transplant				
BMI (kg/m^2), median (range)	25.2 (15.3 - 44.6)	27.1 (18.8 - 44.6)	24.7 (15.3 - 44.3)	**0.0001**
Overweight (25 kg/m^2 ≤ BMI >30 kg/m^2), %	34.7	39.0	33.0	**<0.001**
Obese (BMI ≥ 30 kg/m^2), %	17.0	27.0	14.0	
HDL (mmol/L), median (range)	3.5	1.3 (0.5-4.1)	1.3 (0.2-7.0)	0.08
LDL (mmol/L), median (range)	1.3 (0.21-7)	2.6 (0.8-5.8)	2.6 (0.3-8.7)	0.8
Cholesterol (mmol/L), median (range)	2.6 (0.3-8.7)	5.0 (2.3-9.2)	4.8 (1.7-12.0)	**0.01**
Incidence of NODAT (%)$	27.1	25.9	28.1	0.6
CMV infection (%)				
Recipient CMV infection (R+)	57.1	21.8	23.7	
Donor CMV infection (D+)	53.0	20.8	20.6	0.9
Recipient and Donor CMV infection (R+D+)	32.6	33.2	33.1	
Calcineurin inhibitors (%)				
TAC	45.1	42.2	48.6	
CSA	19.6	21.1	19.6	0.3
None	35.2	36.8	31.8	

wg: weight gain, CMV: Citomegalovirus, TAC: Tacrolimus, CSA: Cyclosporine, NODAT: New Onset Diabetes After Transplant

comparison between wg≥10% and wg<10%

*at 12 months after transplantation, missing n=118

$NODAT excluded those patients with diabetes previous to transplant

Table 2: Characteristics of Sample B (all and by 10% weight gain one year after transplantation)

Characteristic	**All** **156**	**wg≥10%*** **42**	**wg<10%*** **78**	**p-value #**
Recipient age at transplantation (years), median (range)	48 (22-68)	47 (26-66)	49 (22-68)	0.4
Recipient men (%)	60.9	59.5	61.5	0.8
Period of follow up (months), median (range)	12 (1-60)	12 (1-60)	12 (1-60)	1
Living donor (%)	11.5	11.9	7.7	0.4
Donor age (years), median (range)	43.5 (10-73)	45 (10-65)	43 (11-69)	0.7
Transplanted organ (%)				
kidney	65.4	76.2	60.3	
Liver	23.7	7.1	26.9	**0.03**
Lung	10.9	16.7	12.8	
Before transplant				
BMI (kg/m2), median (range)	23.4 (15.8-37.3)	22.9 (18.7-33.5)	24.2 (15.8-37.3)	0.06
Overweight (25 kg/m^2 ≤ BMI >30 kg/m^2), %	24.1	14.3	30.8	0.08
Obese (BMI ≥ 30 kg/m^2), %	10.9	9.5	12.8	
At 12 months after transplant				
BMI (kg/m2), median (range)	25.2 (16.5-39.3)	26.8 (20.9-39.3)	24.3 (16.5-35.4)	**0.0006**
Overweight (25 kg/m^2 ≤ BMI >30 kg/m^2), %	35.1	45.2	28.2	**0.004**
Obese (BMI ≥ 30 kg/m^2), %	18.5	28.6	14.1	
Incidence of NODAT (%)	28.8	30.9	35.9	0.6
CMV infection (%)				
Recipient CMV infection (R+)	49.3	30.8	36.1	
Donor CMV infection (D+)	61.5	23.1	15.3	0.6
Recipient and Donor CMV infection (R+D+)	27.6	30.8	27.8	
Calcineurin inhibitors (%)				
TAC	34.6	26.2	47.4	**0.02**
CSA	65.4	73.8	52.7	

wg: weight gain, CMV: Citomegalovirus, TAC: Tacrolimus, CSA: Cyclosporine, NODAT: New Onset Diabetes After Transplant.
comparison between wg≥10% and wg<10%
*at 12 months after transplantation

Table 3: Weighted Genetic Risk Scores from GWAS SNPs and their associations with BMI in Sample A.

	n	Effect on BMI per additional risk allele [CI 95%]	p-value*	E. Var (%)
SNP group#1				
All population	881	0.16 [0.11 - 0.23]	**p<0.008**	1.46
Age [18 - 54] years	444	0.10 [0.01 - 0.17]	*0.08*	0.56
Age > 54 years	437	0.23 [0.14 – 0.32]	**p<0.008**	2.74
SNP group#2				
All population	854	0.11 [0.07 - 0.15]	**p<0.008**	2.08
Age [18 - 54] years	452	0.08 [0.03 - 0.13]	**p<0.008**	1.10
Age > 54 years	426	0.13 [0.07 - 0.19]	**p<0.008**	2.90

E. Var: Explained Variability

CI: Confidence Interval

SNP : Single Nucleotide Polymorphism

BMI: Body Mass Index

** p-value corrected by multiple test*

Table 4: Weighted Genetic Risk Scores from GWAS SNPs and their associations with BMI in Sample B.

	n	Effect on BMI per additional risk allele [CI 95%]	p-value*	E. Var (%)
SNP group#1				
All population	124	0.20 [0.07 - 0.35]	**0.02**	2.40
Men	73	n.c	n.c	n.c
Women	61	0.28 [-0.05 - 0.63]	0.3	n.c
SNP group#2				
All population	117	0.02 [-0.08 - 0.11]	1.0	n.c
Men	69	-0.03 [-0.18 - 0.07]	1.0	n.c
Women	48	n.c	n.c	n.c

E. Var: Explained Variability

CI: Confidence Interval

BMI: Body Mass Index

SNP : Single Nucleotide Polymorphism

n.c: not calculated because of non significant association and/or low sample size

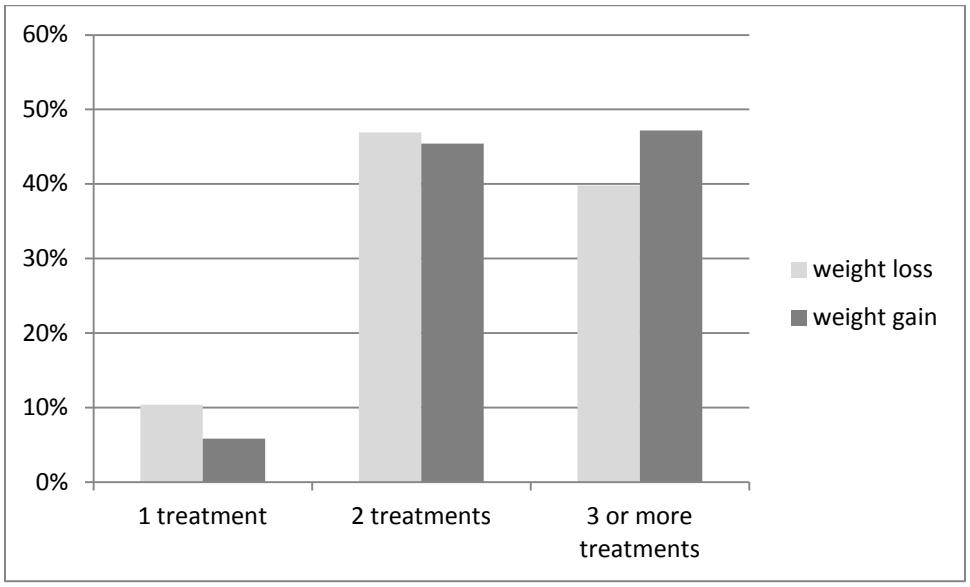
** p-value corrected by multiple test*

Table 5. Comparison of genetic versus non-genetic model for 10% weight gain prediction at one year after transplantation.

		AUROC [95% CI]	Specificity	Sensitivity	Accuracy	LRT-p	IDI [95% CI]*	NNG
Sample A	**SNP group#1**							
	non genetic model	0.66 [0.58 – 0.72]	0.59	0.66	0.61	0.0004	0.08 [0.06 - 0.10]	24
	genetic model	0.74 [0.70 – 0.83]	0.61	0.77	0.65			
	SNP group#2							
	non genetic model	0.66 [0.54 – 0.69]	0.65	0.62	0.64	0.008	0.17 [0.14 – 0.20]	13
	genetic model	0.80 [0.71 – 0.84]	0.70	0.77	0.72			
Sample B	**SNP group#1**							
	non genetic model	0.67 [0.61 – 0.88]	0.55	0.76	0.63	0.04	0.36 [0.28 - 0.45]	6
	genetic model	0.89 [0.79 – 0.97]	0.78	0.88	0.81			

AUROC: Area Under the Receiver Operating Characteristic Curve
IDI : Integrated Discrimination Improvement
NNG: Number Needed to Genotype
LRT: Likelihood Ratio tests
SNP : Single Nucleotide Polymorphism
CI: Confidence Interval
**p-value < 0.01*

Figure 1. Percentage of weight gain in Sample A at one year after transplantation by number of immunosuppressant treatments (cyclosporine, tacrolimus, glucocorticoids, azatioprine and/or mycophenolate).



4. Project IV

Genetic and clinic predictors of New Onset Diabetes Mellitus after Transplantation

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Summary

The present work describes how several genetic risk factors are associated with New Onset Diabetes After Transplantation (NODAT) and how they predict NODAT 12 months after transplantation in two samples of SOT. Three genetic risk scores (GRS) were constructed with SNPs obtained from GWAS studies and were tested first in a main sample (n_{main}= 725) and in another sample ($n_{replication}$= 156). Two out of the three GRS were significantly associated with NODAT in the main sample. In addition, a clinical risk score including several clinical variables was found to be associated with NODAT in the main and replication samples. Finally, the discrimination parameters showed that adding genetic factors in the clinical model improved the ability of the model to discriminate NODAT events one year after transplantation.

Genetic and clinic predictors of New Onset Diabetes Mellitus after Transplantation

Abstract (words: 241)

New Onset Diabetes after Transplantation (NODAT) is a frequent complication after solid organ transplantation, with higher incidence during the first year. Several clinical and genetic factors have been described as risk factors of Type 2 Diabetes that shares some genetic factors with NODAT. We investigated if three genetic risk scores (w-GRS) and clinical factors were associated with NODAT and how they predicted NODAT development one year after transplantation. In both main (n=725) and replication (n=156) samples the clinical risk score was significantly associated with NODAT (OR_{main}: 1.60 [1.36-1.90], $p=3.72*10^{-8}$ and $OR_{replication}$: 2.14 [1.39-3.41], p=0.0008, respectively). Two w-GRS were significantly associated with NODAT in the main sample ($OR_{w\text{-}GRS\ 2}$:1.09 [1.04-1.15], p=0.001 and $OR_{w\text{-}GRS\ 3}$:1.14 [1.01-1.29], p=0.03) and a similar $OR_{w\text{-}GRS\ 2}$ was found in the replication sample, although it did not reach significance probably due to a power issue. Despite the low OR of w-GRS on NODAT compared to clinical covariates, when integrating w-GRS 2 and w-GRS 3 in the clinical model, the Area under the Receiver Operating Characteristics curve (AUROC), specificity, sensitivity and accuracy were 0.69, 0.71, 0.58, 0.68, respectively, with significant Likelihood Ratio test discrimination index (p-value 0.0004), performing better in NODAT discrimination than the clinical model alone. Twenty-five patients needed to be genotyped in order to detect one misclassified case that developed NODAT one year after transplantation if using only clinical covariates. To our knowledge, this is the first study extensively examining genetic risk scores contributing to NODAT development.

INTRODUCTION

In the past decades, diabetes mellitus has become a growing public health problem. New Onset Diabetes after Transplantation (NODAT) is a frequent complication after Solid Organ Transplant (SOT) (1). The incidence of NODAT varies from 4% to 25% in kidney, 2.5 to 25% in liver, 4 to 40% in heart and 30 to 35% in lung transplant recipients (2). A high incidence of NODAT (i.e. 15% to 30%) occurs the first year after kidney transplant, decreasing by 5 to 6 times thereafter (3). NODAT leads to high risk complications, such as infections and cardiovascular diseases, reducing patient and graft survival (4, 5).

Risk factors for the development of NODAT include old age, obesity (6), African-American (3, 7) and Hispanic ethnicities (3), family history of diabetes, presence of hepatitis C and receipt of a deceased donor transplant (7). In addition, immunosuppressant treatments such as corticosteroids (8), calcineurin inhibitors (9) and/or sirolimus (10) contribute to the development of NODAT.

Type 2 Diabetes Mellitus (T2DM) is a polygenic disease. Until now, several genetic factors have been associated with T2DM. NODAT shares similarities with T2DM, with both diseases characterized by a combination of insulin resistance and insulin hyposecretion and with some shared genetic risk factors (11). Therefore, we hypothesized that genetic risk factors that have recently been associated with T2DM might also influence the risk of NODAT one year after transplantation. Thus, a large-scale association analysis identified 12 new locis related to diabetes (12) and some of the results were further replicated (13, 14). In addition, a recent study aggregating published meta-analyses of Genome Wide Association Studies (GWAS) from four major ethnic groups found new single nucleotide polymorphisms (SNPs) associated with

T2DM and replicated results from previous reports (15). Finally, a first exploratory GWAS of NODAT in kidney transplanted recipients revealed 8 significant SNPs (16).

The aim of the present study was to investigate whether several GRS were associated with NODAT development and how clinical and genetic factors could predict the development of NODAT one year after transplantation. We assessed whether adding genetic factors improved the clinical-based model. Such an integrated model would allow clinicians to identify individuals at high risk of NODAT before transplantation and, therefore, to provide early intervention and/or prevention.

METHODS

Main Sample

The Swiss Transplant Cohort Study (STCS) is an ongoing prospective multicenter study (Basel, Bern, Geneva, Lausanne, St. Gallen and Zurich) started in May 2008 which enrolls SOT recipients with no particular eligibility or exclusion criteria. The present study included individuals with a functional graft for at least 12 months after transplantation (transplantation between May 2008- May 2011). A total of 1294 patients were followed up in their respective transplant centers at baseline and at 6, 12, 24, 36 and 48 months after transplantation. Lipid profile, BMI, blood pressure and patient characteristics were collected at the different time-points of the follow up. NODAT was diagnosed if patients were taking an antidiabetic treatment after transplantation or if diabetes was reported in their case report forms. Patients with diabetes or prediabetes previous to transplant were excluded from analysis, as well as those with multi-organ transplants. Further details have been published elsewhere (17, 18). Only Caucasians and recipients of 18 years or older were retained. If an individual was subject

to more than one transplant, only the first SOT was considered. A total of 725 Caucasian patients for whom NODAT status was assessed were considered for analysis.

Replication Sample

One hundred and ninety-seven patients were enrolled between 2003 and 2005 from the outpatient clinic of the transplant center of the University Hospital of Lausanne, Switzerland. Only patients with a functional graft for more than 12 months were eligible to participate in the study. Further details can be found elsewhere (17, 19, 20). Briefly, data such as patient's age, gender, BMI, ethnicity, and immunosuppressive treatments were collected retrospectively from the medical files. Additionally, weight, fasting blood glucose levels, glycated hemoglobin, 2 hours oral glucose tolerance test, insulin and oral anti-diabetic treatment at baseline, at 1, 3, 6, 9, 12 and at the yearly follow-up during the 5 years after transplantation were collected retrospectively from the medical files between October 2011 and April 2012. All patients gave their written informed consent. Blood samples were collected for further genotyping analysis. Altogether, 156 Caucasians of 18 years or older from whom clinical and genetic data were available were included in the analysis.

Selection of genetic polymorphisms and genotyping

Fourteen and 30 GWAS significant SNPs ($p<5*10^{-8}$) were selected from two GWAS Meta analysis associated with T2DM in Caucasian (12) and four multi-ethnic general populations (15). In addition, 8 SNPs were retained from a GWAS of NODAT conducted in patients with kidney transplantation (16). For the main sample, genotypes were obtained from the Human OmniExpress-24 BeadChip Kit as described by the manufacturers' protocol (Illumina, San Diego, CA). For the replication sample, genotyping was performed using the Illumina 200K

Cardiometabochip (Illumina, San Diego, CA) at the iGE3 genomics platform of the University of Geneva (http://www.ige3.unige.ch/genomics-platform.php). Briefly, the CardioMetabochip is a custom Illumina iSelect genotyping array designed to test DNA variation of 200'000 SNPs from regions identified by large scale meta-analyses of Genome Wide Association Studies (GWAS) for metabolic and cardiovascular traits (32). Polymorphisms or proxies were chosen based on genotype availability. A quality control was done for the genotyped SNPs. Samples were excluded from the analysis if sex was inconsistent with genetic data from X-linked markers, and when genotype call rate was <0.96 and gene call score <0.15. GenomeStudio Data Analysis Software was used to export results generated by Illumina CardiometaboChip. Tables S1-S3 show a detailed description of the polymorphisms. Of note, most of the genotypes from the NODAT GWAS (16) could not be obtained for the replication sample; therefore these SNPs could not be tested for replication.

Construction of Clinical and Genetic Risk Scores

Clinical Risk Score

Construction of clinical risk score was done according to risk factors previously described (1, 21) and one point was assigned to each of the following covariates: male gender, age above the mean (53 years), BMI ≥ 30 kg/m^2, glucocorticoid treatment, tacrolimus immunosuppressant treatment, deceased donor status, antibodies Hepatitis C Virus positive (antiHCV+). In the main sample, the score ranged from 0 to 7 and was grouped as low risk score (0-1), intermediate risk score (2-3) and high risk score (4-7). The clinic risk score was also tested in the replication sample.

Genetic Risk Score

Three weighted genetic risk scores (GRS) were calculated based on the 3 previously mentioned GWAS studies (12, 15, 16). A score was assigned to each individual based on the number (0, 1 or 2) of risk alleles carried, weighted by the risk coefficient reported as Odds Ratios (OR) (22).

Statistical analysis

Descriptive analysis presented quantitative data as median and range unless otherwise specified whereas qualitative data is expressed as percentages. Chi-squared tests or rank sum tests were used to compare categorical or non-parametric continuous variables, respectively, in people who developed NODAT and those who did not. Significant threshold of p-value was set at 0.05 and multiple test correction was applied when necessary. Two SNPs were excluded from the main and replication samples since they were not in Hardy-Weinberg Equilibrium (HWE) (further description in Table S1 and S2). We first assessed the association of individual covariates with NODAT development. For both samples, GRS and significant covariates with p-values<0.10 were then included in a multivariate logistic model and the corresponding OR with its 95% Confidence Interval (CI) were calculated.

In a second step, for those GRS significantly associated with NODAT in the main sample, we assessed the ability of the genetic model to discriminate between patients with and without NODAT one year after transplantation. Genetic factors were integrated in the model as w-GRS. A clinical risk score as described above was also tested. Area under the Receiver Operating Characteristic Curve (AUROC) was calculated for a model containing only clinical covariates and another integrating clinical and genetic factors. An AUROC lower than 0.70 indicates low discriminative accuracy (23). In addition, sensitivity (percentage of correctly predicted individuals with NODAT among all NODAT individuals), specificity (percentage of correctly predicted individuals without NODAT among all truly individuals without NODAT) and accuracy

(percentage of correctly classified NODAT among all subjects) were obtained for each model using "pROC" R package (24). As previously described in the literature, (25, 26) and in order to compare the genetic and non-genetic model, Likelihood Ratio Tests (LRT) and Integrated Discrimination Improvement (IDI) estimates with their respective p-values were calculated. We used the Net Reclassification Index (NRI) to assess to which extent adding genetic factors in the non-genetic model resulted in classifications of individuals into risk categories which better reflect their actual outcome. Finally, the number needed to genotype (NNG) (i.e. the average number of patients who need to be genotyped to detect one misclassified case of NODAT if using only clinical covariates) was calculated based on the inverse of the difference between the accuracy of clinical and genetic models (27).

RESULTS

Population description

Sixty-five percent were men in the main sample (Table 1) and the median age was 53 years, which was significantly higher than in the replication sample (48 years, $p<0.001$, Table 2). Higher percentages of living donors and elderly donors were found in the main sample when compared to the replication sample (32% versus 12%, respectively and 52 years versus 44 years, Tables 1 and 2, respectively). Tacrolimus was more prescribed in the main sample (67% vs 35%). Median of baseline BMI and BMI at 12 months after transplantation were similar in both samples.

NODAT individuals were generally older, had higher baseline and 12-months BMI after transplantation and higher percentage of obesity when compared to non-NODAT individuals, both in the main and in the replication samples (Tables 1 and 2).

Clinical factors and Clinical risk score

In the main sample, when conducting multivariate analysis (Table 3), induction treatment and baseline BMI were significantly associated with NODAT at one year after transplantation, with induction treatment protecting against NODAT development. In the replication sample (Table 4), baseline BMI and tacrolimus treatment were significantly associated with NODAT. A clinical risk score was constructed combining several clinical covariates which have been described in the literature as risk factors of NODAT. The score ranged from low risk to high risk; 0 to 7 in the main sample and from 1 to 6 in the replication sample. Table S4 shows associations of clinical risk scores with NODAT development, which was significant for both main and replication samples. Figure 1 shows the NODAT incidence according to the clinic risk score in the main sample. Sixty-six percent of NODAT had a high clinic risk score compared to 43% of non-NODAT ($p=2.5*10^{-7}$).

Genetic risk scores

Three w-GRS were tested in the main sample (Table 3). Significant associations were found for w-GRS 2 and 3 with NODAT development ($OR_{w\text{-}GRS\ 2}$:1.09 [1.04-1.15], p=0.001 and $OR_{w\text{-}GRS\ 3}$:1.14 [1.01-1.29], p=0.03). Finally, when combining all SNPs in a w-GRS, the odds of developing NODAT increased by 8% per one unit increase of the w-GRS (OR: 1.08 [1.03-1.12], p=0.0005). When testing the association of w-GRS with NODAT in the replication sample (N=156), similar OR was found for w-GRS 2, although it did not reach statistical significance (Table 4, w-GRS 3 not available, see Material and Methods). A power analysis showed that the minimum sample size needed in order to observe an effect in cohorts with proportion of NODAT ranging from 0.27 to 0.29 is N=320 and N=335, respectively, with a confidence level of 95% and a width of CI set at 0.1.

Additional analysis concerning the distribution of genotypes frequencies among NODAT and non-NODAT individuals in the main sample differed significantly for the *rs2020902*, a SNP previously associated with NODAT in a GWAS study (16) and located in the *CASP9* gene (p-value: 0.04, not significant after multiple test correction). This gene is implicated in several processes such as hyperglycemia or lipotoxicity via induction of β-cell apoptosis (28, 29).

NODAT discrimination assessment, Number Needed to Genotype (NNG)

The ability of a model integrating clinical and genetic factors to discriminate NODAT versus non-NODAT one year after transplantation was assessed in the main sample. Adding genetic components to the clinical model improved the final model, with LRT-p values being significant for w-GRS 2 (p=0.001) and when combining w-GRS 2 and w-GRS 3 (p=0.0004) (Table 5). The final model (i.e. including SNPs from both w-GRS 2 and w-GRS 3) showed an IDI of 0.02, meaning that the difference in predicted risks of developing NODAT after one year increased by 2% in the genetic model (p=0.004). In addition, the correctly reclassified individuals increased by 24% in the final model (NRI: 0.24 [0.05-0.43], p=0.01), with AUROC, specificity, sensitivity and accuracy values being 0.69, 0.71, 0.58 and 0.68, respectively.

Additionally, the ability to discriminate between NODAT and non-NODAT at one year after transplantation was assessed for a model keeping the same genetic factors than previously described (i.e. w-GRS 2 and w-GRS 3) but integrating clinical predictors in a clinical risk score (Table S5). Several discrimination parameters became significant when comparing clinical risk score model versus clinical plus genetic risk scores model (i.e. LRT, IDI, NRI). Such is the case for the IDI which shows the difference of predicted risks between p50 of NODAT and non-NODAT in both clinical and clinical plus genetic models, with a better discrimination in the later model. However the AUROC did not exceed the 0.70 value generally considered as indicator of

for clinical significance and the accuracy did not improve when adding GRS in the clinical risk score. On the other hand, the clinical relevance of the combined model was also estimated by calculating the NNG, in order to detect a misclassified case of NODAT one year after transplantation using only clinical covariates. The global percentage of correctly classified patients (i.e. accuracy) within the model integrating clinical and genetic factors (w-GRS 2 and w-GRS 3 combined) was 68%. With an accuracy of the clinical model alone being 64% (Table 5), the NNG was 25. Since the models integrating w-GRS 2 and w-GRS 3 alone did not improve in accuracy, the NNG could not be calculated.

DISCUSSION

To our knowledge, this is the first study extensively examining the influence of w-GRS on NODAT development. Our results showed that two out of three w-GRS tested were significantly associated with NODAT in the main sample. The fact that the two significant w-GRS were calculated from T2DM GWAS studies supports the hypothesis that T2DM and NODAT share some common genetic risk factors (11). These results were found in the main sample and similar results were found in the replication sample, although they did not reach significance threshold. As shown by the sample size calculation, in the replication sample the minimum sample size needed to observe an effect is not reached, suggesting that the replication sample is underpowered to detect the small effect size of each genetic risk score. In addition, patients in the main sample were older than in the replication sample (53 years versus 48 years), with a higher proportion of individuals in the main sample treated with TAC (67% versus 35%). Both TAC treatment and an elderly age have been described as risk factors of NODAT (6, 30). As expected, the clinical risk score was significantly associated with NODAT

in both main and replication samples, with a higher score being associated with higher NODAT incidence.

Although the clinical risk score had higher impact on NODAT, adding genetic factors as a w-GRS in the clinical model improved NODAT discrimination one year after transplantation although accuracy and AUROC of the model were lower than 0.70 considered as the threshold for low discriminative accuracy (23). The low AUROC found could be explained by the few number of SNPs included in the GRS since the highest AUROC was found for w-GRS 2 (N=30 SNPs). Nevertheless, the AUROC parameter has been criticized because it does not show risk prediction, (31, 32). Thus, when looking at other parameters evaluating the discrimination of the genetic versus clinical model, risk prediction and reclassification (i.e. LRT, IDI and NRI) they consistently suggest that genetics improve the clinical model. Despite the small contribution of genetics in improving the model, the combined genetic and clinical model allows to obtain a NNG of 25.

Genetic variants tested in the present study came from different sources. Those polymorphisms associated with T2DM in a Caucasian population (i.e. w-GRS 1) were not significantly associated with NODAT when combined in a w-GRS in our sample. Often the non replication of results is attributed to limited sample size and/or other missing variants contributing to the outcome. The polymorphisms which were associated with NODAT in our cohort came from a large T2DM GWAS multiethnic meta-analysis (26,488 cases and 83,964 controls) (i.e w-GRS 2) (15). Also, significant results were found with polymorphisms from the first NODAT GWAS conducted within kidney transplanted recipients (57 cases and 370 controls) (i.e w-GRS 3) but these results need to be tested for replication in another sample (16).

Some limitations of the present study should be acknowledged. The size of the replication sample should be increased. Indeed, since NODAT outcome is mainly affected by numerous genetic variants of small effects, replication samples should be large enough in order to avoid being underpowered. In addition, a replication sample including patients with similar age and TAC prescription is needed. Also, due to the moderate accuracies, the NNG could only be calculated when integrating a larger number of SNPs in a GRS. For the clinical risk score, history of diabetes was not available in our samples. This factor should be included in the clinical risk score construction despite the conflicting literature on the influence of family history of diabetes (33). In addition, some of the genotypes published in the GWAS studies (12, 15, 16) could not be obtained in the present study. Finally, our findings cannot be extrapolated to other ethnicities.

To date, little research has been conducted on genetic factors and NODAT development. Although the number of SNPs should be increased in order to tentatively improve model prediction, this study found two GRS significantly associated with NODAT, thus providing a first approach integrating genetic and clinical factors. The present results must be replicated in future studies integrating other additional genetic variants, with such studies possibly allowing researchers to translate a risk assessment using clinical and genetic variables into clinical practice.

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Declaration of interests:

CBE received honoraria for conferences or teaching CME courses from Advisis, Astra Zeneca, Lundbeck, MSD, Sandoz, Servier and Vifor-Pharma in the past 3 years. He has received an unrestricted educational grant from Takeda in the past 3 years. JFD Advisory committees: Bayer, BMS, Gilead Science, Janssen Cilag, Jennerex, Merck, Novartis, Roche. Speaking and teaching: Bayer, Boehringer-Ingelheim, Novartis, Roche. SC received honoraria for teaching CME courses from Astra Zeneca and Lundbeck. The authors of this manuscript have no conflicts of interest to disclose.

Footnote

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REFERENCES

1. Pham PT, Pham PC, Lipshutz GS, Wilkinson AH. New onset diabetes mellitus after solid organ transplantation. Endocrinol Metab Clin North Am. 2007;36(4):873-90; vii.
2. Gomes MB, Cobas RA. Post-transplant diabetes mellitus. Diabetology & metabolic syndrome. 2009;1(1):14.
3. Kasiske BL, Snyder JJ, Gilbertson D, Matas AJ. Diabetes mellitus after kidney transplantation in the United States. Am J Transplant. 2003;3(2):178-85.
4. Fernandez-Fresnedo G, Escallada R, de Francisco AL, Rodrigo E, Zubimendi JA, Ruiz JC, et al. Posttransplant diabetes is a cardiovascular risk factor in renal transplant patients. Transplant Proc. 2003;35(2):700.
5. Cosio FG, Kudva Y, van der Velde M, Larson TS, Textor SC, Griffin MD, et al. New onset hyperglycemia and diabetes are associated with increased cardiovascular risk after kidney transplantation. Kidney Int. 2005;67(6):2415-21.
6. Reisaeter AV, Hartmann A. Risk factors and incidence of posttransplant diabetes mellitus. Transplant Proc. 2001;33(5A Suppl):8s-18s.
7. Sumrani NB, Delaney V, Ding ZK, Davis R, Daskalakis P, Friedman EA, et al. Diabetes mellitus after renal transplantation in the cyclosporine era--an analysis of risk factors. Transplantation. 1991;51(2):343-7.
8. Arner P, Gunnarsson R, Blomdahl S, Groth CG. Some characteristics of steroid diabetes: a study in renal-transplant recipients receiving high-dose corticosteroid therapy. Diabetes Care. 1983;6(1):23-5.
9. Knoll GA, Bell RC. Tacrolimus versus cyclosporin for immunosuppression in renal transplantation: meta-analysis of randomised trials. BMJ. 1999;318(7191):1104-7.
10. Johnston O, Rose CL, Webster AC, Gill JS. Sirolimus is associated with new-onset diabetes in kidney transplant recipients. J Am Soc Nephrol. 2008;19(7):1411-8.
11. Kurzawski M, Dziewanowski K, Lapczuk J, Wajda A, Drozdzik M. Analysis of common type 2 diabetes mellitus genetic risk factors in new-onset diabetes after transplantation in kidney transplant patients medicated with tacrolimus. Eur J Clin Pharmacol. 2012;68(12):1587-94.
12. Voight BF, Scott LJ, Steinthorsdottir V, Morris AP, Dina C, Welch RP, et al. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. Nat Genet. 2010;42(7):579-89.
13. Song M, Zhao F, Ran L, Dolikun M, Wu L, Ge S, et al. The Uyghur population and genetic susceptibility to type 2 diabetes: potential role for variants in CDKAL1, JAZF1, and IGF1 genes. OMICS. 2015;19(4):230-7.
14. Hivert MF, Jablonski KA, Perreault L, Saxena R, McAteer JB, Franks PW, et al. Updated genetic score based on 34 confirmed type 2 diabetes Loci is associated with diabetes incidence and regression to normoglycemia in the diabetes prevention program. Diabetes. 2011;60(4):1340-8.
15. Mahajan A, Go MJ, Zhang W, Below JE, Gaulton KJ, Ferreira T, et al. Genome-wide trans-ancestry meta-analysis provides insight into the genetic architecture of type 2 diabetes susceptibility. Nat Genet. 2014;46(3):234-44.
16. McCaughan JA, McKnight AJ, Maxwell AP. Genetics of new-onset diabetes after transplantation. J Am Soc Nephrol. 2014;25(5):1037-49.
17. Quteineh L, Bochud PY, Golshayan D, Crettol S, Venetz JP, Manuel O, et al. CRTC2 polymorphism as a riks factor for the incidence of metabolic syndrome traits in patients with solid organ transplantation. The pharmacogenomics Journal, Doi:101038/tpj201582. 2015.
18. Koller MT, van Delden C, Muller NJ, Baumann P, Lovis C, Marti HP, et al. Design and methodology of the Swiss Transplant Cohort Study (STCS): a comprehensive prospective nationwide long-term follow-up cohort. Eur J Epidemiol. 2013;28(4):347-55.

19. Crettol S, Venetz JP, Fontana M, Aubert JD, Ansermot N, Fathi M, et al. Influence of ABCB1 genetic polymorphism on cyclosporine intracellular concentrations in transplant recipients. Pharmacogen Genomics. 2008;18:307-15.
20. Crettol S, Venetz JP, Fontana M, Aubert JD, Pascual M, Eap CB. CYP3A7, CYP3A5, CYP3A4, and ABCB1 genetic polymorphisms, cyclosporine concentration, and dose requirement in transplant recipients. Ther Drug Monit. 2008;30(6):689-99.
21. Pham PT, Pham PM, Pham SV, Pham PA, Pham PC. New onset diabetes after transplantation (NODAT): an overview. Diabetes Metab Syndr Obes. 2011;4:175-86.
22. Speliotes EK, Willer CJ, Berndt SI, Monda KL, Thorleifsson G, Jackson AU, et al. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. Nat Genet. 2010;42(11):937-48.
23. Janssens AC, Moonesinghe R, Yang Q, Steyerberg EW, van Duijn CM, Khoury MJ. The impact of genotype frequencies on the clinical validity of genomic profiling for predicting common chronic diseases. Genet Med. 2007;9(8):528-35.
24. Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez JC, et al. pROC: display and analyze ROC curves. 1.7.3 ed2014.
25. Pencina MJ, D'Agostino RB, Sr., D'Agostino RB, Jr., Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med. 2008;27(2):157-72; discussion 207-12.
26. Pencina MJ, D'Agostino RB, Pencina KM, Janssens AC, Greenland P. Interpreting incremental value of markers added to risk prediction models. Am J Epidemiol. 2012;176(6):473-81.
27. Borobia AM, Lubomirov R, Ramirez E, Lorenzo A, Campos A, Muñoz-Romo R, et al. An acenocoumarol dosing algorithm using clinical and pharmacogenetic data in Spanish patients with thromboembolic disease. PLoS ONE. 2012;7(7):e41360.
28. Hui H, Dotta F, Di Mario U, Perfetti R. Role of caspases in the regulation of apoptotic pancreatic islet beta-cells death. J Cell Physiol. 2004;200(2):177-200.
29. Maedler K, Spinas GA, Lehmann R, Sergeev P, Weber M, Fontana A, et al. Glucose induces beta-cell apoptosis via upregulation of the Fas receptor in human islets Diabetes. 2001;50(8):1683-90.
30. Rathi M, Rajkumar V, Rao N, Sharma A, Kumar S, Ramachandran R, et al. Conversion from tacrolimus to cyclosporine in patients with new-onset diabetes after renal transplant: an open-label randomized prospective pilot study. Transplant Proc. 2015;47(4):1158-61.
31. Kerr KF, McClelland RL, Brown ER, Lumley T. Evaluating the incremental value of new biomarkers with integrated discrimination improvement. American journal of epidemiology. 2011;174(3):364-74.
32. Pepe MS, Janes HE. Gauging the performance of SNPs, biomarkers, and clinical factors for predicting risk of breast cancer. Journal of the National Cancer Institute. 2008;100(14):978-9.
33. Montori VM, Basu A, Erwin PJ, Velosa JA, Gabriel SE, Kudva YC. Posttransplantation diabetes: a systematic review of the literature. Diabetes Care. 2002;25(3):583-92.

Table 1. Main sample characteristics

Characteristic	Main Sample n=725	NODAT n=198	non-NODAT n=527	p-value #
Recipient age at transplantation, median (range)	53 (18-79)	55 (19-73)	51 (18-79)	**0.002**
Recipient males, %	65	70	63	0.06
Period of follow up (months), median (range)	12 (0-48)	12 (0-48)	12 (0-48)	0.3
Living donor, %	32	27	34	0.07
Donor age (years), median (range)	52 (1 - 86)	53 (14-86)	52 (1-85)	0.8
Transplanted organ, %				
Kidney	68	63	69	
Liver	15	17	15	
Lung	9	10	9	0.4
Heart	6	9	5	
Multi-organ transplantation	2	2	2	
Before transplantation				
BMI, median (range)	24.4 (13.7 - 41.2)	25.6 (15.2-41.2)	24 (13.7-39.1)	**0.0005**
Overweight (25 kg/m^2 ≤ BMI >30 kg/m^2), %	29	32	28	**<0.001**
Obese (BMI ≥ 30 kg/m^2), %	13	23	10	
HDL (mmol/L)	1.16 (0.01 - 8)	1.14 (0.01 - 8)	1.18 (0.1 - 7.6)	0.5
LDL (mmol/L)	2.4 (0.08 - 10.0)	2.4 (0.1 - 6.3)	2.3 (0.1 - 10.0)	0.9
Cholesterol (mmol/L)	4.2 (0.6 - 11.7)	4.2 (0.6 - 9.2)	4.2 (0.8 - 11.7)	0.4
At 12 months after transplantation				
BMI, median (range)	25.1 (15.4 - 44.4)	26.1 (17.2-44.3)	24.7 (15.4-44.4)	**0.003**
Overweight (25 kg/m^2 ≤ BMI >30 kg/m^2), %	36	37	35	**0.003**
Obese (BMI ≥ 30 kg/m^2), %	15	22	13	
HDL (mmol/L)	1.4 (0.21-7)	1.2 (0.21-3.0)	1.3 (0.5-7.0)	**0.01**
LDL (mmol/L)	2.7 (0.4-7.0)	2.5 (0.4-7.0)	2.7 (0.7-5.8)	0.07
Cholesterol (mmol/L)	4.9 (2.3-12.0)	4.7 (2.3-12.0)	5 (2.6-10.3)	0.05
Hypolipemiant treatment*, %	33	41	31	**0.032**
Percentage of 10% weight gain, %	23	22	24	0.6
Weight gain (mean), %	3.7	3.5	3.7	0.7
CMV infection, %				
Recipient CMV infection (R+)	26	28	25	
Donor CMV infection (D+)	20	20	20	0.5
Recipient and Donor CMV infection (R+D+)	32	33	32	
Calcineurin inhibitors, %				
TAC	67	76	63	
CSA	26	16	30	**0.001**
None	7	8	7	
Incidence of NODAT, %	27	-	-	

CMV: Citomegalovirus, TAC: Tacrolimus, CSA: Cyclosporine, NODAT: New Onset Diabetes After Transplant.

* Patients considered had neither diabetes nor hypercholesterolemia before trasplantation. Patients were treated with statins.

comparison between NODAT and non-NODAT

Table 2. Replication sample characteristics

Characteristic	Replication Sample n=156	NODAT n= 45	non-NODAT n= 111	p-value #
Recipient age at transplantation, median (range)	48 (22-68)	53 (28-68)	46 (22-68)	**0.002**
Recipient males (%)	61	73	56	**0.048**
Period of follow up (months), median (range)	60 (1-60)	-	-	
Living donor (%)	12	11	12	0.9
Donor age (years), median (range)	44 (10-73)	48 (13-69)	41 (10-73)	**0.04**
Transplanted organ, %				
Kidney	65	27	74	
Liver	24	30	70	0.46
Lung	11	41	59	
Before transplant				
BMI, median (range)	23.4 (15.8-37.3)	26.4 (18.8-37.3)	22.3 (15.8-36.2)	**0.0003**
Overweight (25 kg/m^2 ≤ BMI >30 kg/m^2), %	24.1	37.8	17.4	**<0.001**
Obese (BMI ≥ 30 kg/m^2), %	10.9	20	6.5	
At 12 months after transplant				
BMI, median (range)	25.2 (16.5-39.3)	26.8 (20.3-39.3)	24.7 (16.5-37.5)	**0.003**
Overweight (25 kg/m^2 ≤ BMI >30 kg/m^2), %	35.1	37.8	33.9	**0.012**
Obese (BMI ≥ 30 kg/m^2), %	18.5	31.1	13.2	
Hypolipemiant treatment*, %	28.8	39.4	24.7	0.08
Percentage of 10% weight gain, %	35	32	37	0.6
Weight gain (mean), %	6.3	3.2	6.6	0.3
CMV infection, %				
Recipient CMV infection (R+)	49.3	42.2	52.5	0.2
Donor CMV infection (D+)	61.5	66.7	59.2	0.4
Recipient and Donor CMV infection (R+D+)	27.6	28.9	27	0.8
Calcineurin inhibitors, %				
TAC	35	56	26	**<0.001**
CSA	65	44	74	
Incidence of NODAT, %	29	-	-	

CMV: Citomegalovirus, TAC: Tacrolimus, CSA: Cyclosporine, NODAT: New Onset Diabetes After Transplant.

comparison between NODAT and non-NODAT

* Patients considered had neither diabetes nor hypercholesterolemia before trasplantation. Patients were treated with statins.

Table 3. Odds Ratio of NODAT development in the main sample for a model integrating clinical and genetic factors

	Non-genetic model			w-GRS 1			w-GRS 2			w-GRS 3			w-GRS All SNPs		
Covariate	**OR**	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
Clinical															
Age	1.01	1.00-1.03	0.08	1.01	1.00-1.03	0.09	1.01	1.00-1.03	0.08	1.01	1.00-1.03	0.07	1.01	1.00-1.03	0.07
Women	0.77	0.52-1.14	0.2	0.78	0.52-1.15	0.22	0.84	0.56-1.25	0.4	0.78	0.52-1.15	0.21	0.83	0.55-1.23	0.36
Kidney transplanted	0.67	0.44-1.04	0.07	0.67	0.43-1.03	0.07	0.65	0.42-1.01	0.06	0.67	0.43-1.03	0.07	0.63	0.40-0.98	**0.04**
Baseline BMI	1.05	1.01-1.10	**0.01**	1.06	1.01-1.10	**0.01**	1.07	1.02-1.11	**0.004**	1.06	1.01-1.10	**0.01**	1.06	1.02-1.11	**0.008**
Living donor	0.76	0.49-1.17	0.22	0.78	0.49-1.20	0.26	0.85	0.54-1.32	0.46	0.78	0.50-1.20	0.27	0.85	0.54-1.34	0.49
Induction treatment	0.62	0.41-0.94	**0.02**	0.62	0.41-0.95	**0.02**	0.62	0.40-0.95	**0.03**	0.6	0.39-0.92	**0.02**	0.62	0.40-0.96	**0.03**
Treatment															
CSA	0.65	0.31-1.41	0.26	0.59	0.28-1.29	0.17	0.66	0.31-1.49	0.3	0.69	0.32-1.51	0.33	0.68	0.31-1.53	0.33
TAC	1.72	0.87-3.59	0.13	1.62	0.81-3.39	0.18	1.65	0.81-3.54	0.18	1.71	0.86-3.61	0.14	1.73	0.85-3.73	0.14
Antihepatitis C	1.22	0.63-2.31	0.54	1.23	0.63-2.33	0.53	1.28	0.65-2.45	0.46	1.18	0.60-2.23	0.62	1.27	0.65-2.43	0.48
Genetic															
w-GRS				1.01	0.94-1.10	0.67	1.09	1.04-1.15	**0.001**	1.14	1.01-1.29	**0.03**	1.08	1.03-1.12	**0.0005**

OR: Odds Ratio. CI: Confidence Interval. w-GRS: Weighted Genetic Risk Score. CSA: Cyclosporine, TAC: Tacrolimus
w-GRS 1: *Voight et al., Nat Genet. 2010;42(7):579-89* ; w-GRS 2 : *Mahajan et al., Nat Genet. 2014;46(3):234-44* ; w-GRS 3 : *McCaughan et al., J Am Soc Nephrol. 2014;25(5):1037-49*

Table 4. Odds Ratio of NODAT development in the replication sample for a model integrating clinical and genetic factors

Covariate	Non-genetic model			w-GRS 1			w-GRS 2			w-GRS 3			w-GRS All SNPs[#]		
	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value
Clinical															
Age	1.04	1.00-1.10	**0.04**	1.04	1.00-1.09	**0.04**	1.04	0.99-1.09	0.08				1.04	0.99-1.09	0.07
Women	0.43	0.17-1.05	0.07	0.43	0.17-1.05	0.07	0.38	0.14-1.00	0.06				0.39	0.14-1.02	0.06
Baseline BMI	1.13	1.03-1.25	**0.02**	1.13	1.03-1.25	**0.02**	1.17	1.05-1.31	**0.004**				1.18	1.06-1.32	**0.003**
TAC (vs CSA)	3.13	1.31-7.78	**0.01**	3.2	1.32-8.04	**0.01**	2.89	1.14-7.6	**0.03**				2.97	1.17-7.8	0.02
Kidney transplanted	0.84	0.33-2.22	0.73	0.85	0.33-2.25	0.75	0.71	0.25-1.99	0.51				0.73	0.26-2.04	0.54
Genetic															
w-GRS				0.97	0.81-1.15	0.73	1.06	0.94-1.21	0.32				1.02	0.93-1.14	0.58

OR: Odds Ratio, CI: Confidence Interval, w-GRS: Weighted Genetic Risk Score. CSA: Cyclosporine, TAC: Tacrolimus

[#]includes w-GRS 1 and w-GRS 2

w-GRS 1: *Voight et al., Nat Genet. 2010;42(7):579-89* ; w-GRS 2 : *Mahajan et al., Nat Genet. 2014;46(3):234-44* ; w-GRS 3 : *McCaughan et al., J Am Soc Nephrol. 2014;25(5):1037-49*

Table 5. Discrimination parameters of clinical and genetic (i.e. w-GRS 2, w-GRS 3) models for NODAT prediction at one year in the main sample

NODAT: 140 non-NODAT: 498	**AUROC**	**Specificity**	**Sensitivity**	**Accuracy**	**LRT-p**	**IDI [95% CI]**		**NRI (continuous) [95% CI]**		**NNG**
Model							p-value	Net correctly reclassified	p-value	
Clinical model	0.65	0.74	0.53	0.69						
*Clinical and genetic*risk score model*	0.69	0.60	0.74	0.63	**0.001**	0.01 [0.004 - 0.03]	**0.008**	0.38 [0.20 - 0.57]	**0.00004**	n.c
Model										
Clinical model	0.66	0.73	0.55	0.69						
*Clinical and genetic** risk score model*	0.67	0.64	0.66	0.64	0.10	0.004 [-0.001 - 0.009]	0.18	0.13 [-0.06 - 0.31]	0.17	n.c
Model										
Clinical model	0.66	0.64	0.63	0.64						
*Clinical and genetic*** risk score model*	0.69	0.71	0.58	0.68	**0.0004**	0.02 [0.006 - 0.03]	**0.004**	0.24 [0.05 - 0.43]	**0.01**	25

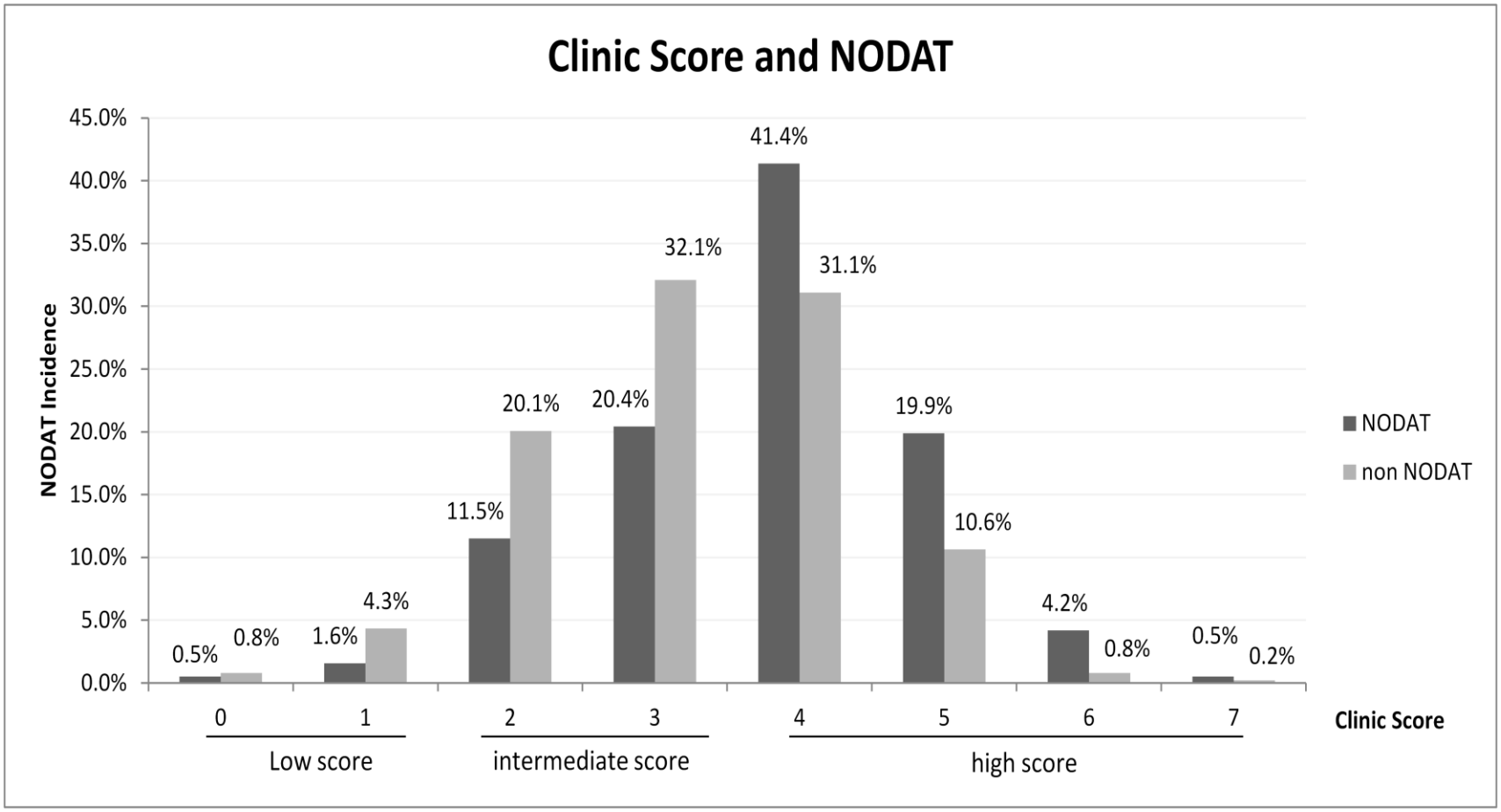
w-GRS: weighted Genetic Risk Score, CI: Confidence Interval, AUROC: Area Under the Receiver Operating Characteristics curve, LRT: Likelihood Ratio Test, IDI: Integrated Discrimination Improvement, NRI: Net Reclassification Improvement, NNG: Number Needed to Genotype

*integrates w-GRS 2 *(Mahajan et al., Nat Genet. 2014;46(3):234-44)*

** integrates w-GRS 3 *(McCaughan et al., J Am Soc Nephrol. 2014;25(5):1037-49)*

***integrates w-GRS 2 + 3

Figure 1. Association of the clinic score with NODAT in the main sample



5. Project V

Association of socioeconomic factors with metabolic parameters in psychiatric patients under psychotropic treatment

Background: Weight gain and metabolic abnormalities are a well known problem in the psychiatric population. This effect has been attributed to the psychotropic treatment, to the illness itself, but also to lifestyle. It is therefore important to explore also social factors in metabolic studies with psychiatric patients. In the general population, socioeconomic status (SES) has been described as well as a moderator of obesity, with clear patterns in women but contradictory results in men. As a way of integrating socioeconomic factors, the Swiss Socioeconomic Position (SEP) index was developed by a group of researchers in the Institute of Social and Preventive Medicine of the University of Bern integrating the socioeconomic position by neighborhood based on income, education, occupation and housing conditions. To our knowledge, no longitudinal studies have yet evaluated the impact of SES on metabolic parameters in psychiatric population under psychotropic treatment.

Aim and Methods: In the present study we wanted to determine how SES, as well as the Swiss SEP index are associated with metabolic outcomes in psychiatric patients. 491 individuals from 18 to 65 years old who gave their informed consent and started or switched to a psychotropic treatment (i.e. aripiprazole, amisulpride, clozapine, olanzapine, quetiapine, risperidone, lithium and/or valproate) were followed up for 12 months. Clinical (i.e. age, sex, treatment, treatment duration) and socioeconomic covariates (i.e. occupational status and living alone or not) were obtained from medical or administrative files. Metabolic-related outcomes (i.e. BMI, weight, height, waist circumference (WC), lipid profile) were obtained for each individual during the routine medical check-ups. From personal postal address, the address was geocoded and a SEP index was assigned to each individual.

Results: Preliminary analysis showed an influence of occupational status, with disability pension and living alone being associated with stronger weight gain. Multivariate analysis integrating clinical (i.e. age, sex, treatment, time of treatment) and socioeconomic covariates (i.e. occupational status, living alone, SEP index) showed that socioeconomic covariates were associated with weight gain, BMI and/or WC over time. Patients who were employed had lower BMI (-1.72 [-2.60 - (-)0.91]kg/m^2) and WC (-4.56 [-6.88 - (-)2.20]cm) when compared to individuals receiving disability pension (p-value <0.001). In turn, weight gain was higher in those employed individuals (1.18%) compared to individuals receiving disability pension. Employed individuals had lower baseline BMI (22.2 kg/m^2) compared to the disability pension group (24.5 kg/m^2); a known risk factor for strong drug induced weight gain during psychotropic treatment. On the same line, the SEP index was inversely correlated with BMI and WC (a 10% increase in the SEP index corresponded to 0.4 kg/m^2 and 1 cm decrease, respectively). SES is a critical factor in psychiatric populations at high risk of weight gain and should be considered when planning interventions to moderate metabolic effects in such populations.

Manuscript in preparation

6. Project VI

Risk factors for increasing weight and BMI after solid organ transplantation – a secondary data analysis of the Swiss Transplant Cohort Study

This is an ongoing collaboration with the Institute of Nursing Science, University of Basel, Switzerland (*S. Beckmann, SM. De Geest*) which integrates the Swiss Transplant Cohort Study Psychosocial Interest Group.

The aim of the present work is to describe the evolution of weight and BMI and to determine the biomedical (i.e. treatment), biological (i.e. age, gender, pre-transplantation BMI), genetic (i.e. genetic risk scores), behavioral (i.e. smoking status), socioeconomic (i.e. level of education, marital and working status), and psychological (i.e. mental health quality of life) risk factors for post-transplanted weight and BMI gain in liver, kidney, heart and lung transplant recipients. The contribution in the present work will focus on providing genetic risk scores (GRS), interpreting and discussing the obtained results concerning GRS.

Ongoing collaboration

DISCUSSION AND FUTURE PERSPECTIVES

In the present thesis work, we wanted to study the factors influencing weight gain and other metabolic side-effects in high risk treated psychiatric and transplanted populations. The first part of the work (**Projects I-IV**) focused on genetic determinants and the second part (**Project V**) explored the SES factors. The main results of this work can be summarized as follows: We showed the effect of a *PCK1* gene polymorphism on BMI, waist circumference and other metabolic-related outcomes (**Project I**). The *PCK1* is a downstream gene of the *CRTC1*; a gene previously studied in our laboratory and which was associated with BMI in psychiatric populations. A pathway analysis combining *CRTC1* and *PCK1* variants confirmed that carriers of protective alleles had lower BMI over time compared to non-carriers. **Projects II, III and IV**, show how several polymorphisms combined into genetic risk scores were associated with BMI and NODAT in psychiatric and transplanted cohorts. These polymorphisms were initially obtained from GWAS and CG studies conducted in large general and diabetic populations. In addition, in transplanted populations, genetic factors improved the ability to predict 10% of weight gain and NODAT development one year after transplantation. Finally, in the **Project V** we showed, over time, how SES factors (i.e. living alone, occupational status and SEP index) are related to weight change, BMI and other obesity-related outcomes in a psychiatric sample under psychotropic treatment. An ongoing study (**Project VI**) will also study how SES, clinical, psychosocial as well as genetic factors influence the evolution of BMI and weight in a SOT cohort.

1. Environmental aspects

1.1 The psychiatric populations

The psychiatric populations studied here showed, as described previously, (120, 129) the susceptibility of metabolic-related side effects compared to the general population. In general, in the present work, the studied metabolic traits had weaker or no significant effects in general population when compared to psychiatric samples (i.e. see **Project I**), suggesting that psychiatric populations are at high risk of obesity and/or metabolic side-effects. Among the three psychiatric groups significant differences were observed concerning obesity prevalence (detailed results presented in the descriptive tables of the manuscripts; see **Project I** and

II). Overall, the Geneva outpatient setting had the highest prevalence of obesity probably due to specific psychotropic drug effect, since they had the highest prescription of olanzapine and clozapine, both molecules being stronger inducers of weight gain among psychotropic drugs (50). Different effect sizes, detected in the *Suivi Metabolique* versus the outpatient setting cohorts (i.e. Geneva and *Ambulatoire*), could be explained by a lower prevalence of obesity at baseline and a shorter treatment duration in the *Suivi Metabolique*, because both baseline BMI and treatment duration are moderators of weight gain (130). However, to exclude a winner's curse event, these results need to be replicated in other short treatment duration samples. Interestingly, in the *Suivi Metabolique*, some socio-economic factors were also related to metabolic outcomes. More specifically, individuals with disability pension (occupational status) had higher BMI than those who work or those unemployed. Similar results were found in a cross-sectional study conducted in schizophrenia patients where individuals in a sheltered employment had higher prevalence of obesity than patients with no earned income (131). Altogether, these results show higher BMI in individuals receiving social assistance. On the other hand, weight gain was lower among individuals under psychotropic treatment with disability pension. This could be explained by the previously reported stronger weight gain in patients with low baseline BMI (130). In addition, living alone was positively associated with weight gain. This finding is in line with a previous study in general population showing that living alone predicts weight gain among women (132). Finally, when analyzing the SEP index (which integrates the socioeconomic position by neighborhood based on income, education, occupation and housing conditions) an inverse correlation was found with BMI during treatment. These results are in agreement with a previous study which found positive associations between a low SEP index and some causes of death (133).

1.2 The transplanted populations

Two transplanted populations are described in the present thesis work and although the prevalence of NODAT one year after transplantation is similar between both samples, some differences have been observed. The immunosuppressant cohort had higher percentage of weight gain recipients which could be explained by the fact that individuals were younger and had higher percentage of deceased donors when

compared to STCS. Also, high percentage of cyclosporine prescription was found in the Immunosuppressant cohort. As described in the **Introduction** chapter, young age, deceased donors and cyclosporine treatment have a negative impact on BMI, increasing the risk of gaining weight.

Concerning the organ, kidney was the first transplanted organ in both samples followed by liver and lung. Although associated with a deleterious effect on graft survival and graft function, weight gain after transplantation has also been described as a positive event. This is the case of lung transplanted recipients where recipients gaining more weight in the first year (11%-84% proportion of baseline weight) had a better survival compared to those with less weight gain (-32%-10%) (134). Due to our sample sizes, we could only conduct stratified analysis by kidney / non kidney recipients.

Altogether these findings show that environmental factors play a role on metabolic outcomes and factors such as treatment, treatment duration, transplanted organ and/or socioeconomic determinants should be considered in future studies.

2. Genetic aspects

Concerning the genetic factors, several approaches have been used in the present work: Firstly, a candidate gene approach (monogenic analysis) and secondly, a genetic risk score approach (polygenic analysis). Common obesity and diabetes are polygenic diseases. Thus, a combination of several genes which can better represent the genetic profile is warranted. However, a monogenic and candidate gene analysis is also of interest if the aim of the study is to focus precisely on that gene or pathway and its association with the outcome. The motivation of the monogenic analysis (in **Project I**) was the fact that *PCK1* is a downstream gene of *CRTC1*; a gene playing a role on weight regulation in mice (135) and a gene associated with BMI in psychiatric populations (120). In addition, PCK1 is a key enzyme of the gluconeogenesis regulation. In this manuscript, we integrated both *CRTC1* and *PCK1* protective alleles finding lower effects on the BMI compared to the *PCK1* protective alleles alone.

The **Projects II, III and IV**, focused on BMI-related and diabetes-related polymorphisms/genes integrated in w-GRS. Most of the genes were obtained from GWAS studies (31, 34, 110, 136, 137), but also from CG approaches (i.e. antipsychotic-induced weight gain-related polymorphisms (66)) and from gene expression analysis (108). One of the major issues of GWAS is to elucidate the biological pathways behind the variants associated with the phenotype, since they are hypothesis free. Regarding BMI, when exploring further the role of the genes in or nearby the region of polymorphisms, the implicated genes are related to glutamate signaling (*CADM2, GRID1, NEGR1*) (138, 139) which integrates pathways responding to changes in feeding and fasting (140), or genes causing monogenic obesity syndromes (*MC4R, POMC*) (141) or eventually genes related to insulin secretion, energy metabolism and/or adipogenesis (*TCF7L2, GIPR, IRS1*) (31). Finally, these genes are mostly expressed in the hypothalamus; one of the key-regulation sites of body mass (31). Genes whose expression was associated with weight change in kidney transplant recipients (108) were related to diabetes, obesity and neurological concepts such as dopamine, nicotine, and cognition (108).

Concerning polymorphisms related to diabetes, most of them have been associated with indices of beta-cell function (i.e. *TCF7L2, KCNQ1*) and insulin sensitivity (i.e. *KLF14*). *FTO* has been also associated with reduced insulin sensitivity; an action that would be driven by obesity (136). Regarding the GWAS NODAT-related variants presented in **Project IV**, they would be mainly related to beta-cell dysfunction and more specifically to beta-cell apoptosis. Insulin resistance may contribute to NODAT but this has not been conclusively proven (142).

All these results taken together confirm that genetic factors contribute significantly to the complex nature of the studied metabolic outcomes. The fact that the BMI explained variability remains low suggests the contribution of other factors potentially influencing the BMI (discussed further in **limitations and strengths**, **Future perspectives**). Finally, the w-GRS which was significantly associated with BMI in the psychiatric samples was also significantly associated with BMI in the transplanted population, validating this score in other specific populations.

3. Strengths and Limitations

All the research conducted in the present work is based on observational studies, resembling more real life situations. Also, many factors (genetic and environmental) influenced the studied outcomes but we could not control all of them. Thus, measuring exact food and nutrient intake and individuals' physical activity in order to assess energy expenditure is a difficult issue in an observational setting.

The follow-up design increases power and allows establishing causality. More longitudinal studies are needed; although they are expensive and there is a high risk of drop out.

Working with gene candidates implies several issues which must be considered. Although hypothesis-driven, studying only one gene at a time may lead to non-replication of the results since this may not be representative enough of the variability and of the effect of a complex phenotype. GWAS, on the contrary, uses a hypothesis-free approach and allows for identification of several novel variants. However, results interpretation of GWAS can be a complicated issue, since no a priori biological approach is considered. In the present work, the best replicated GRS was obtained from a GWAS (i.e. GRS in **Project II, Project III**) with the BMI association results replicated in both psychiatric and transplanted populations.

Since allele frequencies can greatly vary among ethnicities, the genetic results in the present work are only valid for Caucasian subjects. Nevertheless, it is worth mentioning that the results obtained in the present work with T2DM Caucasians came from variants tested in a large trans-ethnic meta-analysis (137).

Replication of the results remains one of the main issues in pharmacogenetic research contributing to the challenging translation into clinical practice. GRS have the advantage of including a wider spectrum of genetic variants, with results remaining valid for various populations. This is the case of the 32 BMI-related polymorphisms which have been replicated in several populations including different ethnicities (143-145) in addition to the specific transplanted and psychiatric populations presented here.

4. Future perspectives

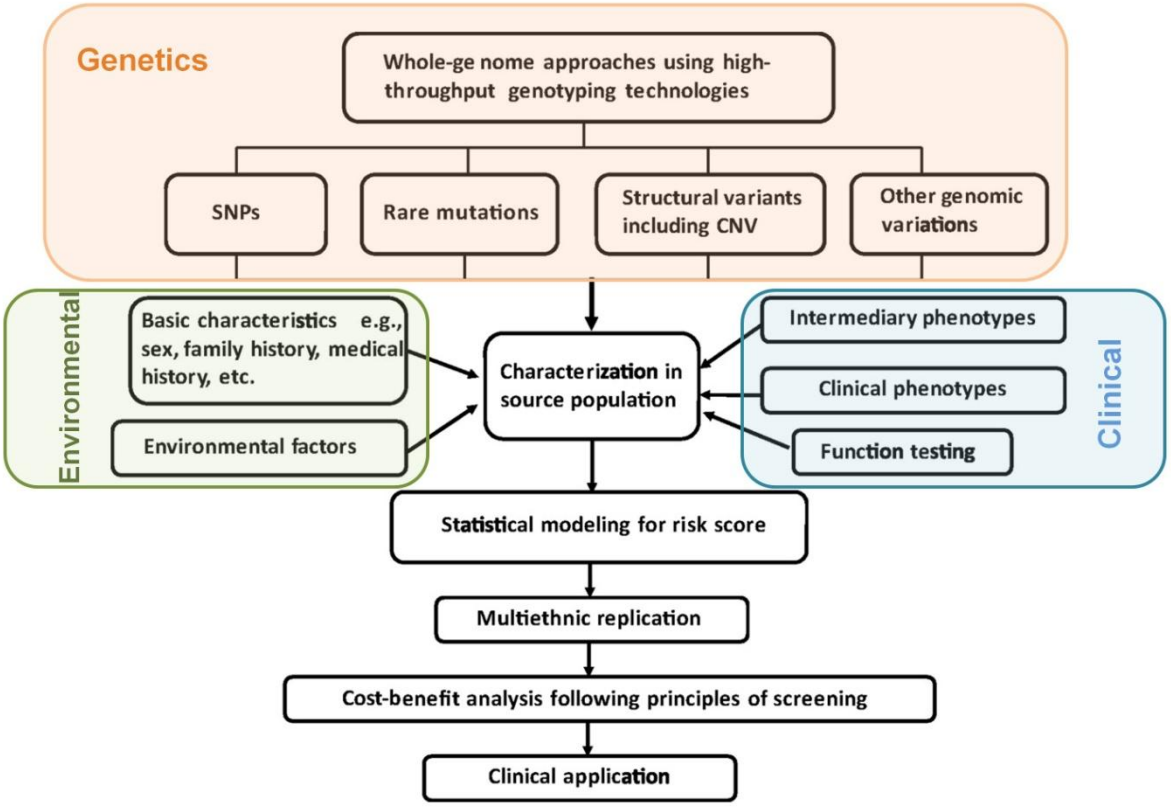
Several attempts to further explore the biological impact of different genes have been conducted in our laboratory, for instance the influence of polymorphisms on gene expression in human peripheral blood mononuclear cells or in fibroblasts. However, this is laborious work and only a limited number of samples could be analyzed. In addition, some positive results could not be replicated (data not shown). The non-replication or the negative results could be attributed to small sample size or the non-adequacy of tissue tested. Nowadays, powerful platforms concerning genotype specific-tissue expression, such as the GTEx project, are easily accessible and can save a lot of laborious work, while offering, at the same time, results on a large number of samples (146). GTEx project aims to collect and analyze multiple human tissues from general population donors who are extensively genotyped. This helps to identify genes whose expression is affected by genetic variation and/or to provide a better understanding of the gene regulation. Recently, other projects such as the Encyclopedia of DNA Elements (ENCODE) identify all functional regions in the human genome. The data generated from this project is accessible through public databases (147, 148). It is described that 90% of the SNPs linked to diseases and detected through GWAS are found outside of protein-coding region (i.e. non-coding DNA) (148). Nowadays, it is well-known that much of this functional non-coding DNA participates in the regulation of the expression of coding genes (149). Thus, with the availability of such databases, molecular genetic studies will better characterize the role of enhancers, amplifiers, regulators.

The significant results concerning GRS methodology referred to well established polymorphisms found in large meta-analysis of GWAS. From a biological point of view, many of these genes functions are unknown. The analysis of a whole metabolic pathway (integrating the genes in a polygenic score) and its association with metabolic parameters would be another way of exploring genetic influences on specific phenotypes. A study based on genetic architecture of circulating lipid levels showed that pathway analysis based on the best predictive polygenic score was more informative than the pathway analysis based on the genome-wide significant findings (150). Interestingly, another study about folate levels and negative symptoms severity in schizophrenia based part of the analysis on genetic risk scores constructed with polymorphisms implicated in the folate metabolic pathway (151). In addition, a GRS association analysis of gene variants implicated in

oxidative stress and inflammation pathways (potentially linked to BMI, obesity and psychiatric illness, see **Introduction**) needs to be further explored. From a methodological point of view, the fact of including all polymorphisms of the pathway in a polygenic score will enrich the GRS for positive associations; however, the GRS will certainly also include false-positive associations. Therefore, a pre-selection of the SNPs to include in the final GRS needs to be done in an independent discovery sample; large enough to have power to detect such association.

Finally, many questions must be answered before genetic information can be appropriately translated into clinical practice for preventing complex metabolic disorders. From the genetic point of view, only little BMI variability is explained by genetic polymorphisms. This missing variability could be potentially explained by other variants than SNPs such as CNV or rare alleles, which are typically not included in the gene-candidate associations or GWAS. Data obtained by next generation sequencing techniques would allow taking into account other variants. Epigenetic can also contribute to explaining the genetic variance. In addition, gene-gene (or SNP-SNP) and gene-environment interaction effects represent potential sources of variance that need to be explored. To date, several gene polymorphisms have been associated with the variation on how people respond to environment (i.e. physical activity, hunger and intake of high-calorie meals) (152), emphasizing the fact that genetic factors should be considered together with environmental and clinical factors in order to better characterize the target population. Finally, before implementing genetic tests into clinical practice, a cost-benefit analysis is warranted in order to provide recommendations for policy and decision-making strategies. Figure 5, shows an integrative view of the key elements contributing towards personalized treatment.

Figure 5: An integrative view of personalized treatment (adapted from: (153))



5. Final conclusions

Many aspects of genetics of obesity and of other metabolic outcomes still remain uncovered. Presently, genetic knowledge cannot yet be translated from bench to bedside given that more understanding is needed on their implication in energy balance regulation.

In order to curb the obesity epidemic, a population-based multisectorial (i.e. by improving health policies, health services and preventive actions such as dietary advice) multi-disciplinary (e.g. from physicians, nurses, dieticians to biologists, bioinformatics or pharmacologists) and also culturally (e.g. developed versus developing world, westernized versus oriental cultures) approach is warranted. The present work suggests some clues for next steps towards personalized medicine in high risk populations.

REFERENCES

References

1. WHO, obesity and overweight (Fact sheet N° 311): World Health Organization; 2015 [updated January 2015; cited 2015 July 2015]. World Health Organization]. Available from: http://www.who.int/mediacentre/factsheets/fs311/en/.

2. Berrington de Gonzalez A, Hartge P, Cerhan JR, Flint AJ, Hannan L, MacInnis RJ, et al. Body-mass index and mortality among 1.46 million white adults. The New England journal of medicine. 2010;363(23):2211-9.

3. Pi-Sunyer X. The medical risks of obesity. Postgraduate medicine. 2009;121(6):21-33.

4. Overseas Development Institute (ODI). Overweight and obese adults reaching almost a billion in developing countries, as numbers continue to grow in richer nations 2014 [22.02.2016]. Available from: http://www.odi.org/news/703-overweight-obese-adults-reaching-almost-billion-developing-countries-as-numbers-continue-grow-richer-nations.

5. Centers for Disease Control and Prevention. Adult Obesity Causes & Consequences 2015 [01.02.2016]. Available from: http://www.cdc.gov/obesity/adult/causes.html.

6. Specchia ML, Veneziano MA, Cadeddu C, Ferriero AM, Mancuso A, Ianuale C, et al. Economic impact of adult obesity on health systems: a systematic review. European journal of public health. 2015;25(2):255-62.

7. Neel JV. The "thrifty genotype" in 1998. Nutrition reviews. 1999;57(5 Pt 2):S2-9.

8. Elmquist JK, Scherer PE. The cover. Neuroendocrine and endocrine pathways of obesity. Jama. 2012;308(11):1070-1.

9. Emond M, Schwartz GJ, Ladenheim EE, Moran TH. Central leptin modulates behavioral and neural responsivity to CCK. The American journal of physiology. 1999;276(5 Pt 2):R1545-9.

10. de Kloet AD, Woods SC. Molecular neuroendocrine targets for obesity therapy. Current opinion in endocrinology, diabetes, and obesity. 2010;17(5):441-5.

11. Fan W, Boston BA, Kesterson RA, Hruby VJ, Cone RD. Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. Nature. 1997;385(6612):165-8.

12. Spiegelman BM, Flier JS. Obesity and the regulation of energy balance. Cell. 2001;104(4):531-43.

13. Mutch DM, Clement K. Unraveling the genetics of human obesity. PLoS genetics. 2006;2(12):e188.

14. Kopelman PG. Obesity as a medical problem. Nature. 2000;404(6778):635-43.

15. Stevens J, Katz EG, Huxley RR. Associations between gender, age and waist circumference. European journal of clinical nutrition. 2010;64(1):6-15.

16. Sheehan TJ, DuBrava S, DeChello LM, Fang Z. Rates of weight change for black and white Americans over a twenty year period. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity. 2003;27(4):498-504.

17. Ravussin E. Metabolic differences and the development of obesity. Metabolism: clinical and experimental. 1995;44(9 Suppl 3):12-4.

18. Beydoun MA, Wang Y. Pathways linking socioeconomic status to obesity through depression and lifestyle factors among young US adults. Journal of affective disorders. 2010;123(1-3):52-63.

19. National Obesity Observatory. Obesity and Mental Health 2011 [20.03.2016]. Available from: http://www.noo.org.uk/uploads/doc/vid_10266_Obesity%20and%20mental%20health_FINAL_070311_MG.pdf.

20. Fruhstorfer BH, Mousoulis C, Uthman OA, Robertson W. Socio-economic status and overweight or obesity among school-age children in sub-Saharan Africa - a systematic review. Clinical obesity. 2016;6(1):19-32.

21. van Vliet JS, Gustafsson PA, Duchen K, Nelson N. Social inequality and age-specific gender differences in overweight and perception of overweight among Swedish children and adolescents: a cross-sectional study. BMC public health. 2015;15:628.

22. Wolf KJ, Lorenz RG. Gut Microbiota and Obesity. Current obesity reports. 2012;1(1):1-8.

23. Shen J, Obin MS, Zhao L. The gut microbiota, obesity and insulin resistance. Molecular aspects of medicine. 2013;34(1):39-58.

24. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature. 2006;444(7122):1022-3.

25. Jumpertz R, Le DS, Turnbaugh PJ, Trinidad C, Bogardus C, Gordon JI, et al. Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans. The American journal of clinical nutrition. 2011;94(1):58-65.

26. Tilg H, Kaser A. Gut microbiome, obesity, and metabolic dysfunction. The Journal of clinical investigation. 2011;121(6):2126-32.

27. Farooqi S, O'Rahilly S. Genetics of obesity in humans. Endocrine reviews. 2006;27(7):710-18.

28. Bouchard C, Demirjian A, Mongeau E. Sibling correlations and genetic estimates for selected blood variables in French Canadian children. Human genetics. 1980;54(2):259-63.

29. Stunkard AJ, Sorensen TI, Hanis C, Teasdale TW, Chakraborty R, Schull WJ, et al. An adoption study of human obesity. The New England journal of medicine. 1986;314(4):193-8.

30. Loktionov A. Common gene polymorphisms and nutrition: emerging links with pathogenesis of multifactorial chronic diseases (review). The Journal of nutritional biochemistry. 2003;14(8):426-51.

31. Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, et al. Genetic studies of body mass index yield new insights for obesity biology. Nature. 2015;518(7538):197-206.

32. Loos RJ, Lindgren CM, Li S, Wheeler E, Zhao JH, Prokopenko I, et al. Common variants near MC4R are associated with fat mass, weight and risk of obesity. Nat Genet. 2008;40(6):768-75.

33. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science. 2007;316(5826):889-94.

34. Speliotes EK, Willer CJ, Berndt SI, Monda KL, Thorleifsson G, Jackson AU, et al. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. Nat Genet. 2010;42(11):937-48.

35. Wu L, Xi B, Zhang M, Shen Y, Zhao X, Cheng H, et al. Associations of six single nucleotide polymorphisms in obesity-related genes with BMI and risk of obesity in Chinese children. Diabetes. 2010;59(12):3085-9.

36. Waalen J. The genetics of human obesity. Translational research : the journal of laboratory and clinical medicine. 2014;164(4):293-301.

37. Andreasen CH, Stender-Petersen KL, Mogensen MS, Torekov SS, Wegner L, Andersen G, et al. Low physical activity accentuates the effect of the FTO rs9939609 polymorphism on body fat accumulation. Diabetes. 2008;57(1):95-101.

38. Mammes O, Aubert R, Betoulle D, Pean F, Herbeth B, Visvikis S, et al. LEPR gene polymorphisms: associations with overweight, fat mass and response to diet in women. European journal of clinical investigation. 2001;31(5):398-404.

39. Santoro N, Perrone L, Cirillo G, Raimondo P, Amato A, Brienza C, et al. Effect of the melanocortin-3 receptor C17A and G241A variants on weight loss in childhood obesity. The American journal of clinical nutrition. 2007;85(4):950-3.

40. Hunter DJ. Gene-environment interactions in human diseases. Nature reviews Genetics. 2005;6(4):287-98.

41. De Hert M, Dekker JM, Wood D, Kahl KG, Holt RI, Moller HJ. Cardiovascular disease and diabetes in people with severe mental illness position statement from the European Psychiatric Association (EPA), supported by the European Association for the Study of Diabetes (EASD) and the European Society of Cardiology (ESC). European psychiatry : the journal of the Association of European Psychiatrists. 2009;24(6):412-24.

42. Laursen TM, Munk-Olsen T, Vestergaard M. Life expectancy and cardiovascular mortality in persons with schizophrenia. Current opinion in psychiatry. 2012;25(2):83-8.

43. M DEH, Schreurs V, Vancampfort D, R VANW. Metabolic syndrome in people with schizophrenia: a review. World psychiatry : official journal of the World Psychiatric Association. 2009;8(1):15-22.

44. Stahl SM. Stahl's Essential Psychopharmacology - Neuroscientific Basis and Practical Applications. Third edition. New York: Cambridge University Press; 2008.

45. van Os J, Kapur S. Schizophrenia. Lancet. 2009;374(9690):635-45.

46. Farde L, Nordstrom AL, Wiesel FA, Pauli S, Halldin C, Sedvall G. Positron emission tomographic analysis of central D1 and D2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. Relation to extrapyramidal side effects. Archives of general psychiatry. 1992;49(7):538-44.

47. Nasrallah HA. Atypical antipsychotic-induced metabolic side effects: insights from receptor-binding profiles. Molecular psychiatry. 2008;13(1):27-35.

48. Balt SL, Galloway GP, Baggott MJ, Schwartz Z, Mendelson J. Mechanisms and genetics of antipsychotic-associated weight gain. Clinical pharmacology and therapeutics. 2011;90(1):179-83.

49. American Diabetes Association, American Psychiatric Association, American Association of Clinical Endocrinologists, North American Association for the Study of Obesity. Consensus development conference on antipsychotic drugs and obesity and diabetes. Diabetes care. 2004;27(2):596-601.

50. Leucht S, Cipriani A, Spineli L, Mavridis D, Orey D, Richter F, et al. Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: a multiple-treatments meta-analysis. Lancet. 2013;382(9896):951-62.

51. Vancampfort D, Vansteelandt K, Correll CU, Mitchell AJ, De Herdt A, Sienaert P, et al. Metabolic syndrome and metabolic abnormalities in bipolar disorder: a meta-analysis of prevalence rates and moderators. The American journal of psychiatry. 2013;170(3):265-74.

52. Verrotti A, D'Egidio C, Mohn A, Coppola G, Chiarelli F. Weight gain following treatment with valproic acid: pathogenetic mechanisms and clinical implications. Obesity reviews : an official journal of the International Association for the Study of Obesity. 2011;12(5):e32-43.

53. McKnight RF, Adida M, Budge K, Stockton S, Goodwin GM, Geddes JR. Lithium toxicity profile: a systematic review and meta-analysis. Lancet. 2012;379(9817):721-8.

54. Lopresti AL, Drummond PD. Obesity and psychiatric disorders: commonalities in dysregulated biological pathways and their implications for treatment. Prog Neuropsychopharmacol Biol Psychiatry. 2013;45:92-9.

55. Song X, Fan X, Song X, Zhang J, Zhang W, Li X, et al. Elevated levels of adiponectin and other cytokines in drug naive, first episode schizophrenia patients with normal weight. Schizophrenia research. 2013;150(1):269-73.

56. Myint AM, Kim YK. Network beyond IDO in psychiatric disorders: revisiting neurodegeneration hypothesis. Progress in neuro-psychopharmacology & biological psychiatry. 2014;48:304-13.

57. Cowen PJ. Serotonin and depression: pathophysiological mechanism or marketing myth? Trends Pharmacol Sci. 2008;29(9):433-6.

58. Millan MJ, Agid Y, Brune M, Bullmore ET, Carter CS, Clayton NS, et al. Cognitive dysfunction in psychiatric disorders: characteristics, causes and the quest for improved therapy. Nature reviews Drug discovery. 2012;11(2):141-68.

59. Carriere I, Fourrier-Reglat A, Dartigues JF, Rouaud O, Pasquier F, Ritchie K, et al. Drugs with anticholinergic properties, cognitive decline, and dementia in an elderly general population: the 3-city study. Archives of internal medicine. 2009;169(14):1317-24.

60. Kasper S, Resinger E. Cognitive effects and antipsychotic treatment. Psychoneuroendocrinology. 2003;28 Suppl 1:27-38.

61. McIntyre RS, Cha DS, Jerrell JM, Soczynska JK, Woldeyohannes HO, Taylor V, et al. Obesity and mental illness: implications for cognitive functioning. Advances in therapy. 2013;30(6):577-88.

62. Craft S, Watson GS. Insulin and neurodegenerative disease: shared and specific mechanisms. The Lancet Neurology. 2004;3(3):169-78.

63. Reynolds GP, Zhang ZJ, Zhang XB. Association of antipsychotic drug-induced weight gain with a 5-HT2C receptor gene polymorphism. Lancet. 2002;359(9323):2086-7.

64. Reynolds GP, Kirk SL. Metabolic side effects of antipsychotic drug treatment--pharmacological mechanisms. Pharmacology & therapeutics. 2010;125(1):169-79.

65. Yuan X, Yamada K, Ishiyama-Shigemoto S, Koyama W, Nonaka K. Identification of polymorphic loci in the promoter region of the serotonin 5-HT2C receptor gene and their association with obesity and type II diabetes. Diabetologia. 2000;43(3):373-6.

66. Lett TA, Wallace TJ, Chowdhury NI, Tiwari AK, Kennedy JL, Muller DJ. Pharmacogenetics of antipsychotic-induced weight gain: review and clinical implications. Molecular psychiatry. 2012;17(3):242-66.

67. Henderson DC, Vincenzi B, Andrea NV, Ulloa M, Copeland PM. Pathophysiological mechanisms of increased cardiometabolic risk in people with schizophrenia and other severe mental illnesses. The lancet Psychiatry. 2015;2(5):452-64.

68. Samaan Z, Anand SS, Zhang X, Desai D, Rivera M, Pare G, et al. The protective effect of the obesity-associated rs9939609 A variant in fat mass- and obesity-associated gene on depression. Mol Psychiatry. 2013;18(12):1281-6.

69. Quteineh L, Preisig M, Rivera M, Milaneschi Y, Castelao E, Gholam-Rezaee M, et al. Association of CRTC1 polymorphisms with obesity markers in subjects from the general population with lifetime depression. Submitted. 2015.

70. Suarez A, Lathi J, Kajantie E, Eriksson JG, Räikkönen K. Early life stress, FKBP5 polymorphisms and type 2 diabetes. World Congress of Psychiatric Genetics; Toronto, Canada: Insititute of Behavioural Sciences, University of Helsinki, Finland; 2015.

71. Davies G, Armstrong N, Bis JC, Bressler J, Chouraki V, Giddaluru S, et al. Genetic contributions to variation in general cognitive function: a meta-analysis of genome-wide association studies in the CHARGE consortium (N=53949). Molecular psychiatry. 2015;20(2):183-92.

72. Pham PT, Pham PM, Pham SV, Pham PA, Pham PC. New onset diabetes after transplantation (NODAT): an overview. Diabetes, metabolic syndrome and obesity : targets and therapy. 2011;4:175-86.

73. Davidson JA, Wilkinson A, International Expert Panel on New-Onset Diabetes after T. New-Onset Diabetes After Transplantation 2003 International Consensus Guidelines: an endocrinologist's view. Diabetes Care. 2004;27(3):805-12.

74. Baid S, Cosimi AB, Farrell ML, Schoenfeld DA, Feng S, Chung RT, et al. Posttransplant diabetes mellitus in liver transplant recipients: risk factors, temporal relationship with hepatitis C virus allograft hepatitis, and impact on mortality. Transplantation. 2001;72(6):1066-72.

75. Ye X, Kuo HT, Sampaio MS, Jiang Y, Bunnapradist S. Risk factors for development of new-onset diabetes mellitus after transplant in adult lung transplant recipients. Clinical transplantation. 2011;25(6):885-91.

76. Gomes MB, Cobas RA. Post-transplant diabetes mellitus. Diabetology & metabolic syndrome. 2009;1(1):14.

77. Hart A, Weir MR, Kasiske BL. Cardiovascular risk assessment in kidney transplantation. Kidney Int. 2015;87(3):527-34.

78. Kasiske BL, Snyder JJ, Gilbertson D, Matas AJ. Diabetes mellitus after kidney transplantation in the United States. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2003;3(2):178-85.

79. Hur KY, Kim MS, Kim YS, Kang ES, Nam JH, Kim SH, et al. Risk factors associated with the onset and progression of posttransplantation diabetes in renal allograft recipients. Diabetes care. 2007;30(3):609-15.

80. Pischon T, Sharma AM. Obesity as a risk factor in renal transplant patients. Nephrol Dial Transplant. 2001;16(1):14-7.

81. Halme L, Eklund B, Kyllonen L, Salmela K. Is obesity still a risk factor in renal transplantation? Transpl Int. 1997;10(4):284-8.

82. Pfeiffer T, Albrecht KH, Wagner K, Herget S, Eigler FW. [The effect of obesity on outcome of kidney transplantation]. Med Klin (Munich). 1994;89(12):635-9.

83. Richards J, Gunson B, Johnson J, Neuberger J. Weight gain and obesity after liver transplantation. Transplant international : official journal of the European Society for Organ Transplantation. 2005;18(4):461-6.

84. Everhart JE, Lombardero M, Lake JR, Wiesner RH, Zetterman RK, Hoofnagle JH. Weight change and obesity after liver transplantation: incidence and risk factors. Liver transplantation and surgery : official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society. 1998;4(4):285-96.

85. Krasnoff JB, Vintro AQ, Ascher NL, Bass NM, Paul SM, Dodd MJ, et al. A randomized trial of exercise and dietary counseling after liver transplantation. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2006;6(8):1896-905.

86. Rodrigo E, Fernandez-Fresnedo G, Valero R, Ruiz JC, Pinera C, Palomar R, et al. New-onset diabetes after kidney transplantation: risk factors. Journal of the American Society of Nephrology : JASN. 2006;17(12 Suppl 3):S291-5.

87. Martinez-Castelao A, Hernandez MD, Pascual J, Morales JM, Marcen R, Errasti P, et al. Detection and treatment of post kidney transplant hyperglycemia: a Spanish multicenter cross-sectional study. Transplantation proceedings. 2005;37(9):3813-6.

88. Palepu S, Prasad GV. New-onset diabetes mellitus after kidney transplantation: Current status and future directions. World journal of diabetes. 2015;6(3):445-55.

89. Luan FL, Langewisch E, Ojo A. Metabolic syndrome and new onset diabetes after transplantation in kidney transplant recipients. Clinical transplantation. 2010;24(6):778-83.

90. Marcen R. Immunosuppressive drugs in kidney transplantation: impact on patient survival, and incidence of cardiovascular disease, malignancy and infection. Drugs. 2009;69(16):2227-43.

91. Rafacho A, Ortsater H, Nadal A, Quesada I. Glucocorticoid treatment and endocrine pancreas function: implications for glucose homeostasis, insulin resistance and diabetes. The Journal of endocrinology. 2014;223(3):R49-62.

92. Leal-Cerro A, Soto A, Martinez MA, Dieguez C, Casanueva FF. Influence of cortisol status on leptin secretion. Pituitary. 2001;4(1-2):111-6.

93. Depczynski B, Daly B, Campbell LV, Chisholm DJ, Keogh A. Predicting the occurrence of diabetes mellitus in recipients of heart transplants. Diabetic medicine : a journal of the British Diabetic Association. 2000;17(1):15-9.

94. Abboudi H, Macphee IA. Individualized immunosuppression in transplant patients: potential role of pharmacogenetics. Pharmacogenomics and personalized medicine. 2012;5:63-72.

95. Razeghi E, Heydarian P, Amerian M, Pourmand G. The risk factors for diabetes mellitus after kidney transplantation. Saudi journal of kidney diseases and transplantation : an official publication of the Saudi Center for Organ Transplantation, Saudi Arabia. 2010;21(6):1038-43.

96. Schiel R, Heinrich S, Steiner T, Ott U, Stein G. Post-transplant diabetes mellitus: risk factors, frequency of transplant rejections, and long-term prognosis. Clinical and experimental nephrology. 2005;9(2):164-9.

97. Parikh CR, Klem P, Wong C, Yalavarthy R, Chan L. Obesity as an independent predictor of posttransplant diabetes mellitus. Transplantation proceedings. 2003;35(8):2922-6.

98. Spinelli GA, Felipe CR, Park SI, Mandia-Sampaio EL, Tedesco-Silva H, Jr., Medina-Pestana JO. Lipid profile changes during the first year after kidney transplantation: risk factors and influence of the immunosuppressive drug regimen. Transplantation proceedings. 2011;43(10):3730-7.

99. Robert N, Wong GW, Wright JM. Effect of cyclosporine on blood pressure. The Cochrane database of systematic reviews. 2010(1):CD007893.

100. Pirsch JD, Miller J, Deierhoi MH, Vincenti F, Filo RS. A comparison of tacrolimus (FK506) and cyclosporine for immunosuppression after cadaveric renal transplantation. FK506 Kidney Transplant Study Group. Transplantation. 1997;63(7):977-83.

101. Meier-Kriesche HU, Li S, Gruessner RW, Fung JJ, Bustami RT, Barr ML, et al. Immunosuppression: evolution in practice and trends, 1994-2004. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2006;6(5 Pt 2):1111-31.

102. Ponticelli C. The pros and the cons of mTOR inhibitors in kidney transplantation. Expert review of clinical immunology. 2014;10(2):295-305.

103. Johnston O, Rose CL, Webster AC, Gill JS. Sirolimus is associated with new-onset diabetes in kidney transplant recipients. Journal of the American Society of Nephrology : JASN. 2008;19(7):1411-8.

104. Padiyar A, Augustine JJ, Hricik DE. Induction antibody therapy in kidney transplantation. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2009;54(5):935-44.

105. Toniutto P, Fabris C, Minisini R, Apollonio L, Fumo E, Caldato M, et al. Weight gain after liver transplantation and the insertion/deletion polymorphism of the angiotensin-converting enzyme gene. Transplantation. 2005;79(10):1338-43.

106. Fougeray S, Loriot MA, Nicaud V, Legendre C, Thervet E, Pallet N. Increased body mass index after kidney transplantation in activating transcription factor 6 single polymorphism gene carriers. Transplantation proceedings. 2011;43(9):3418-22.

107. Watt KD, Dierkhising R, Fan C, Heimbach JK, Tillman H, Goldstein D, et al. Investigation of PNPLA3 and IL28B genotypes on diabetes and obesity after liver transplantation: insight into mechanisms of disease. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2013;13(9):2450-7.

108. Cashion A, Stanfill A, Thomas F, Xu L, Sutter T, Eason J, et al. Expression levels of obesity-related genes are associated with weight change in kidney transplant recipients. PloS one. 2013;8(3):e59962.

109. Kurzawski M, Dziewanowski K, Lapczuk J, Wajda A, Drozdzik M. Analysis of common type 2 diabetes mellitus genetic risk factors in new-onset diabetes after transplantation in kidney transplant patients medicated with tacrolimus. European journal of clinical pharmacology. 2012;68(12):1587-94.

110. McCaughan JA, McKnight AJ, Maxwell AP. Genetics of new-onset diabetes after transplantation. Journal of the American Society of Nephrology : JASN. 2014;25(5):1037-49.

111. Mayo Clinic. Personalized medicine and pharmacogenomics 2015 [23.02.2016]. Available from: http://www.mayoclinic.org/healthy-lifestyle/consumer-health/in-depth/personalized-medicine/art-20044300.

112. US Food and Drug Administration. Table of Pharmacogenomic Biomarkers in Drug Labeling 2015 [23.02.2016]. Available from: http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm.

113. Kirchheiner J, Brosen K, Dahl ML, Gram LF, Kasper S, Roots I, et al. CYP2D6 and CYP2C19 genotype-based dose recommendations for antidepressants: a first step towards subpopulation-specific dosages. Acta psychiatrica Scandinavica. 2001;104(3):173-92.

114. Ferrell PB, Jr., McLeod HL. Carbamazepine, HLA-B*1502 and risk of Stevens-Johnson syndrome and toxic epidermal necrolysis: US FDA recommendations. Pharmacogenomics. 2008;9(10):1543-6.

115. Ikediobi ON, Shin J, Nussbaum RL, Phillips KA, Translational UCf, Policy Research on Personalized M, et al. Addressing the challenges of the clinical application of pharmacogenetic testing. Clinical pharmacology and therapeutics. 2009;86(1):28-31.

116. Gervasini G, Benitez J, Carrillo JA. Pharmacogenetic testing and therapeutic drug monitoring are complementary tools for optimal individualization of drug therapy. European journal of clinical pharmacology. 2010;66(8):755-74.

117. Utecht KN, Hiles JJ, Kolesar J. Effects of genetic polymorphisms on the pharmacokinetics of calcineurin inhibitors. American journal of health-system pharmacy : AJHP : official journal of the American Society of Health-System Pharmacists. 2006;63(23):2340-8.

118. Kirchheiner J, Nickchen K, Bauer M, Wong ML, Licinio J, Roots I, et al. Pharmacogenetics of antidepressants and antipsychotics: the contribution of allelic variations to the phenotype of drug response. Molecular psychiatry. 2004;9(5):442-73.

119. American Diabetes A, American Psychiatric A, American Association of Clinical E, North American Association for the Study of O. Consensus development conference on antipsychotic drugs and obesity and diabetes. Diabetes care. 2004;27(2):596-601.

120. Choong E, Quteineh L, Cardinaux JR, Gholam-Rezaee M, Vandenberghe F, Dobrinas M, et al. Influence of CRTC1 polymorphisms on body mass index and fat mass in psychiatric patients and in the general adult population. JAMA Psychiatry 2013;70(10):1011-9.

121. Koller MT, van Delden C, Muller NJ, Baumann P, Lovis C, Marti HP, et al. Design and methodology of the Swiss Transplant Cohort Study (STCS): a comprehensive prospective nationwide long-term follow-up cohort. Eur J Epidemiol. 2013;28(4):347-55.

122. Quteineh L, Bochud PY, Golshayan D, Crettol S, Venetz JP, Manuel O, et al. CRTC2 polymorphism as a riks factor for the incidence of metabolic syndrome traits in patients with solid organ transplantation. The pharmacogenomics Journal, Doi:101038/tpj201582. 2015.

123. Crettol S, Venetz JP, Fontana M, Aubert JD, Ansermot N, Fathi M, et al. Influence of ABCB1 genetic polymorphism on cyclosporine intracellular concentrations in transplant recipients. Pharmacogen Genomics. 2008;18:307-15.

124. Crettol S, Venetz JP, Fontana M, Aubert JD, Pascual M, Eap CB. CYP3A7, CYP3A5, CYP3A4, and ABCB1 genetic polymorphisms, cyclosporine concentration, and dose requirement in transplant recipients. Ther Drug Monit. 2008;30(6):689-99.

125. Firmann M, Mayor V, Vidal PM, Bochud M, Pecoud A, Hayoz D, et al. The CoLaus study: a population-based study to investigate the epidemiology and genetic determinants of cardiovascular risk factors and metabolic syndrome. BMC Cardiovasc Disord. 2008;8:6.

126. Teslovich TM, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, Koseki M, et al. Biological, clinical and population relevance of 95 loci for blood lipids. Nature. 2010;466(7307):707-13.

127. Lango Allen H, Estrada K, Lettre G, Berndt SI, Weedon MN, Rivadeneira F, et al. Hundreds of variants clustered in genomic loci and biological pathways affect human height. Nature. 2010;467(7317):832-8.

128. Heid IM, Jackson AU, Randall JC, Winkler TW, Qi L, Steinthorsdottir V, et al. Meta-analysis identifies 13 new loci associated with waist-hip ratio and reveals sexual dimorphism in the genetic basis of fat distribution. Nat Genet. 2010;42(11):949-60.

129. Choong E, Bondolfi G, Etter M, Jermann F, Aubry JM, Bartolomei J, et al. Psychotropic drug induced weight gain and other metabolic complications in a Swiss Psychiatric population. Journal of Psychiatric Research. 2012;46:540-48.

130. De Hert M, Detraux J, van Winkel R, Yu W, Correll CU. Metabolic and cardiovascular adverse effects associated with antipsychotic drugs. Nature reviews Endocrinology. 2012;8(2):114-26.

131. Limosin F, Gasquet I, Leguay D, Azorin JM, Rouillon F. Body mass index and prevalence of obesity in a French cohort of patients with schizophrenia. Acta psychiatrica Scandinavica. 2008;118(1):19-25.

132. Gram Quist H, Christensen U, Christensen KB, Aust B, Borg V, Bjorner JB. Psychosocial work environment factors and weight change: a prospective study among Danish health care workers. BMC public health. 2013;13:43.

133. Panczak R, Galobardes B, Voorpostel M, Spoerri A, Zwahlen M, Egger M, et al. A Swiss neighbourhood index of socioeconomic position: development and association with mortality. Journal of epidemiology and community health. 2012;66(12):1129-36.

134. Singer LG, Brazelton TR, Doyle RL, Morris RE, Theodore J, International Lung Transplant Database Study G. Weight gain after lung transplantation. The Journal of heart and lung transplantation : the official publication of the International Society for Heart Transplantation. 2003;22(8):894-902.

135. Breuillaud L, Rossetti C, Meylan EM, Merinat C, Halfon O, Magistretti PJ, et al. Deletion of CREB-regulated transcription coactivator 1 induces pathological aggression, depression-related behaviors, and neuroplasticity genes dysregulation in mice. Biol Psychiatry. 2012;72(7):528-36.

136. Voight BF, Scott LJ, Steinthorsdottir V, Morris AP, Dina C, Welch RP, et al. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. Nature genetics. 2010;42(7):579-89.

137. Mahajan A, Go MJ, Zhang W, Below JE, Gaulton KJ, Ferreira T, et al. Genome-wide trans-ancestry meta-analysis provides insight into the genetic architecture of type 2 diabetes susceptibility. Nature genetics. 2014;46(3):234-44.

138. Ibrahim-Verbaas CA, Bressler J, Debette S, Schuur M, Smith AV, Bis JC, et al. GWAS for executive function and processing speed suggests involvement of the CADM2 gene. Molecular psychiatry. 2016;21(2):189-97.

139. Griswold AJ, Ma D, Cukier HN, Nations LD, Schmidt MA, Chung RH, et al. Evaluation of copy number variations reveals novel candidate genes in autism spectrum disorder-associated pathways. Human molecular genetics. 2012;21(15):3513-23.

140. Sohn JW, Elmquist JK, Williams KW. Neuronal circuits that regulate feeding behavior and metabolism. Trends in neurosciences. 2013;36(9):504-12.

141. Farooqi IS, O'Rahilly S. Monogenic obesity in humans. Annual review of medicine. 2005;56:443-58.

142. Nam JH, Mun JI, Kim SI, Kang SW, Choi KH, Park K, et al. beta-Cell dysfunction rather than insulin resistance is the main contributing factor for the development of postrenal transplantation diabetes mellitus. Transplantation. 2001;71(10):1417-23.

143. Zhu J, Loos RJ, Lu L, Zong G, Gan W, Ye X, et al. Associations of genetic risk score with obesity and related traits and the modifying effect of physical activity in a Chinese Han population. PloS one. 2014;9(3):e91442.

144. Peterson RE, Maes HH, Lin P, Kramer JR, Hesselbrock VM, Bauer LO, et al. On the association of common and rare genetic variation influencing body mass index: a combined SNP and CNV analysis. BMC genomics. 2014;15:368.

145. Goumidi L, Cottel D, Dallongeville J, Amouyel P, Meirhaeghe A. Effects of established BMI-associated loci on obesity-related traits in a French representative population sample. BMC genetics. 2014;15:62.

146. Keen JC, Moore HM. The Genotype-Tissue Expression (GTEx) Project: Linking Clinical Data with Molecular Analysis to Advance Personalized Medicine. J Pers Med. 2015;5(1):22-9.

147. ENCODE Consortium. The Encyclopedia of DNA Elements (ENCODE) Consortium [17.03.2016]. Available from: https://www.encodeproject.org/.

148. Maher B. ENCODE: The human encyclopaedia. Nature. 2012;489(7414):46-8.

149. Encode Project Consortium. An integrated encyclopedia of DNA elements in the human genome. Nature. 2012;489(7414):57-74.

150. Demirkan A, Amin N, Isaacs A, Jarvelin MR, Whitfield JB, Wichmann HE, et al. Genetic architecture of circulating lipid levels. European journal of human genetics : EJHG. 2011;19(7):813-9.

151. Roffman JL, Brohawn DG, Nitenson AZ, Macklin EA, Smoller JW, Goff DC. Genetic variation throughout the folate metabolic pathway influences negative symptom severity in schizophrenia. Schizophrenia bulletin. 2013;39(2):330-8.

152. Choquet H, Meyre D. Genetics of Obesity: What have we Learned? Current genomics. 2011;12(3):169-79.

153. Liu S, Song Y. Building genetic scores to predict risk of complex diseases in humans: is it possible? Diabetes. 2010;59(11):2729-31.

APPENDIX

Supplementary files. Project I: Association of *PCK1* with Body Mass Index and other metabolic features in patients with psychotropic treatments

eTable 1. Description of demographic and clinical psychiatric Caucasian samples.

Characteristics	Discovery Sample n = 478	Replication Sample 1 n = 168	Replication Sample 2 n = 188	Combined sample n= 834
Male,%	44	53	62	50
Age, median (range), years	50 (12-96)	42 (19-64)	42 (19-69)	45 (12-96)
Diagnosis				
Psychotic disorders,%	33.3	27.5	43.4	34.5
Schizo-affective disorders,%	6.5	15.6	12.1	10
Bipolar disorders,%	19.9	32.9	17	22.2
Depression disorders,%	20.4	16.8	13.7	17.9
Others diagnosis,%	19.9	7.2	13.7	15.4
Initial BMI status ‡				
BMI, median (range), kg/m^2	24 (13-44)	25 (15-46)	25 (16-46)	24 (13-46)
Overweight (25≥ Initial BMI<30), %	23	36	32	28
Obese (Initial BMI≥ 30), %	14	15	15	14
Current BMI status #				
BMI, median (range), kg/m^2	25 (15-50)	28 (16-42)	27 (17-44)	25 (15-50)
Overweight (25≥ Current BMI<30), %	26	30	34	27
Obese (Current BMI≥ 30), %	18	40	27	24
Initial waist circumference ‡				
WC, median (range), cm	90 (54-138)	--	--	87 (54-138)
High WC ≥ 94cm (male), 88cm (female), %	43 (n=315)	--	--	43 (n=315)
Current waist circumference #				
WC, median (range), cm	93 (48 – 162)	--	98 (51-148)	95 (48-162)
High WC ≥ 94 (male), 88 (female), %	54 (n=592)	--	64 (n=182)	57 (n=774)
Initial Lipid status ‡				
High LDL, % (n)[a]	9 (n=224)	--	--	9 (n=224)
High TG, % (n)[b]	19 (n=234)	--	--	19 (n=234)
Low HDL, % (n)[c]	25 (n=222)	--	--	25 (n=222)
Current Lipid status #				
High LDL, % (n)[a]	14 (n=383)	--	--	15 (n=363)
High TG, % (n)[b]	28 (n=402)	--	--	28 (n=402)
Low HDL, % (n)[c]	27 (n=359)	28 (n=164)	19 (n=160)	26 (n=665)
Smoker, %	41	60	75	50

	Discovery Sample n = 478	**Replication Sample 1 n = 168**	**Replication Sample 2 n = 188**	**Combined sample n= 834**
Prescribed psychotropic drug				
Amisulpride, %	8	-	10	7
Aripirazole, %	10	-	8	8
Clozapine, %	8	14	9	9
Olanzapine, %	10	16	12	11
Quetiapine, %	31	18	23	28
Risperidone, %	16	17	16	16
Lithium, %	7	20	12	10
Valproate, %	4	14	8	6
Treatment duration, median (range), months	6 (1-12)	27.4 (3-333)	36 (1-390)	9 (1-390)

‡ Before the current psychotropic treatment

For sample 1,3 : current observation ; for sample 2 : last follow-up

-- Missing clinical values or obtained in non fasting conditions

a. High LDL cholesterol : equal or higher than 4.1 mmol/L

b. High triglycerides : equal or higher than 2.2 mmol/L

c. Low HDL cholesterol : lower than 1 mmol/L

BMI: body mass index, WC: waist circumference, LDL: low density lipoprotein, TG: triglycerides, HDL: high density lipoprotein

eTable 2. HWE and *PCK1* genotypes distribution among three psychiatric cohorts.

rs11552145	**Discovery sample**	**Replication 1**	**Replication 2**	**Combined Sample**
GG	478	141	173	792
GA	197	49	72	318
AA	30	8	11	49
HWE ($p^{\$}$-value)	0.40	0.68	1.00	0.08
rs707555	**Discovery sample**	**Replication 1**	**Replication 2**	**Combined Sample**
CC	547	166	190	903
CG	142	29	61	232
GG	16	3	6	25
HWE ($p^{\$}$-value)	0.28	0.80	1.00	0.12
rs8123020	**Discovery sample**	**Replication 1**	**Replication 2**	**Combined Sample**
CC	546	140	193	879
CT	149	55	62	266
TT	11	3	2	16
HWE ($p^{\$}$-value)	1.00	1.00	0.84	1.00
rs2071023	**Discovery sample**	**Replication 1**	**Replication 2**	**Combined Sample**
CC	217	52	69	338
CG	333	103	122	558
GG	153	41	53	247
HWE ($p^{\$}$-value)	0.96	1.00	1.00	1.00

$^{\$}$p-corrected value

eTable 3. Marginal analysis of the influence of *PCK1* polymorphisms on BMI in the three psychiatric samples.

		Discovery Sample[#]			Replication 1			Replication 2			Combined Sample*		
Baseline BMI	***rs11552145***	**AA**	**G-allele**	**p-value[$]**	**AA**	**G-allele**	**p-value**	**AA**	**G-allele**	**p-value**	**AA**	**G-allele**	**p-value**
	n	22	354	0.36	8	131	0.49	10	169	0.46	40	654	*0.05*
	BMI [kg/m^2] (SE)	22.4 (0.7)	24.3 (0.3)		24.3 (1.4)	25.5 (0.4)		23.8 (0.7)	25 (0.4)		23.1 (0.5)	24.7 (0.2)	
	rs707555	**GG**	**C-allele**	**p-value[$]**									
	n	10	366	1.00									
	BMI [kg/m^2] (SE)	23.6 (6.7)	24.2 (5.1)										
	rs8123020	**TT**	**C-allele**	**p-value[$]**									
	n	10	366	1.00									
	BMI [kg/m^2] (SE)	23.4 (3.1)	24.2 (5.2)										
	rs2071023	**CC**	**G-allele**	**p-value[$]**	**CC**	**G-allele**	**p-value**	**CC**	**G-allele**	**p-value**	**CC**	**G-allele**	**p-value**
	n	122	277	0.28	33	106	0.66	46	130	0.58	194	496	**0.048**
	BMI [kg/m^2] (SE)	23.6 (0.5)	24.4 (0.3)		24.8 (0.6)	25.6 (0.5)		24.5 (0.7)	25.0 (0.5)		24.0 (0.4)	24.8 (0.2)	
		Discovery Sample[#]			**Replication 1**			**Replication 2**			**Combined Sample***		
Current BMI	***rs11552145***	**AA**	**G-allele**	**p-value[$]**	**AA**	**G-allele**	**p-value**	**AA**	**G-allele**	**p-value**	**AA**	**G-allele**	**p-value**
	n	12	421	***0.08***	8	160	0.57	11	170	0.80	30	742	**0.01**
	BMI [kg/m^2] (SE)	22.8 (2.9)	25.4 (5.4)		27.1 (1.3)	28.2 (0.4)		26.9 (1.6)	27.3 (0.4)		23.3 (0.6)	25.7 (0.2)	
	rs707555	**CC**	**G-allele**	**p-value[$]**									
	n	12	421	1.00									
	BMI [kg/m^2] (SE)	25.1 (6.1)	25.3 (5.4)										
	rs8123020	**TT**	**C-allele**	**p-value[$]**									
	n	10	423	1.00									
	BMI [kg/m^2] (SE)	25.8 (2.6)	25.3 (5.4)										
	rs2071023	**CC**	**G-allele**	**p-value[$]**	**CC**	**G-allele**	**p-value**	**CC**	**G-allele**	**p-value**	**CC**	**G-allele**	**p-value**
	n	143	333	**0.018**	39	128	0.41	49	132	0.88	287	722	**0.003**
	BMI [kg/m^2] (SE)	24.5 (0.5)	25.7 (0.3)		27.5 (0.7)	28.3 (0.5)		26.9 (0.7)	27.3 (0.5)		25.3 (0.3)	26.4 (0.2)	

For current BMI, only significant findings in the discovery sample were further tested for replication. The same SNPs were also tested for replication at the baseline BMI.

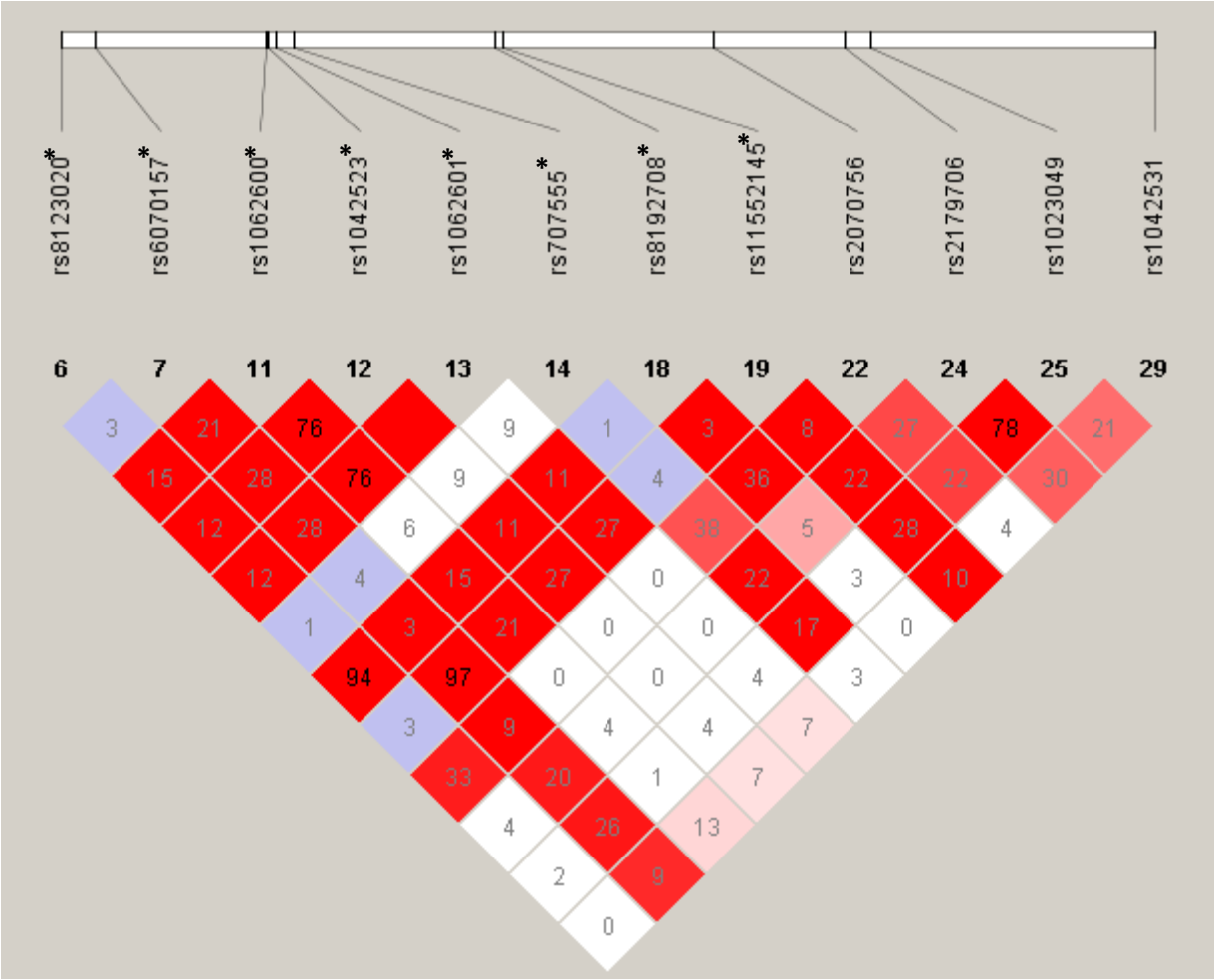
*Only patients treated for up to 24 months.

[$]p-corrected value for the discovery sample.

eTable 4. Selected polymorphisms description and Minor Allele Frequencies (MAF).

variant	position in gene	type of variation	major / minor allele	MAF in combined psychiatric sample	MAF in Caucasians
rs11552145	chr 20:56138648	missense Glu>Lys	G/A	0.17	0.16
rs707555	chr 20:56137895	missense Leu>Val	G/C	0.12	0.14
rs8123020	chr 20:56137061	intron variant	C/T	0.12	0.12
rs2071023	chr 20:56135934	5' near gene	C/G	0.46	0.48

eFigure 1: Pairwise linkage disequilibrium (LD) in CEU HapMap samples for *PCK1* polmorphisms. LD expressed as r^2.



** SNPs tested in the present study, including SNPs in LD with one of the four analyzed SNPs. rs2071023 (not present in the figure) is in LD with rs1062600 (r^2=1), rs1062601 (r^2=0.81) and rs1042523 (r^2=0.82). rs11552145 is in LD with rs6070157 (r^2=0.97). rs8123020 is in LD with rs8192708 (r^2=0.94).*

Supplementary files. Project II: Association of Genetic Risk Scores (GRS) with Body Mass Index in Swiss Psychiatric Cohorts

S1 Table. SNP description and HWE analysis of 32 SNPs previously associated with BMI in a Genome Wide Association Study [1].

nearest gene	SNP	Major/minor allele	Chr position	MAF (Caucasian)	HWE in the Sample 1 (p-value*)	HWE in all psychiatric samples (p-value*)
CADM2	*rs13078807*	A/G	3:85884150	0.20	0.34	0.08
FTO	*rs1558902*	T/C	16:53800954	0.44	0.88	0.45
GPRC5B	*rs12444979*	C/T	16:19933600	0.12	0.99	0.55
LRP1B	*rs2890652*	T/C	2:142959931	0.16	0.63	0.88
BDNF	*rs10767664*	C/A	11:27728539	0.24	0.19	0.28
TFAP2B	*rs987237*	A/G	6:50803050	0.20	0.02	0.08
NRXN3	*rs10150332*	T/C	14:79936964	0.22	0.09	0.01
MC4R	*rs571312*	C/A	18:57839769	0.23	0.19	0.05
MAP2K5	*rs2241423*	G/A	15:68086838	0.23	0.21	0.12
PRKD1	*rs11847697*	C/T	14:30501885	0.05	0.06	0.13
TNNI3K	*rs1514175*	G/A	1:74991644	0.44	0.86	0.91
SEC16B	*rs543874*	A/G	1:177889480	0.20	0.79	0.99
SLC39A8	*rs13107325*	C/T	4:103188709	0.08	0.42	0.16
NUDT3	*rs206936*	A/G	6:34302869	0.20	0.07	0.35
ZNF608	*rs4836133*	G/A	5:124330522	0.47	0.36	0.07
MTIF3	*rs4771122*	A/G	13:28020180	0.26	0.91	0.87
MTCH2	*rs3817334*	C/T	11:47650993	0.42	0.53	0.67
FLJ35779	*rs2112347*	T/G	5:75015242	0.38	0.08	0.04
TMEM18	*rs2867125*	C/T	2:622827	0.18	0.10	0.23
TMEM160	*rs3810291*	A/G	19:47569003	0.34	0.82	0.01
RBJ / POMC	*rs713586*	T/C	2:25158008	0.46	0.33	0.14
NEGR1	*rs2815752*	A/G	1:72812440	0.37	0.35	0.58
KCTD15	*rs29941*	G/A	19:34309532	0.32	0.58	0.30
PTBP2	*rs1555543*	C/A	1:96944797	0.42	0.40	0.14
ETV5	*rs9816226*	C/T	3:185834290	0.22	0.10	0.98
GNPDA2	*rs10938397*	A/G	4:45182527	0.42	0.66	0.32
RPL27A	*rs4929949*	T/C	11:8605739	0.50	0.78	0.89
FAIM2	*rs7138803*	G/A	12:50247468	0.34	0.22	0.38
FANCL	*rs887912*	C/T	2:59302877	0.31	0.14	0.14
QPCTL	*rs2287019*	C/T	19:46202172	0.19	0.23	0.10
LRRN6C	*rs10968576*	A/G	9:28414339	0.31	0.13	0.31
SH2B1	*rs7359397*	C/T	16:28885659	0.34	0.89	0.41

*HWE: Hardy-Weinberg Equilibrium. MAF: Minor Allele Frequency.*p-value corrected threshold < 0.001*

S2 Table. SNP description and HWE analyses of 20 Candidate Gene SNPs associated with antipsychotic induced weight gain.

nearest gene	SNP	Major/Minor Allele	MAF (Caucasian)	HWE in the Sample 1 (p-value*)	HWE in all psychiatric samples (p-value*)	mutation type	effect allele	Effect on BMI	animal / in vitro studies related to obesity or metabolic parameters	clinical studies
CRTC1	*rs6510997*	C>T	0.17	0.16	0.23	Intron variant	T-allele	decreased weight	[2]	[3]
HSD11β1	*rs3753519*	C>T	0.10	0.56	0.86	Intron variant	T-allele	decreased weight	[4]	[5]
MCHR2	*rs6925272*	C>T	0.37	0.13	0.20	Intron variant	T-allele	decreased weight	[6]	[7]
PCK1	*rs11552145*	G>A	0.16	0.10	0.02	Missense variant (Glu -> Lys)	AA	decreased weight	[8]	[9]
CRTC2	*rs8450*	G>A	0.30	0.71	0.03	3 prime UTR variant	AA	increased weight	[10]	[11]
IRS2	*rs1411766*	G>A	0.36	0.06	0.11	Intergenic variant	A-allele	increased weight	[12]	[13]
PPARGC1A	*rs8192678*	C>T	0.36	0.52	0.20	Missense variant (Gly -> Ser)	T-allele	decreased weight	[14]	[15]
FAAH	*rs324420*	C>A	0.21	0.60	0.75	Missense variant (Pro -> Thr)	A-allele	More frequent in patients with 7% of weight gain	[16]	[17]
INSIG2	*rs17587100*	A>C	0.10	0.68	0.47	Intergenic variant	C-allele	change in BMI	[18]	[19]
PPARG	*rs1801282*	G>A	0.12	0.15	0.24	Missense variant (Pro -> Ala)	A-allele	weight loss	[20]	[21, 22]
PRKAA1	*rs10074991*	G>A	0.29	0.09	0.08	Intron variant	A-allele	change in weight	[23]	[24]
SCARB1	*rs4765623*	C>T	0.32	0.78	0.50	Intron variant	T-allele	weight gain in the olanzapine-treated group	[25]	[26]
TNF	*rs1800629*	G>A	0.14	0.04	0.07	Upstream gene variant	GG	weight gain	[27]	[28]
ADRA2A	*rs1800544*	C>G	0.26	0.52	0.63	Upstream gene variant	C-allele	weight gain	[29]	[30, 31]
CNR1	*rs806378*	C>T	0.27	0.31	0.65	Intron variant	T-allele	weight gain	[32]	[33, 34]
DRD2	*rs1800497*	G>A	0.18	0.12	0.32	Intron variant	C-allele	weight gain	[35]	[36]
HTR2A	*rs6313*	G>A	0.44	0.32	0.32	Synonymous variant (Ser -> Ser)	A-allele	weight gain	[37]	[38, 39]
LEPR	*rs1137101*	A>G	0.49	0.12	0.11	Missense variant (Gln -> Arg)	G allele	weight gain	[40]	[41]
ADIPOQ	*rs17300539*	G>A	0.07	0.63	0.64	Upstream gene variant	G-allele	decreased risk of obesity	[37]	[24, 42]
LEP	*rs7799039*	G>A	0.46	0.18	0.24	Upstream gene variant	A-allele	weight gain	[37]	[37]

*HWE: Hardy-Weinberg Equilibrium. MAF: Minor Allele Frequency.*p-value corrected threshold < 0.001*

S3 Table. Description of SNPs previously associated with Diabetes in GWAS [43].

Chr position	SNP	Major/Minor Alleles	MAF in Caucasians	Gene	Position
10:114758349	rs7903146	C>T	0.17	*TCF7L2*	intron-variant
11:72433098	rs1552224	A>C	0.07	*ARAP1*	utr-variant-5-prime
2:227020653	rs7578326	A>G	0.30	*IRS1*	intron-variant
10:94465559	rs5015480	T>C	0.42	-	intergenic
2:60584819	rs243021	A>G	0.48	-	intergenic
11:92673828	rs1387153	C>T	0.41	-	intergenic
11:2691471	rs231362	G>A	0.25	*KCNQ1*	intron-variant
5:76424949	rs4457053	A>G	0.12	*ZBED3*	intron-variant
9:22133284	rs10965250	G>A	0.23	-	intergenic
X:152899922	rs5945326	A>G	0.25	-	intergenic
10:104844872	rs7092200	T>C	0.38	-	intergenic
6:152790573	rs9371601	T>G	0.37	*SYNE1*	intron-variant
8:95960511	rs896854	C>T	0.46	*TP53INP1*	intron-variant
3:185529080	rs1470579	A>C	0.46	*IGF2BP2*	intron-variant
7:28196222	rs849134	A>G	0.30	*JAZF1*	intron-variant
12:66174894	rs1531343	G>C	0.22	*HMGA2*	intron-variant
8:118185025	rs3802177	G>A	0.29	*SLC30A8*	utr-variant-3-prime
16:53845487	rs11642841	C>A	0.17	*FTO*	intron-variant
17:36098040	rs4430796	A>G	0.46	*HNF1B*	intron-variant
12:71634794	rs4760790	G>A	0.24	-	intergenic
6:20686996	rs9368222	C>A	0.30	*CDKAL1*	intron-variant
7:130438214	rs13234407	G>A	0.34	-	intergenic
9:107669073	rs13284054	T>C	0.12	*ABCA1*	intron-variant
4:6293350	rs10012946	C>T	0.19	*WFS1*	intron-variant

Chr: Chromosome. MAF: Minor Allele Frequency

S4 Table. Description of SNPs previously associated with Psychiatric disease in GWAS [44].

chr: position	SNP	Major/Minor Alleles	MAF in Caucasians	Genes	Position
11:125550049	rs556884	A>G	0.12	*ACRV1*	intron-variant
3:52818579	rs2239551	G>A	0.41	*ITIH1*	intron-variant
10:104844872	rs7092200	T>C	0.38	-	intergenic
6:152790573	rs9371601	T>G	0.37	*SYNE1*	intron-variant
8:4188511	rs10866968	C>T	0.41	*CSMD1*	intron-variant
10:62181128	rs10994338	G>A	0.13	*ANK3*	intron-variant
10:104660004	rs11191454	A>G	0.12	*AS3MT*	intron-variant
10:104906211	rs11191580	T>C	0.14	*NT5C2*	intron-variant
8:89574375	rs13263450	G>T	0.13	-	intergenic

Chr: Chromosome. MAF: Minor Allele Frequency

S5 Table. Allele effects (β-coefficients) calculated from the general population for the 52 SNPs.

Gene	SNP	Allele Effect	Per allele effect (β-coefficient*)	p-value
BDNF	*rs10767664*	A	0.048	1.2E-19
CADM2	*rs13078807*	G	0.033	5.4E-10
ETV5	*rs9816226*	T	0.048	4.7E-18
FAIM2	*rs7138803*	A	0.035	5.2E-16
FANCL	*rs887912*	T	0.026	2.4E-08
FLJ35779	*rs2112347*	T	0.028	1.6E-10
FTO	*rs1558902*	A	0.080	2.9E-75
GNPDA2	*rs10938397*	G	0.042	5.4E-21
GPRC5B	*rs12444979*	C	0.050	2.7E-15
KCTD15	*rs29941*	G	0.032	2.6E-12
LRP1B	*rs2890652*	C	0.036	2.0E-10
LRRN6C	*rs10968576*	G	0.029	3.8E-10
MAP2K5	*rs2241423*	G	0.037	5.4E-13
MC4R	*rs571312*	A	0.056	2.0E-28
MTCH2	*rs3817334*	T	0.030	2.0E-12
MTIF3	*rs4771122*	G	0.029	1.3E-08
NEGR1	*rs2815752*	A	0.038	1.7E-18
NRXN3	*rs10150332*	C	0.031	1.4E-09
NUDT3	*rs206936*	G	0.022	2.2E-05
PRKD1	*rs11847697*	T	0.070	1.0E-09
PTBP2	*rs1555543*	C	0.024	1.5E-08
QPCTL	*rs2287019*	C	0.037	2.0E-09
RBJ POMC	*rs713586*	C	0.026	6.9E-10
RPL27A	*rs4929949*	C	0.024	3.2E-08
SEC16B	*rs543874*	G	0.044	2.4E-16
SH2B1	*rs7359397*	T	0.028	1.5E-10
SLC39A8	*rs13107325*	T	0.055	2.9E-08
TFAP2B	*rs987237*	G	0.049	3.9E-19
TMEM160	*rs3810291*	A	0.029	2.8E-09
TMEM18	*rs2867125*	C	0.060	2.2E-26
TNNI3K	*rs1514175*	A	0.030	4.9E-12
ZNF608	*rs4836133*	A	0.023	3.0E-07
CRTC1	*rs3746266*[#]	T	0.015	2.2E-02
HSD	*rs3753519*	C	0.003	6.5E-01
PCK1	*rs6070157*[#]	T	0.003	6.3E-01
CRTC2	*rs8450*	C	0.004	3.7E-01
IRS2	*rs1411766*	A	0.001	8.9E-01
PPARGC1A	*rs8192678*	T	0.0001	9.9E-01
PRKAA1	*rs10074991*	A	0.006	2.3E-01

Gene	SNP	Allele Effect	Per allele effect (β-coefficient*)	p-value
LEPR	*rs1137101*	A	-0.006	0.14
INSIG2	*rs17587100*	A	-0.006	0.42
DRD2	*rs1800497*	A	0.014	0.01
TNF	*rs1800629*	A	0.003	0.60
PPARG	*rs2197423*[#]	A	0.015	0.02
FAAH	*rs324420*	A	0.002	0.68
ADRA2A	*rs1800544*	A	0.003	0.51
HTR2A	*rs6313*	A	-0.006	0.14
SCARB1	*rs7954697*[#]	A	0.006	0.18
CNR1	*rs806378*	T	-0.014	0.00
MCHR2	*rs7749425*[#]	T	0.003	0.47
ADIPOQ	*rs17300539*	A	0.013	0.18
LEP	*rs7799039*	A	-0.003	0.56

** β-coefficients are obtained from GIANT consortia [#] rs3746266 is a proxy of rs6510997 (r^2=0.70), rs6070157 is a proxy of rs11552145 (r^2=1), rs2197423 is a proxy of rs1801282 (r^2=1), rs7954697 is a proxy of rs4765623 (r^2=0.62), rs7749425 is a proxy of rs6925272 (r^2=0.93)*

S6 Table. Detailed characteristics of the combined sample stratified by gender.

	Men 375	**Women** 375	p-value
Score, mean (SD)	1.02 (0.13)	1.02 (0.13)	0.8
1st quartile of GRS, %	24	26	
2nd quartile of GRS, %	26	20	
3th quartile of GRS, %	22	28	
4th quartile of GRS, %	29	26	0.1
Newly diagnosed and first episode, (%)**	23	30	0.1
Age, median (range), years	40 (13-97)	49 (15-96)	**0.0001**
Baseline BMI (kg/m^2) *	24.6 (16-44)	24.1 (13-46)	**0.004**
Current BMI (kg/m^2) #	25.5 (17-50)	24.2 (15-47)	0.1
Treatment prescription			
Ami, Ari, Li, Quet, Risp	70	70	
Clo, Olan, Valp	30	30	0.9
Treatment duration, median (range), months	9 (1-24)	6 (1-23)	**0.05**
High waist circumference (WC ≥94 cm men, 88 cm women); %	50	53	0.5
Diagnostic, %			
Psychotic disorders	49	34	
Bipolar disorders	22	21	**<0.001**
Depression	11	21	

Ami: amisulpride, Ari: aripiprazole, Li: lithium, Quet: quetiapine, Risp: risperidone, Clo: clozapine, Olan: olanzapine, Valp: valproate. WC: waist circumference

** Before the current psychotropic treatment*

*** Only for Sample 1*

 # Last observed data

S7 Table. Detailed characteristics of the combined sample by first episode and newly diagnosed (FEND) patients.

	FEND 116	**Others** 309	p-value
Score, mean (SD)	1.02 (0.12)	1.01 (0.13)	0.2
1st quartile of GRS, %	21	26	
2nd quartile of GRS, %	22	22	
3th quartile of GRS, %	26	25	
4th quartile of GRS, %	30	26	0.4
Men, %	37	46	0.10
Age, median (range), years	58 (14-96)	51 (13-97)	0.4
Baseline BMI (kg/m^2) *	22.3 (13.4-38.2)	24.2 (14.3-44.5)	0.09
Current BMI (kg/m^2) #	23.4 (16.5-37.7)	26.0 (14.7-50.2)	**0.01**
Treatment prescription			
Ami, Ari, Li, Quet, Risp	79	73	
Clo, Olan, Valp	20	27	0.2
Treatment duration, median (range), months	3 (1-12)	4 (1-23.8)	**0.002**
High waist circumference (WC ≥94 cm men, 88 cm women); %	41	50	0.2
Diagnostic, %			
Psychotic disorders	32	40	
Bipolar disorders	8	22	**<0.001**
Depression	20	16	

Ami: amisulpride, Ari: aripiprazole, Li: lithium, Quet: quetiapine, Risp: risperidone, Clo: clozapine, Olan: olanzapine, Valp: valproate.WC: waist circumference

** Before the current psychotropic treatment*

 # Last observed data

S8 Table. Weighted GRS association with BMI obtained from 32 SNPs of Genome Wide Association Studies.

	n	BMI difference between GRS (p90) and GRS (p10) [95% CI]			p-value
		at baseline	at 12 months	at 24 months	
Sample 1*	425	1.38 [0.21 – 2.57]	1.55 [0.21 – 2.88]		**0.01**
Sample 2 **	148	-0.42 [-2.75 – 1.91]	-0.49 [-3.29 – 2.29]	-0.59 [-4.3 – 3.11]	0.8
Sample 3 **	177	2.02 [-0.002 – 4.04]	2.19 [-0.06 – 4.44]	2.38 [-0.35 – 5.13]	**0.04**
Samples 2 and 3 **	325	1.14 [-0.38 – 2.68]	1.29 [-0.47 – 3.06]	1.46 [-0.76 – 3.69]	0.06
All samples combined	750	1.31 [0.39 – 2.24]	1.47 [0.42 – 2.52]		**0.001**
FEND patients*	116	2.52 [0.31 – 4.73]	2.91 [0.32 – 5.50]		**0.01**
Men	375	2.05 [1.04 – 3.05]	2.29 [1.15 – 3.45]		**0.0001**
Women	375	0.59 [-0.53 – 1.71]	0.65 [-0.62 - 1.93]		0.3

GRS: Genetic Risk Score, p90: percentile 90 of GRS, p10: percentile 10 of GRS.
**follow-up to 12 months of treatment. **follow-up to 24 months of treatment.*
FEND: First Episode and Newly Diagnosed Patients

S9 Table. Weighted GRS association with BMI obtained from 20 Candidate Genes SNPs.

	n	BMI difference between GRS (p95) and GRS (p5) [95% CI]			p-value
		at baseline	at 12 months	at 24 months	
Sample 1*	425	-0.03 [-1.39 – 1.32]	-0.03 [-1.55 – 1.48]		0.96
Sample 2 **	143	1.66 [-1.22 – 4.55]	1.97 [-1.48 – 5.43]	2.37 [-2.10 – 6.85]	0.28
Sample 3 **	175	1.26 [-1.03 – 3.54]	1.36 [-1.17 – 3.89]	1.48 [-1.53 – 4.48]	0.31
Samples 2 and 3 **	318	1.19 [-0.59 – 2.97]	1.33 [-0.71 – 3.38]	1.51 [-1.00 – 4.04]	0.21
All samples combined	743	0.53 [-0.90 – 1.99]	0.42 [-0.65 – 1.51]		0.46
FEND patients*	116	-1.53 [-4.00 – 0.94]	-1.75 [-4.62 – 1.11]		0.22
Men	374	1.16 [-0.05 – 2.38]	1.30 [-0.08 – 2.69]		0.11
Women	369	-0.37 [-1.76 – 1.02]	-0.41 [-1.97 – 1.15]		0.66

GRS: Genetic Risk Score, p95: percentile 95 of GRS, p5: percentile 5 of GRS.
**follow-up to 12 months of treatment. **follow-up to 24 months of treatment.*
FEND: First Episode and Newly Diagnosed Patients

S10 Table. Weighted GRS association with BMI obtained from 20 SNPs of Candidate gene approach and 32 SNPs of Genome Wide Association Studies (52 SNPs).

	n	BMI difference between GRS (p95) and GRS (p5) [95% CI]			p-value
		at baseline	at 12 months	at 24 months	
Sample 1*	425	1.87 [0.49-3.26]	2.08 [0.53 - 3.63]		**0.01**
Sample 2 **	143	-0.20 [-2.79 – 2.39]	-0.24 [-3.35 – 2.87]	-0.29 [-4.36 – 3.79]	0.8
Sample 3 **	175	2.37 [0.13-4.61]	2.57 [0.08-5.06]	2.79 [-0.19-5.78]	**0.04**
Samples 2 and 3 **	318	1.71 [-0.03 – 3.45]	1.92 [-0.07 – 3.92]	2.18 [-0.29 – 4.66]	0.06
All samples combined	743	1.74 [0.68-2.80]	1.94 [0.75-3.14]		**0.001**
FEND patients*	116	3.19 [0.54-5.84]	3.66 [0.58-6.73]		**0.01**
Men	374	2.75 [1.57-3.93]	3.09 [1.74-4.45]		**0.0001**
Women	369	0.85 [-0.49 – 2.21]	0.94 [-0.57 – 2.47]		0.3

GRS: Genetic Risk Score, p95: percentile 95 of GRS, p5: percentile 5 of GRS.
**follow-up to 12 months of treatment. **follow-up to 24 months of treatment.*
FEND: First Episode and Newly Diagnosed Patients

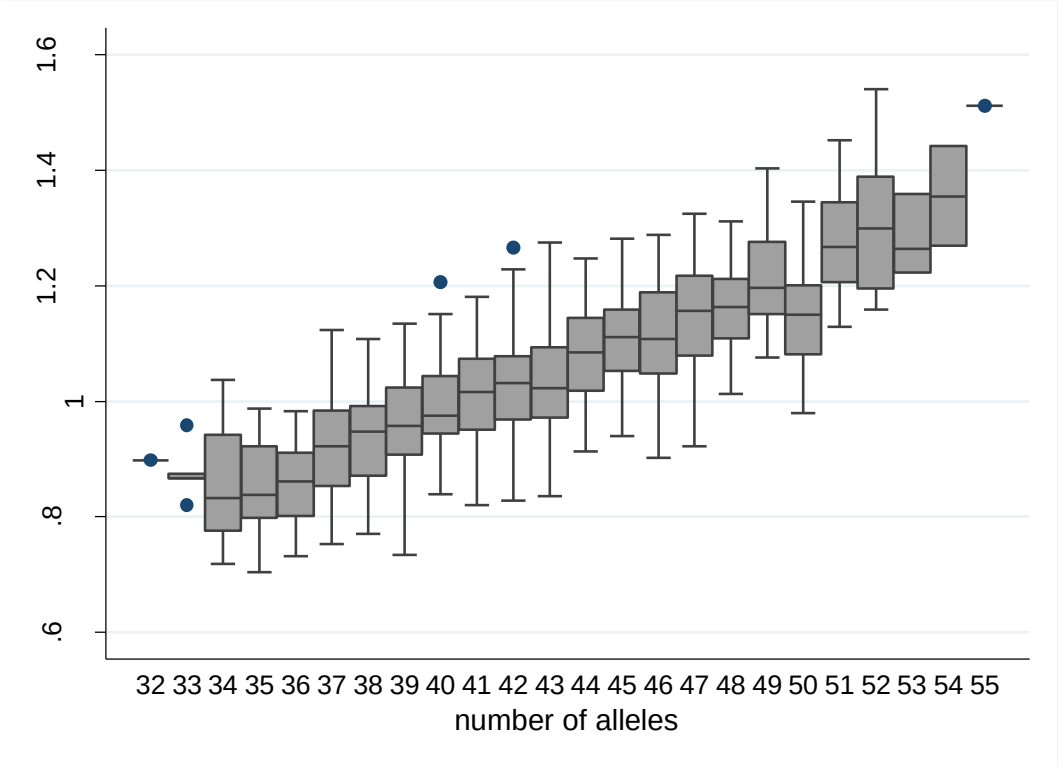
REFERENCES

1. Speliotes EK, Willer CJ, Berndt SI, Monda KL, Thorleifsson G, Jackson AU, et al. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nat Genet*. 2010;42(11):937-48.
2. Altarejos JY, Goebel N, Conkright MD, Inoue H, Xie J, Arias CM, et al. The Creb1 coactivator Crtc1 is required for energy balance and fertility. *Nat Med*. 2008;14(10):1112-7.
3. Choong E, Quteineh L, Cardinaux JR, Gholam-Rezaee M, Vandenberghe F, Dobrinas M, et al. Influence of CRTC1 polymorphisms on body mass index and fat mass in psychiatric patients and in the general adult population. *Jama Psychiatry* 2013;70(10):1011-9.
4. Paterson JM, Morton NM, Fievet C, Kenyon CJ, Holmes MC, Staels B, et al. Metabolic syndrome without obesity: Hepatic overexpression of 11beta-hydroxysteroid dehydrogenase type 1 in transgenic mice. *Proc Natl Acad Sci U S A*. 2004;101(18):7088-93.
5. Quteineh L, Vandenberghe F, Saigi Morgui N, Delacretaz A, Choong E, Gholam-Rezaee M, et al. Impact of HSD11B1 polymorphisms on BMI and components of the metabolic syndrome in patients receiving psychotropic treatments. *Pharmacogenet Genomics*. 2015;25(5):246-58.
6. Chee MJ, Pissios P, Prasad D, Maratos-Flier E. Expression of melanin-concentrating hormone receptor 2 protects against diet-induced obesity in male mice. *Endocrinology*. 2014;155(1):81-8.
7. Ghoussaini M, Vatin V, Lecoeur C, Abkevich V, Younus A, Samson C, et al. Genetic study of the melanin-concentrating hormone receptor 2 in childhood and adulthood severe obesity. *J Clin Endocrinol Metab*. 2007;92(11):4403-9.
8. Franckhauser S, Munoz S, Elias I, Ferre T, Bosch F. Adipose overexpression of phosphoenolpyruvate carboxykinase leads to high susceptibility to diet-induced insulin resistance and obesity. *Diabetes*. 2006;55(2):273-80.
9. Saigi Morgui N, Vandenberghe F, Delacrétaz A, Quteineh L, Choong E, Gholam-Rezaee M, et al. Association of PCK1 with Body Mass Index and Other Metabolic Features in patients with psychotropic treatments. *J Clin Psychopharmacol*. 2015;35(5):544-52.
10. Wang Y, Vera L, Fischer WH, Montminy M. The CREB coactivator CRTC2 links hepatic ER stress and fasting gluconeogenesis. *Nature*. 2009;460(7254):534-7.
11. Keshavarz P, Inoue H, Nakamura N, Yoshikawa T, Tanahashi T, Itakura M. Single nucleotide polymorphisms in genes encoding LKB1 (STK11), TORC2 (CRTC2) and AMPK alpha2-subunit (PRKAA2) and risk of type 2 diabetes. *Mol Genet Metab*. 2008;93(2):200-9.
12. Bruning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, et al. Role of brain insulin receptor in control of body weight and reproduction. *Science*. 2000;289(5487):2122-5.
13. Butte NF, Voruganti VS, Cole SA, Haack K, Comuzzie AG, Muzny DM, et al. Resequencing of IRS2 reveals rare variants for obesity but not fasting glucose homeostasis in Hispanic children. *Physiol Genomics*. 2011;43(18):1029-37.
14. Bournat JC, Brown CW. Mitochondrial dysfunction in obesity. *Curr Opin Endocrinol Diabetes Obes*. 2010;17(5):446-52.
15. Consitt LA, Bell JA, Koves TR, Muoio DM, Hulver MW, Haynie KR, et al. Peroxisome proliferator-activated receptor-gamma coactivator-1alpha overexpression increases lipid oxidation in myocytes from extremely obese individuals. *Diabetes*. 2010;59(6):1407-15.
16. Kempf K, Hector J, Strate T, Schwarzloh B, Rose B, Herder C, et al. Immune-mediated activation of the endocannabinoid system in visceral adipose tissue in obesity. *Horm Metab Res*. 2007;39(8):596-600.

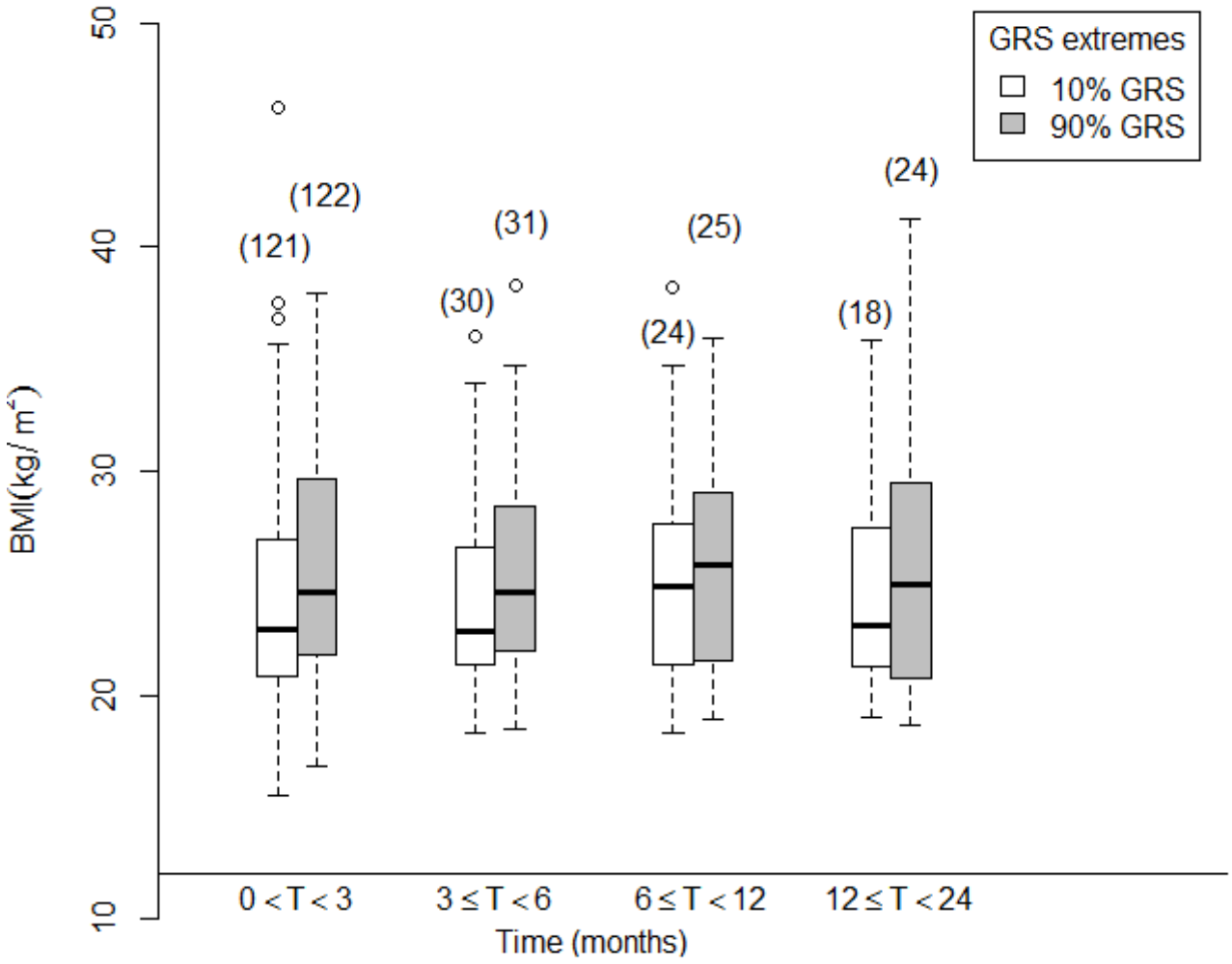
17. Monteleone P, Milano W, Petrella C, Canestrelli B, Maj M. Endocannabinoid Pro129Thr FAAH functional polymorphism but not 1359G/A CNR1 polymorphism is associated with antipsychotic-induced weight gain. *J Clin Psychopharmacol*. 2010;30(4):441-5.
18. Krapivner S, Popov S, Chernogubova E, Hellenius ML, Fisher RM, Hamsten A, et al. Insulin-induced gene 2 involvement in human adipocyte metabolism and body weight regulation. *J Clin Endocrinol Metab*. 2008;93(5):1995-2001.
19. Ingelman-Sundberg M. Pharmacogenomic biomarkers for prediction of severe adverse drug reactions. *The New England journal of medicine*. 2008;358(6):637-9.
20. Wu Z, Rosen ED, Brun R, Hauser S, Adelmant G, Troy AE, et al. Cross-regulation of C/EBP alpha and PPAR gamma controls the transcriptional pathway of adipogenesis and insulin sensitivity. *Mol Cell*. 1999;3(2):151-8.
21. Herken H, Erdal M, Aydin N, Sengul C, Karadag F, Barlas O, et al. The association of olanzapine-induced weight gain with peroxisome proliferator-activated receptor-gamma2 Pro12Ala polymorphism in patients with schizophrenia. *DNA Cell Biol*. 2009;28(10):515-9.
22. Delahanty LM, Pan Q, Jablonski KA, Watson KE, McCaffery JM, Shuldiner A, et al. Genetic predictors of weight loss and weight regain after intensive lifestyle modification, metformin treatment, or standard care in the Diabetes Prevention Program. *Diabetes Care*. 2012;35(2):363-6.
23. Minokoshi Y, Kim YB, Peroni OD, Fryer LG, Muller C, Carling D, et al. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature*. 2002;415(6869):339-43.
24. Jassim G, Ferno J, Theisen FM, Haberhausen M, Christoforou A, Havik B, et al. Association study of energy homeostasis genes and antipsychotic-induced weight gain in patients with schizophrenia. *Pharmacopsychiatry*. 2011;44(1):15-20.
25. Braun A, Trigatti BL, Post MJ, Sato K, Simons M, Edelberg JM, et al. Loss of SR-BI expression leads to the early onset of occlusive atherosclerotic coronary artery disease, spontaneous myocardial infarctions, severe cardiac dysfunction, and premature death in apolipoprotein E-deficient mice. *Circ Res*. 2002;90(3):270-6.
26. Libert S, Pointer K, Bell EL, Das A, Cohen DE, Asara JM, et al. SIRT1 activates MAO-A in the brain to mediate anxiety and exploratory drive. *Cell*. 2011;147(7):1459-72.
27. Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB. The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. *J Clin Invest*. 1995;95(5):2111-9.
28. Wang YC, Bai YM, Chen JY, Lin CC, Lai IC, Liou YJ. Genetic association between TNF-alpha -308 G>A polymorphism and longitudinal weight change during clozapine treatment. *Hum Psychopharmacol*. 2010;25(4):303-9.
29. Hellstrom L, Rossner S, Hagstrom-Toft E, Reynisdottir S. Lipolytic catecholamine resistance linked to alpha 2-adrenoceptor sensitivity--a metabolic predictor of weight loss in obese subjects. *Int J Obes Relat Metab Disord*. 1997;21(4):314-20.
30. Sickert L, Muller DJ, Tiwari AK, Shaikh S, Zai C, De Souza R, et al. Association of the alpha 2A adrenergic receptor -1291C/G polymorphism and antipsychotic-induced weight gain in European-Americans. *Pharmacogenomics*. 2009;10(7):1169-76.
31. Park YM, Chung YC, Lee SH, Lee KJ, Kim H, Byun YC, et al. Weight gain associated with the alpha2a-adrenergic receptor -1,291 C/G polymorphism and olanzapine treatment. *Am J Med Genet B Neuropsychiatr Genet*. 2006;141B(4):394-7.
32. Cota D, Marsicano G, Tschop M, Grubler Y, Flachskamm C, Schubert M, et al. The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *J Clin Invest*. 2003;112(3):423-31.

33. Tiwari AK, Zai CC, Likhodi O, Lisker A, Singh D, Souza RP, et al. A common polymorphism in the cannabinoid receptor 1 (CNR1) gene is associated with antipsychotic-induced weight gain in Schizophrenia. *Neuropsychopharmacology*. 2010;35(6):1315-24.
34. Vazquez-Roque MI, Camilleri M, Vella A, Carlson P, Laugen J, Zinsmeister AR. Association of genetic variation in cannabinoid mechanisms and gastric motor functions and satiation in overweight and obesity. *Neurogastroenterol Motil*. 2011;23(7):637-e257.
35. Kuo DY. Co-administration of dopamine D1 and D2 agonists additively decreases daily food intake, body weight and hypothalamic neuropeptide Y level in rats. *J Biomed Sci*. 2002;9(2):126-32.
36. Hong CJ, Liou YJ, Bai YM, Chen TT, Wang YC, Tsai SJ. Dopamine receptor D2 gene is associated with weight gain in schizophrenic patients under long-term atypical antipsychotic treatment. *Pharmacogenet Genomics*. 2010;20(6):359-66.
37. Gasque G, Conway S, Huang J, Rao Y, Vosshall LB. Small molecule drug screening in Drosophila identifies the 5HT2A receptor as a feeding modulation target. *Sci Rep*. 2013;3:srep02120.
38. Jeong H, Cohen DE, Cui L, Supinski A, Savas JN, Mazzulli JR, et al. Sirt1 mediates neuroprotection from mutant huntingtin by activation of the TORC1 and CREB transcriptional pathway. *Nature medicine*. 2011;18(1):159-65.
39. Parker AC, Pritchard P, Preston T, Smyth RL, Choonara I. Enhanced drug metabolism in young children with cystic fibrosis. *Archives of disease in childhood*. 1997;77(3):239-41.
40. Dubern B, Clement K. Leptin and leptin receptor-related monogenic obesity. *Biochimie*. 2012;94(10):2111-5.
41. Ellingrod VL, Bishop JR, Moline J, Lin YC, Miller dD. Leptin and leptin receptor gene polymorphisms and increases in body mass index (BMI) from olanzapine treatment in persons with schizophrenia. *Psychopharmacol Bull*. 2007;40(1):57-62.
42. Lu JF, Zhou Y, Huang GH, Jiang HX, Hu BL, Qin SY. Association of ADIPOQ polymorphisms with obesity risk: A meta-analysis. *Hum Immunol*. 2014;75(10):1062-8.
43. Voight BF, Scott LJ, Steinthorsdottir V, Morris AP, Dina C, Welch RP, et al. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. *Nat Genet*. 2010;42(7):579-89.
44. Cross-Disorder Group of the Psychiatric Genomics C, Smoller JW, Craddock N, Kendler K, Lee PH, Neale BM, et al. Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. *Lancet*. 2013;381(9875):1371-9.

S1 Fig. Relationship between weighted genetic risk score and number of alleles (unweighted genetic risk score)



S2 Fig. Evolution of Body Mass Index between Genetic Risk Score extreme percentiles (10% and 90%):



Boxplots show median values of BMI for each time of the treatment duration (solid horizontal line), 25th and 75th percentile values (box outline), the lowest and upper value within 1.5 Interquartile range (whiskers) and outlier values (open circles). (n) corresponds to individuals.

Supplementary files. Project III: Clinical and Genetic factors influencing Body Mass Index and risk prediction of weight gain in Solid Organ Transplant populations

Table S1. SNP group#1 description (1)

SNP	Gene(s)	Sample A Proxy (LD, r^2)	Sample B Proxy (LD, r^2)	Alleles	Effect allele	β	SE	*P* value
rs13078807	*CADM2*			A/G	G	0.1	0.02	3.94E-11
rs1558902	*FTO*	rs1421085 (1)	rs1421085 (1)	T/A	A	0.39	0.02	4.8E-120
rs12444979	*GPRC5B*			C/T	C	0.17	0.03	2.91E-21
rs2890652	*LRP1B*	not found in Sample A	rs17834293 (0.7)	T/C	C	0.09	0.03	1.35E-10
rs10767664	*BDNF*	rs7103411 (1)	rs2030323 (1)	A/T	A	0.19	0.03	4.69E-26
rs987237	*TFAP2B*			A/G	G	0.13	0.03	2.9E-20
rs10150332	*NRXN3*	rs17109256 (1)	rs17109256 (1)	T/C	C	0.13	0.03	2.75E-11
rs571312	*MC4R*			C/A	A	0.23	0.03	6.43E-42
rs2241423	*MAP2K5*			G/A	G	0.13	0.02	1.19E-18
rs11847697	*PRKD1*	not found in Sample A	rs10134820 (0.74)	C/T	T	0.17	0.05	5.76E-11
rs1514175	*TNNI3K*			G/A	A	0.07	0.02	8.16E-14
rs543874	*SEC16B*			A/G	G	0.22	0.03	3.56E-23
rs13107325	*SLC39A8*			C/T	T	0.19	0.04	1.5E-13
rs206936	*NUDT3*			A/G	G	0.06	0.02	3.02E-08
rs4836133	*ZNF608*	rs6864049 (1)	rs6864049 (1)	C/A	A	0.07	0.02	1.97E-09
rs4771122	*MTIF3*	rs9512699 (0.87)	rs1006353 (0.74)	A/G	G	0.09	0.03	9.48E-10
rs3817334	*MTCH2*	rs7124681 (1)		C/T	T	0.06	0.02	1.59E-12
rs2112347	*FLJ35779*	rs10057967 (1)		T/G	T	0.1	0.02	2.17E-13
rs2867125	*TMEM18*			C/T	C	0.31	0.03	2.77E-49
rs3810291	*TMEM160*			A/G	A	0.09	0.02	1.64E-12
rs713586	*RBJ / POMC*	rs713587 (0.97)	rs10182181 (1)	T/C	C	0.14	0.02	6.17E-22
rs2815752	*NEGR1*			A/G	A	0.13	0.02	1.61E-22
rs29941	*KCTD15*	rs29942 (1)		G/A	G	0.06	0.02	3.01E-09
rs1555543	*PTBP2*	rs10489741 (1)	rs11165643 (1)	C/A	C	0.06	0.02	3.68E-10
rs9816226	*ETV5*	not found in Sample A	rs7647305 (0.71)	T/A	T	0.14	0.03	1.69E-18
rs10938397	*GNPDA2*			A/G	G	0.18	0.02	3.78E-31
rs4929949	*RPL27A*	rs11041994 (0.97)	rs7127684 (0.93)	C/T	C	0.06	0.02	2.8E-09
rs7138803	*FAIM2*			G/A	A	0.12	0.02	1.82E-17
rs887912	*FANCL*	rs1016287 (1)		C/T	T	0.1	0.02	1.79E-12
rs2287019	*QPCTL*			C/T	C	0.15	0.03	1.88E-16
rs10968576	*LRRN6C*			A/G	G	0.11	0.02	2.65E-13
rs7359397	*SH2B1*	rs3888190 (0.97)		C/T	T	0.15	0.02	1.88E-20

Table S2. SNP group#2 description (2)

SNP	Gene(s)	Proxy Sample A (LD, r^2)	Proxy Sample B (LD, r^2)	Alleles	Effect allele	β	SE	*P* value
rs17024393	*GNAT2*; *AMPD2*			C/T	T	0.066	0.009	7.03E-14
rs11847697	*PRKD1*	not found in Sample A	rs10134820 (0.74)	T/C	C	0.049	0.008	3.99E-09
rs7899106	*GRID1*	rs11201714 (1)		G/A	A	0.04	0.007	2.96×10^{-8}
rs16851483	*RASA2*		rs2035935 (0.91)	T/G	G	0.048	0.008	3.55×10^{-10}
rs13107325	*SLC39A8*			T/C	C	0.048	0.007	1.83E-12
rs11191560	*NT5C2; CYP17A1*; *SFXN2*			C/T	T	0.031	0.005	8.45×10^{-9}
rs12429545	*OLFM4*			A/G	G	0.033	0.005	1.09E-12
rs13201877	*IFNGR1*; *OLIG3*	not found in Sample A		G/A	A	0.024	0.004	4.29×10^{-8}
rs2121279	*LRP1B*			T/C	C	0.025	0.004	2.31E-08
rs17001654	*NUP54*; *SCARB2*	rs17001561 (1)	rs17001561 (1)	G/C	C	0.031	0.005	7.76×10^{-9}
rs2207139	*TFAP2B*	rs943005 (1)	rs734597 (0.90)	G/A	A	0.045	0.004	4.13E-29
rs1460676	*FIGN*	rs10192119 (1)		C/T	T	0.021	0.004	4.98×10^{-8}
rs2245368	*PMS2L11*	not found in Sample A	bad genotype quality	C/T	T	0.032	0.006	3.19×10^{-8}
rs543874	*SEC16B*			G/A	A	0.048	0.004	2.62E-35
rs17203016	*CREB1*; *KLF7*			G/A	A	0.021	0.004	3.41×10^{-8}
rs13078960	*CADM2*	rs9852127 (0.95)	rs7622475 (0.95)	G/T	T	0.03	0.004	1.74E-14
rs12016871	*MTIF3*; *GTF3A*		rs1885988 (0.82)	T/C	C	0.03	0.005	2.29E-10
rs17094222	*HIF1AN*	rs17113301 (0.90)		C/T	T	0.025	0.004	5.94×10^{-11}
rs9914578	*SMG6*; *N29617*	rs8082647 (1)		G/C	C	0.02	0.004	2.07×10^{-8}
rs6567160	*MC4R*			C/T	T	0.056	0.004	3.93E-53
rs2176598	*HSD17B12*			T/C	C	0.02	0.004	2.97×10^{-8}
rs758747	*NLRC3*			T/C	C	0.023	0.004	7.47×10^{-10}
rs205262	*C6orf106*; *SNRPC*			G/A	A	0.022	0.004	1.75E-10
rs11126666	*KCNK3*			A/G	G	0.021	0.003	1.33×10^{-9}
rs1016287	*LINC01122*			T/C	C	0.023	0.003	2.25E-11
rs2033529	*TDRG1; LRFN2*			G/A	A	0.019	0.003	1.39×10^{-8}
rs2650492	*SBK1*; *APOBR*	not found in Sample A		A/G	G	0.021	0.004	1.92×10^{-9}
rs6465468	*ASB4*	rs2375019 (0.80)		T/G	G	0.025	0.005	4.98×10^{-8}
rs10968576	*LINGO2*			G/A	A	0.025	0.003	6.61E-14

rs1000940	*RABEP1*	rs3026101 (1)		G/A	A	0.019	0.003	1.28×10^{-8}
rs12401738	*FUBP1*; *USP33*	rs17381664 (0.83)		A/G	G	0.021	0.003	1.15E-10
rs3849570	*GBE1*	rs3772883 (1)	rs6792696 (0.70)	A/C	C	0.019	0.003	2.60×10^{-8}
rs6477694	*EPB41L4B; C9orf4*	not found in Sample A		C/T	T	0.017	0.003	2.67×10^{-8}
rs2176040	*LOC646736*; *IRS1*	rs2943641 (0.96)	rs2972143 (0.96)	A/G	G	0.024	0.004	9.99×10^{-9}
rs7138803	*BCDIN3D*; *FAIM2*			A/G	G	0.032	0.003	8.15E-24
rs7239883	*LOC284260; RIT2*			G/A	A	0.023	0.004	1.51×10^{-8}
rs657452	*AGBL4*			A/G	G	0.023	0.003	5.48×10^{-13}
rs11583200	*ELAVL4*			C/T	T	0.018	0.003	1.48×10^{-8}
rs3888190	*ATXN2L*; *SBK1*; *SULT1A2*; TUFM			A/C	C	0.031	0.003	3.14E-23
rs977747	*TAL1*			T/G	G	0.017	0.003	2.18×10^{-8}
rs3817334	*MTCH2*; *C1QTNF4*; *SPI1*; CELF1	rs7124681 (1)		T/C	C	0.026	0.003	5.15E-17
rs2080454	*CBLN1*			C/A	A	0.017	0.003	8.60×10^{-9}
rs1558902	*FTO*	rs1421085 (1)	rs1421085 (1)	A/T	T	0.082	0.003	7.51E-153
rs7715256	*GALNT10*	rs7719067 (1)		G/T	T	0.017	0.003	8.85×10^{-9}
rs492400	*PLCD4*; *CYP27A1*; USP37; *TTLL4*;*STK36*; *ZNF142*; *RQCD1*			C/T	T	0.024	0.004	6.78×10^{-9}
rs9641123	*CALCR*; *hsa-miR-653*	rs10488551 (0.93)	rs5014937 (0.70)	C/G	G	0.029	0.005	2.08×10^{-10}
rs10938397	*GNPDA2*; *GABRG1*			G/A	A	0.04	0.003	3.21E-38
rs12566985	*FPGT-TNNI3K*	rs6604872 (1)	rs1514175 (0.97)	G/A	A	0.024	0.003	3.28E-15
rs9540493	*MIR548X2*; *PCDH9*			A/G	G	0.021	0.004	4.97×10^{-8}
rs3736485	*SCG3*; *DMXL2*			A/G	G	0.018	0.003	7.41×10^{-9}
rs10182181	*ADCY3*; *POMC*; NCOA1;SH2B1; APOBR	rs713587 (1)		G/A	A	0.031	0.003	8.78E-24
rs10733682	*LMX1B*			A/G	G	0.017	0.003	1.83×10^{-8}
rs4787491	*MAPK3*; *KCTD13*; *INO80E*; *TAOK2*; *YPEL3*; *DOC2A*; *FAM57B*			G/A	G	0.022	0.004	2.70×10^{-8}
rs12286929	*CADM1*			G/A	G	0.022	0.003	1.31×10^{-12}
rs11688816	*EHBP1*	rs360791 (0.90)		G/A	G	0.017	0.003	1.89×10^{-8}
rs7141420	*NRXN3*			T/C	T	0.024	0.003	1.23E-14
rs1808579	*NPC1*; *C18orf8*	rs11663558 (1)		C/T	T	0.017	0.003	4.17E-08
rs4740619	*C9orf93*			T/C	C	0.018	0.003	4.56×10^{-9}
rs1928295	*TLR4*			T/C	C	0.019	0.003	7.91×10^{-10}
rs1167827	*HIP1*; *PMS2L3*; *PMS2P5*; *WBSCR16*			G/A	A	0.02	0.003	6.33×10^{-10}
rs2820292	*NAV1*	rs1032524 (1)		C/A	A	0.02	0.003	1.83×10^{-10}

rs12940622	*RPTOR*			G/A	A	0.018	0.003	2.49E-09
rs6804842	*RARB*			G/A	A	0.019	0.003	2.48×10^{-9}
rs2365389	*FHIT*	rs815710 (0.96)		C/T	T	0.02	0.003	1.63×10^{-10}
rs11165643	*PTBP2*	rs10489741 (1)		T/C	C	0.022	0.003	2.07E-12
rs2836754	*ETS2*			C/T	T	0.017	0.003	1.61×10^{-8}
rs3101336	N*EGR1*			C/T	T	0.033	0.003	2.66E-26
rs1441264	*MIR548A2*			A/G	G	0.017	0.003	2.96×10^{-8}
rs9925964	*KAT8;ZNF646*; *VKORC1*; *ZNF668*; *STX1B*;*FBXL19*	rs1978487 (0.98)		A/G	G	0.019	0.003	8.11×10^{-10}
rs2112347	*POC5*; *HMGCR*; *COL4A3BP*	rs10057967 (1)		T/G	G	0.026	0.003	6.19E-17
rs1528435	*UBE2E3*	rs6727573 (1)		T/C	C	0.018	0.003	1.20×10^{-8}
rs12885454	*PRKD1*	rs11625899 (1)		C/A	A	0.021	0.003	1.94×10^{-10}
rs4256980	*TRIM66*; *TUB*	rs4929927 (1)	rs7113874 (1)	G/C	C	0.021	0.003	2.90E-11
rs3810291	*ZC3H4*			A/G	G	0.028	0.004	4.81E-15
rs29941	*KCTD15*	rs29942 (1)		G/A	A	0.018	0.003	2.41E-08
rs7164727	*LOC100287559*; *BBS4*	rs9460 (0.84)		T/C	C	0.019	0.003	3.92×10^{-9}
rs10132280	*STXBP6*			C/A	A	0.023	0.003	1.14×10^{-11}
rs9400239	*FOXO3*; *HSS00296402*	not found in Sample A		C/T	T	0.019	0.003	1.61×10^{-8}
rs17405819	*HNF4G*	rs12679314 (1)		T/C	C	0.022	0.003	2.07E-11
rs7903146	*TCF7L2*			C/T	T	0.023	0.003	1.11×10^{-11}
rs6091540	*ZFP64*	rs6096969 (1)		C/T	T	0.03	0.004	2.15×10^{-11}
rs7599312	*ERBB4*			G/A	A	0.022	0.003	1.17×10^{-10}
rs9374842	*LOC285762;*	rs1329530 (1)		T/C	C	0.023	0.004	2.67×10^{-8}
rs17724992	*GDF15*; *PGPEP1*			A/G	G	0.019	0.004	3.42×10^{-8}
rs2033732	*RALYL*			C/T	T	0.019	0.004	4.89×10^{-8}
rs16951275	*M4P2K5*; *LBXCOR1*	rs4776982 (1)	rs2241420 (1)	T/C	C	0.031	0.004	1.91E-17
rs11030104	*BDNF*		rs7103411 (0.91)	A/G	G	0.041	0.004	5.56E-28
rs2287019	*QPCTL*; *GIPR*			C/T	T	0.036	0.004	4.59E-18
rs7243357	*GRP*			T/G	G	0.022	0.004	3.86×10^{-8}
rs13021737	*TMEM18*	not found in Sample A		G/A	A	0.06	0.004	1.11E-50
rs2075650	*TOMM40*; *APOE*; *APOC1*			A/G	G	0.026	0.005	1.25E-08
rs12446632	*GPRC5B*; *IQCK*	rs12444979 (0.88)		G/A	A	0.04	0.005	1.48E-18
rs1516725	*E7V5*	rs10513801 (1)	rs4234589 (1)	C/T	T	0.045	0.005	1.89E-22
rs13191362	*PARK2*	rs13202339 (1)		A/G	G	0.028	0.005	7.34×10^{-9}

rs11057405	*CLIP1*		G/A	A	0.031	0.006	2.02×10^{-8}
rs11727676	*HHIP*		T/C	C	0.036	0.006	2.55×10^{-8}
rs16907751	*ZBTB10*	not found in Sample A	C/T	T	0.047	0.009	3.89×10^{-8}

SE: Standard Error

Table S3. SNP group#3 description (3)

Gene	SNP	Position	Major/minor allele	Effect allele	β-coefficients *	Proxy Sample A (LD, r^2)	Proxy Sample B (LD, r^2)	# SNPs tagged
MSRA	rs2001338	intron-variant(dbSNP)	A/G	A	-0.0108		rs13254942 (0.89)	9
NMUR2	rs982716	utr-variant-3-prime(dbSNP)	C/T	T	-0.0033		rs17113291 (0.82)	5
FSD2	rs12592976	intron-variant(dbSNP)	T/C	T	-0.0046		rs17158366 (0.80)	1
REPIN1	rs1051760	utr-variant-3-prime(dbSNP)	A/G	A	0.0119		rs17173681 (1)	2
ANGPTL2	rs999092	intron-variant(dbSNP)	A/G	A	0.0096	rs11789486 (1)	rs2789507 (0.78)	5
LEP	rs4236625	intron-variant(dbSNP)	A/T	A	-0.0133	rs7795794 (0.88)	rs4731427 (0.80)	3
GLIS3	rs7870193	intron-variant(dbSNP)	C/T	T	0.0016	rs2791757 (0.86)	rs605571 (1)	4
GRB14	rs13000232	intron-variant(dbSNP)	G/C	C	0.0022	rs4130269 (0.95)	rs6754749 (0.90)	7
TAS2R38	rs1726866	missense(GVS)	A/G	A	0.0013		rs713598 (0.73)	2
PTRF	rs12948909	intron-variant(dbSNP)	A/C	A	-0.005	rs7223784 (1)	rs7223784 (1)	1
BCMO1	rs11865869	intron-variant(dbSNP)	A/G	A	-0.0032			1
CRP	rs1205	utr-variant-3-prime(dbSNP)	C/T	T	0.0077			0
CPE	rs1438114	intron-variant(dbSNP)	T/G	T	-0.0048			3
EXT2	rs2067787	intron-variant(dbSNP)	T/C	T	-0.0046			0
MTCH2	rs3817334	intron-variant(dbSNP)	T/C	C	0.026	rs7124681 (1)		2
SERPINA12	rs4905211	intron-variant(dbSNP)	G/A	A	-0.0027			0
H6PD	rs732950	intron-variant(dbSNP)	G/T	T	-0.0037	rs2268175 (0.93)		2
TFAP2B	rs987237	intron-variant(dbSNP)	A/G	G	0.045			0
HRASLS2	rs9943597	intron-variant(dbSNP)	A/C	A	0.0032			2

** β-coefficients are obtained from GIANT consortia*

Table S4: Weighted Genetic Risk Scores from candidate gene SNPs (SNP group#3) and their associations with BMI.

	n	Effect on BMI per additional risk allele [CI 95%]	p-value*	E. Var (%)
Sample A	938	0.01 [-0.01 - 0.03]	1.0	n.c
Sample B	118	0.05 [0.01 - 0.10]	**0.048**	1.72

E. Var: Explained Variability

CI: Confidence Interval

BMI: Body Mass Index

n.c: not calculated because of non significant association

** multiple test correction p-value*

Table S5. Weighted genetic risk scores association with BMI in Sample B when combining GWAS with candidate gene SNPs.

	n	effect on BMI per additional risk allele [CI 95%]	p-value	E. Var (%)
SNP group#1 + SNP group#3	115	0.16 [0.08 - 0.24]	0.001	4.1
SNP group#2 + SNP group#3	108	0.04 [-0.04 - 0.11]	0.11	n.c

E. Var: Explained Variability

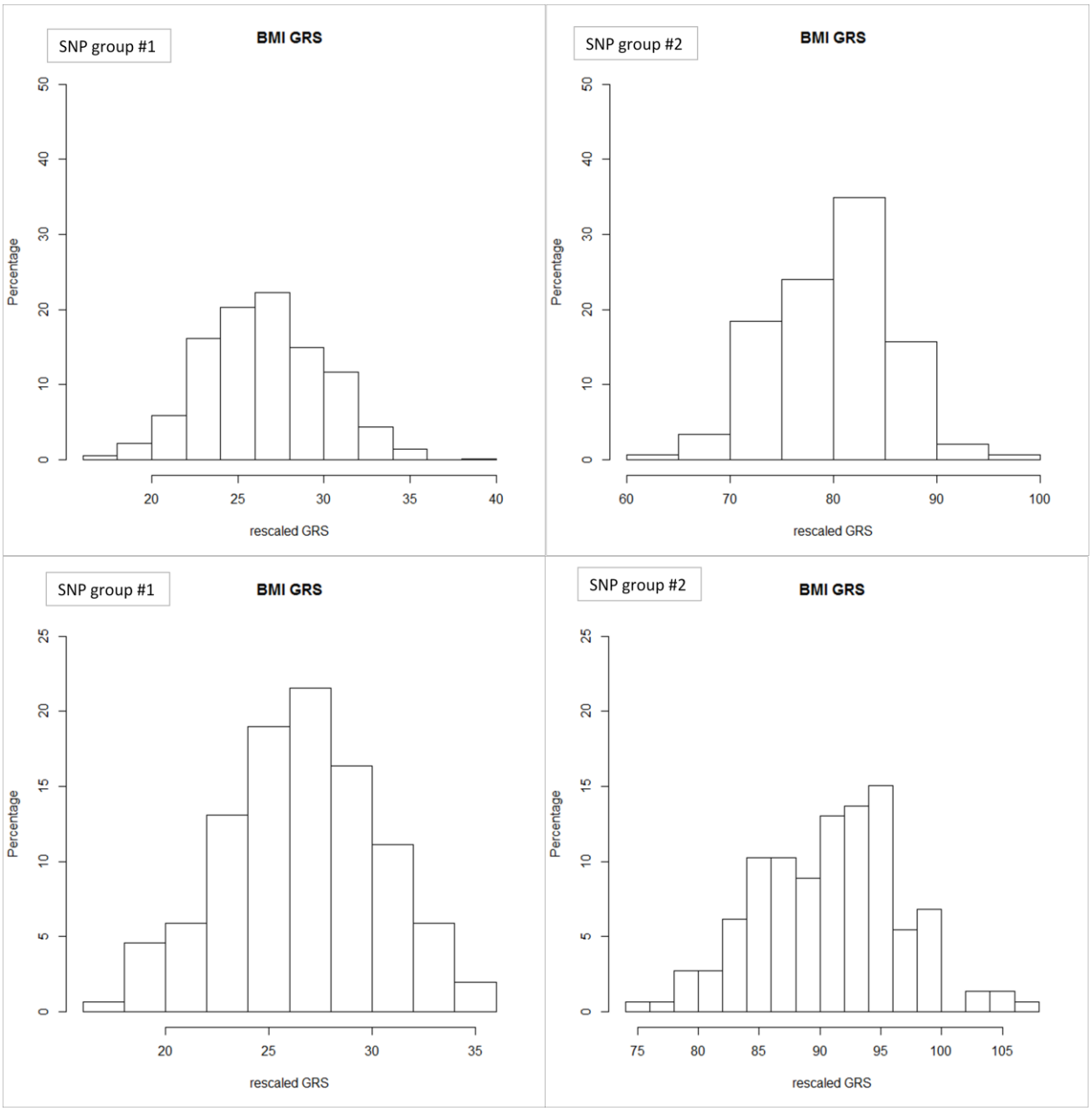
CI: Confidence Interval

BMI: Body Mass Index

SNP: Single Nucleotide Polymorphism

n.c: not calculated because of non significant association

Figure S1. Distribution of w-GRS within Samples A and B using SNP group#1 and #2



Upper: Sample A; Lower: Sample B

REFERENCES

1. Speliotes EK, Willer CJ, Berndt SI, Monda KL, Thorleifsson G, Jackson AU, et al. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. Nat Genet. 2010;42(11):937-48.
2. Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, et al. Genetic studies of body mass index yield new insights for obesity biology. Nature. 2015;518(7538):197-206.
3. Cashion A, Stanfill A, Thomas F, Xu L, Sutter T, Eason J, et al. Expression levels of obesity-related genes are associated with weight change in kidney transplant recipients. PloS one. 2013;8(3):e59962.

Supplementary files. Project IV: Genetic and clinic predictors of New Onset Diabetes Mellitus after Transplantation

Table S1. SNPs description according to the study of Mahajan et al. (1)

Gene	SNP-Risk Allele	proxy Main Sample	LD (r2)	proxy Replication Sample	LD (r2)	Major>Minor allele	MAF	position	OR	95% CI
UBE2E2	rs7612463-C			no proxy found		C>A	20.8	intron-variant(dbSNP)	1.1	[1.04 - 1.16]
TCF7L2	rs7903146-T					C>T	17.5	intron-variant(dbSNP)	1.40	[1.35-1.46]
CDKAL1	rs7756992-G					A>G	45.5	intron-variant(dbSNP)	1.20	[1.16-1.25]
HHEX - EXOC6	rs1111875-C	rs10882099	0.97			C>T	42.9	intergenic(GVS)	1.15	[1.11-1.19]
SLC30A8	rs3802177-G					G>A	29.1	utr-variant-3-prime(dbSNP)	1.16	[1.11-1.22]
IGF2BP2	rs4402960-T					G>T	34.7	intron-variant(dbSNP)	1.13	[1.09-1.17]
KCNQ1	rs163184-G					T>G	34.7	intron-variant(dbSNP)	1.09	[1.04-1.13]
HMG20A	rs7178572-G	rs1022172	0.96			G>A	49.8	intron-variant(dbSNP)	1.08	1.04-1.13
KCNJ11	rs5215-C					T>C	27.2	missense(dbSNP)	1.08	[1.04-1.12]
ZMIZ1	rs12571751-A	rs703980	1			A>G	46.7	intron-variant(dbSNP)	1.09	1.06-1.13
JAZF1	rs849135-G	rs849142	0.97			G>A	22.5	intron-variant(dbSNP)	1.12	[1.08-1.17]
CDC123 - CAMK1D	rs11257655-T					C>T	37.4	intergenic(GVS)	1.06	[1.01-1.11]
FAF1	rs17106184-G	rs1278516	1			G>A	11	intron-variant(dbSNP)	1.1	1.07-1.14
BCL6 - LPP-AS2	rs6808574-C					C>T	11.3	intergenic(GVS)	1.07	1.04-1.09
ARL15	rs702634-A					A>G	21.8	intron-variant(dbSNP)	1.06	1.04-1.09
NYAP2 - MIR5702	rs2943640-C	rs2943641	0.96			C>A	14.9	intergenic(GVS)	1.09	1.05-1.13
ADCY5	rs11717195-T	rs2877716	0.9			T>C	9.2	intron-variant(dbSNP)	1.09	[1.05-1.14]
KRT18P24 - CHCHD2P9	rs17791513-A					A>G	6	intergenic(GVS)	1.06	[1.04-1.08]
RPSAP52	rs2261181-T					C>T	10.6	intron-variant(dbSNP)	1.16	[1.10-1.23]
HNF4A	rs4812829-A	rs2144908	1			G>A	26.4	intron-variant(dbSNP)	1.07	[1.01-1.12]
TMEM154	rs6813195-C	no proxy found				C>T	0.41	intergenic(GVS)	1.08	[1.06-1.10]
SSR1	rs9505118-A					A>G	40.9	intron-variant(dbSNP)	1.06	1.04-1.08
POU5F1	rs3130501-G					G>A	27.1	intron-variant(dbSNP)	1.07	1.04-1.09
CDKN2A/B	rs10811661-T	no proxy found				T>C	0.18	intergenic(GVS)	1.18	1.13-1.24
WFS1	rs4458523-G			rs10012946	1	G>T	20.1	intron-variant(dbSNP)	1.09	1.06-1.13
HNF1B	rs4430796-G			rs11651755	0.97	A>G	46.4	intron-variant(dbSNP)	1.13	1.07-1.09
RPS3AP49 - MC4R	rs12970134-A			rs11663816 - not	1	G>A	20.9	intergenic(GVS)	1.08	1.03-1.12

				in HWE						
MPHOSPH9	rs1727313-C	rs1463877	0.9	rs1727294	1	G>C	9.9	near-gene-3(GVS)	1.21	[1.13-1.31]
PPARG	rs1801282-C	rs17036160	0.9	rs2197423	1	C>G	4.9	missense(dbSNP)	1.16	1.10-1.23
FTO	rs9936385-C			rs9923233	0.94	T>C	30.7	intron-variant(dbSNP)	1.13	1.09-1.18

MAF: Minor Allele Frequency, LD: Linkage Disequilibrium, OR: Odds Ratio, CI: Confidence Interval

Table S2. SNPs description according to the study of Voight et al. (2)

Gene	SNP-Risk Allele	proxy Main Sample	LD (r2)	proxy Replication Sample	LD (r2)	Major>Minor allele	MAF	position	OR	95% CI
KRT18P48 - DUSP9	rs5945326-A	not in HWE		no proxy found		A>G	0.21	intergenic(GVS)	1.27	[1.18-1.37]
ZBED3-AS1	rs4457053-G	rs7708285	0.93			A>G	12.4	intron-variant(dbSNP)	1.08	[1.06-1.11]
TP53INP1; LOC101927002	rs896854-T	rs7845219	0.85			C>T	45.7	intron-variant(dbSNP)	1.06	[1.04-1.09]
KCNQ1;KCNQ1OT1	rs231362-G					G>A	24.7	intron-variant(dbSNP)	1.08	[1.06-1.10]
ARAP1	rs1552224-A					A>C	7.1	utr-variant-5-prime(dbSNP)	1.14	[1.11-1.17]
ZFAND6 - FAH	rs11634397-G					G>A	33.2	near-gene-3(GVS)	1.06	[1.04-1.08]
LOC646736	rs7578326-A	rs13405357	0.85			A>G	29.8	intron-variant(dbSNP)	1.11	[1.08-1.13]
RPS3AP42 - MTNR1B	rs1387153-T					C>T	41.2	intergenic(GVS)	1.09	[1.06-1.11]
KLF14 - MIR29A	rs972283-G			rs13234407	0.97	G>A	27.9	intergenic(GVS)	1.07	[1.05-1.10]
KRT18P24 - CHCHD2P9	rs13292136-C	rs10512085	0.87	rs17791513	0.76	C>T	9.3	intergenic(GVS)	1.11	[1.07-1.15]
RNA5SP94 - MIR4432	rs243021-A			rs243083	1	G>A	47.7	intergenic(GVS)	1.08	[1.06-1.10]
RPSAP52	rs1531343-C	rs2612067	1.00	rs2612035	0.82	G>C	22.2	intron-variant(dbSNP)	1.10	[1.07-1.14]
PRC1;PRC1-AS1	rs8042680-A			rs4932182	0.64	C>A	23.5	intron-variant(dbSNP)	1.07	[1.05-1.09]
OASL	rs7957197-T	no proxy found		rs7965349	0.87	T>A	0.11	intron-variant(dbSNP)	1.07	[1.05-1.10]

MAF: Minor Allele Frequency, LD: Linkage Disequilibrium, OR: Odds Ratio, CI: Confidence Interval

Table S3. SNPs description according to the study of McCaughan et al. (3)

Gene	SNP-Risk Allele	proxy Main Sample	LD (r2)	proxy Replication Sample	LD (r2)	Major>Minor allele	MAF	position	OR	95% CI
	rs10484821	no proxy found		rs10484820	0.78	T>C	0.14	regulatory region variant	3.5	[2.1-5.8]
DNAJC16	rs7533125-C	rs4646092	1.00	no proxy found		T>C	0.26	intron-variant(dbSNP)	2.4	[1.5-3.6]
CELA2B	rs2861484-T	rs4646092	0.81	no proxy found		G>T	0.15	intron-variant(dbSNP)	2.4	[1.5-3.7]
AGMAT	rs11580170-T	rs4646092	0.81	no proxy found		C>T	0.28	missense(dbSNP)	2.2	[1.4-3.4]
CASP9	rs2020902-G			no proxy found		A>G	0.09	intron-variant(dbSNP)	2.3	[1.5-3.6]
NOX4	rs1836882-C			no proxy found		T>C	0.22	intron-variant(dbSNP)	2.7	[1.5-4.8]
none	rs198372-A			rs5065	0.56	G>A	0.08	upstream-variant-2KB(dbSNP)	2.5	[1.5-4.2]
none	rs4394754-T			no proxy found		C>T	0.2	intergenic(GVS)	2.1	[1.4-3.2]

MAF: Minor Allele Frequency, LD: Linkage Disequilibrium, OR: Odds Ratio, CI: Confidence Interval

Table S4. Odds Ratio of NODAT development in the model integrating clinical risk score and genetic risk score

covariate	Main Sample			Replication Sample		
	OR	95% CI	p-value	OR	95% CI	p-value
Clinical risk score*	1.60	1.36 – 1.90	**3.72E-08**	2.14	1.39 – 3.41	**0.0008**
Genetic risk score $^{\$}$	1.08	1.03 – 1.12	**0.0002**	1.06	0.98 – 1.16	0.17

OR: Odds Ratio, CI: Confidence Interval, w-GRS: Weighted Genetic Risk Score

* includes age, BMI≥30 kg/m^2, living donor status, glucocorticoid treatment, immunosuppressant treatment and anti Hepatitis C Virus status

$ includes weighted Genetic Risk Score with 45 SNPs in the Main Sample and 40 SNPs in the Replication Sample

Table S5. Discrimination parameters of clinical risk score and of clinical plus genetic risk scores in the main sample

NODAT: 138 non-NODAT: 492	**AUROC**	**Specificity**	**Sensitivity**	**Accuracy**	**LRT-p**	**IDI [95% CI]**		**NRI (continuous) [95% CI]**		**NNG**
Models							p-value	Net correctly reclassified	p-value	
clinical risk score model	0.66	0.73	0.52	0.69						
clinical and genetic risk scores model*	0.68	0.54	0.79	0.60	**0.005**	0.01 [0.0007 - 0.02]	**0.03**	0.27 [0.09 - 0.46]	**0.004**	n.c

n.c not calculated, LRT: Likelihood Ratio Test, IDI: Integrated Discrimination Improvement, NRI: Net Reclassification Improvement, NNG: Number Needed to Genotype, AUROC: Area Under the Receiver Operating Characteristics curve

* integrates w-GRS 2 + w-GRS 3

REFERENCES

1. Mahajan A, Go MJ, Zhang W, Below JE, Gaulton KJ, Ferreira T, et al. Genome-wide trans-ancestry meta-analysis provides insight into the genetic architecture of type 2 diabetes susceptibility. Nat Genet. 2014;46(3):234-44.
2. Voight BF, Scott LJ, Steinthorsdottir V, Morris AP, Dina C, Welch RP, et al. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. Nat Genet. 2010;42(7):579-89.
3. McCaughan JA, McKnight AJ, Maxwell AP. Genetics of new-onset diabetes after transplantation. J Am Soc Nephrol. 2014;25(5):1037-49.

246 Original article

Impact of *HSD11B1* polymorphisms on BMI and components of the metabolic syndrome in patients receiving psychotropic treatments

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Background **Metabolic syndrome (MetS) associated with psychiatric disorders and psychotropic treatments represents a major health issue. 11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) is an enzyme that catalyzes tissue regeneration of active cortisol from cortisone. Elevated enzymatic activity of 11β-HSD1 may lead to the development of MetS.**

Methods **We investigated the association between seven *HSD11B1* gene (encoding 11β-HSD1) polymorphisms and BMI and MetS components in a psychiatric sample treated with potential weight gain-inducing psychotropic drugs ($n = 478$). The polymorphisms that survived Bonferroni correction were analyzed in two independent psychiatric samples ($n_{R1} = 168$, $n_{R2} = 188$) and in several large population-based samples ($n_1 = 5338$; $n_2 = 123\,865$; $n_3 > 100\,000$).**

Results ***HSD11B1 rs846910-A*, *rs375319-A*, and *rs4844488-G* allele carriers were found to be associated with lower BMI, waist circumference, and diastolic blood pressure compared with the reference genotype ($P_{corrected} < 0.05$). These associations were exclusively detected in women ($n = 257$) with more than 3.1 kg/m^2, 7.5 cm, and 4.2 mmHg lower BMI, waist circumference, and diastolic blood pressure, respectively, in *rs846910-A*, *rs375319-A*, and *rs4844488-G* allele carriers compared with noncarriers ($P_{corrected} < 0.05$). Conversely, carriers of the *rs846906-T* allele had significantly higher waist circumference and triglycerides and lower high-density lipoprotein-cholesterol exclusively in men ($P_{corrected} = 0.028$). The *rs846906-T* allele was also associated with a higher risk of MetS at 3 months of follow-up (odds ratio: 3.31, 95% confidence interval: 1.53–7.17, $P_{corrected} = 0.014$). No association was observed between *HSD11B1* polymorphisms and BMI and MetS components in the population-based samples.**

Conclusions **Our results indicate that *HSD11B1* polymorphisms may contribute toward the development of MetS in psychiatric patients treated with potential weight gain-inducing psychotropic drugs, but do not play a significant role in the general population.** ***Pharmacogenetics and Genomics*** **25:246–258**

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Introduction

Weight gain and obesity are major health problems associated with psychiatric disorders and/or with psychotropic drug treatments, and in particular, atypical antipsychotics (AP) and some mood stabilizers (MS) [1,2]. This may have major clinical consequences considering that obesity can lead to the development of other components of the metabolic syndrome (MetS) such as dyslipidemia, hypertension, and type 2 diabetes [1], which may ultimately lead to the development of cardiovascular diseases (CVDs), reducing patients' quality of life and increasing mortality in psychiatric populations [3]. Indeed, schizophrenic patients are reported to have excess risk of mortality and 20% shorter life span compared with the general population,

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with CVDs being the leading cause of death [4]. Meta-analyses also showed nearly two times increased risk of mortality and CVDs in depressive patients [5] and regular follow-up of all components of the MetS is therefore strongly recommended in psychiatric patients receiving psychotropic drug treatments [6].

Heritability has been shown to influence individual susceptibility to overweight or obesity, both in the general population [7,8] and in psychiatric patients treated with weight-inducing psychotropic drugs [9–11]. Genome-wide association studies (GWAS) carried out to date only explain a small fraction of BMI heritability [8] and more obesity susceptibility genes remain to be discovered. Whereas genome-wide association study meta-analyses have been very useful, other approaches are also needed to further understand the biology of human obesity.

The 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) has been associated with the MetS in the general population (reviewed in Wamil and Seckl [12]). This microsomal enzyme catalyzes tissue regeneration of active cortisol from the inactive form cortisone and is highly expressed in metabolic tissues such as liver and adipose tissue and also in the central nervous system, where it amplifies the action of endogenous cortisol, which binds to glucocorticosteroids receptors [13]. Although not all patients with MetS have increased levels of cortisol [14], it is well known that increased plasma cortisol levels (as in Cushing's syndrome) are associated with visceral obesity and with other features of the MetS. Thus, increased enzymatic activity of 11β-HSD1 may also lead to the development of MetS. Indeed, mice with transgenic overexpression of 11β-HSD1 in liver or adipose tissue are hyperphagic, obese, and show other features of the MetS, especially under a high-fat diet [15,16], whereas inhibition of 11β-HSD1 ameliorates the features of MetS in obese mice [17,18]. In obese humans, there is an association between 11β-HSD1 activity in abdominal subcutaneous fat/adipose tissue and central obesity [19,20].

Human population-based studies suggest that polymorphisms within the *HSD11B1* gene, which encodes 11β-HSD1, are associated with MetS and/or its different components [21–25]. Two single nucleotide polymorphisms (SNPs), *HSD11B1 rs846910G>A* in the 5′-flanking region and *rs12086634T>G* in the third intron, were associated independently with type 2 diabetes [21], hypertension [22,23], waist circumference (WC) [23], and the MetS overall [23], but not with BMI [21,22,26–28]. Other SNPs within the *HSD11B1* showed inconsistent results with the MetS [26,28–30]. Importantly, no pharmacogenetic studies, to our knowledge, have investigated the association between *HSD11B1* SNPs and obesity or MetS components in psychiatric samples treated with psychotropic weight gain-inducing drugs.

We aimed to study the association of seven *HSD11B1* variants (*rs12565406G>T*, *rs10863782G>A*, *rs846910G>A*, *rs3753519G>A*, *rs12086634T>G*, *rs4844488A>G*, and *rs846906C>T*) with BMI, MetS, and its different components in psychiatric patients taking potential weight gain-inducing psychotropic drugs, a population known to have the higher prevalence of obesity, and hence MetS, compared with the general population.

Materials and methods

The association of *HSD11B1* variants with BMI, MetS, and its different components as defined by the National Cholesterol Education Program's Adult Treatment Panel III report (ATP III) [31] including WC, systolic and diastolic blood pressure (SBP and DBP), fasting glucose, triglycerides, and high-density lipoprotein-cholesterol (HDL-C) was investigated in the main study sample. A full description of this sample has been published elsewhere [32]. Briefly, 478 White psychiatric patients with newly prescribed aripiprazole, amisulpride, clozapine, olanzapine, quetiapine, risperidone, lithium, valproate, and/or mirtazapine were recruited prospectively since 2007 from all psychiatric wards of the Lausanne University Hospital. Sixty-two percent had already received other psychotropic treatments and were included after having switched medication. No wash-out period was required. Body weight, WC, blood pressure, and the other components of the MetS were prospectively recorded at several time points during the first 12 months of psychotropic treatment according to published recommended monitoring guidelines (i.e. before starting the current psychotropic drugs, and then at months 1, 2, 3, 6, 9, and 12) [6]. The newly introduced psychotropic drug was considered the main psychotropic medication and any other potential weight gain-inducing drugs of interest, including typical and atypical AP and MS, were classified as comedications possibly causing weight gain. The study was approved by the Psychiatry Ethics Committee of Lausanne University hospital and written informed consent was provided by all participants or by their legal representatives.

Replication samples

We attempted to replicate the results in two independent samples of White psychiatric patients [32]. The first replication sample was from a retrospective study carried out in outpatient psychiatric centers of Geneva University Hospital from 2006 to 2008. A total of 168 patients treated for more than 3 months with clozapine, olanzapine, quetiapine, risperidone, lithium, and/or valproate were included. Seventy-two percent had already received other psychotropic treatments before the current treatment. The second replication sample was also recruited from a retrospective study carried out since 2010 in two outpatient psychiatric centers of Lausanne (Lausanne University Hospital and a private psychiatric center). A total of 188 patients treated with aripiprazole, amisulpride, clozapine, olanzapine, quetiapine, risperidone, sertindole lithium, and/or valproate were included. Fifty-two percent of the

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studied sample had already received another psychotropic drug before the current treatment. For both samples, only BMI was available at different time-points during treatment; body weight and height were measured in all patients at inclusion, whereas their baseline weight before the initiation of the current treatment and/or at different times during treatment was obtained from the medical file or was self-reported (baseline weight was self-reported in 76% of the cases). As shown previously in our replication samples [32], self-reported weight was found to be a very reliable estimate of measured weight obtained from the medical files. Both studies consisted of one single visit performed during the usual clinical psychiatric follow-up. The medication with the longest treatment duration was entered in the model as the main psychotropic medication. Both studies were approved by their respective ethical committees and written informed consent was provided by all participants or by their legal representatives.

Population-based samples

Cohorte Lausannoise (CoLaus and PsyCoLaus)

Participants aged 35–75 years in this population-based study (CoLaus) were recruited between June 2003 and May 2006 as described previously [33]. The assessment included cardiovascular risk factors such as the BMI, fat mass, WC, blood pressure, blood glucose, triglycerides, and HDL-C. In addition, all Whites (91% of the sample) underwent a genetic exam ($n = 5338$). All participants of CoLaus in the age range of 35–66 years were asked to also participate in a psychiatric evaluation (PsyCoLaus) based essentially on a semistructured diagnostic interview [34]. Combined genetic and psychiatric data were available for 2990 participants. Genotyping for the CoLaus/PsyCoLaus participants was performed using the Affymetrix GeneChipR Human Mapping 500K array set.

Genetic Investigation of ANtropometric Traits (GIANT) consortium

The GIANT consortium carried out a meta-analysis of GWAS data with a discovery set of 123 865 individuals of European ancestry from 46 studies for height [35], BMI [8], and waist-to hip ratio [36].

Genome-wide associations scans for total cholesterol, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, and triglycerides

Data on lipid traits have been downloaded from the 'Genome Wide Associations Scans for Total Cholesterol, HDL-C, LDL-C and triglycerides' website [37,38], which is a meta-analysis of 46 lipid GWASs. These studies together comprise around 100 000 individuals of European descent (maximum sample size 100 184 for total cholesterol, 95 454 for LDL-C, 99 900 for HDL-C, and 96 598 for triglycerides), ascertained in the USA, Europe, or Australia.

Of note, CoLaus is part of both GIANT and 'Genome Wide Associations Scans for Total Cholesterol, HDL-C, LDL-C and triglycerides'.

Selection and genotyping of *HSD11B1* polymorphisms

Genomic DNA of psychiatric patients' was extracted from whole blood. Selection and genotyping of *HSD11B1* SNPs were carried out in two steps: first *rs846910G > A*, *rs12086634T > G* polymorphisms, which were investigated previously in the general population, were selected and genotyped using the TaqMan allelic discrimination assay (ABI PRISM 7000 Sequence Detection System; Applied Biosystems, Rotkreuz, Switzerland). TaqMan SNP Genotyping Assays ID: C___8887157_10 and ID: C__22275467_10 were used for *rs846910G > A* and *rs12086634T > G* SNPs, respectively. All reagents were obtained from Applied Biosystems, and genotyping was performed according to the manufacturer's protocol. In a second step, selection of tagging SNPs within the *HSD11B1* gene using the hapMap Genome Browser (release 28) and analyzed by haploview [39] was applied. Eight tagging SNPs (*rs12565406G > T*, *rs10863782G > A*, *rs846910G > A*, *rs3753519G > A*, *rs12086634T > G*, *rs11119328C > A*, *rs4844488A > G*, and *rs846906C > T*) were found by limiting the search to SNPs with a minor allele frequency more than 5% in the White population and an r^2 cutoff of 0.8, covering 100% of genetic variations within the *HSD11B1* gene in the HapMap Genome Browser and 87% of *HSD11B1* genetic variations in the 1000genome database [40]. Of note, both *rs846910G > A* and *rs12086634T > G* were among the tagging SNPs. These eight SNPs were customized and added to the Illumina 200K cardiometabochip [41]. All the SNPs were tested for Hardy–Weinberg equilibrium and linkage disequilibrium (LD), the latter measured by both D' and r^2. It is worth mentioning that genotypes for *rs846910G > A* and *rs12086634T > G* performed using the TaqMan method were identical to those genotyped using the cardiometabochip. For technical reasons, *HSD11B1 rs11119328* SNP could not be genotyped in the cardiometabochip; therefore, seven *HSD11B1* SNPs were finally analyzed (covering 86% of genetic variations within the *HSD11B1* gene in the HapMap Genome Browser).

Gene expression analysis

The functional effect of the two promoter SNPs (*rs846910G > A* and *rs3753519G > A*) on *HSD11B1* gene expression was investigated in a peripheral model using peripheral blood mononuclear cells (details in Supplementary data, Supplemental digital content 1, *http://links.lww.com/FPC/A815*).

Statistical analysis

Psychiatric samples

The impact of *HSD11B1* SNPs on BMI, MetS, and its components was investigated in the main psychiatric

follow-up study, in which multiple observations for each clinical variable for each patient at different time-points were measured. Because of the nonlinearity of our models and the absence of any linear transformation, these associations were assessed by fitting a Generalized Additive Mixed Model (GAMM) [42,43] to allow a smooth trend for the response in time on the basis of multiple observations for each patient (using a thin plate regression spline basis) adjusting for age, sex, smoking status, current psychotropic drug, and comedications possibly causing weight gain for BMI and WC analyses (lists of these comedications have been published in Choong *et al.* [32]), adjusted for antihypertensive drug intake for blood pressure analyses, antidiabetics for glucose analyses, and hypolipidemic drug intake for triglycerides and HDL-C analyses. A random effect at the participant level was also introduced to take the dependence structure of the observed data into account. GAMMs were fitted using the mgcv package of R (settings were fixed at package defaults). To be more conservative, the uncertainty of estimated parameters was assessed by 1000 bootstraps [44] at the participant level and the results were similar to those obtained by 10 000 bootstraps. Whenever the *P*-value for the 1000 bootstrap was lower than 0.001 ($P < 0.001$), 10 000 bootstrap analysis was carried out. If the *P*-value for the 10 000 bootstrap was lower than 0.0001 ($P < 0.0001$), 100 000 bootstrap was applied. The model is fitted on all observations of patients; thus, model coefficients provide information on both the direction and the magnitude of the overall association between BMI and different components of the MetS and the genotypes for the specific period of treatment studied. The psychotropic drugs were classified according to their therapeutic class (AP vs. MS vs. mirtazapine) [45]. Similar GAMM models were applied to test the association between *HSD11B1* SNPs and BMI in the replication samples and in the combined sample.

Because of the small number of individuals homozygous for *HSD11B1* variant alleles, the associations were analyzed using a dominant model. Stratified sex analyses were carried out systematically when analyzing the effect of *HSD11B1* polymorphisms on BMI or MetS components. The *P*-values of these models were adjusted for multiple comparisons using Bonferroni correction; for each outcome tested in the main study sample, the *P*-values were corrected by the seven studied *HSD11B1* SNPs. Both the empirical *P*-values for the GAMM models and the adjusted *P*-values are cited in the Tables and Supplementary Tables (*http://links.lww.com/FPC/A818*, *http://links.lww.com/FPC/A819*, *http://links.lww.com/FPC/A820*, *http://links.lww.com/FPC/A821*, *http://links.lww.com/FPC/A822*, *http://links.lww.com/FPC/A826*).

The χ^2-test was used to assess the risk of MetS as a whole between *HSD11B1* genotypes at baseline, 3, and 12 months of follow-up. Logistic regression was then applied adjusting for age and sex.

All the analyses were carried out using Stata 12 (StataCorp, College Station, Texas, USA) and R version 2.13.0 software (*http://www.R-project.org*). Haploview 4.2 (Daly Lab at the Broad Institute, Cambridge, Massachusetts, USA) [39] was used to define haplotype blocks and LD between different *HSD11B1* SNPs (D' and r^2).

Population-based studies

The associations of *HSD11B1* SNPs with adiposity traits (BMI, weight, WC, and fat mass), blood pressure, and glucose and lipid traits were analyzed using multivariate linear regression with allele dosage in which potential confounding factors such as age, sex, and smoking status were added as covariates in the CoLaus study. In addition, to determine whether the SNPs of interest were differentially associated with the components of the MetS in patients with and without major depressive disorder (MDD), we tested the two-way interactions between each *HSD11B1* SNP and MDD in the PsyCoLaus subsample. BMI, WC, and waist-to hip ratio were the only adiposity traits analyzed by the GIANT Consortium. Triglycerides and HDL-C were analyzed in the 'Genome Wide Associations Scans for Total Cholesterol, HDL-C, LDL-C and triglycerides' study. In both meta-analysis GWAS studies, we determined the association *P*-values for the four SNPs. For the GIANT study, sex-specific BMI associations were also available [46]. As the three population-based samples have large samples sizes and to measure the influence of each copy of the protective/risk variant allele, the association between *HSD11B1* SNPs and metabolic traits were tested in an additive model.

Results

Table 1 shows the clinical characteristics of the White samples of the main study ($n = 478$) and the two replication studies ($n_1 = 168$ and $n_2 = 188$). The prevalence of obesity was higher in the replication studies compared with the main study, which could be explained by the very long treatment duration in the former studies (Table 1). MetS was detected in almost 17% of the main psychiatric sample at baseline, and 27 and 27.5% at 3 and 12 months of the follow-up, respectively.

Table 2 shows the analyzed *HSD11B1* SNPs, their positions, and minor allele frequencies observed in the main psychiatric study sample ($n = 478$). None of the SNPs deviates from Hardy–Weinberg equilibrium and the minor allele frequencies in the psychiatric sample was comparable with those reported in HapMap for Whites (Table 2). Haploview analyses defined two haplotype blocks formed from *rs12565406–rs10863782* and *rs846910–rs3753519* SNPs (Supplementary Fig. 1, Supplemental digital content 2, *http://links.lww.com/FPC/A816*). Only *rs10863782* and *rs3753519* SNPs were in considerable LD ($r^2 = 0.58$) (Supplementary Fig. 1b, Supplemental digital content 2, *http://links.lww.com/FPC/A816*).

Genotype frequencies in the main psychiatric sample, the replication samples, and the combined sample are shown in Supplementary Table 1 (Supplemental digital content 3, *http://links.lww.com/FPC/A817*).

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Table 1 Characteristics of the three psychiatric study samples: main study and replication studies

Characteristics	Psychiatric study sample ($n=478$)	First replication sample ($n=168$)	Second replication sample ($n=188$)
Men (%)	43.7	52.9	62.2
Age [median (range)] years	50 (12–97)	42.2 (19.5–64)	42.3 (19–69)
Diagnosis (%)			
Psychotic disorders	28.7	27.4	42.0
Mood disorders	35.4	49.4	29.8
Schizoaffective disorder	6.5	15.5	11.7
Other diagnosis	19.2	7.1	13.3
Unknown diagnosis	10.2	0.6	3.2
BMI			
Initial BMI [median (range)] (kg/m^2)[a]	23.5 (13.3–44.5)	25.2 (15.4–45.5)	24.4 (15.5–46.2)
25 ≥ initial BMI < 30 (%)[a]	22.7	36.7	31.7
Initial BMI ≥ 30 (%)[a]	15.7	15.1	15
Current BMI [median (range)] (kg/m^2)	24.2 (15.2–50.2)	28.0 (16.2–42.3)	26.5 (16.8–43.9)
25 ≥ current BMI < 30 (%)	25.6	29.8	33.5
Current BMI ≥ 30 (%)	18.7	39.9	27.6
Smoker (%)	42.0	59.5	76.4
Prescribed psychotropic drug			
Amisulpride (%)	8.2	0	10.7
Aripirazole (%)	8.8	0	7.5
Clozapine (%)	7.3	14.3	9.1
Olanzapine (%)	10.5	16.1	12.3
Quetiapine (%)	32.2	18.4	22.4
Risperidone (%)	15.9	17.3	17.6
Lithium (%)	6.9	20.2	11.8
Valproate (%)	4.8	13.7	8.6
Mirtazapine (%)	5.4	0	0
Treatment duration, [median (range)] (months)	6.0 (1.0–12.0)	27.4 (2.9–332.6)	35.7 (1.0–390.3)
Comedication possibly causing weight gain (%)	48.3	29.2	26.1

[a]Before the current psychotropic treatment.

Table 2 Genomic positions, minor allele frequencies, and deviation from Hardy–Weinberg equilibrium of *HSD11B1* single nucleotide polymorphisms in the main psychiatric study

HSD11B1 SNP	Alleles W/m	Position	MAF	HWE	MAF HapMap
rs12565406	G/T	209861086	0.088	0.33	0.085
rs10863782	G/A	209872590	0.175	0.65	0.164
rs846910	G/A	209875254	0.058	0.23	0.077
rs3753519	G/A	209875515	0.110	0.56	0.097
rs12086634	T/G	209880259	0.178	0.73	0.206
rs4844488	A/G	209885509	0.040	0.77	0.075
rs846906	C/T	209887718	0.151	0.76	0.136

HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequencies.

HSD11B1 polymorphisms in the main psychiatric study sample

Only complete observations and data on the tested variables were included in the GAMM model (different sample sizes were obtained for each clinical variable). Carriers of the variant *rs846910-A*, *rs375319-A*, and *rs4844488-G* alleles showed 2.3, 2.3, and 2.2 kg/m^2 lower BMI values, respectively, compared with patients with the wild-type genotypes [$n=450$, Bonferroni-corrected P-values ($P_{corrected}$) = 0.0014, < 0.00007, and 0.007, respectively] (Table 3). This association was exclusively detected in women ($n=257$), with more than 3.1 kg/m^2 lower BMI in *HSD11B1 rs846910-A*, *rs375319-A*, and *rs4844488-G* carriers compared with noncarriers ($P_{corrected} < 0.00007$, < 0.00007, and 0.04, respectively, explaining 3.6, 4.8, and 1.5% of BMI variance in women), whereas no association was observed among men ($n=193$, $P_{corrected} > 0.05$) (Table 3). No significant association was observed between *HSD11B1 rs12086634T > G*, *rs10863782G > A*, *rs12565406G > T*, and *rs846906C > T* SNPs and BMI, also when analyzing men and women separately ($P_{corrected} > 0.05$).

Because of sex differences of WC, the GAMM was applied for each sex separately. Similar to the findings for BMI, women ($n=255$) had 8.2 cm ($P_{corrected} = 0.00007$), 8.1 cm ($P_{corrected} < 0.00007$), and 7.5 cm ($P_{corrected} = 0.028$) lower WC among carriers of the *HSD11B1 rs846910-A*, *rs375319-A*, and *rs4844488-G* alleles, respectively, compared with noncarriers, explaining 2.8, 5.1, and 1.7% of the variance in WC (Table 4). No association was observed between these three SNPs and WC in men. Interestingly, for the *rs846906C > T* SNP, only men carrying the *T*-allele showed 4.7 cm higher WC compared with noncarriers ($n=204$, $P_{corrected} = 0.014$), explaining 2.3% of the variance in WC. No significant association was observed between *HSD11B1 rs12086634T > G*, *rs10863782G > A* and *rs12565406G > T* and WC in both sexes ($P_{corrected} > 0.05$).

No significant association was observed between *HSD11B1* SNPs and SBP in the main psychiatric group ($n=386$, $P_{corrected} > 0.05$) or on analyzing the men and women subgroups (Supplementary Table 2, Supplemental digital content 4, *http://links.lww.com/FPC/A818*). However, *rs846910G > A*, *rs375319G > A*, and *rs4844488A > G* were associated significantly with DBP. Among women ($n=219$), carriers of the *rs846910-A*, *rs375319-A*, and *rs4844488-G* alleles had 4.7 mmHg ($P_{corrected} = 0.028$), 4.2 mmHg ($P_{corrected} = 0.004$), and 7.0 mmHg ($P_{corrected} = 0.001$) lower DBP compared with noncarriers, explaining 1.3, 1.9, and 2.2% of the variance in DBP for each SNP, respectively (Supplementary Table 3, Supplemental digital content 5, *http://links.lww.com/FPC/A819*).

No significant association was observed between *HSD11B1* SNPs and fasting blood glucose in the main psychiatric group ($n=294$, $P_{corrected} > 0.05$) or on analyzing subgroups of men and women (Supplementary Table 4, Supplemental digital content 6, *http://links.lww.com/FPC/A820*). However, lipid analyses showed a significant association between *rs846906C > T* and triglycerides in the entire sample, with *T*-allele carriers having 0.29 mmol/l higher triglyceride levels compared with noncarriers ($n=312$, $P_{corrected} = 0.007$, explained variance = 1.9%). This association was exclusively observed in men, among whom carriers of the *T*-allele had 0.53 mmol/l higher triglyceride levels compared with

Table 3 Associations between *HSD11B1* single nucleotide polymorphisms in a dominant model and body mass index during follow-up in the main psychiatric study

	Main psychiatric sample				Men			Women			
BMI	*n*	*β* (95% CI)	*P*-value ($P_{corrected}$)	E. var.	*n*	*β* (95% CI)	*P*-value ($P_{corrected}$)	E. var.	*n*	*β* (95% CI)	*P*-value ($P_{corrected}$)	E. var.
rs12565406	450				193				257			
GG		Reference				Reference				Reference		
GT/TT		−0.855 (−1.71 to 0.03)	0.03 (>0.05)			−0.62 (−1.52 to 0.35)	0.16 (>0.05)			−1.01 (−2.36 to 0.72)	0.07 (>0.05)	
rs10863782	450				193				257			
GG		Reference				Reference				Reference		
GA/AA		−0.97 (−1.80 to −0.20)	0.02 (>0.05)			−0.54 (−1.49 to 0.26)	0.12 (>0.05)			−1.33 (−2.19 to −0.49)	0.01 (>0.05)	
rs846910	450				193				257			
GG		Reference				Reference				Reference		
GA/AA		−2.28 (−3.49 to −1.12)	**0.0002 (0.0014)[§]**	1.68		−0.18 (−1.70 to 1.55)	0.44 (>0.05)			−3.94 (−5.77 to −2.37)	**<0.00001 (<0.00007)[§]**	3.64
rs3753519	450				193				257			
GG		Reference				Reference				Reference		
GA/AA		−2.27 (−3.08 to −1.59)	**<0.00001 (<0.00007)[§]**	2.91		−0.92 (−2.02 to −0.02)	0.03 (>0.05)			−3.29 (−4.61 to −2.23)	**<0.00001 (<0.00007)[§]**	4.79
rs12086634	450				193				257			
TT		Reference				Reference				Reference		
TG/GG		−0.16 (−0.98 to 0.74)	0.46 (>0.05)			0.06 (−1.17 to 0.96)	0.46 (>0.05)			−0.22 (−1.34 to 0.95)	0.42 (>0.05)	
rs4844488	450				193				257			
AA		Reference				Reference				Reference		
AG/GG		−2.24 (−3.67 to −0.76)	**0.001 (0.007)**	1.17		−1.38 (−2.75 to 0.24)	0.06 (>0.05)			−3.11 (−5.76 to −1.24)	**0.006 (0.042)**	1.53
rs846906	450				193				257			
CC		Reference				Reference				Reference		
CT/TT		0.75 (−0.03 to 1.55)	0.03 (>0.05)			1.35 (0.22–2.43)	0.01 (>0.05)			0.30 (−0.80 to 1.55)	0.20 (>0.05)	

Results were obtained by fitting Generalized Additive Mixed Models for patients, controlling for age, sex (whenever appropriate), smoking status, current psychotropic drug, and comedications possibly causing weight-gain.
P-values that survived Bonferroni correction are presented in bold.
CI, confidence interval; E. Var., explained variance by the polymorphism (%), only calculated for tests that survived Bonferroni correction; $P_{corrected}$, Bonferroni-corrected *P*-value.
[§]100 000 bootstraps were used for this analysis. One thousand bootstraps were performed for the rest of the analyses.

Table 4 Associations between *HSD11B1* single nucleotide polymorphisms in a dominant model and waist circumference during follow-up in the main psychiatric study

	Main psychiatric sample				Men				Women			
Waist circumference	*n*	*β* (95% CI)	*P*-value ($P_{corrected}$)	E. var.	*n*	*β* (95% CI)	*P*-value ($P_{corrected}$)	E. var.	*n*	*β* (95% CI)	*P*-value ($P_{corrected}$)	E. var.
rs12565406					204				255			
GG		NA				Reference				Reference		
GT/TT						−1.51 (−4.03 to 2.30)	0.18 (>0.05)			−1.56 (−4.72-2.36)	0.20 (>0.05)	
rs10863782					204				255			
GG		NA				Reference				Reference		
GA/AA						−1.61 (−3.91 to 1.91)	0.19 (>0.05)			−3.59 (−6.05 to −1.07)	0.01 (>0.05)	
rs846910					204				255			
GG		NA				Reference				Reference		
GA/AA						−2.33 (−6.34 to 3.12)	0.27 (>0.05)			−8.22 (−11.64 to −4.63)	**0.00001 (0.00007)[§]**	2.82
rs3753519					204				255			
GG		NA				Reference				Reference		
GA/AA						−2.99 (−5.85 to 1.48)	0.11 (>0.05)			−8.05 (−11.11 to −4.75)	**< 0.00001 (< 0.00007)[§]**	5.09
rs12086634					204				255			
TT		NA				Reference				Reference		
TG/GG						−0.78 (−5.61 to 1.90)	0.25 (>0.05)			−0.29 (−2.96 to 2.82)	0.43 (>0.05)	
rs4844488					204				255			
AA		NA				Reference				Reference		
AG/GG						−4.83 (−9.75 to 0.91)	0.07 (>0.05)			−7.49 (−12.66 to −2.06)	**0.004 (0.028)**	1.66
rs846906					204				255			
CC		NA				Reference				Reference		
CT/TT						4.69 (1.88–8.68)	**0.002 (0.014)**	2.34		0.31 (−3.32 to 3.23)	0.39 (>0.05)	

Results were obtained by fitting Generalized Additive Mixed Models for patients, controlling for age, smoking status, current psychotropic drug, and comedications possibly causing weight-gain.
P-values that survived Bonferroni correction are presented in bold.
CI, confidence interval; E. var., explained variance by the polymorphism (%), only calculated for tests that survived Bonferroni correction; NA, non-applicable; $P_{corrected}$, Bonferroni-corrected *P*-value.
[§]100 000 bootstraps were used for this analysis. One thousand bootstraps were performed for the rest of the analyses.

Table 5 Associations between *HSD11B1* single nucleotide polymorphisms in a dominant model and body mass index during follow-up in the three combined psychiatric study samples

	Combined psychiatric samples				Men				Women			
BMI	*n*	β (95% CI)	*P*-value	E. var.	*n*	β (95% CI)	*P*-value	E. var.	*n*	β (95% CI)	*P*-value	E. var.
rs846910	802				396				406			
GG		Reference				Reference				Reference		
GA/AA		−1.42 (−2.22 to −0.56)	**0.001**	0.59		−0.25 (−1.19 to 0.65)	0.36			−2.45 (−3.66 to −1.33)	**< 0.0001**[§]	1.49
rs3753519	802				396				406			
GG		Reference				Reference				Reference		
GA/AA		−1.87 (−2.46 to −1.15)	**< 0.0001**[§]	1.89		−1.13 (−1.91 to −0.50)	**0.002**	0.85		−2.57 (−3.32 to −1.63)	**< 0.0001**[§]	3.00
rs4844488	802				396				406			
AA		Reference				Reference				Reference		
AG/GG		−0.87 (−1.89 to 0.29)	0.08			−0.97 (−2.15 to −0.05)	**0.02**	0.34		−0.78 (−2.25 to 0.82)	0.25	

Results were obtained by fitting Generalized Additive Mixed Models for patients, controlling for age, sex (whenever appropriate), smoking status, current psychotropic drug, and comedications possibly causing weight gain.
Significant *P*-values are presented in bold.
CI, confidence interval; E. Var., explained variance by the polymorphism (%), only calculated for significant tests.
[§]10 000 bootstraps were used for this analysis. 1000 bootstraps were performed for the rest of the analyses.

noncarriers ($n = 128$, $P_{corrected} = 0.028$), explaining 5.4% of variance in triglycerides (Supplementary Table 5, Supplemental digital content 7, *http://links.lww.com/FPC/A821*). No significant association was observed between the other *HSD11B1* SNPs and triglycerides, either for women or for men ($P_{corrected} > 0.05$). Because of differences in HDL-C levels between men and women, the GAMM model was used for each sex separately (Supplementary Table 6, Supplemental digital content 8, *http://links.lww.com/FPC/A822*). Interestingly, men carrying the *T*-allele of *rs846906C > T* showed 0.14 mmol/l lower HDL-C levels compared with noncarriers ($n = 126$, $P_{corrected} = 0.006$), explaining 3.4% of the variance in HDL-C. No significant association was observed between the other *HSD11B1* SNPs and HDL-C ($P_{corrected} > 0.05$).

HSD11B1 polymorphisms in the psychiatric replication studies

Only BMI data were available for the two psychiatric replication samples. The three *HSD11B1* SNPs *rs846910G > A*, *rs3753519G > A*, and *rs4844488A > G* that survived Bonferroni correction for BMI were analyzed in the replication samples (Supplementary Table 7, Supplemental digital content 9, *http://links.lww.com/FPC/A823*). For the rs*3753519G > A*, a significant association was only found in the second replication sample ($n = 184$), in which carriers of the *A*-allele had 1.3 kg/m^2 lower BMI compared with noncarriers (95% confidence interval: −2.28 to −0.31, $P = 0.01$) (Supplementary Table 7, Supplemental digital content 9, *http://links.lww.com/FPC/A823*).

No association was observed between *HSD11B1 rs846910G > A* or *rs4844488A > G* SNPs and BMI in the two replication samples. The lower frequency of female participants in both replication samples (Table 1) did not explain the lack of association between these two SNPs and BMI, given that there was also no such association among women in these samples.

On combining the three psychiatric samples, a significant association was observed between *HSD11B1 rs846910G > A* and BMI in the entire sample ($n = 802$, $P = 0.001$) and in women ($n = 406$, $P < 0.0001$) (Table 5). Significant associations were also found for *rs3753519G > A* in the entire sample, as well as in men and women, whereas *rs4844488A > G* was no longer associated with BMI after adding the replication samples to the main study sample (Table 5).

We further studied the effect of *HSD11B1* SNPs on BMI between different psychotropic drugs. *HSD11B1* SNPs were mostly associated with BMI in the subgroup of patients treated with olanzapine/clozapine or with risperidone/quetiapine. No influence of *HSD11B1* SNPs was found in the subgroup of patients treated with MS (more details in Supplementary Table 8, Supplemental digital content 10, *http://links.lww.com/FPC/A824*).

HSD11B1 haplotype blocks and combinations

A haplotype block was created from *HSD11B1 rs846910* and *rs3753519*. A small increase in the effect was observed in patients carrying the variant alleles of the two SNPs compared with the other genotypes and also compared with the carriers of the variant allele of each SNP separately. More details in Supplementary Table 9, Supplemental digital content 11 (*http://links.lww.com/FPC/A825*).

HSD11B1 single nucleotide polymorphisms in newly diagnosed patients

The effect of *HSD11B1* SNPs on BMI or MetS components (mainly WC and DBP) was more pronounced in a subgroup of patients from the main psychiatric study sample who were newly diagnosed with a psychiatric disorder. Details in Supplementary Tables 10, Supplemental digital content 12 (*http://links.lww.com/FPC/A826*) and 11, Supplemental digital content 12 (*http://links.lww.com/FPC/A826*).

HSD11B1 polymorphisms and the risk of metabolic syndrome

The risk of Mets as a whole was assessed between different *HSD11B1* genotypes at three time-points: at baseline, and at 3 and 12 months of follow-up (Table 6). *rs846906C>T* was associated with a higher risk of MetS at 3 months of follow-up (21 and 43% for *rs846906-CC* and *T*-allele carriers, respectively, odds ratio: 3.31, 95% confidence interval: 1.53–7.17, $P_{corrected} = 0.014$). The same association was observed for this SNP and MetS at 12 months of follow-up, but did not survive Bonferroni correction (Table 6). None of the other *HSD11B1* SNPs were associated with the MetS. The same results were also obtained applying the criteria of the International Diabetes Federation consensus [47] (data not shown).

HSD11B1 polymorphisms in the population-based samples

No significant associations were observed between *HSD11B1* SNPs that survived Bonferroni corrections (*rs846910G>A*, *rs3753519G>A*, *rs4844488A>G*, and *rs846906C>T*) and BMI or MetS components in the CoLaus sample, including sex analyses (Supplementary Table 12 Supplemental digital content 13, *http://links.lww.com/FPC/A827*). Moreover, in PsyCoLaus, there were no two-way interactions between *HSD11B1* SNPs and MDD for the risk of the MetS or its components, that is there was no evidence for differential associations between these SNPs and MetS components according to the patients' depression status. *HSD11B1* SNPs were not associated with obesity traits in the GIANT study sample or with lipid traits in the 'Genome Wide Associations Scans for Total Cholesterol, HDL-C, LDL-C and triglycerides' study. Moreover, GIANT's sex-specific meta-analyses ($N_{men} = 60\,586$ and $N_{women} = 73\,137$) [46] do not show significant associations between the four *HSD11B1* SNPs and BMI (Supplementary Table 13, Supplemental digital content 14, *http://links.lww.com/FPC/A828*). Interestingly, even if the results were not significant, the direction of the association was similar to the psychiatric samples in most of the population-based analyses.

Gene expression analyses and *HSD11B1* polymorphism

No influence of *rs846910G>A* and *rs3753519G>A* was observed on *HSD11B1* gene expression. Details including discussion are available in Supplementary data and Supplementary Figs 2–4 (Supplemental digital content 1, *http://links.lww.com/FPC/A815*).

Discussion

Several conflicting results have been found between *HSD11B1* SNPs and MetS in the general population. However, these studies were carried out in relatively small samples with different ethnicities. The present study aimed to test whether common SNPs within the *HSD11B1* gene are associated with BMI and the MetS in a sample of psychiatric patients receiving potential weight gain-inducing psychotropic drugs, which has, to our knowledge, never been investigated. In addition, we extended our analyses to several large community samples to elucidate the real impact of *HSD11B1* SNPs in nonclinical individuals. Carriers of the variant alleles of three *HSD11B1* SNPs (*rs846910-A*, *rs3753519-A*, *and rs4844488-G*) showed lower BMI, WC, and DBP compared with the wild-type genotypes in the main psychiatric study sample. These associations were exclusively observed in women. A small increase in the effect on BMI and/or MetS components was also observed by combining two SNPs, *rs846910G>A*, and *rs3753519G>A*. In addition, men carrying the variant allele of *rs846906C>T* showed higher WC, higher triglycerides, and lower HDL-C blood levels compared with wild-type genotype. *HSD11B1* SNPs were investigated previously in population-based samples and related to the MetS, but with contradictory results. In American Indians, the variant *rs846910-A* allele was associated with diabetes mellitus ($n = 706$) [21] and higher blood pressure ($n = 918$) [22]. In contrast, in a study in Bosnian patients ($n = 86$), the *rs846910-A* allele showed a protective effect against high blood pressure [48]. This SNP also showed a protective effect in 248 White families ascertained through a proband with hypertension ($n > 800$) as it was associated with lower left ventricular mass, an independent risk factor for cardiovascular mortality [25]. In a sample of 600 women, patients who were heterozygous for *rs846910-A* and homozygous for *rs12086634T* had a higher risk of MetS; however, no data were presented for the influence of *rs846910* SNP solely with the MetS [23]. Finally, this SNP was not associated with obesity or other metabolic traits in other studies ($n = 448$, 534, 1880) [27,28,49]. These contradictory results could be explained by differences in methodology and tested samples as well as differences in the outcome in each study. *Rs3753519G>A* was investigated in only one study and *rs3753519-A* was associated strongly with obesity in

Table 6 Association between *HSD11B1* genotypes and the metabolic syndrome as defined by the National Cholesterol Education Program's Adult Treatment Panel III report in the main psychiatric sample

	At baseline			3 months			12 months		
	n (%)	OR (95% CI)	*P*-value ($P_{corrected}$)	*n* (%)	OR (95% CI)	*P*-value ($P_{corrected}$)	*n* (%)	OR (95% CI)	*P*-value ($P_{corrected}$)
rs12565406									
GG	23/135 (17)	Reference		37/137 (27)	Reference		23/87 (26)	Reference	
GT/TT	4/26 (15)	1.34 (0.39–4.602)	0.64 (>0.05)	7/26 (27)	1.18 (0.44–3.14)	0.75 (>0.05)	7/22 (32)	1.25 (0.44–3.53)	0.68 (>0.05)
rs10863782									
GG	20/111 (18)	Reference		31/116 (27)	Reference		19/75 (25)	Reference	
GA/AA	7/50 (14)	1.16 (0.42–3.19)	0.78 (>0.05)	13/47 (28)	1.11 (0.50–2.44)	0.79 (>0.05)	11/34 (32)	1.36 (0.55–3.37)	0.51 (>0.05)
rs846910									
GG	26/145 (18)	Reference		41/147 (28)	Reference		27/100 (27)	Reference	
GA/AA	1/16 (6)	0.46 (0.06–3.88)	0.48 (>0.05)	3/16 (19)	0.69 (0.18–2.66)	0.59 (>0.05)	3/9 (33)	1.32 (0.30–5.74)	0.71 (>0.05)
rs3753519									
GG	24/129 (19)	Reference		40/138 (29)	Reference		23/84 (27)	Reference	
GA/AA	3/32 (9)	0.61 (0.16–2.26)	0.46 (>0.05)	4/25 (16)	0.52 (0.16–1.68)	0.28 (>0.05)	7/25 (28)	1.02 (0.37–2.80)	0.96 (>0.05)
rs12086634									
TT	16/107 (15)	Reference		29/119 (24)	Reference		20/72 (28)	Reference	
TG/GG	11/54 (20)	1.47 (0.61–3.58)	0.39 (>0.05)	15/44 (34)	1.38 (0.63–3.03)	0.42 (>0.05)	10/37 (27)	0.88 (0.35–2.19)	0.78 (>0.05)
rs4844488									
AA	27/152 (18)	Reference		43/157 (27)	Reference		28/102 (27)	Reference	
AG/GG	0/9 (0)	NA		1/6 (17)	0.44 (0.05–4.27)	0.48 (>0.05)	2/7 (29)	0.90 (0.16–5.09)	0.91 (>0.05)
rs846906									
CC	18/117 (15)	Reference		24/117 (21)	Reference		17/79 (22)	Reference	
CT/TT	9/44 (20)	1.59 (0.62–4.09)	0.34 (>0.05)	20/46 (43)	3.31 (1.53–7.18)	**0.002 (0.014)**	13/30 (43)	3.17 (1.25–8.06)	**0.02 (>0.05)**

Odds ratios (OR) and *P*-values were adjusted for age and sex.
P-values that survived Bonferroni correction are presented in bold.
The NCEP ATP III panel defined metabolic syndrome as the presence of three or more of the following risk determinants: (a) increased waist circumference (>102 cm for men, >88 cm for women); (b) increased triglycerides (≥150 mg/dl) or treatment with hypolipidemic agents; (c) low HDL cholesterol (<40 mg/dl in men, <50 mg/dl in women); (d) hypertension (≥130/≥85 mmHg) or treatment with antihypertensive; and (e) impaired fasting glucose (≥110 mg/dl) or treatment with antidiabetics.

children (n=534) [28]. The results on obesity are inconsistent with our results in which carriers of the *rs3753519-A* allele showed a protective effect against obesity and was associated with lower blood pressure. In addition, unlike our results, other SNPs within the *HSD11B1* gene such as *rs846910*, *rs4844488*, and *rs846906* were not associated with obesity in the former study [28]. This discrepancy could be explained by the fact that the former study was carried out in healthy children, whereas ours included mostly adult psychiatric patients treated with potential weight gain-inducing drugs. *HSD11B1 rs4844488* was analyzed in few studies, and no significant association was found between this SNP and BMI and/or MetS components [25,28,50]. Finally, in our study, *HSD11B1 rs846906C>T* was the only SNP associated with increased WC, triglycerides and decreased HDL-C, and exclusively in men. In addition, the *rs846906C>T* was associated with an increased risk of MetS at 3 months of follow-up. Only a few publications have analyzed this intronic SNP and no association was found with the tested phenotypes [25,28,51,52].

In the present study, three *HSD11B1* SNPs were associated strongly with BMI and MetS components in the subgroup of psychiatric women taking psychotropic drugs. The adipose tissue is a well-known source of estrogen production through aromatization of androgens [53,54]. A direct relationship between aromatase activity and body weight was also proposed [55,56]. In addition, a dual relationship in the production of estrogen and cortisol in the adipose tissue was suggested [56], in which estrogen may increase cortisone to cortisol conversion mediated by 11β-HSD1 and cortisol may increase aromatase activity, producing more estrogen in the tissues [57]. However, we cannot explain the findings between *rs846906C>T* and lipid traits and WC in men.

The association between *HSD11B1* SNPs and BMI was mainly observed in the main psychiatric study and was only partially observed in the replication samples. The main psychiatric sample has a shorter treatment duration, includes relatively newly treated patients, and has lower initial BMI compared with the replication samples, which have a longer treatment duration and a longer history of psychiatric disorder (Table 1). Interestingly, when investigating specifically the subgroup of newly diagnosed patients with psychiatric illness at the same year of study inclusion and started psychotropic treatment within the first year following the first psychiatric diagnosis, a stronger association was observed between *HSD11B1* SNPs and BMI or MetS components during the follow-up, suggesting a role of *HSD11B1* SNPs early in the psychiatric disorder and/or during the psychotropic medication. The effect of these SNPs might disappear after years of psychiatric illness and/or treatment with psychotropic drugs, with the majority of patients being overweight or obese.

In the present paper, we observed a significant association between the *HSD11B1* SNPs and BMI or MetS components in the clinical, but not in the population-based samples. Previous data suggest a role of glucocorticoids

and the hypothalamic–pituitary–adrenal (HPA) axis in the development of psychosis and/or depression. Animal studies showed an influence of 11ß-HSD1 on the regulation of the HPA axis [58,59]. In humans, 11ß-HSD1 was found to be expressed in the hypothalamus, suggesting not only a role in the modulation of glucocorticoids feedback of the HPA axis but also a possible regulatory effect on metabolism and appetite [60]. In addition, *HSD11B1 rs11119328* SNP was found to be associated with increased susceptibility to depression and with increased late-night cortisol levels and in postmenopausal women with higher androstenedione levels [52]. Altogether, these data suggest a possible role of the 11ß-HSD1 in the development of psychiatric disorders. Given the low proportion of patients with severe psychiatric disorders and psychotropic medication in the community, the discrepant results according to the recruitment source suggest that the effect of the *HSD11B1* gene on BMI and MetS is restricted to severe psychiatric disorders and/or patients treated with AP or MS. This hypothesis is in line with our recent study showing a stronger association between polymorphisms within the *cAMP-regulated transcriptional coactivator 1* (*CRTC1*) gene and obesity markers (BMI and fat mass) in psychiatry compared with population-based samples, even though the former sample size is much smaller than the latter [32]. The *CRTC1* genetic polymorphism explains up to 9% of BMI variance in young psychiatric women [32]. Another example is the *fat mass and obesity associated* (*FTO*) gene in which polymorphisms within this gene showed significant associations with obesity in two cohorts of depressive patients, but not in healthy controls [61]. SNPs in the *melanocortin 4 receptor* (*MC4R*) gene were also associated significantly with weight gain in four independent small psychiatric populations [11] and showed a small effect in the population-based samples [8]. Altogether, these data suggest that psychiatric disorders and/or psychotropic treatments seem to unravel the importance of selected genes involved in obesity and the effect of these polymorphisms could be observed even in small psychiatric sample sizes compared with the population-based samples.

Several limitations of this study need to be acknowledged. Hormonal measurements were not available for our samples; thus, the interaction between estrogen and *HSD11B1* variants could not be explored. This study was restricted to patients of White origin and the results cannot be generalized to other ethnic groups. Finally, our gene expression analysis did not show a functional activity of the two SNPs and further studies, in particular, with adipocytes and/or peripheral blood mononuclear cells from psychiatric patients, are needed to elucidate the biochemical mechanisms underlying the associations observed.

In conclusion, this is the first pharmacogenetic study relating genetic polymorphisms within *HSD11B1* and BMI and/or MetS and its components in psychiatric patients. Previous studies failed to associate *HSD11B1* SNPs with BMI and/or WC in different population-based samples and showed many conflicting results for the other MetS traits. In the present psychiatric sample treated with potential weight gain-inducing psychotropic drugs, *HSD11B1* SNPs were significantly associated with BMI and metabolic traits, especially in women and in newly drug-treated patients. In addition, in several very large population-based samples, we could not show an impact of *HSD11B1* SNPs on BMI and MetS traits, showing that these SNPs do not play an important role in the general population. Further studies are needed to determine the mechanism by which *HSD11B1* SNPs influence obesity and other metabolic disturbances in psychiatric patients treated with psychotropic drugs.

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Conflicts of interest

C.B.E. has received honoraria for conferences or teaching CME courses from Advisis, Astra Zeneca, Essex Chemie, Lundbeck, MSD, Sandoz, Servier, and Vifor-Pharma in the past 3 years. A.V.G. has received honoraria for a conference or workshop participation from Vifor and Bayer Sheringer in the past 3 years. G.W. has received honoraria from Lilly, Novartis, GSK, and MSD for talks. M.P. has received honoraria for conferences or teaching CME courses from Astra Zeneca, Lundbeck, Servier SA, and swissprofessionalmedia AG in the past 3 years. For the remaining authors there are no conflicts of interest.

References

1 Yumru M, Savas HA, Kurt E, Kaya MC, Selek S, Savas E, *et al.* Atypical antipsychotics related metabolic syndrome in bipolar patients. *J Affect Disord* 2007; **98**:247–252.

2 Berkowitz RI, Fabricatore AN. Obesity, psychiatric status, and psychiatric medications. *Psychiatr Clin North Am* 2011; **34**:747–764.
3 Isomaa B, Almgren P, Tuomi T, Forsén B, Lahti K, Nissén M, *et al.* Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care* 2001; **24**:683–689.
4 Brown S. Excess mortality of schizophrenia. A meta-analysis. *Br J Psychiatry* 1997; **171**:502–508.
5 Cuijpers P, Vogelzangs N, Twisk J, Kleiboer A, Li J, Penninx BW. Differential mortality rates in major and subthreshold depression: meta-analysis of studies that measured both. *Br J Psychiatry* 2013; **202**:22–27.
6 American Diabetes Association, American Psychiatric Association, American Association of Clinical Endocrinologists, North American Association for the Study of Obesity. Consensus development conference on antipsychotic drugs and obesity and diabetes. *Diabetes Care* 2004; **27**:596–601.
7 Dubois L, Ohm Kyvik K, Girard M, Tatone-Tokuda F, Pérusse D, Hjelmborg J, *et al.* Genetic and environmental contributions to weight, height, and BMI from birth to 19 years of age: an international study of over 12,000 twin pairs. *PLoS One* 2012; **7**:e30153.
8 Speliotes EK, Willer CJ, Berndt SI, Monda KL, Thorleifsson G, Jackson AU, *et al.* Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nat Genet* 2010; **42**:937–948.
9 Ryan MC, Flanagan S, Kinsella U, Keeling F, Thakore JH. The effects of atypical antipsychotics on visceral fat distribution in first episode, drug-naive patients with schizophrenia. *Life Sci* 2004; **74**:1999–2008.
10 Correll CU, Malhotra AK. Pharmacogenetics of antipsychotic-induced weight gain. *Psychopharmacology (Berl)* 2004; **174**:477–489.
11 Malhotra AK, Correll CU, Chowdhury NI, Müller DJ, Gregersen PK, Lee AT, *et al.* Association between common variants near the melanocortin 4 receptor gene and severe antipsychotic drug-induced weight gain. *Arch Gen Psychiatry* 2012; **69**:904–912.
12 Wamil M, Seckl JR. Inhibition of 11beta-hydroxysteroid dehydrogenase type 1 as a promising therapeutic target. *Drug Discov Today* 2007; **12**:504–520.
13 Tomlinson JW, Walker EA, Bujalska IJ, Draper N, Lavery GG, Cooper MS, *et al.* 11beta-hydroxysteroid dehydrogenase type 1: a tissue-specific regulator of glucocorticoid response. *Endocr Rev* 2004; **25**:831–866.
14 Walker BR, Soderberg S, Lindahl B, Olsson T. Independent effects of obesity and cortisol in predicting cardiovascular risk factors in men and women. *J Intern Med* 2000; **247**:198–204.
15 Masuzaki H, Paterson J, Shinyama H, Morton NM, Mullins JJ, Seckl JR, Flier JS. A transgenic model of visceral obesity and the metabolic syndrome. *Science* 2001; **294**:2166–2170.
16 Paterson JM, Morton NM, Fievet C, Kenyon CJ, Holmes MC, Staels B, *et al.* Metabolic syndrome without obesity: hepatic overexpression of 11beta-hydroxysteroid dehydrogenase type 1 in transgenic mice. *Proc Natl Acad Sci U S A* 2004; **101**:7088–7093.
17 Morton NM, Holmes MC, Fiévet C, Staels B, Tailleux A, Mullins JJ, Seckl JR. Improved lipid and lipoprotein profile, hepatic insulin sensitivity, and glucose tolerance in 11beta-hydroxysteroid dehydrogenase type 1 null mice. *J Biol Chem* 2001; **276**:41293–41300.
18 Wang SJ, Birtles S, de Schoolmeester J, Swales J, Moody G, Hislop D, *et al.* Inhibition of 11beta-hydroxysteroid dehydrogenase type 1 reduces food intake and weight gain but maintains energy expenditure in diet-induced obese mice. *Diabetologia* 2006; **49**:1333–1337.
19 Desbriere R, Vuaroqueaux V, Achard V, Boullu-Ciocca S, Labuhn M, Dutour A, Grino M. 11beta-hydroxysteroid dehydrogenase type 1 mRNA is increased in both visceral and subcutaneous adipose tissue of obese patients. *Obesity (Silver Spring)* 2006; **14**:794–798.
20 Paulsen SK, Pedersen SB, Fisker S, Richelsen B. 11Beta-HSD type 1 expression in human adipose tissue: impact of gender, obesity, and fat localization. *Obesity (Silver Spring)* 2007; **15**:1954–1960.
21 Nair S, Lee YH, Lindsay RS, Walker BR, Tataranni PA, Bogardus C, *et al.* 11beta-hydroxysteroid dehydrogenase type 1: genetic polymorphisms are associated with Type 2 diabetes in Pima Indians independently of obesity and expression in adipocyte and muscle. *Diabetologia* 2004; **47**:1088–1095.
22 Franks PW, Knowler WC, Nair S, Koska J, Lee YH, Lindsay RS, *et al.* Interaction between an 11beta HSD1 gene variant and birth era modifies the risk of hypertension in Pima Indians. *Hypertension* 2004; **44**:681–688.
23 Gambineri A, Tomassoni F, Munarini A, Stimson RH, Mioni R, Pagotto U, *et al.* A combination of polymorphisms in HSD11B1 associates with in vivo 11{beta}-HSD1 activity and metabolic syndrome in women with and without polycystic ovary syndrome. *Eur J Endocrinol* 2011; **165**:283–292.
24 Gambineri A, Vicennati V, Genghini S, Tomassoni F, Pagotto U, Pasquali R, Walker BR. Genetic variation in 11beta-hydroxysteroid dehydrogenase type 1 predicts adrenal hyperandrogenism among lean women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2006; **91**:2295–2302.
25 Rahman TJ, Mayosi BM, Hall D, Avery PJ, Stewart PM, Connell JM, *et al.* Common variation at the 11-beta hydroxysteroid dehydrogenase type 1 gene is associated with left ventricular mass. *Circ Cardiovasc Genet* 2011; **4**:156–162.
26 Moon SS, Lee YS, Kim JG, Kim SW, Jeong JY, Jeon EJ, *et al.* Relationship of 11beta-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase gene polymorphisms with metabolic syndrome and type 2 diabetes. *Endocr J* 2011; **58**:949–959.
27 Duran-Gonzalez J, Ortiz I, Gonzales E, Ruiz N, Ortiz M, Gonzalez A, *et al.* Association study of candidate gene polymorphisms and obesity in a young Mexican-American population from South Texas. *Arch Med Res* 2011; **42**:523–531.
28 Olza J, Gil-Campos M, Leis R, Ruperez AI, Tojo R, Canete R, *et al.* A gene variant of 11beta-hydroxysteroid dehydrogenase type 1 is associated with obesity in children. *Int J Obes* 2012; **36**:1558–1563.
29 Smit P, Dekker MJ, de Jong FJ, van den Beld AW, Koper JW, Pols HA, *et al.* Lack of Association of the 11beta-hydroxysteroid dehydrogenase type 1 gene 83,557insA and hexose-6-phosphate dehydrogenase gene R453Q polymorphisms with body composition, adrenal androgen production, blood pressure, glucose metabolism, and dementia. *J Clin Endocrinol Metab* 2007; **92**:359–362.
30 Ku YH, Koo BK, Kwak SH, Cho YM, Shin HD, Lee HK, *et al.* Regulatory effect of common promoter polymorphisms on the expression of the 11beta-hydroxysteroid dehydrogenase type 1 gene. *Horm Res* 2009; **72**:25–32.
31 Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C. American Heart Association, *et al.* Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation* 2004; **109**:433–438.
32 Choong E, Quteineh L, Cardinaux JR, Gholam-Rezaee M, Vandenberghe F, Dobrinas M, *et al.* Influence of CRTC1 polymorphisms on body mass index and fat mass in psychiatric patients and the general adult population. *JAMA Psychiatry* 2013; **70**:1011–1019.
33 Firmann M, Mayor V, Vidal PM, Bochud M, Pécoud A, Hayoz D, *et al.* The CoLaus study: a population-based study to investigate the epidemiology and genetic determinants of cardiovascular risk factors and metabolic syndrome. *BMC Cardiovasc Disord* 2008; **8**:6.
34 Preisig M, Waeber G, Vollenweider P, Bovet P, Rothen S, Vandeleur C, *et al.* The PsyCoLaus study: methodology and characteristics of the sample of a population-based survey on psychiatric disorders and their association with genetic and cardiovascular risk factors. *BMC Psychiatry* 2009; **9**:9.
35 Lango Allen H, Estrada K, Lettre G, Berndt SI, Weedon MN, Rivadeneira F, *et al.* Hundreds of variants clustered in genomic loci and biological pathways affect human height. *Nature* 2010; **467**:832–838.
36 Heid IM, Jackson AU, Randall JC, Winkler TW, Qi L, Steinthorsdottir V, *et al.* Meta-analysis identifies 13 new loci associated with waist-hip ratio and reveals sexual dimorphism in the genetic basis of fat distribution. *Nat Genet* 2010; **42**:949–960.
37 Genome wide associations scans for total cholesterol, HDL-C, LDL-C and triglycerides. Available at: *http://www.sph.umich.edu/csg/abecasis/public/lipids2010/*. [Accessed 14 January 2014].
38 Teslovich TM, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, Koseki M, *et al.* Biological, clinical and population relevance of 95 loci for blood lipids. *Nature* 2010; **466**:707–713.
39 Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; **21**:263–265.
40 Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, *et al.* 1000 Genomes Project Consortium. An integrated map of genetic variation from 1,092 human genomes. *Nature* 2012; **491**:56–65.
41 Voight BF, Kang HM, Ding J, Palmer CD, Sidore C, Chines PS, *et al.* The metabochip, a custom genotyping array for genetic studies of metabolic, cardiovascular, and anthropometric traits. *PLoS Genet* 2012; **8**:e1002793.
42 Lin X, Zhang D. Inference in generalized additive mixed models by using smoothing splines. *J R Statist Soc B* 1999; **61**:381–400.
43 Wood SN. GAMMs with R. In: Carlin BP, Chatfield C, Tanner M, Zidek J, editors. *Generalized additive models – an introduction with R Broken*. Boca Raton, Florida, USA: Chapman & Hall/CRC; 2006. pp. 319–324.
44 Davison AC, Hinkley DV. *Bootstrap methods and their application*. Cambridge, NY: Cambridge University Press; 1997.
45 Morant J. Swiss Drug Compendium, Vol 31e. Basel, Switzerland: Documed S. A; 2010. p. 3-A 5257.
46 Randall JC, Winkler TW, Kutalik Z, Berndt SI, Jackson AU, Monda KL, *et al.* Sex-stratified genome-wide association studies including 270,000 individuals show sexual dimorphism in genetic loci for anthropometric traits. *PLoS Genet* 2013; **9**:e1003500.

47 The IDF consensus woldwide definition of the metabolic syndrome. 2009. Available at: *http://www.idf.org*. [Accessed January 2014].

48 Dujic T, Bego T, Mlinar B, Semiz S, Malenica M, Prnjavorac B, *et al*. Association between 11beta-hydroxysteroid dehydrogenase type 1 gene polymorphisms and metabolic syndrome in Bosnian population. *Biochem Med (Zagreb)* 2012; **22**:76–85.

49 Iwai N, Tago N, Yasui N, Kokubo Y, Inamoto N, Tomoike H, Shioji K. Genetic analysis of 22 candidate genes for hypertension in the Japanese population. *J Hypertens* 2004; **22**:1119–1126.

50 Feldman K, Szappanos A, Butz H, Grolmusz V, Majnik J, Likó I, *et al*. The rs4844880 polymorphism in the promoter region of the HSD11B1 gene associates with bone mineral density in healthy and postmenopausal osteoporotic women. *Steroids* 2012; **77**:1345–1351.

51 Chua AK, Azziz R, Goodarzi MO. Association study of CYP17 and HSD11B1 in polycystic ovary syndrome utilizing comprehensive gene coverage. *Mol Hum Reprod* 2012; **18**:320–324.

52 Dekker MJ, Tiemeier H, Luijendijk HJ, Kuningas M, Hofman A, de Jong FH, *et al*. The effect of common genetic variation in 11beta-hydroxysteroid dehydrogenase type 1 on hypothalamic-pituitary-adrenal axis activity and incident depression. *J Clin Endocrinol Metab* 2012; **97**:E233–E237.

53 Longcope C, Pratt JH, Schneider SH, Fineberg SE. Aromatization of androgens by muscle and adipose tissue in vivo. *J Clin Endocrinol Metab* 1978; **46**:146–152.

54 Perel E, Killinger DW. The interconversion and aromatization of androgens by human adipose tissue. *J Steroid Biochem* 1979; **10**:623–627.

55 Edman CD, MacDonald PC. Effect of obesity on conversion of plasma androstenedione to estrone in ovulatory and anovulator young women. *Am J Obstet Gynecol* 1978; **130**:456–461.

56 Mattsson C, Olsson T. Estrogens and glucocorticoid hormones in adipose tissue metabolism. *Curr Med Chem* 2007; **14**:2918–2924.

57 Yang K, Khalil MW, Strutt BJ, Killinger DW. 11 beta-hydroxysteroid dehydrogenase 1 activity and gene expression in human adipose stromal cells: effect on aromatase activity. *J Steroid Biochem Mol Biol* 1997; **60**:247–253.

58 Harris HJ, Kotelevtsev Y, Mullins JJ, Seckl JR, Holmes MC. Intracellular regeneration of glucocorticoids by 11beta-hydroxysteroid dehydrogenase (11beta-HSD)-1 plays a key role in regulation of the hypothalamic-pituitary-adrenal axis: analysis of 11beta-HSD-1-deficient mice. *Endocrinology* 2001; **142**:114–120.

59 Paterson JM, Holmes MC, Kenyon CJ, Carter R, Mullins JJ, Seckl JR. Liver-selective transgene rescue of hypothalamic-pituitary-adrenal axis dysfunction in 11beta-hydroxysteroid dehydrogenase type 1-deficient mice. *Endocrinology* 2007; **148**:961–966.

60 Bisschop PH, Dekker MJ, Osterthun W, Kwakkel J, Anink JJ, Boelen A, *et al*. Expression of 11beta-hydroxysteroid dehydrogenase type 1 in the human hypothalamus. *J Neuroendocrinol* 2013; **25**:425–432.

61 Rivera M, Cohen-Woods S, Kapur K, Breen G, Ng MY, Butler AW, *et al*. Depressive disorder moderates the effect of the FTO gene on body mass index. *Mol Psychiatry* 2012; **17**:604–611.

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RESEARCH ARTICLE

Influence of *MCHR2* and *MCHR2-AS1* Genetic Polymorphisms on Body Mass Index in Psychiatric Patients and In Population-Based Subjects with Present or Past Atypical Depression

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Abstract

Obesity development during psychotropic treatments represents a major health issue in psychiatry. Melanin-concentrating hormone receptor 2 (MCHR2) is a central receptor involved in energy homeostasis. *MCHR2* shares its promoter region with *MCHR2-AS1*, a long antisense non-coding RNA. The aim of this study was to determine whether tagging single nucleotide polymorphisms (tSNPs) of *MCHR2* and *MCHR2-AS1* are associated with the body mass index (BMI) in the psychiatric and in the general population. The influence of *MCHR2* and *MCHR2-AS1* tSNPs on BMI was firstly investigated in a discovery psychiatric sample ($n_1 = 474$). Positive results were tested for replication in two other psychiatric samples ($n_2 = 164$, $n_3 = 178$) and in two population-based samples (CoLaus, $n_4 = 5409$; GIANT, $n_5 = 113809$). In the discovery sample, TT carriers of rs7754794C>T had 1.08 kg/m^2 ($p = 0.04$) lower BMI as compared to C-allele carriers. This observation was replicated in an independent psychiatric sample (-2.18 kg/m^2; $p = 0.009$). The association of rs7754794C>T and BMI seemed stronger in subjects younger than 45 years (median of age). In the population-based sample, a moderate association was observed (-0.17 kg/m^2; $p = 0.02$) among

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younger individuals (<45y). Interestingly, this association was totally driven by patients meeting lifetime criteria for atypical depression, i.e. major depressive episodes characterized by symptoms such as an increased appetite. Indeed, patients with atypical depression carrying rs7754794-TT had 1.17 kg/m^2 ($p = 0.04$) lower BMI values as compared to C-allele carriers, the effect being stronger in younger individuals (-2.50 kg/m^2; $p = 0.03$; interaction between rs7754794 and age: p-value = 0.08). This study provides new insights on the possible influence of *MCHR2* and/or *MCHR2-AS1* on obesity in psychiatric patients and on the pathophysiology of atypical depression.

Introduction

Compared to the general population, patients with chronic severe mental disorders have an estimated shorter life expectancy of 15 to 25 years due to the psychiatric disorder and/or physical comorbidities (i.e obesity or other metabolic disorders) but also in the use of psychotropic treatments[1]. Indeed, many antipsychotics, in particular atypical antipsychotics, and some mood stabilizers and antidepressants are associated with important weight gain[2]. The variability of weight gain observed in patients sharing similar clinical risk factors (i.e gender, age and psychotropic treatment)[3], together with the heritability of weight regulation observed in twin, adoption and family studies[4] support the key role of genetic factors in the development of obesity. Moreover, recent changes in Western lifestyle (ubiquitous access of industrial/palatable food and poor physical activity) strongly increase the influence of genetic risk factors towards the development of obesity. However, genome-wide association (GWAS) and candidate gene studies have only explained a small variance of the body mass index (BMI)[5]. Therefore, the identification of new genetic predictors for the development of obesity in psychiatric patients is not only of great interest for a better understanding of the mechanisms underlying excessive weight increase, but also for the future personalized prescription of psychotropic drugs.

The regulation of food intake, a major component in energy balance, is achieved in part by highly specialized hypothalamic neurons that are able to sense and integrate peripheral feeding cues. The exact mechanisms by which peripheral cues-related signals interact within the hypothalamus to modulate the response are only partially understood. However, in this highly complex system of regulation, some specific pathways have been characterized[6, 7]. The melanocortin pathway, in the arcuate nucleus of the hypothalamus, is a major axis through which peripheral peptides and hormones converge and act to modulate the energy balance. Recent studies have enlightened the involvement of the melanin-concentrating hormone receptor 2 (MCHR2) in the transduction of central orexigenic signals. More specifically, melanin-concentrating hormone (MCH), the agonist of MCHR2, has been shown to be a critical hypothalamic regulator involved in energy homeostasis in mammals[8, 9]. Mice lacking *MCH* gene have been observed to be lean, having decreased feeding behavior and increased energy expenditure[10]. Even though *MCHR2* is not expressed in rodents, a recent study showed that induction of *MCHR2* expression in mice protected against diet-induced obesity[11]. In humans, *MCH* was shown to be expressed in neurons of the lateral hypothalamus, an area that coincides with *MCH* receptors sites of expression[9]. Moreover, in a French general population, a linkage with childhood obesity was identified on chromosome 6q16.3-q24.2[12] and two single nucleotide polymorphisms (SNPs) within *MCHR2* were further associated with childhood obesity[13]. Of note, some atypical antipsychotics have been reported to affect

neuropeptide hormone levels involved in energy homeostasis[14–16]. Specifically, the expression of *MCH* as well as its receptors may be upregulated during antipsychotic treatments, which may enhance rewarding aspects of food[17]. Moreover, the first genome scan targeting obesity as a side effect of antipsychotics has observed an implication of the pro-melanin-concentrating hormone (*PMCH*), the precursor of *MCH*[18].

Interestingly, during the preparation of the present study, gene region analyses revealed that the *MCHR2* SNP associated with BMI in a Caucasian population-based sample (i.e *MCHR2/MCHR2-AS1* rs6925272)[13], lies not only in the promoter of *MCHR2* but also in the promoter of another gene transcribed in an antisense way, *MCHR2-AS1* (*MCHR2*-antisense RNA). *MCHR2-AS1* is a RNA gene affiliated to the long non-coding RNA (lncRNA) class. Although this class of genes is still poorly understood, recent studies have linked some lncRNAs with the development of different diseases[19–21].

Because of the high prevalence of obesity, of metabolic abnormalities and of mortality rate within the psychiatric population, the probable involvement of MCHR2 in the phenotype of obesity and the absence of studies examining the possible influence of genetic polymorphisms of *MCHR2* on BMI in psychiatric patients, we examined associations between tagging SNPs of *MCHR2* and of *MCHR2-AS1* with BMI in three independent psychiatric samples treated with psychotropic drugs that were likely to induce weight (i.e clozapine, olanzapine, quetiapine, risperidone, lithium, valproate, mirtazapine, aripiprazole and/or amisulpride). In order to further investigate whether these above-mentioned associations are valid in the general population as well or are only specific to psychiatry, we then attempted to replicate the results in two population-based samples, one of which had subjects with psychiatric evaluations.

Results

Demographic and clinical characteristics of three psychiatric Caucasian populations are presented in S1 Table. In the discovery sample, the prevalence of obesity at the end of the follow-up was lower (17%) than in both replication samples (39% and 28%), which could in part be explained by the longer treatment duration in the latter samples. The median age of patients in the discovery sample was higher (50 years) than in both replication samples (43 and 42 years), the former sample containing geriatric patients, which is not the case for both replication samples. In each of these three independent psychiatric samples, almost half of patients gained more than 5% of initial weight during the current psychotropic treatment (41%, 56% and 51%), with a median duration of treatment of 6, 27 and 35 months, respectively.

MCHR2 and *MCHR2-AS1* tagging SNPs are presented in S2 Table. rs9403322 and rs4559096 deviated from Hardy-Weinberg equilibrium in the discovery sample (p-values$\leq$0.05). These two SNPs were therefore not further analyzed. Therefore, a total of twelve SNPs were analyzed in this study. Minor allele frequencies (MAF) in our combined sample were comparable to those reported in HapMap (Caucasians).

Associations between *MCHR2* and *MCHR2-AS1* Tagging Polymorphisms and BMI in the Psychiatric Sample

In the discovery sample, three tagging SNPs of *MCHR2* (i.e. *MCHR2* rs4840109, *MCHR2* rs2001456 and *MCHR2* rs7754794) were significantly associated with BMI, with carriers of the G allele (for rs4840109), G allele (for rs2001456) and TT genotype (for rs7754794) having lower BMI values as compared to others, respectively (more details in S3 Table). Multiple comparison tests in the discovery sample using the false discovery rate method correcting for 12 independent tests revealed p-corrected-values of 0.04 for each of these three SNPs. The remaining *MCHR2* and *MCHR2-AS1* tagging SNPs were not associated with BMI. *MCHR2*

rs4840109, *MCHR2* rs2001456 and *MCHR2* rs7754794 were tested for replication in replication samples 1 and 2. P-values of replication analyses were corrected for 3 independent tests using false discovery rate correction. Both rs4840109 and rs2001456 were not replicated and were therefore not considered for further analyses. rs7754794 was significantly associated with BMI in the replication sample 1, for which carriers of TT genotype had 2.18 kg/m^2 lower BMI as compared to C-allele carriers ($p_{corrected}$ = 0.009). A significant association was also observed within the combined sample, with TT carriers having 0.84 kg/m^2 lower BMI as compared to others ($p_{corrected}$ = 0.02; Table 1). Fig 1 presents the evolution of BMI during psychotropic treatment in patients of the combined sample according to rs7754794 genotype. In carriers with the TT genotype, the BMI remained stable over time, whereas the BMI of CC or CT carriers increased along the treatment duration. The difference across genotypes reached the threshold of significance after six months of treatment.

Associations between *MCHR2* rs7754794C>T and BMI in Age-Stratified Subgroups of the Combined Sample

The influence of rs7754794 polymorphism on BMI was assessed in age-stratified psychiatric subgroups of the combined sample (interaction between rs7754794 and age: p-value = 0.08). Only patients younger than 45 years (the median of age in the psychiatric sample) appeared to be concerned with this genetic effect on BMI, with carriers of TT genotype having 1.59 kg/m^2 lower BMI as compared to others (p = 0.003) (Table 2). Of note, in the discovery sample, rs7754794-TT carriers younger than 45 years had significantly lower waist circumference (WC) as compared to others (-4.34 cm; p = 0.02; S4 Table).

MCHR2 and *MCHR2-AS1* Tagging SNPs Haplotype Analysis

Four haplotype blocks were observed (S1 Fig). Combinations formed from the first three haplotype blocks did not show any significant association with BMI in the combined psychiatric sample (data not shown). Regarding the block 4 (within *MCHR2-AS1*), by combining SNPs rs11155243, rs9484646 and rs12214805, four different combinations were formed. Wild-type carriers for these three SNPs (i.e. GGC, frequency of this combination: 0.5) had 2.02 lower unit BMI as compared to others (p = 0.04). Age-stratified analyses could not be conducted due to an insufficient number of observations.

Replication of *MCHR2* rs7754794C>T Association with BMI in Population-Based Samples

The association of *MCHR2* rs7754794 with BMI was further investigated for replication in two population-based samples (CoLaus and GIANT) using rs7749425, a proxy of rs7754794 (r^2 = 0.97). The rs7754794 association with BMI was not replicated in these samples (Table 3). However, in CoLaus, age-stratified analyses revealed that individuals younger than 45 years and carrying rs7754794-TT had a significantly lower BMI and WC than others. Of note, in order to avoid bias, the same threshold (i.e. 45 years old) was used both in psychiatric and in population-based samples. Age-stratified data were not available in GIANT.

In PsyCoLaus, the subset of CoLaus with psychiatric evaluations, stratifications according to depression subtypes revealed some differences between atypical and non-atypical subgroups. Women were more prevalent among those with atypical depression (74%) than among the other depressives (63%; S5 Table). As expected, in the former subgroup, the proportion of subjects with increased appetite, which is one of the 5 diagnostic criteria for this depression subtype, was much higher (41%) than in the latter subgroup (5%). Moreover, subjects with

Table 1. Association of *MCHR2* rs7754794C>T with BMI in three independent Caucasian psychiatric samples.

MCHR2 rs7754794C>T	Discovery sample n = 441			Replication sample 1 n = 153			Replication sample 2 n = 142			Combined sample n = 736		
	β (95% CI) (kg/m^2)	p-value	Ex. var (%)	β (95% CI) (kg/m^2)	p-value	Ex. var (%)	β (95% CI) (kg/m^2)	p-value	Ex. var (%)	β (95% CI) (kg/m^2)	p-value	Ex. var (%)
CC/CT	ref			ref			ref			ref		
TT	-1.08 (-2.11–(-) 0.35)	**0.04**	0.46	-2.18 (-3.87–(-) 1.01)	**0.009**	2.78	0.79 (-0.81–3.01)	0.42		-0.84 (-1.52–(-) 0.32)	**0.02**	0.28

Results were obtained by fitting Generalized Additive Mixed Models for patients, controlling for age, sex, smoking status, current psychotropic drug and comedications possibly causing weight-gain. β: estimate. p-value: corrected for multiple tests.Ex. var (%): explained variance by the polymorphism, only calculated for significant results. ref: reference.

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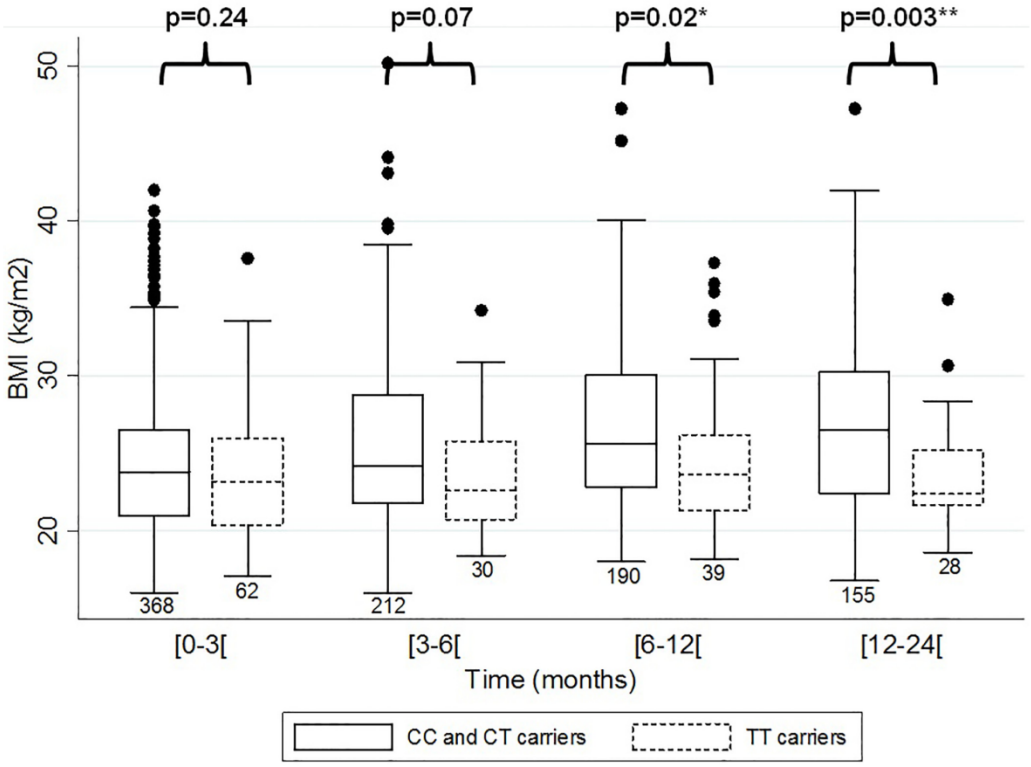

Fig 1. BMI evolution during psychotropic treatment according to protective or risk *MCHR2* rs7754794C>T genotype. Caucasian patients carrying protective (TT) or risk (CC or CT) rs7754794C>T variant. Median, interquartiles and number of observations for each box are indicated.

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Table 2. Age-stratified analysis for *MCHR2* rs7754794C>T association with BMI.

MCHR2 rs7754794C>T	Combined sample			
	n	β (95% CI) (kg/m^2)	p-value	Ex. var (%)
Age≤45	374			
CC/CT		ref		
TT		-1.59 (-2.65–(-)0.46)	**0.003**	1.11
Age>45	366			
CC/CT		ref		
TT		-0.23 (-1.09–0.75)	0.35	

Results were obtained by fitting Generalized Additive Mixed Models for patients, controlling for age (whenever appropriate), sex (whenever appropriate), smoking status, current psychotropic drug and comedications possibly causing weight-gain. β: estimate. Ex. var (%): explained variance by the polymorphism, only calculated for significant results. ref: reference.

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atypical depression were more frequently in a current episode at the moment of the evaluation than the other subjects with a major depressive disorder (28% versus 13%), which is likely to explain the higher proportion of antidepressant use (23% versus 13%, respectively). In the PsyCoLaus sample, there was no association between rs7754794 and BMI (Table 4). However, there was an interaction (p = 0.04) between the rs7754794-TT genotype and a lifetime major depressive episode with DSM-IV atypical depression regarding the BMI. Indeed, among subjects with atypical depression features, those carrying rs7754794-TT had a significantly lower BMI (-1.17 kg/m^2) as compared to C-allele carriers (p = 0.04), whereas the BMI of individuals with no history of an atypical depression was not influenced by this genetic polymorphism. Age-stratified analyses revealed that this association was also observed in individuals younger than 45 years with atypical depression (-2.50 kg/m^2; p = 0.03). Of note, no significant association was found between rs7754794 and BMI in subjects without lifetime major depressive episode (data not shown). Finally, no difference of rs7754794 frequency was observed between subgroups of diagnosis of PsyCoLaus (S6 Table). Subgroups of diagnosis were not available in the combined sample.

Discussion

The present results suggest a contribution of *MCHR2* and/or *MCHR2-AS1* in the regulation of human body weight, which is consistent with the proposed role of MCH and MCHR2 pathway in the literature[9] and with the only other genetic study on *MCHR2* which reported an association of *MCHR2* genetic polymorphism with obesity in the general population[13]. To our knowledge, this is the first study performed in psychiatric subjects, i.e. a population with a high prevalence of obesity or overweight phenotypes. Specifically, this study showed a significant association of *MCHR2/MCHR2-AS1* genetic polymorphisms with BMI in the psychiatric population as well as in a psychiatric subgroup of a population-based sample. Moreover, a haplotype combination of three *MCHR2-AS1* tagging SNPs was also significantly associated with BMI in the psychiatric population.

Interestingly, the association of *MCHR2* rs7754794 with BMI was only observed in patients younger than 45 years old. Because a first treatment exposure has been previously described as an important risk factor for important weight gain[22], it could be hypothesized that age would be a proxy of first-treatment exposure in younger patients. However, most of the young patients had already received previous psychiatric drug treatment before inclusion and there

Table 3. Replication analyses in Caucasian population-based samples.

rs7749425C>T (proxy of rs7754794C>T)	ALL SUBJECTS						≤45 years subjects					
	BMI			WC			BMI			WC		
	n	β (kg/m^2)	p-value	n	β (cm)	p-value	n	β (kg/m^2)	p-value	n	β (cm)	p-value
GIANT	113809	ref										
		0.0032	0.47		NA			NA			NA	
CoLaus	5409	ref		5409	ref		1463	ref		1463	ref	
		-0.0492	0.22		-0.0381	0.34		-0.17	**0.02**		-0.15	**0.04**

Results were obtained by using robust regression, adjusted for age and sex. BMI: body mass index; WC: waist circumference. ref: reference (i.e carriers of the C allele at rs7749425 locus). β: estimate. NA: non applicable.

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was no association between *MCHR2* rs7754794 and BMI in a subgroup of the discovery sample with a newly diagnosed psychiatric disorder (see Supporting information for further details). Therefore, *MCHR2* rs7754794 seems to be associated with BMI in younger patients independently of the psychotropic treatment exposure status. *MCHR2* has been described as one of the components acting in the hypothalamic regulation of food intake[23, 24], a system of regulation involved early in the development of obesity. Additionally, age has been found to affect appetite regulation with elderly individuals having a less efficient hypothalamic regulation of food intake[25, 26]. These elements may suggest that genetic risk factors involved in the regulation of food intake in young individuals may be more important than in the elderly. Interestingly, replication analyses in the population-based sample (CoLaus) was in accordance with this hypothesis, where the association between *MCHR2* genetic polymorphism and BMI was only significant in younger individuals. Moreover, the only reported study that associated *MCHR2* with obesity also observed an age-dependent genetic susceptibility in obesity, with the younger being more concerned[13]. The association between *MCHR2/MCHR2-AS1* with BMI found in the discovery sample was confirmed in replication sample 1 but not in replication

Table 4. Replication analyses in PsyCoLaus, the subset of CoLaus with psychiatric evaluations.

rs7749425C>T (proxy of rs7754794C>T)	ALL SUBJECTS						≤45 years subjects					
	BMI			WC			BMI			WC		
	n	β (kg/m^2)	p-value	n	β (cm)	p-value	n	β (kg/m^2)	p-value	n	β (cm)	p-value
PsyCoLaus:	3938	ref		3938	ref		907	ref		907	ref	
All subjects		-0.15	0.42		-0.58	0.26		-0.28	0.38		-1.33	0.13
PsyCoLaus:	1580	ref		1580	ref		404	ref		404	ref	
Depression		-0.21	0.46		-0.52	0.5		-0.35	0.45		-1.73	0.16
PsyCoLaus:	1127	ref		1127	ref		278	ref		278	ref	
Non-atypical depression		0.18	0.56		0.17	0.85		0.37	0.44		-0.36	0.79
PsyCoLaus:	453	ref		453	ref		126	ref		126	ref	
Atypical depression		-1.17	**0.04***			0.12		-2.5	**0.03***		-5.59	**0.05**

Results were obtained by using robust regression, adjusted for age and sex. BMI: body mass index; WC: waist circumference. ref: reference (i.e carriers of the C allele at rs7749425 locus). β: estimate.

*Interaction between atypical depression and rs7749425 significant (p = 0.04).

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sample 2. In the latter sample, the positive association between rs7754794 and BMI in patients older than 45 years may have hampered replication when considering the whole replication sample 2 (see Supporting information for further details). Additionally, the longer treatment duration in replication sample 2 (median of 35 months) as compared to the two other samples (6 and 27 months, respectively) may have also contributed to the observed differences.

Interestingly, within the PsyColaus replication sample, MDD (major depressive disorder) subtypes stratification revealed that individuals with present or past depression with atypical features were concerned with the protective effect of rs7754794 on BMI, whereas other individuals (i.e those without depression or those with depression with non-atypical features) were not. Atypical depression has been characterized by an improved mood in response to positive events, featuring some symptoms such as an increased appetite, weight gain and hypersomnia [27]. In PsyCoLaus, the atypical subtype of MDD has been described as a strong predictor of obesity[28]. Of note, several common biological states linking obesity and depression have been determined, such as the dysregulation of the hypothalamic-pituitary-adrenocortical axis [29, 30]. Moreover, the *MCH* pathway has been involved in both body weight and mood status regulation in rats[31]. In the present study, no difference of rs7754794 frequency was observed in the atypical depression subgroup of PsyCoLaus, as compared to others (data not shown), suggesting that *MCHR2/MCHR2-AS1* variant is not a risk factor for atypical depression but rather for BMI increase during atypical depression. BMI increase in patients with atypical depression may result from several factors, including illness symptoms, such as an increased appetite and/or a sleeping dysregulation. Interestingly, *MCH* has been associated with the regulation of both atypical depression features in humans[32, 33]. Exploratory association analyses of *MCHR2* rs7754794 with appetite conducted in the discovery sample and in the atypical depression subgroup revealed no significant association (see Supporting information for further details). It would be interesting to investigate in the future whether this genetic variant is associated with sleep regulation. Of note, *MCHR2* rs7754794 effect on BMI was higher in the psychiatric population than in replication population-based samples. In addition, positive results found in the general population appear to be totally driven by the subgroup of subjects with present or past atypical depression. It can however not be excluded that *MCHR2* rs7754794 does contribute to BMI regulation in other populations, including non psychiatric individuals as well.

Interestingly, *MCHR2* rs7754794 is not only a tagging SNP of *MCHR2*, but is also a proxy of rs6925272 ($r^2 = 0.97$), a *MCHR2-AS1* tagging SNP lying in the promoter region of both *MCHR2* and *MCHR2-AS1*. Therefore, our results could be directly linked with a differential *MCHR2* and/or *MCHR2-AS1* genotype-dependent expression. Analyses of *MCHR2* and *MCHR2-AS1* expression have been conducted in peripheral blood mononuclear cells in a subset of the discovery sample. Unfortunately, these two genes were not expressed in these cells (data not shown). Even when focusing on patients receiving olanzapine, a medication having been described as a potent inducer of MCH receptor[17], no expression of these two genes could be detected in peripheral cells (data not shown). Further expression analyses of *MCHR2* and *MCHR2-AS1* within their functional tissue are of particular interest and will help to understand their implication in the development of obesity. Regarding the possible biological function of long antisense non-coding genes, a recent study observed an epigenetic-conducted transcription of a gene (i.e *APOA1*; apolipoprotein A1) by a lncRNA in its antisense direction (*APOA1-AS1*)[34]. However, further studies are needed to better characterize the role of long noncoding antisense RNAs in the pathophysiology of obesity.

Several limitations of this study need to be acknowledged. Firstly, this study was restricted to Caucasian patients and results cannot be extrapolated to other ethnicities. Secondly, we could not link any *MCHR2* and/or *MCHR2-AS1* tagging-variants with their expression to

functionally validate our hypotheses. Thirdly, we were not able to determine whether the polymorphism associated with BMI lies in *MCHR2*, *MCHR2-AS1* or in the promoter region of both genes. On the other hand, the fact that the results were replicated in one independent sample and in a psychiatric subgroup of a population-based sample, the latter used as a proof of concept of the polymorphism effect, strengthens the validity of our data.

In conclusion, this is the first genetic study linking *MCHR2* and/or *MCHR2-AS1* tagging polymorphisms and BMI in psychiatric patients under psychotropic treatments. The present results are in agreement and expand those from the lone study preformed until now, showing a significant association between *MCHR2* and BMI in the general population[13]. Moreover, the significant interaction found between *MCHR2/MCHR2-AS1* and BMI in population-based subjects with present and/or previous history of atypical depression but not non-atypical depression provides new clues to the pathophysiology of atypical depression.

Materials and Methods

Psychiatric Samples

Discovery sample (Lausanne follow-up prospective psychiatric study). Since 2007, a prospective cohort study is ongoing in the Psychiatric University Hospital of Lausanne including 474 Caucasian patients with newly prescribed psychotropic drugs (see Supporting Information). Clinical variables and body weight were prospectively recorded at several time points during the first 12 months of treatment, according to published recommended monitoring guidelines (i.e before starting the psychotropic treatment and at months 1,2,3,6 and 12)[35].

Replication sample 1 (Geneva retrospective psychiatric study). From 2006 to 2008, a study was conducted in out-patient psychiatric centers of Geneva University Hospital. 163 Caucasian patients treated for more than 3 months with psychotropic drugs were included (see Supporting Information).

Replication sample 2 Lausanne retrospective study). From 2010 to 2011, a study was conducted in two out-patient psychiatric centers of Lausanne (Lausanne University Hospital and a private psychiatric center). 178 Caucasian patients treated with psychotropic drugs were recruited (see Supporting Information).

In the three samples, demographic data, history of treatment and comedications were obtained from medical files. At inclusion, body weight and height were measured with participants standing without shoes in light clothes. Body weight was measured in kilograms to the nearest kg. Height was measured to the nearest cm using a height gauge. Body mass index (BMI) was defined as weight/height^2 (kg/m^2). BMI values between 25–30 kg/m^2 and equal or higher than 30 kg/m^2 were used to define overweight and obese patients, respectively. Psychiatric diagnoses were established by physicians according to the ICD-10 classification. Most patients had already received other psychotropic treatments before the current treatment. For patients in replication samples 1 and 2, clinical variables, body weight and height were measured during the interview, while their previous weight data (i.e. weight before the beginning of the current treatment and/or weight at different times during the current treatment) were either collected from medical files or self-reported. Full description of these samples was published elsewhere[36]. Written informed consents were obtained from patients or their legal representatives for the three psychiatric cohorts and these studies were approved by the Ethics Committee of Geneva and Lausanne University Hospitals.

Population-Based Samples

Results were replicated in two population-based samples: CoLaus/PsyCoLaus, n = 5 409 [37, 38] and Genetic Investigation of ANtropometric Traits (GIANT, n = 123 865)[5].

CoLaus/PsyCoLaus. Participants aged from 35 to 75 years were recruited between June 2003 and May 2006, as previously described for CoLaus[37]. The assessment included cardiovascular risk factors such as body mass index (BMI), fat mass, waist circumference (WC), blood pressure, blood glucose, triglycerides and high density lipoprotein cholesterol. In addition, all Caucasians (91% of the sample) underwent a genetic exam (GWAS; n = 5409). All participants of CoLaus in the age range of 35 to 66 years were also asked to participate in a psychiatric evaluation (PsyCoLaus) based essentially on a semi-structured diagnostic interview [38]. In PsyCoLaus, we could subtype depressive individuals by atypical features according to the DSM-IV. Combined genetic and psychiatric data were available for 3938 participants. Genotyping for the CoLaus/PsyCoLaus subjects was performed using the Affymetrix GeneChipR Human Mapping 500K array set. Demographic and clinical characteristics of PsyCoLaus are shown in results.

Genetic Investigation of ANtropometric Traits (GIANT) consortium. The GIANT consortium performed a meta-analysis of GWAS data with a discovery set of 123 865 individuals of European ancestry from 46 studies for height [39], BMI [5] and waist-to hip ratio [40].

Genotyping and Candidate Gene Polymorphisms

rs6925272 was first selected based on a previous study[13] and genotyped using Taqman allelic discrimination assay (ABI PRISM 7000 Sequence Detection System; Applied Biosystems, Rotkreuz, Switzerland). Tagging SNPs within *MCHR2* and *MCHR2-AS1* where then selected using HapMap Genome Browser (release #28, NCBI build 36, dbSNP b126). Ten tagging polymorphisms within *MCHR2* and twelve within *MHCR2-AS1* were obtained by limiting the search to SNPs with a minor allele frequency >5% in the Caucasian population and r^2 cutoff of 0.8. *MCHR2* tagging SNPs were customized and added to the Illumina 200K cardiometabochip [41], whereas four among twelve *MCHR2-AS1* tagging SNPs were added to the cardiometabochip. Due to technical issues, proxies of SNPs were chosen in some cases (S7 Table). A good concordance was observed between genotypes obtained using Taqman and those obtained in the Cardiometabochip.

Statistical Analysis

Associations between tagging SNPs of *MCHR2* and of *MCHR2-AS1* and BMI were first tested in the discovery sample. Only SNPs significantly associated with BMI were tested for replication in the two replication psychiatric samples and the population based samples. For the assessment of association between BMI and tagging SNPs in psychiatric samples, a generalized additive mixed model (GAMM) was fitted, adjusting for age, gender, smoking status, current psychotropic drug and comedications potentially inducing weight gain (S8 Table), allowing a smooth trend for the response in time based on multiple observations for each patient. GAMMs were fitted using the mgcv package of R, in which parameter uncertainties (confidence intervals and p-values) were computed using 1000 bootstrap replicates with replacement, performed on patient level. Replication analyses in population-based samples were conducted using robust regression adjusted for age and sex. P-values of these two-sided models ≤ 0.05 were considered as statistically significant. All the analyses were performed using Stata 12 (StataCorp, College Station TX, USA) and R version 2.13.0 software. Haploview 4.2 [42] was used to define haplotype blocks and linkage disequilibrium (LD) between different *MCHR2* or *MCHR2-AS1* SNPs (D' and r^2). The haplo.stat package of R was used for haplotype analysis. P-values were defined using asymptotic chi-squared tests of haplo scores.

Supporting Information

S1 Fig. *MCHR2* and *MCHR2-AS1* SNPs haplotype blocks.
(DOCX)

S1 Table. Characteristics of psychiatric Caucasian samples: discovery, replication and combined samples.
(DOCX)

S2 Table. Genotype frequencies of *MCHR2* and *MCHR2-AS1* SNPs in three Caucasian psychiatric samples.
(DOCX)

S3 Table. Associations of *MCHR2* and *MCHR2-AS1* tagging SNPs with BMI in the Caucasian discovery psychiatric sample.
(DOCX)

S4 Table. *MCHR2* rs7754794C>T tagging SNP association with waist circumference in the discovery sample*.
(DOCX)

S5 Table. Characteristics of PsyCoLaus sample.
(DOCX)

S6 Table. Genotype frequencies of *MCHR2* rs7749425C>T according to subgroups of diagnosis in PsyCoLaus sample.
(DOCX)

S7 Table. *MCHR2* and *MCHR2-AS1* tagging SNPs referenced in HapMap.
(DOCX)

S8 Table. Comedications considered as weight-inducers in statistical analyses.
(DOCX)

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Author Contributions

Conceived and designed the experiments: CBE. Performed the experiments: AD FV NSM LQ. Analyzed the data: AD ZK EC. Contributed reagents/materials/analysis tools: AD MP FV NSM LQ EC MGR ZK PM JMA AVG EC PV GW PC CBE. Wrote the paper: AD MP MGR CBE.

References

1. Casey DE, Haupt DW, Newcomer JW, Henderson DC, Sernyak MJ, Davidson M, et al. Antipsychotic-induced weight gain and metabolic abnormalities: implications for increased mortality in patients with schizophrenia. The Journal of clinical psychiatry. 2004; 65 Suppl 7:4–18; quiz 9–20. PMID: 15151456.

2. Leucht S, Cipriani A, Spineli L, Mavridis D, Orey D, Richter F, et al. Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: a multiple-treatments meta-analysis. Lancet. 2013; 382 (9896):951–62. doi: 10.1016/S0140-6736(13)60733-3 PMID: 23810019.
3. Malhotra AK, Correll CU, Chowdhury NI, Muller DJ, Gregersen PK, Lee AT, et al. Association between common variants near the melanocortin 4 receptor gene and severe antipsychotic drug-induced weight gain. Archives of general psychiatry. 2012; 69(9):904–12. doi: 10.1001/archgenpsychiatry.2012.191 PMID: 22566560; PubMed Central PMCID: PMC4166499.
4. Comuzzie AG, Allison DB. The search for human obesity genes. Science. 1998; 280(5368):1374–7. PMID: 9603720.
5. Speliotes EK, Willer CJ, Berndt SI, Monda KL, Thorleifsson G, Jackson AU, et al. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. Nature genetics. 2010; 42 (11):937–48. doi: 10.1038/ng.686 PMID: 20935630; PubMed Central PMCID: PMC3014648.
6. Abbott CR, Kennedy AR, Wren AM, Rossi M, Murphy KG, Seal LJ, et al. Identification of hypothalamic nuclei involved in the orexigenic effect of melanin-concentrating hormone. Endocrinology. 2003; 144 (9):3943–9. doi: 10.1210/en.2003-0149 PMID: 12933668.
7. Gao Q, Horvath TL. Neurobiology of feeding and energy expenditure. Annual review of neuroscience. 2007; 30:367–98. doi: 10.1146/annurev.neuro.30.051606.094324 PMID: 17506645.
8. Kishi T, Elmquist JK. Body weight is regulated by the brain: a link between feeding and emotion. Molecular psychiatry. 2005; 10(2):132–46. doi: 10.1038/sj.mp.4001638 PMID: 15630408.
9. Pissios P, Bradley RL, Maratos-Flier E. Expanding the scales: The multiple roles of MCH in regulating energy balance and other biological functions. Endocrine reviews. 2006; 27(6):606–20. Epub 2006/06/22. doi: 10.1210/er.2006-0021 PMID: 16788162.
10. Shimada M, Tritos NA, Lowell BB, Flier JS, Maratos-Flier E. Mice lacking melanin-concentrating hormone are hypophagic and lean. Nature. 1998; 396(6712):670–4. doi: 10.1038/25341 PMID: 9872314
11. Chee MJ, Pissios P, Prasad D, Maratos-Flier E. Expression of melanin-concentrating hormone receptor 2 protects against diet-induced obesity in male mice. Endocrinology. 2013. Epub 2013/10/31. doi: 10.1210/en.2013-1738 PMID: 24169555.
12. Meyre D, Lecoeur C, Delplanque J, Francke S, Vatin V, Durand E, et al. A genome-wide scan for childhood obesity-associated traits in French families shows significant linkage on chromosome 6q22.31-q23.2. Diabetes. 2004; 53(3):803–11. PMID: 14988267.
13. Ghoussaini M, Vatin V, Lecoeur C, Abkevich V, Younus A, Samson C, et al. Genetic study of the melanin-concentrating hormone receptor 2 in childhood and adulthood severe obesity. The Journal of clinical endocrinology and metabolism. 2007; 92(11):4403–9. doi: 10.1210/jc.2006-2316 PMID: 17698913.
14. Perez-Iglesias R, Vazquez-Barquero JL, Amado JA, Berja A, Garcia-Unzueta MT, Pelayo-Teran JM, et al. Effect of antipsychotics on peptides involved in energy balance in drug-naive psychotic patients after 1 year of treatment. Journal of clinical psychopharmacology. 2008; 28(3):289–95.
15. Sentissi O, Epelbaum J, Olie JP, Poirier MF. Leptin and ghrelin levels in patients with schizophrenia during different antipsychotics treatment: a review. Schizophrenia bulletin. 2008; 34(6):1189–99. doi: 10.1093/schbul/sbm141 PMID: 18165262; PubMed Central PMCID: PMC2632509.
16. Esen-Danaci A, Sarandol A, Taneli F, Yurtsever F, Ozlen N. Effects of second generation antipsychotics on leptin and ghrelin. Progress in neuro-psychopharmacology & biological psychiatry. 2008; 32 (6):1434–8. doi: 10.1016/j.pnpbp.2008.03.015 PMID: 18579280.
17. Guesdon B, Denis RG, Richard D. Additive effects of olanzapine and melanin-concentrating hormone agonism on energy balance. Behavioural brain research. 2010; 207(1):14–20. doi: 10.1016/j.bbr.2009.09.032 PMID: 19788900.
18. Chagnon YC, Merette C, Bouchard RH, Emond C, Roy MA, Maziade M. A genome wide linkage study of obesity as secondary effect of antipsychotics in multigenerational families of eastern Quebec affected by psychoses. Molecular psychiatry. 2004; 9(12):1067–74. doi: 10.1038/sj.mp.4001537 PMID: 15224101.
19. Congrains A, Kamide K, Ohishi M, Rakugi H. ANRIL: Molecular Mechanisms and Implications in Human Health. International journal of molecular sciences. 2013; 14(1):1278–92. doi: 10.3390/ijms14011278 PMID: 23306151; PubMed Central PMCID: PMC3565320.
20. Congrains A, Kamide K, Oguro R, Yasuda O, Miyata K, Yamamoto E, et al. Genetic variants at the 9p21 locus contribute to atherosclerosis through modulation of ANRIL and CDKN2A/B. Atherosclerosis. 2012; 220(2):449–55. doi: 10.1016/j.atherosclerosis.2011.11.017 PMID: 22178423.
21. Pasic I, Shlien A, Durbin AD, Stavropoulos DJ, Baskin B, Ray PN, et al. Recurrent focal copy-number changes and loss of heterozygosity implicate two noncoding RNAs and one tumor suppressor gene at chromosome 3q13.31 in osteosarcoma. Cancer research. 2010; 70(1):160–71. Epub 2010/01/06. doi: 10.1158/0008-5472.CAN-09-1902 PMID: 20048075.

22. De Hert M, Detraux J, van Winkel R, Yu W, Correll CU. Metabolic and cardiovascular adverse effects associated with antipsychotic drugs. Nature reviews Endocrinology. 2012; 8(2):114–26. doi: 10.1038/nrendo.2011.156 PMID: 22009159.

23. Rodriguez M, Beauverger P, Naime I, Rique H, Ouvry C, Souchaud S, et al. Cloning and molecular characterization of the novel human melanin-concentrating hormone receptor MCH2. Molecular pharmacology. 2001; 60(4):632–9. PMID: 11562423.

24. Sailer AW, Sano H, Zeng Z, McDonald TP, Pan J, Pong SS, et al. Identification and characterization of a second melanin-concentrating hormone receptor, MCH-2R. Proceedings of the National Academy of Sciences of the United States of America. 2001; 98(13):7564–9. doi: 10.1073/pnas.121170598 PMID: 11404457; PubMed Central PMCID: PMC34708.

25. MacIntosh CG, Andrews JM, Jones KL, Wishart JM, Morris HA, Jansen JB, et al. Effects of age on concentrations of plasma cholecystokinin, glucagon-like peptide 1, and peptide YY and their relation to appetite and pyloric motility. The American journal of clinical nutrition. 1999; 69(5):999–1006. PMID: 10232642.

26. Donini LM, Savina C, Cannella C. Eating habits and appetite control in the elderly: the anorexia of aging. International psychogeriatrics / IPA. 2003; 15(1):73–87. PMID: 12834202.

27. Lamers F, de Jonge P, Nolen WA, Smit JH, Zitman FG, Beekman AT, et al. Identifying depressive subtypes in a large cohort study: results from the Netherlands Study of Depression and Anxiety (NESDA). The Journal of clinical psychiatry. 2010; 71(12):1582–9. doi: 10.4088/JCP.09m05398blu PMID: 20673552.

28. Lasserre AM, Glaus J, Vandeleur CL, Marques-Vidal P, Vaucher J, Bastardot F, et al. Depression With Atypical Features and Increase in Obesity, Body Mass Index, Waist Circumference, and Fat Mass: A Prospective, Population-Based Study. JAMA psychiatry. 2014. doi: 10.1001/jamapsychiatry.2014.411 PMID: 24898270.

29. Bornstein SR, Schuppenies A, Wong ML, Licinio J. Approaching the shared biology of obesity and depression: the stress axis as the locus of gene-environment interactions. Molecular psychiatry. 2006; 11(10):892–902. doi: 10.1038/sj.mp.4001873 PMID: 16880826.

30. Musselman DL, Evans DL, Nemeroff CB. The relationship of depression to cardiovascular disease: epidemiology, biology, and treatment. Archives of general psychiatry. 1998; 55(7):580–92. PMID: 9672048.

31. Borowsky B, Durkin MM, Ogozalek K, Marzabadi MR, DeLeon J, Lagu B, et al. Antidepressant, anxiolytic and anorectic effects of a melanin-concentrating hormone-1 receptor antagonist. Nature medicine. 2002; 8(8):825–30. doi: 10.1038/nm741 PMID: 12118247

32. Barson JR, Morganstern I, Leibowitz SF. Complementary roles of orexin and melanin-concentrating hormone in feeding behavior. International journal of endocrinology. 2013; 2013:983964. doi: 10.1155/2013/983964 PMID: 23935621; PubMed Central PMCID: PMC3727095.

33. Blouin AM, Fried I, Wilson CL, Staba RJ, Behnke EJ, Lam HA, et al. Human hypocretin and melanin-concentrating hormone levels are linked to emotion and social interaction. Nature communications. 2013; 4:1547. doi: 10.1038/ncomms2461 PMID: 23462990; PubMed Central PMCID: PMC3595130.

34. Halley P, Kadakkuzha BM, Faghihi MA, Magistri M, Zeier Z, Khorkova O, et al. Regulation of the apolipoprotein gene cluster by a long noncoding RNA. Cell reports. 2014; 6(1):222–30. doi: 10.1016/j.celrep.2013.12.015 PMID: 24388749; PubMed Central PMCID: PMC3924898.

35. American Diabetes A, American Psychiatric A, American Association of Clinical E, North American Association for the Study of O. Consensus development conference on antipsychotic drugs and obesity and diabetes. Obesity research. 2004; 12(2):362–8.

36. Choong E, Quteineh L, Cardinaux JR, Gholam-Rezaee M, Vandenberghe F, Dobrinas M, et al. Influence of CRTC1 polymorphisms on body mass index and fat mass in psychiatric patients and the general adult population. JAMA psychiatry. 2013; 70(10):1011–9. doi: 10.1001/jamapsychiatry.2013.187 PMID: 23925723.

37. Firmann M, Mayor V, Vidal PM, Bochud M, Pecoud A, Hayoz D, et al. The CoLaus study: a population-based study to investigate the epidemiology and genetic determinants of cardiovascular risk factors and metabolic syndrome. BMC cardiovascular disorders. 2008; 8:6. Epub 2008/03/28. doi: 10.1186/1471-2261-8-6 PMID: 18366642; PubMed Central PMCID: PMC2311269.

38. Preisig M, Waeber G, Vollenweider P, Bovet P, Rothen S, Vandeleur C, et al. The PsyCoLaus study: methodology and characteristics of the sample of a population-based survey on psychiatric disorders and their association with genetic and cardiovascular risk factors. BMC psychiatry. 2009; 9:9. doi: 10.1186/1471-244X-9-9 PMID: 19292899; PubMed Central PMCID: PMC2667506.

39. Lango Allen H, Estrada K, Lettre G, Berndt SI, Weedon MN, Rivadeneira F, et al. Hundreds of variants clustered in genomic loci and biological pathways affect human height. Nature. 2010; 467(7317):832–8. doi: 10.1038/nature09410 PMID: 20881960; PubMed Central PMCID: PMC2955183.

40. Heid IM, Jackson AU, Randall JC, Winkler TW, Qi L, Steinthorsdottir V, et al. Meta-analysis identifies 13 new loci associated with waist-hip ratio and reveals sexual dimorphism in the genetic basis of fat distribution. Nature genetics. 2010; 42(11):949–60. doi: 10.1038/ng.685 PMID: 20935629; PubMed Central PMCID: PMC3000924.

41. Voight BF, Kang HM, Ding J, Palmer CD, Sidore C, Chines PS, et al. The metabochip, a custom genotyping array for genetic studies of metabolic, cardiovascular, and anthropometric traits. PLoS genetics. 2012; 8(8):e1002793. doi: 10.1371/journal.pgen.1002793 PMID: 22876189; PubMed Central PMCID: PMC3410907.

42. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21(2):263–5. doi: 10.1093/bioinformatics/bth457 PMID: 15297300.