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Impact of *HSD11B1* polymorphisms on body mass index and components of the metabolic syndrome in patients with psychotropic treatments

Pharmacogenetics of the metabolic syndrome in psychiatry

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Abstract:

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 catalyses tissue regeneration of active cortisol from cortisone. Elevated enzymatic activity of 11β-HSD1 may lead to the development of MetS. We investigated the association between 7 *HSD11B1* gene (encoding 11β-HSD1) polymorphisms and BMI and MetS components in a psychiatric sample treated with potentially weight-gain inducing psychotropic drugs (n=478). The polymorphisms that survived Bonferroni correction were analyzed in two independent psychiatric samples (n_{R1}=168 and n_{R2}=188) and in several large population-based samples (n_1=5338; n_2=123'865; n_3>100,000). *HSD11B1 rs846910-A*, *rs375319-A* and *rs4844488-G* allele carriers were found to be associated with lower BMI, waist circumference and diastolic blood pressure as compared to 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 to non-carriers ($P_{corrected}$<0.05). Conversely, carriers of *rs846906-T* allele had significantly higher waist circumference, triglycerides and lower HDL-cholesterol, exclusively in men ($P_{corrected}$=0.028). R*s846906-T* allele was also associated with higher risk of MetS at 3 months of follow-up (odd ratio: 3.31, 95%C.I.: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. Our results indicate that *HSD11B1* polymorphisms may contribute to the development of MetS in psychiatric patients treated with weight-gain inducing psychotropic drugs but do not play a significant role in the general population.

Keywords: Metabolic syndrome, Body mass index, psychotropic drugs, pharmacogenetics.

Introduction:

Weight gain and obesity are important 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 mortality risk and 20% shorter life span as compared to the general population, with CVDs being the leading cause of death[4]. Meta-analyses also showed nearly 2 times increased mortality risk 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) conducted to date only explain a small fraction of body mass index (BMI) heritability[8] and more obesity susceptibility genes remain to be discovered. Whereas genome-wide association study meta-analyses have been extremely valuable, 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 ref[12]). This microsomal enzyme catalyses 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 (GC) receptors[13]. Although not all subjects with MetS have increased level 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, elevated 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 high fat diet[15, 16], while 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 independently associated 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 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 7 *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 potentially weight-gain inducing psychotropic drugs, a population known to have higher obesity prevalence, and hence MetS, compared to the general population.

Material 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. Full description of this sample was published elsewhere[32]. Briefly, 478 Caucasian 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, then at months 1, 2, 3, 6, 9, and 12)[6]. The newly introduced psychotropic drug was considered as the main psychotropic medication and any other potential weight gain-inducing drugs of interest, including typical and atypical AP and MS, were classified as co-medications possibly causing weight gain. The study was approved by the

Psychiatry Ethics Committee of Lausanne University hospital and written informed consent was given by all subjects or by their legal representatives.

Replication samples:

We tried to replicate the results in two independent samples of Caucasian psychiatric patients[32]. The first replication sample stemmed from a retrospective study conducted in out-patient 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 derived from a retrospective study conducted since 2010 in two out-patient 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 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, while their baseline weight before the initiation of the current treatment and/or at different times during treatment was collected 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 given by all subjects or by their legal representatives.

Population based samples:

- **Cohorte Lausannoise (CoLaus and PsyCoLaus)**

Participants aged 35 to 75 years in this population-based study (CoLaus) were recruited between June 2003 and May 2006 as previously described[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 Caucasians (91% of the sample) underwent a genetic exam (n=5338). All participants of CoLaus in the age range of 35 to 66 years were asked to also participate in a psychiatric evaluation (PsyCoLaus) based essentially on a semi-structured diagnostic interview[34]. Combined genetic and psychiatric data were available for 2990 participants. Genotyping for the CoLaus/PsyCoLaus subjects was performed using the Affymetrix GeneChipR Human Mapping 500K array set.

- **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[35], BMI[8] and waist-to hip ratio (WHR)[36].

- **Genome Wide Associations Scans for Total Cholesterol, HDL-C, LDL-C and triglycerides**

Data on lipid traits have been downloaded from "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 >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 United States, 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

Psychiatric patients' genomic DNA was extracted from whole blood. Selection and genotyping of *HSD11B1* SNPs were done in 2 steps: first *rs846910G>A*, *rs12086634T>G* polymorphisms, which were previously investigated in the general population, were selected and genotyped using 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 (Rotkreuz, Switzerland), and genotyping was performed according to the manufacturer's protocol. In a second step, selection of tagging SNPs within the *HSD11B1* gene using 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 > 5% in the Caucasian population and

r^2 cutoff of 0.8, covering 100% of genetic variations within *HSD11B1* gene in the HapMap Genome Browser and 87% of *HSD11B1* genetic variations in 1000genome database[40]. Of note, both *rs846910G>A* and *rs12086634T>G* were among the tagging SNPs. These 8 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.

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 (PBMC)(details in supplementary data).

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. Due to 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 based on multiple observations for each patient (using a thin plate regression spline basis) adjusting for age, sex, smoking status, current psychotropic drug, and co-medications possibly causing weight-

gain for BMI and WC analyses (list of these co-medications are published in[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 subject level was also introduced to take the dependence structure of observed data into account. GAMMs were fitted using the mgcv package of R (settings were fixed at package defaults). In order to be more conservative, the uncertainty of estimated parameters were assessed by 1000 bootstraps[44] at the subject level and results were similar with those gained by 10'000 bootstraps. Whenever the p-value for the 1000 bootstrap was lower than 0.001 ($p<0.001$), 10000 bootstrap analysis was performed. If 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, so model coefficients provide information on both the direction and 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 gender analyses were systematically conducted 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 7 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.

Chi^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 performed using Stata 12 (StataCorp, College Station TX, USA) and R version 2.13.0 software (http://www.R-project.org). Haploview 4.2[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), with blood pressure and with 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, in order to determine whether the SNPs of interest were differentially associated with the components of the MetS in subjects with and without major depressive disorder (MDD), we tested the 2-way interactions between each *HSD11B1* SNP and MDD in the PsyCoLaus subsample. BMI, WC and WHR 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 looked up 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 in order 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 Caucasian samples of the main study (n=478) and the two replication studies (n_1=168 and n_2=188). Obesity prevalence was higher in the replication studies as compared to the main study, which could be explained by the very long treatment duration in the former studies (Table 1). MetS was detected in nearly 17% of the main psychiatric sample at baseline, 27% and 27.5% at 3 and 12 months of the follow-up, respectively.

Supplementary Table 1 shows the analyzed *HSD11B1 SNPs,* their positions and minor allele frequencies (MAF) observed in the main psychiatric study sample (n=478). For a technical reason, *HSD11B1 rs11119328* SNP could not be genotyped in the cardiometabochip. None of the SNPs deviates from Hardy-Weinberg equilibrium and the MAF in the psychiatric sample was comparable to those reported in HapMap for Caucasians (supplementary Table 1). Haploview analyses defined 2 haplotype blocks formed from *rs12565406-rs10863782* and *rs846910-rs3753519* SNPs (Supplementary Figure 1). Only *rs10863782* and *rs3753519* SNPs were in considerable linkage disequilibrium LD (r^2=0.58)(Supplementary Figure 1b).

Genotype frequencies in the main psychiatric sample, the replication samples and the combined sample are shown in supplementary Table 2.

***HSD11B1* polymorphisms in the main psychiatric study sample**

Only complete observations and data regarding 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, as compared to patients with the wildtype genotypes (n=450, Bonferroni corrected p-values ($P_{corrected}$)=0.0014, <0.00007 and 0.007, respectively)(Table 2). This association was exclusively detected within women (n=257), with more than 3.1 kg/m^2 lower BMI in *HSD11B1 rs846910-A*, *rs375319-A*, and *rs4844488-G* carriers compared to non-carriers ($P_{corrected}$<0.00007, <0.00007 and 0.04, respectively explaining 3.6, 4.8 and 1.5% of BMI variance in women), while no association was observed among men (n=193, $P_{corrected}$>0.05)(Table 2). 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).

Due to gender differences of WC, the GAMM was applied for each gender separately. Similarly to the findings regarding 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 in carriers of the *HSD11B1 rs846910-A, rs375319-A and rs4844488-G* alleles, respectively, as compared to non-carriers, explaining 2.8, 5.1 and 1.7% of WC variance (Table 3). No association was observed between these 3 SNPs and WC in men. Interestingly, for the *rs846906C>T* SNP, only men carrying the *T-allele* showed 4.7 cm higher WC compared to non-carriers (n=204, $P_{corrected}$=0.014), explaining 2.3% of WC variance. No significant association was observed between *HSD11B1 rs12086634T>G*, *rs10863782G>A* and *rs12565406G>T* and WC in both genders ($P_{corrected}$>0.05).

No significant association was observed between *HSD11B1* SNPs and SBP in the main psychiatric group (n=386, $P_{corrected}$>0.05) nor on analyzing the men and women subgroups (supplementary Table 3). On the other hand, *rs846910G>A*, *rs375319G>A and rs4844488A>G* were significantly associated with DBP. Among women (n=219) carriers of the *rs846910-A, rs375319-A and rs4844488-G* alleles have 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 to non-carriers, explaining 1.3, 1.9 and 2.2% of DBP variance for each SNP respectively (supplementary Table 4).

No significant association was observed between *HSD11B1* SNPs and fasting blood glucose in the main psychiatric group (n=294, $P_{corrected}$>0.05) nor on analyzing men and women subgroups (supplementary Table 5). However, lipid analyses showed a significant association between *rs846906C>T* and triglycerides in the total sample, with *T-allele* carriers having 0.29 mmol/L higher triglycerides levels compared to non-carriers (n=312, $P_{corrected}$=0.007, explained variance=1.9%). This association was exclusively observed in men, in which carriers of the *T-allele* having 0.53 mmol/L higher triglyceride compared to non-carriers (n=128, $P_{corrected}$=0.028), explaining 5.4% of triglycerides variance (supplementary Table 6). No significant association was observed between the other *HSD11B1 SNPs* and triglycerides, neither for women, nor for men ($P_{corrected}$>0.05). Due to differences of HDL-C levels between men and women, the GAMM model was applied for each gender separately (supplementary Table 7). Interestingly, men carrying the *T-allele* of *rs846906C>T* showed 0.14 mmol/L lower HDL-C levels compared to non-carriers (n=126, $P_{corrected}$=0.006), explaining 3.4% of HDL-C variance. 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 was available for the 2 psychiatric replication samples. The 3 *HSD11B1 SNPs rs846910G>A*, *rs3753519G>A* and *rs4844488A>G* that survived Bonferroni correction for BMI were analyzed in the replication samples (Supplementary Table 8). For the rs*3753519 G>A,* a significant association was only found in the second replication sample (n=184), in which carriers of the *A*-allele having 1.3 kg/m^2 lower BMI compared to non-carriers (95%CI: -2.28 - -0.31, p=0.01)(Supplementary Table 8).

No association was observed between *HSD11B1 rs846910G>A* or *rs4844488A>G* SNPs and BMI in the two replication samples. The lower frequency of women subjects in both replication samples (Table 1) did not explain the lack of association between these 2 SNPs and BMI, given that there was also no such association among women in these samples.

By combining the three psychiatric samples, a significant association was observed between *HSD11B1 rs846910G>A* and BMI in the total sample (n=802, p=0.001) and in women (n=406, p<0.0001)(Table 4). Significant associations were also found for *rs3753519G>A* in the total samples, 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 4).

We further studied the effect of *HSD11B1* SNPs on BMI between different psychotropic drugs, and 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 in the subgroup of patients treated with MS (more details in Supplementary data and **Supplementary** table …).

***HSD11B1* haplotype blocks and combinations:**

A haplotype block was formed from *HSD11B1 rs846910* and *rs3753519*. A small increase of the effect was observed in patients carrying the variant alleles of the 2 SNPs compared to the other genotypes and also as compared to the carriers of the variant allele of each SNP separately. More details in supplementary data and supplementary Table 9.

***HSD11B1* SNPs 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 that were newly diagnosed with a psychiatric disorder. Details in supplementary data and supplementary Tables 10 and 11.

***HSD11B1* polymorphisms and the risk of MetS:**

The risk of Mets as a whole was assessed between different *HSD11B1* genotypes at 3 time-points: at baseline, 3 and 12 months of follow-up (Table 5). *rs846906C>T* was associated with higher risk of MetS at 3 months of follow-up (21% and 43% for *rs846906-CC* and *T-allele* carriers, respectively, odd ratio (OR): 3.31, 95%C.I.: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 5). None of the other HSD11B1 SNPs were associated with the MetS. The same results were also obtained by applying the criteria of the International Diabetes Federation (IDF) 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 gender analyses (supplementary Table 12). Moreover, in PsyCoLaus, there were no 2-way interactions between *HSD11B1* SNPs and MDD regarding the risk of the MetS or its components, i.e. there was no evidence for differential associations between these SNPs and MetS components according to the subjects' 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 gender specific meta-analyses (N_{men}= 60,586 and N_{women}= 73,137)[46] do not reveal a significant associations between the four *HSD11B1* SNPs and BMI (supplementary Table 13). 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 Figures 2-4.

Discussion

Several conflicting results have been found between *HSD11B1* SNPs and MetS in the general population. However, these studies were performed in relatively small samples with different ethnicities. The present study aimed to test whether common SNPs within *HSD11B1*

gene are associated with BMI and the MetS in a sample of psychiatric patients receiving potentially 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 non-clinical subjects. Carriers of the variant alleles of three *HSD11B1* SNPs (*rs846910-A, rs3753519-A and rs4844488-G*) showed lower BMI, WC and DBP compared to the wild type genotypes in the main psychiatric study sample. These associations were exclusively observed in women. A small increase of the effect on BMI and/or MetS components was also observed by combining 2 SNPs, *rs846910G>A* and *rs3753519G>A*. Additionally, men carrying the variant allele of *rs846906C>T* showed higher WC, higher triglycerides and lower HDL-C blood levels compared to wild-type genotype. *HSD11B1* SNPs were previously investigated 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 subjects (n=86), the *rs846910-A* allele showed a protective effect against high blood pressure[48]. This SNP also showed a protective effect in 248 Caucasian 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, subjects who were heterozygous for *rs846910-A* and homozygous for *rs12086634-T* had a higher risk of MetS, however, no data was 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; n=534; n=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 strongly associated with obesity in children (n=534)[28]. The results on obesity are inconsistent with our results in which carriers of *rs3753519-A allele* showed a protective effect against obesity and was associated with lower blood pressure. Additionally, unlike our results, other SNPs within *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 performed in healthy children whereas ours included mostly adult psychiatric patients treated with potentially 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 increased risk of MetS at 3 months of follow-up. Only few publications 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 strongly associated 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]. Additionally, 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]. On the other hand, we are unable to 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 shorter treatment duration, includes relatively newly treated patients and has lower initial BMI as compared to the replication samples which have longer treatment duration and 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 1st 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 HPA axis[58, 59]. In humans, 11ß-HSD1 was found to be expressed in the hypothalamus, suggesting not only a role in modulation of glucocorticoids feedback of the HPA axis, but also a possible regulatory effect on metabolism and appetite[60]. Additionally, *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 disorder. Given the low proportion of subjects with severe psychiatric disorders and psychotropic medication in the community the discrepant results according to recruitment source suggests 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 as compared to population-based samples, even though the former sample size is much smaller than the latter[32]. *CRTC1* genetic polymorphism explains up to 9% of BMI variance in young psychiatric females[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 4 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 to the population-based samples.

Several limitations of this study need to be acknowledged. Hormonal measurements were not available for our samples, so the interaction between estrogen and *HSD11B1* variants could not be explored. This study was restricted to patients of Caucasian origin and results cannot be generalized to other ethnic groups. Finally, our gene expression analysis did not show a functional activity of the 2 SNPs and further studies, in particular with adipocytes and/or PBMC from

psychiatric patients, are needed to elucidate the biochemical mechanisms underlying the observed associations.

In conclusion, this is the first pharmacogenetic study relating genetic polymorphisms within *HSD11B1* and BMI and/or MetS and it's 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 regarding the other MetS traits. In the present psychiatric sample treated with potentially weight-gain inducing psychotropic drugs, *HSD11B1* SNPs were significantly associated with BMI and metabolic traits, especially in women and in newly drug-treated subjects. Additionally, in several very large population-based samples, we were not able to 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 reveal the mechanism by which *HSD11B1* SNPs influence obesity and other metabolic disturbance in psychiatric patients treated with psychotropic drug.

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

Characteristics	Psychiatric study Sample, n=478	1st replication sample, n =168	2nd 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
Others diagnosis,%	19.2	7.1	13.3
Unknown diagnosis,%	10.2	0.6	3.2
BMI			
Initial BMI, median (range), kg/m^2 ǂ	23.5 (13.3-44.5)	25.2 (15.4-45.5)	24.4 (15.5-46.2)
25≥ Initial BMI<30, % ǂ	22.7	36.7	31.7
Initial BMI≥ 30, % ǂ	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)
Co-medication possibly causing weight gain, %	48.3	29.2	26.1

‡ Before the current psychotropic treatment
BMI : Body mass index

Table 2: Associations between *HSD11B1* SNPs in a dominant model and body mass index during follow-up in the main psychiatric study:

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

[§] 100000 bootstraps were used for this analysis. 1000 bootstraps were done for the rest of the analyses.

E. Var.: explained variance by the polymorphism (%), only calculated for tests that survived Bonferroni correction.

ref: reference, $P_{corrected:}$ Bonferroni corrected p-value, NA: non-applicable.

Table 3: Associations between *HSD11B1* SNPs in a dominant model and waist circumference during follow-up in the main psychiatric study:

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

[§] 100000 bootstraps were used for this analysis. 1000 bootstraps were done for the rest of the analyses.

E. Var.: explained variance by the polymorphism (%), only calculated for tests that survived Bonferroni correction.

ref: reference, $P_{corrected:}$ Bonferroni corrected p-value, NA: non-applicable.

Table 4: Associations between *HSD11B1* SNPs in a dominant model and body mass index during follow-up in the 3 combined psychiatric study samples:

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

[§] 10000 bootstraps were used for this analysis. 1000 bootstraps were done for the rest of the analyses.

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

ref: reference

Table 5: Association between *HSD11B1* genotypes and the metabolic syndrome as defined by the National Cholesterol Education Program's Adult Treatment Panel III report (ATP III) in the main psychiatric sample:

		At baseline			3 months			12 months	
	n (%)	OR (95% C.I.)	p-value ($P_{corrected}$)	n (%)	OR (95% C.I.)	p-value ($P_{corrected}$)	n (%)	OR (95% C.I.)	p-value ($P_{corrected}$)
rs12565406									
GG	23/135 (17%)	ref		37/137 (27%)	ref		23/87 (26%)	ref	
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%)	ref		31/116 (27%)	ref		19/75 (25%)	ref	
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%)	ref		41/147 (28%)	ref		27/100 (27%)	ref	
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%)	ref		40/138 (29%)	ref		23/84 (27%)	ref	
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%)	ref		29/119 (24%)	ref		20/72 (28%)	ref	
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%)	ref		43/157 (27%)	ref		28/102 (27%)	ref	
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%)	ref		24/117 (21%)	ref		17/79 (22%)	ref	
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)

Odd ratios (OR) and P-values were adjusted for age and sex.

The NCEP ATP III panel defined metabolic syndrome as the presence of three or more of the following risk determinants: *1*) increased waist circumference (>102 cm for men, >88 cm for women); *2*) elevated triglycerides (≥150 mg/dl) or treatment with hypolipidemic agents ; *3*) low HDL cholesterol (<40 mg/dl in men, <50 mg/dl in women); *4*) hypertension (≥130/≥85 mmHg) or treatment with antihypertensive; and *5*) impaired fasting glucose (≥110 mg/dl) or treatment with antdiabetics.