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 (PCK1) 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 *PCK1* 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/100mL, 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 old.

INTRODUCTION

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 (e.g. increase in triglycerides, cholesterol, waist circumference) 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 2-3 fold increased mortality rate when compared to healthy populations.[3] Obesity is attributed to the psychiatric illness, to behavioral and environmental factors (i.e. 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 (e.g. serotonin and dopamine receptors) and hormones (e.g. 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 two 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] 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 old.[11]

Rodents who over-express *PCK1* and *PCK2* were obese, hyperglycemic and insulin resistant[12, 13] whereas mice that under-expressed *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, triglycerides (TG) and cholesterol (CHOL) levels in subcutaneous adipose tissue of non-menopausal 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[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, waist circumference (WC) or physical activity.[22] A case-control study in a diabetic versus non diabetic population also found that non diabetic homozygous for the minor allele of a *PCK1* polymorphism (*+4824T>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 (i.e. WC, blood glucose levels (BGL), low density lipoprotein (LDL),

HDL, CHOL and TG in three 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). 478 Caucasian 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 (i.e. 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] (i.e. 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 paper 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. 168 Caucasian 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 Caucasian patients mostly treated for more than one 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. WC was measured to the nearest cm. 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 paper,[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 three population based samples: Participants in CoLaus (n=5'338) 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 genome-wide 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 (i.e. *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, three SNPS which were available in the CardioMetaboChip were also considered for analysis (i.e. *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 genome wide association studies (GWAS) for metabolic and cardiovascular traits. Quality control excluded samples from the analysis if gender was inconsistent with genetic data from X-linked markers, genotype call rate <0.96, Gene Call (GC) score <0.15. GenomeStudio Data Analysis Software was used to export results generated by Illumina CardioMetaboChip. In total, four SNPs were considered for analyses with minor allele frequency (MAF) higher than 0.10 (Table S-1). All of them were in Hardy Weinberg Equilibrium (HWE) (Table S-2). 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 (LD) with our four selected SNPs (see details in Figure S-1).

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, Rotkreuz, Switzerland) and according to the manufacturers protocol as described elsewhere.[11] Genotyping of the CoLaus subjects was performed using the Affymetrix GeneChip Human Mapping 500K 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/100mL]. *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 (i.e. sex, age and ethnicity) as well as history of treatment (type of psychotropic drug and treatment duration). In order to preserve homogeneity of the samples, only patients treated up to 24 months were taken into account in combined (i.e discovery plus replication) psychiatric sample analyses.

Statistical analysis

Psychiatric Samples

HWE was determined for each polymorphism by a chi-square test. Statistical analyses were done using STATA 12.1 (StataCorp, College Station TX, USA) 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

158 tests was applied. Eventually, differences in sample size might be due to missing genotypes and/or
159 covariates. First, exploratory analyses were conducted to explore differences in BMI between genetic
160 groups in the three psychiatric samples using Mann–Whitney U non parametric test. To fit a longitudinal
161 model on the BMI trend, due to complex and non-linear BMI evolution in time and presence of multiple
162 observations per individual which introduces interdependence among observations, a Generalized
163 Additive Mixed Model (GAMM) was used to assess the association of genetic polymorphism with BMI
164 adjusted by sex, age, treatment and treatment duration. This allowed a smooth trend for the response in
165 time based on multiple observations for each patient (using a thin plate regression spline basis). A
166 random effect at the subject level was also introduced to take the dependence structure of observed data
167 into account.[33] The GAMMs were fitted using the mgcv package of R (settings were fixed at package
168 defaults). To be more conservative, the uncertainty of estimated parameters was assessed by 1'000
169 bootstraps on individuals. For those p-values lower than 0.001, 10'000 bootstraps were performed
170 whenever possible. Multivariate analysis used the same methodology as previously described for the
171 upstream *CRTC1* gene:[11] It was first conducted in the discovery sample and the significant results were
172 tested for replication in the two replication samples. In fitted longitudinal models, stratification by sex, and
173 in some cases by age, was applied when analyzing all samples together. Also, analyzes on WC and on
174 other metabolic traits (i.e. BGL and lipid levels) were conducted in the discovery sample (data available
175 only in this sample) and only for *rs11552145* and *rs2071023* polymorphisms. Due to some missing data
176 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 (i.e. *rs11552145, rs707555* and *rs8123020*) showed no significant results (results not shown).

Population-Based Samples

Significant results from *PCK1* polymorphisms in the discovery sample (i.e. *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 (i.e. TG, HDL, CHOL), we looked up association results from the Global Lipid Consortium.[30]

RESULTS

Table S-3 shows the characteristics of the three psychiatric samples. The discovery sample included patients with the shortest treatment duration (median of 6 months versus 27.4 and 36 months in the replication 1 and 2, respectively, p=0.0001), as well as the lowest BMI (current median BMI of 25 versus

194 28 and 27 kg/m^2 for replication 1 and 2, respectively, p=0.0001) and the lowest prevalence of obesity
195 (BMI≥30 kg/m^2) (18% versus 40% and 27%, respectively, p<0.001).

196 **Association of *PCK1* polymorphisms with BMI in psychiatric populations**

197 Table S-2 shows *PCK1* genotype distribution among the three psychiatric samples. No significant
198 associations were found between *PCK1* polymorphisms and baseline BMI when exploratory analyses
199 were conducted (Table S-4). However, a trend and a significant association was found between
200 *rs11552145* and *rs2071023* and current BMI (BMI at the last follow-up assessment) in the discovery (p-
201 corrected 0.08 and 0.018, respectively) and in the combined sample (p-corrected 0.01 and 0.003,
202 respectively). Figure 1 shows the association of PCK1 *rs11552145* polymorphism with BMI.

203 Multivariate analyses were first conducted in the discovery sample for the four SNPs (Table 1). Carriers of
204 *rs11552145-AA* genotype had, on average, 2.20 lower BMI units when compared to carriers of *G-allele*
205 (p= 0.0004). Similar results were found for *rs2071023-CC* genotype which had 1.27 lower BMI units when
206 compared to *G-allele* carriers (p= 0.004). Significant results were replicated for *rs11552145* and BMI
207 when combining the 2 replication samples. *AA* carriers had 1.42 lower BMI units when compared to *G-*
208 *allele* carriers (p= 0.009). When combining the three samples similar results were found for both
209 *rs11552145* and *rs2071023* (estimates -1.89 and -1.11 kg/m^2 and p<0.001 and p<0.001, respectively).
210 Explained variances in the combined sample for *rs11552145* and *rs2071023* were 0.65% and 0.85%,
211 respectively. For both *rs11552145* and *rs2071023*, further analyses stratified by sex and age were

conducted in the three samples combined. *rs2071023* was associated with BMI only in women whereas for *rs11552145* an association was found in both genders, 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 0.009, explained variance 0.77%). No significant results were found for the other two SNPs *rs8123020* and *rs707555*.

PCK1 polymorphisms and metabolic parameters in psychiatric populations

The association of *rs11552145* and *rs2071023* with other metabolic parameters (i.e. 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 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/100mL, p-value <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 (Figure 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 two 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 three population-based samples (GIANT, CoLaus and Global Lipids Genetics Consortium). Significant associations were found between the two *PCK1* polymorphisms and the WHR in the GIANT cohort (N=123'865) for women and for both genders combined. In addition, significant associations were found for *rs2071023* with HDL and TGL in the Global Lipids Genetics Consortium (N=100'184; p-values: 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 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] since 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 type 2 diabetes (T2DM) and gestational diabetes mellitus (GDM) but with

conflicting results in different 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 GDM 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 (Table S-3), since 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 non-gender stratified sample.[11] In addition, a positive correlation was found between PCK1 mRNA expression levels and BMI in a study conducted with non-menopausal 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 blood glucose levels, activating 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 as estrogen circulating levels were not measured.

In order 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 adenosine

monophosphate protein kinase (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. Firstly, 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. Secondly, although the main inclusion criteria for patients in the present study was that they were receiving psychotropic drugs known to induce weight gain (i.e aripiprazole, amisulpride, clozapine, olanzapine, quetiapine, risperidone, mirtazapine, lithium and/or valproate), other drugs possibly inducing weight (psychotropic

318 and/or somatic drugs) were prescribed, the influence of which could not be evaluated. This study was
319 conducted in Caucasians, thus results cannot be extrapolated to other ethnicities. Not all tagging SNPs
320 could be tested due to limited availability of the genotypes. In addition, no significant associations with
321 BMI were found for the two other tested SNPs *(rs707555* and *rs8123020*), either because of a lack of
322 effect or a lack of power due to the low MAF. Further replications of this study should increase sample
323 size in order to test low MAF polymorphisms and to increase the coverage of *PCK1* gene by including
324 other tagging SNPs. Finally, variants obtained through GWAS should be also considered in further
325 analysis, in particular those on gluconeogenic pathway. It has thus been recently shown that *PCK1*
326 expression is regulated by *CAMK1D*,[52] a gene previously related to diabetes in GWAS.[53]

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

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472

Table 1. Multivariate analysis of *PCK1* polymorphisms and BMI.

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

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

**Fasting patients

$^{\$}$p-corrected value for discovery sample.

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

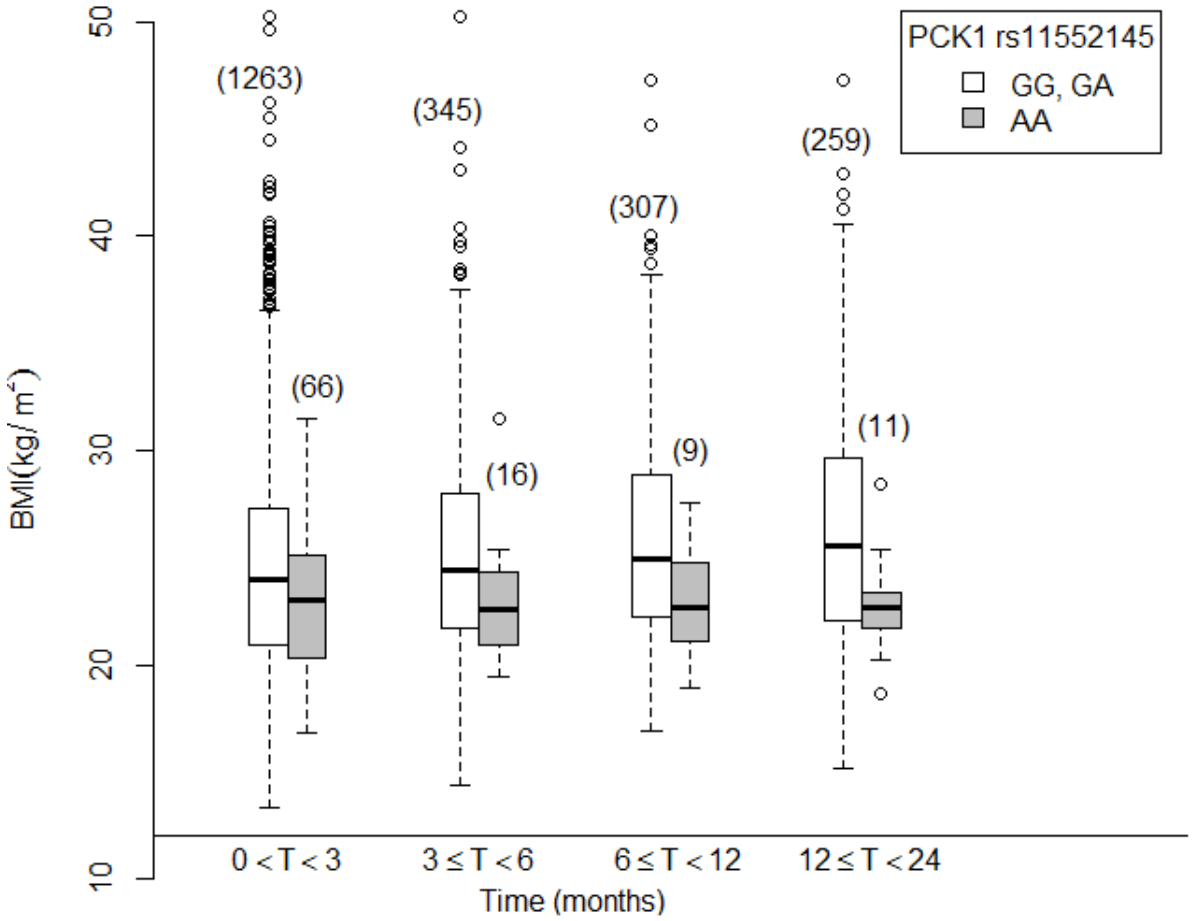
Adjusted by: BMI, age, sex, treatment (antipsychotic or mood stabilizer) and treatment duration. Bootstrap at 1000. WC: waist circumference, HDL: high lipoprotein, TG: triglycerides, CHOL: cholesterol, BGL: blood glucose levels

Table 3. Association of *PCK1* polymorphisms with metabolic traits in population based samples.

	CoLaus (n=5'338)		GIANT (n= 123'865)		Global Lipids Genetics Consortium (n= 100'184)	
rs6070157 **(proxy of *rs11552145*, r^2= 0.99)**	β (SE)	p-value	β (SE)	p-value	β (SE)	p-value
Anthropometric traits						
BMI [kg/m2]	-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-value	β (SE)	p-value	β (SE)	p-value
Anthropometric traits						
BMI [kg/m2]	-0.0196 (0.0198)	0.32	-0.0028 (0.0043)	0.2	N.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

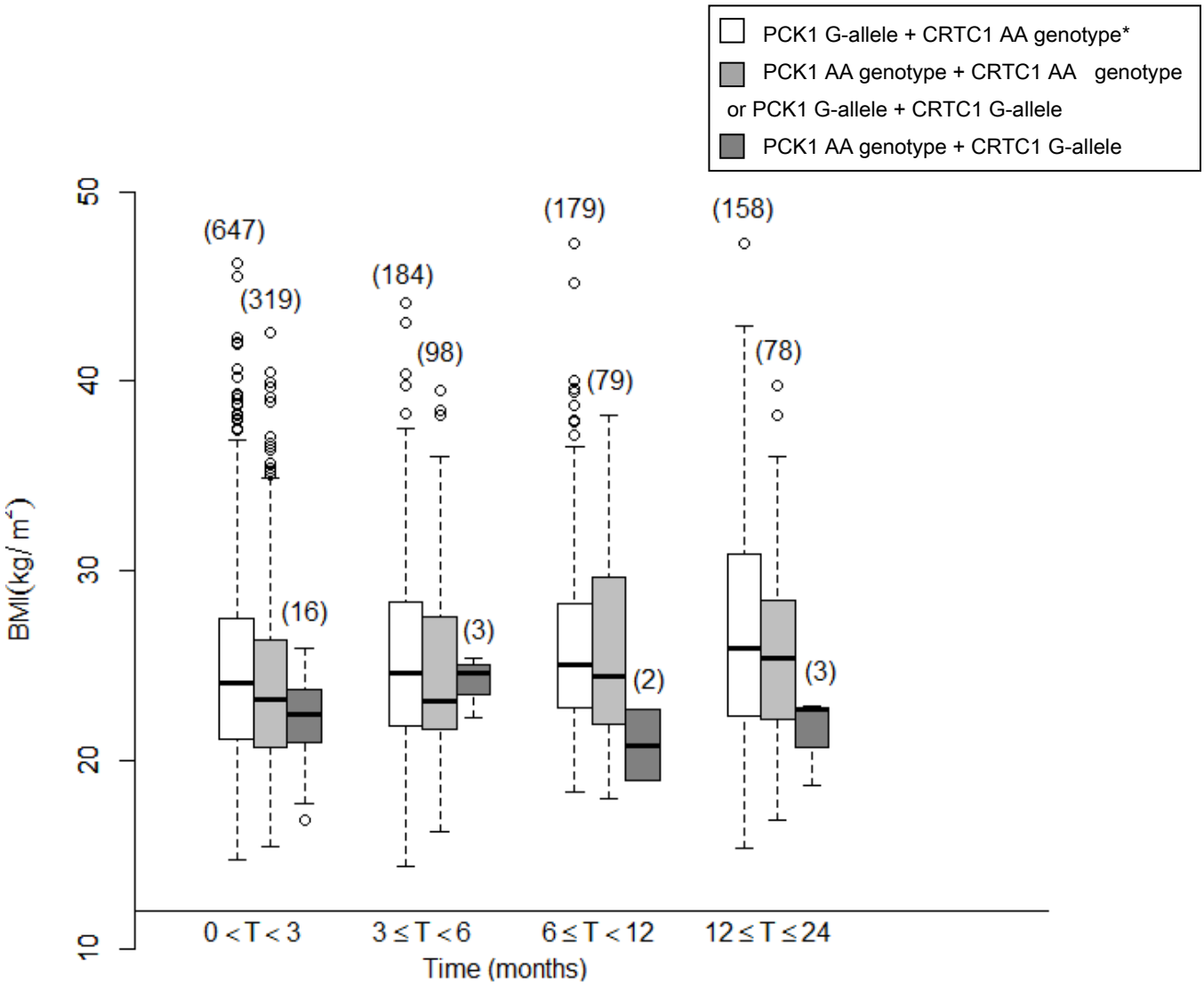
N.A: Data not available. BMI: Body Mass Index, WC: waist circumference, WHR: waist-to-hip ratio, HDL: high lipoprotein cholesterol, CHOL: cholesterol, TG: triglycerides, LDL: low lipoprotein cholesterol, BGL: blood glucose levels.

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).

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 S-1. Selected descriptions of polymorphisms 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

*Source: 1000 Genomes project (http://www.ensembl.org/index.html)

Table S-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

Table S-3. 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 replication Sample 1, 2 : current observation ; for discovery cohort : last follow-up

-- Missing clinical values or obtained in non fasting conditions

a. High LDL cholesterol : equal or higher than 160 mg/100 mL

b. High triglycerides : equal or higher than 196 mg/100 mL

c. Low HDL cholesterol : lower than 39 mg/100 mL

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

Table S-4. Exploratory analysis of the association of *PCK1* polymorphisms with BMI in the three psychiatric samples.

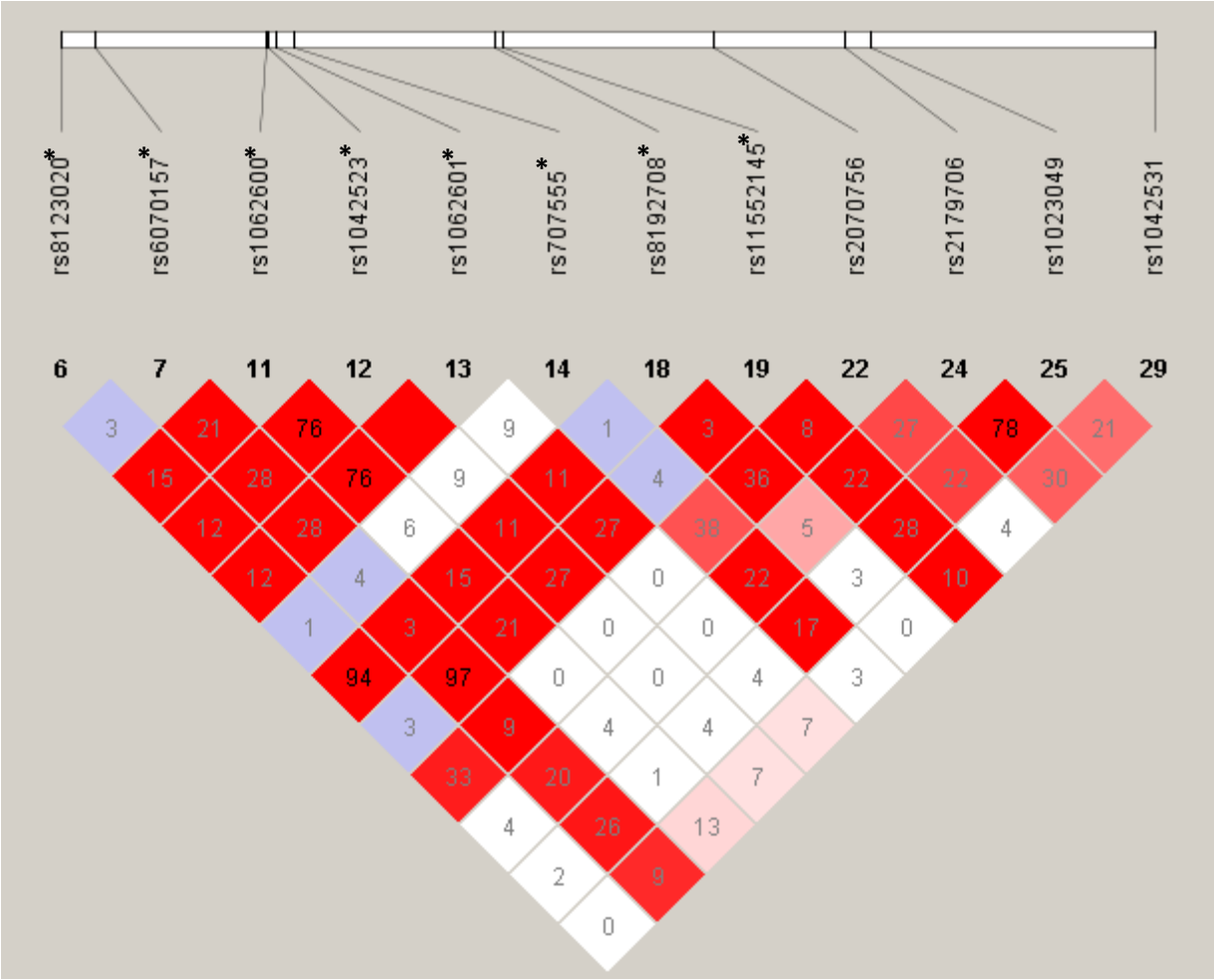
		Discovery Sample[#]			Replication 1			Replication 2			Combined Sample*		
Baseline BMI	***rs11552145***	**AA**	**G-allele**	**p-value[$]**	**AA**	**G-allele**	**p-value**	**AA**	**G-allele**	**p-value**	**AA**	**G-allele**	**p-value**
	n	22	354	0.36	8	131	0.49	10	169	0.46	40	654	*0.05*
	BMI [kg/m^2] (SE)	22.4 (0.7)	24.3 (0.3)		24.3 (1.4)	25.5 (0.4)		23.8 (0.7)	25 (0.4)		23.1 (0.5)	24.7 (0.2)	
	rs707555	**GG**	**C-allele**	**p-value[$]**									
	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.

Figure S-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).*