

## REGULAR RESEARCH ARTICLE

# Genetic Variation in One-Carbon Metabolism and Changes in Metabolic Parameters in First-Episode Schizophrenia Patients

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## Abstract

**Background:** In this study, we aimed to investigate the effects of polymorphisms in genes encoding 1-carbon metabolism enzymes on differential development of metabolic parameters during 12 weeks of treatment with second-generation antipsychotics in first-episode schizophrenia patients.

**Methods:** The following polymorphisms in 1-carbon metabolism genes were genotyped: *MTHFR* (C677T and A1298C), *MTHFD1* (G1958A), *MTRR* (A66G), and *BHMT* (G742A). A broad panel of metabolic parameters including body mass index, waist circumference, total cholesterol low and high density lipoproteins, triglycerides, homocysteine, folate, and vitamin B12 was determined.

**Results:** There was a significant effect of the interaction between the *MTHFR* C677T polymorphism and time on body mass index and waist circumference in the allelic and genotype analyses. Indeed, patients with the *MTHFR* 677CC genotype had higher increase in body mass index and waist circumference compared with other corresponding genotypes or the *MTHFR* 677T allele carriers (CT and TT genotypes). In addition, patients with the *MTHFR* 677TT genotype had higher waist circumference in all time points. Similarly, patients with the *MTHFR* 677TT genotype had higher body mass index in all time points, but this effect was not significant after correction for multiple testing.

**Conclusions:** Our results indicate that the *MTHFR* C677T polymorphism may predict antipsychotic-induced weight gain. Effects of the *MTHFR* C677T polymorphism might be different in initial exposure to antipsychotics compared with long-term perspective.

**Keywords:** antipsychotic-induced weight gain, metabolic syndrome, methylenetetrahydrofolate reductase, *MTHFR*, polymorphism

## Introduction

The development of second-generation antipsychotics (SGA) has been a milestone in clinical psychiatry that has created possibilities of alleviating the burden of extrapyramidal symptoms with

simultaneous maintenance of sufficient efficacy with respect to the treatment of psychotic symptoms. However, it has been observed that SGA exert considerable metabolic side effects,

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## Significance Statement

In this study, we aimed to investigate whether polymorphisms in genes encoding 1-carbon metabolism enzymes might predict changes in metabolic parameters after initiation of treatment with second-generation antipsychotics in first-episode schizophrenia patients. We found that patients with the *MTHFR* 677CC genotype had greater weight gain compared with those with other corresponding genotypes or the *MTHFR* 677T allele carriers (CT and TT genotypes). In addition, patients with the *MTHFR* 677TT genotype had higher body mass index and waist circumference in all time points compared with patients with other corresponding genotypes. Our results indicate that effects of the *MTHFR* C677T polymorphism might be different during the early phase of pharmacological treatment compared with the effects observed in a longer perspective.

including weight gain with its negative cardio-metabolic complications such as diabetes, dyslipidemia, and hypertension (Hasnain et al., 2010). These conditions have been clustered in the definition of metabolic syndrome (MetS), which serves as a risk factor for cardiovascular diseases that are a major contributor of high mortality rates in schizophrenia patients. Weight gain induced by SGA is a major obstacle in maintaining compliance with antipsychotic treatment. However, it has been shown that there is a high inter-individual variability in metabolic adverse effects driven by SGA (Muller et al., 2013). This observation has suggested that genetic variability underlies a differential risk of developing MetS together with its single components in schizophrenia patients. Following this hypothesis, pharmacogenetic studies have appeared to hold a great promise for predicting unfavorable cardio-metabolic outcomes after the initiation of treatment with SGA.

In the past few years, it has been found that genetic variation in the methylenetetrahydrofolate reductase (*MTHFR*) gene might be associated with the risk of antipsychotic-induced weight gain and MetS (Burghardt et al., 2013; Misiak et al., 2013). This enzyme is involved in 1-carbon metabolism and catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is a co-substrate in the conversion of homocysteine (Hcy) to methionine. Two functional polymorphisms have been detected in the *MTHFR* gene (C677T and A1298C). These polymorphisms may lower *MTHFR* activity, leading to hyperhomocysteinemia and low folate levels. Elevated Hcy levels have been reported in various groups of schizophrenia patients (Muntjewerff et al., 2006), including first-episode cases (Kale et al., 2010; Ayesa-Arriola et al., 2012; Garcia-Bueno et al., 2013; Misiak et al., 2014b), and are also recognized as a risk factor for cardiovascular complications. In addition, meta-analyses of studies investigating a specific impact of the *MTHFR* C677T polymorphism revealed that it might increase schizophrenia susceptibility (Peerbooms et al., 2011; Nishi et al., 2014). However, genome-wide association studies have not confirmed these findings (Moustafa et al., 2014).

Three previous cross-sectional studies revealed that the *MTHFR* 677T allele carriers with a diagnosis of schizophrenia are at risk of developing MetS (Ellingrod et al., 2008, 2012). In one cross-sectional study, the *MTHFR* 677T allele was not associated with the risk of MetS in schizophrenia patients; however, the *MTHFR* 1298CC homozygotes had a 2.5-fold increase in the risk of MetS compared with the *MTHFR* 1298AA homozygotes (van Winkel et al., 2010b). However, prospective studies have provided mixed results. In one observational 3-month study, the *MTHFR* C677T polymorphism was not associated with changes in body weight and the levels of glucose, LDL, HDL, and triglycerides (van Winkel et al., 2010a). In the same study, the *MTHFR* 1298CC genotype was related to significantly higher weight gain and greater increase in fasting glucose levels. Other prospective studies have revealed that the *MTHFR* C677T polymorphism might be associated with antipsychotic-induced weight gain (Srisawat et al., 2013; Kao et al.,

2014). In the study by Kao et al. (2014) that included both *MTHFR* polymorphisms, the *MTHFR* 677C allele was associated with greater weight gain; however, no significant association was found at the genotype level. In the subsample of first-episode psychosis patients, the *MTHFR* 677C allele carriers gained approximately twice as much weight, and this allele was associated with a significant decrease in HDL levels. The *MTHFR* 1298C allele was associated with significantly higher weight gain only in first-episode psychosis patients. Finally, in the prospective study by Srisawat et al. (2013), which included 2 samples of Chinese Han and Spanish drug-naïve first-episode schizophrenia (FES) patients, subjects with the *MTHFR* 677CC genotype had significantly higher weight gain in comparison with the *MTHFR* 677T allele carriers. However, this study did not include the analysis of differential evolution of other MetS components. Inconsistent results of previous studies may originate from differences in study design (cross-sectional vs prospective), ethnic heterogeneity, treatment characteristics, and sample size. In addition, previous studies have not focused on other functional polymorphisms in genes encoding enzymes involved in 1-carbon metabolism. In our previous study (Misiak et al., 2016), we investigated whether the *MTHFR* C677T and A1298C polymorphisms together with other functional polymorphisms in genes encoding 1-carbon metabolism enzymes, including the *MTHFD1* (methylenetetrahydrofolate dehydrogenase, methylenetetrahydrofolate cyclohydrolase, and formyltetrahydrofolate synthetase) G1958A polymorphism, the *MTRR* (methionine synthase reductase) A66G polymorphism, and the *BHMT* (betaine-homocysteine methyltransferase) G742A polymorphism, are associated with metabolic dysregulation in FES patients. The *MTHFD1* gene encodes a trifunctional enzyme that catalyzes interconversion of 1-carbon derivatives to tetrahydrofolate. In turn, methionine synthase reductase is involved in reductive methylation of cobalamin that activates methionine synthase, the enzyme converting Hcy to methionine. Similarly, betaine-homocysteine methyltransferase accounts for the synthesis of methionine from Hcy. Although we found a number of associations between polymorphisms in genes encoding 1-carbon metabolism enzymes and metabolic parameters, none of them remained significant after correction for multiple testing. In turn, the aim of this study was to investigate the effects of single nucleotide polymorphisms in 1-carbon metabolism genes (*MTHFR* C677T and A1298C, *MTHFD1* G1958A, *MTRR* A66G, and *BHMT* G742A) on a differential evolution of cardio-metabolic parameters including selected markers of 1-carbon metabolism (Hcy, folate, and vitamin B12) in FES patients after the initiation of treatment with SGA.

## Methods

### Subjects and Procedures

We recruited 135 FES patients (75 males aged  $25.2 \pm 5.0$  years and 60 females aged  $29.7 \pm 7.3$  years) with illness duration over

6 months. A diagnosis of schizophrenia was based on both DSM-IV and ICD-10 criteria validated using the Operational Criteria for Psychotic Illness checklist (McGuffin et al., 1991). All patients were Caucasians and were recruited in the same hospital (Lower Silesian Centre of Mental Health, Wrocław, Poland). The following were the exclusion criteria: general brain disorder, mental retardation, positive urine screening for illicit drugs (amphetamine, cannabis, ecstasy, or opiates), severe physical health impairments, and comorbid drug and/or alcohol use disorder (with exception of nicotine dependence). None of the patients received supplementation of folic acid vitamin B in the course of the study. The study was approved by the local ethics committee, and all participants gave informed consent. At baseline, patients were treated with amisulpride (2 patients), haloperidol (20 patients), olanzapine (58 patients), quetiapine (1 patient), and risperidone (30 patients). There were 24 antipsychotic-naïve patients. The baseline treatment duration was  $6.1 \pm 4.4$  days (up to 17 days) and baseline chlorpromazine equivalent dosage was  $153.4 \pm 111.4$  mg/d. After signing an informed consent, patients receiving haloperidol were switched to olanzapine (15 patients) and risperidone (5 patients), and antipsychotic-naïve patients were assigned to aripiprazole (1 patient), olanzapine (13 patients), and risperidone (10 patients). Other patients remained on a primarily assigned antipsychotic drug. Agitation and hostility in the course of the study were managed using emergency injections of haloperidol and benzodiazepines. Venous blood samples were collected on the day of recruitment and after 12 weeks of antipsychotic treatment. Fasting serum levels of glucose, total cholesterol, LDL, HDL, triglycerides, folate, vitamin B12, and Hcy were determined as described in our previous articles (Misiak et al., 2014a, 2014b). Anthropometric parameters including body mass index (BMI) and waist circumference were measured at baseline and every 4 weeks. Of the total sample, 31 patients were lost to follow-up. Five SNPs were selected for genotyping on the basis of their functional impact: *MTHFR* C677T (rs1801133), *MTHFR* A1298C (rs1801131), *MTHFD1* G1958A (rs2236225), *MTRR* A66G (rs1801394), and *BHMT* G742A (rs3733890) as described in our previous article (Misiak et al., 2016).

## Statistics

Baseline differences in categorical and continuous variables were compared using the Kruskal-Wallis test or the Mann-Whitney U test and the  $\chi^2$  test, where appropriate. In addition, the  $\chi^2$  test was used to test whether genotype distributions were in agreement with the Hardy-Weinberg equilibrium. Due to non-normal distribution of metabolic parameters and missing data, generalized linear mixed models (GLMMs) were applied to data provided in a long format. Metabolic parameters were included as target variables, while particular time points, genotypes, or alleles and interactions between time and genotypes (alleles) represented fixed effects in GLMMs. Baseline chlorpromazine equivalent dosage was added as a random effect to control for pretreatment exposure to antipsychotic treatment. Similarly, baseline folate levels were included as random effects to provide a proxy measure of dietary folate intake. Akaike's Information Criterion and Bayesian Information Criterion were used to choose the best-fitting covariance type. Effects of studied polymorphisms were dichotomized in allelic analysis so that heterozygotes and homozygotes for the minor allele were lumped together (analysis under a dominant genetic model). Similarly, GLMMs were applied to test differences between antipsychotic drugs in terms of metabolic adverse effects. In this analysis,

patients were divided into 2 groups: patients receiving olanzapine ( $n=87$ ) and those receiving other antipsychotics (amisulpride,  $n=2$ ; aripiprazole,  $n=1$ ; quetiapine,  $n=1$ ; and risperidone,  $n=44$ ) after recruitment. Metabolic parameters were included as a target variable, while particular time points, treatment group, and treatment group  $\times$  time interaction represented fixed effects. Baseline chlorpromazine equivalent dosage was also added as a random effect. All tests were 2-tailed with a 0.05 level of significance. Due to multiple comparisons, Bonferroni correction was applied to the level of significance taking into account the number of tested metabolic parameters and polymorphisms. Given that 10 metabolic parameters and 5 polymorphisms were assessed,  $P < .001$  was considered statistically significant after Bonferroni correction. Statistical analysis was performed using the Statistical Package for Social Sciences, version 20 (SPSS Inc.).

## Results

Genotype distribution followed Hardy-Weinberg equilibrium in the initial sample and in patients who completed the study (data not shown). There were no significant differences in general characteristics of patients with respect to genotypes of studied polymorphisms with exception of the *MTRR* A66G polymorphism (Table 1). Indeed, there were significantly more males among the *MTRR* 66AA homozygotes compared with other *MTRR* genotypes. Baseline metabolic parameters with respect to polymorphisms in 1-carbon metabolism genes were presented in our previous article (Misiak et al., 2016). There was a significant worsening of almost all metabolic parameters with exception of Hcy, folate, and vitamin B12 after 12 weeks of antipsychotic treatment (Table 2). The difference in glucose level between baseline and follow-up assessments was not significant after Bonferroni correction ( $P > .005$ ). There were no significant differences between treatment groups in terms of metabolic adverse effects (Table 3).

Supplementary Table 1 presents the results of GLMMs for all polymorphisms. There were significant main effects of time on BMI, waist circumference, total cholesterol, triglycerides, LDL, and folate towards worsening of these metabolic parameters in all GLMMs. After Bonferroni correction, main effects of time on folate levels were not significant in the models including the *MTRR* A66G and the *BHMT* G742A polymorphisms. There was a significant effect of the interaction between the *MTHFR* C677T polymorphism and time on BMI and waist circumference in the allelic and genotype analyses after correction for multiple testing. Indeed, patients with the *MTHFR* 677CC genotype had a higher increase in BMI and waist circumference in comparison with other corresponding genotypes or the *MTHFR* 677T allele carriers (Figure 1). In addition, there was a significant main effect of the *MTHFR* C677T genotype on waist circumference after Bonferroni correction. Patients with the *MTHFR* 677TT genotype had higher waist circumference in all time points (Figure 1). Similarly, these patients had higher BMI in all time points, but this effect was not significant after Bonferroni correction. There were no significant effects of baseline chlorpromazine equivalent dosage and baseline folate levels in all GLMMs. Effects of other polymorphisms were not significant or did not remain significant after Bonferroni correction.

## Discussion

In this study, we found that the *MTHFR* 677CC genotype might be associated with higher antipsychotic-induced weight after 3 months of treatment with SGA in FES patients. These findings

Table 1. General Characteristics with Respect to Gene Polymorphisms

	Age, y	Sex, M (%)	Cigarette smoking, n (%)	Pretreatment duration, d	Baseline chlorpromazine equivalent, mg/d
<i>MTHFR</i> C677T	P = .891	P = .759	P = .402	P = .534	P = .305
CC (n = 64)	27.1 ± 6.3	34 (53.1)	27 (42.2)	5.7 ± 4.4	141.5 ± 116.7
CT (n = 52)	27.0 ± 6.2	30 (57.7)	16 (30.8)	6.7 ± 4.3	167.3 ± 102.4
TT (n = 16)	28.6 ± 8.7	10 (62.5)	5 (31.2)	6.1 ± 5.0	156.2 ± 119.5
<i>MTHFR</i> A1298C	P = .758	P = .208	P = .117	P = .880	P = .499
AA (n = 55)	27.3 ± 7.0	35 (63.6)	20 (36.4)	6.3 ± 4.3	166.7 ± 116.9
AC (n = 64)	27.4 ± 6.2	34 (53.1)	20 (31.2)	6.2 ± 4.7	148.3 ± 111.0
CC (n = 13)	26.1 ± 6.2	5 (38.5)	8 (61.5)	5.5 ± 4.2	123.1 ± 85.7
<i>MTHFD1</i> G1958A	P = .201	P = .169	P = .532	P = .713	P = .365
GG (n = 46)	27.8 ± 6.4	21 (45.6)	14 (30.4)	6.0 ± 4.1	168.6 ± 118.5
GA (n = 61)	26.2 ± 6.4	39 (63.9)	25 (41.0)	6.1 ± 5.0	141.0 ± 131.5
AA (n = 25)	28.6 ± 6.9	14 (56.0)	9 (36.0)	6.6 ± 3.8	156.0 ± 91.6
<i>MTRR</i> A66G	P = .902	P = .049	P = .708	P = .649	P = .867
AA (n = 20)	27.4 6.4	16 (80.0%)	8 (40.0)	5.5 4.4	162.5 138.5
AG (n = 75)	27.6 7.3	37 (49.3%)	25 (33.3)	6.4 4.6	148.9 89.2
GG (n = 37)	26.3 4.9	21 (56.8%)	15 (40.5)	5.9 4.3	157.8 136.4
<i>BHMT</i> G742A	P = .062	P = .525	P = .801	P = .466	P = .233
GG (n = 64)	27.7 6.9	33 (51.6)	24 (37.5)	5.8 4.5	145.9 122.1
GA (n = 50)	27.5 6.0	31 (62.0)	19 (38.0)	6.3 4.3	153.3 104.6
AA (n = 17)	24.1 6.0	10 (58.8)	5 (29.4)	7.3 4.7	179.4 92.0

Data expressed as mean ± SD or the number of cases (%).

Table 2. The Comparison of Baseline and Follow-Up Metabolic Parameters of FES Patients Who Completed the Study

	Baseline	Follow-up	P
BMI, kg/m <sup>2</sup>	23.0 ± 3.1	24.4 ± 3.2	<.001
WC, cm	80.5 ± 11.4	84.9 ± 11.5	<.001
Glucose, mg/dL	85.3 ± 8.0	86.1 ± 6.7	.029 <sup>a</sup>
TC, mg/dL	169.6 ± 33.7	181.0 ± 33.8	<.001
LDL, mg/dL	92.3 ± 26.6	103.0 ± 31.8	<.001
HDL, mg/dL	53.1 ± 14.3	52.7 ± 15.4	<.001
TG, mg/dL	115.6 ± 66.6	143.1 ± 67.0	<.001
Hcy, μmol/L	12.1 ± 4.6	12.2 ± 4.4	.362
Folate, ng/mL	7.0 ± 2.6	6.8 ± 2.4	.280
Vitamin B12, pg/mL	388.2 ± 158.9	379.4 ± 137.2	.056

Abbreviations: BMI, body mass index; Hcy, homocysteine; HDL, high density lipoproteins; LDL, low density lipoproteins; TC, total cholesterol; TG, triglycerides; WC, waist circumference.

Significant differences ( $P < .05$ ) marked in bold characters.

<sup>a</sup>This difference was not significant after Bonferroni correction.

are in agreement with 2 recent longitudinal studies showing that the *MTHFR* 677C allele and/or the *MTHFR* 677CC genotype might predict greater weight gain after initiation of antipsychotic treatment (Srisawat et al., 2013; Kao et al., 2014). It should be noted that opposite results were reported in cross-sectional studies. In these studies, the *MTHFR* 677T allele or the *MTHFR* 677TT genotype was associated with higher risk of MetS in schizophrenia patients (Ellingrod et al., 2008, 2012; Devlin et al., 2012). Interestingly, in our study, the *MTHFR* 677TT genotype was related to higher waist circumference and BMI regardless of antipsychotic treatment. However, the association with BMI was not significant after correction for multiple testing. Srisawat et al. (2013) hypothesized that the impact of the *MTHFR* C677T polymorphism might be different in initial exposure to antipsychotics than that observed in a long-term perspective. Mechanisms underlying the influence of the *MTHFR* gene polymorphisms on antipsychotic-induced weight gain remain unknown. It has been reported that schizophrenic females with the *MTHFR* 677TT have lower methylation

of long interspersed nuclear element-1 sequence, which is a measure of global DNA methylation (Burghardt et al., 2012). In turn, epigenetic phenomena have been widely implicated in the etiology of obesity (van Dijk et al., 2015). In addition, a number of studies have reported that antipsychotics may influence DNA methylation (Melka et al., 2014; Ota et al., 2014; Burghardt et al., 2015; Houtepen et al., 2016; Reynolds et al., 2016). Therefore, it might be assumed that genetic variation in the *MTHFR* gene may influence antipsychotic-induced weight gain via interactions with epigenetic processes induced by antipsychotics.

It should be noted that we found no significant effects of interactions between polymorphisms in 1-carbon metabolism genes on the development of changes in biochemical parameters. To date, in one prospective study, the *MTHFR* C677T predicted changes in HDL levels after initiation of antipsychotic treatment (Kao et al., 2014). In addition, in the study by van Winkel et al. (2010a) the *MTHFR* 1298CC genotype was associated with higher increase in glucose levels. These discrepancies across studies might be associated with differences in ethnicity, exposure to distinct antipsychotics, study duration, dietary intake, or cigarette smoking.

Our study has some limitations that should be taken into account. Firstly, the majority of patients were not antipsychotic-naïve at baseline. However, our genotype and allelic analyses revealed that baseline pretreatment parameters were similar across patients with distinct genotypes. Secondly, our sample size was lower in comparison with other prospective studies evaluating metabolic parameters of first-episode psychosis patients with respect to the *MTHFR* polymorphisms. Nevertheless, we were able to obtain results that are consistent with previous reports. Finally, as similar to previous studies, we did not control for dietary habits, especially regarding folate and vitamins B intake. However, the patients were treated in the same clinical unit and had a similar diet.

In conclusion, our study revealed that the *MTHFR* C677T polymorphism may influence antipsychotic-induced weight gain. However, the impact of the *MTHFR* C677T polymorphism on anthropometric parameters might be different in initial exposure to antipsychotics and in a long-term perspective. Future studies with a longer observation period and more comprehensive



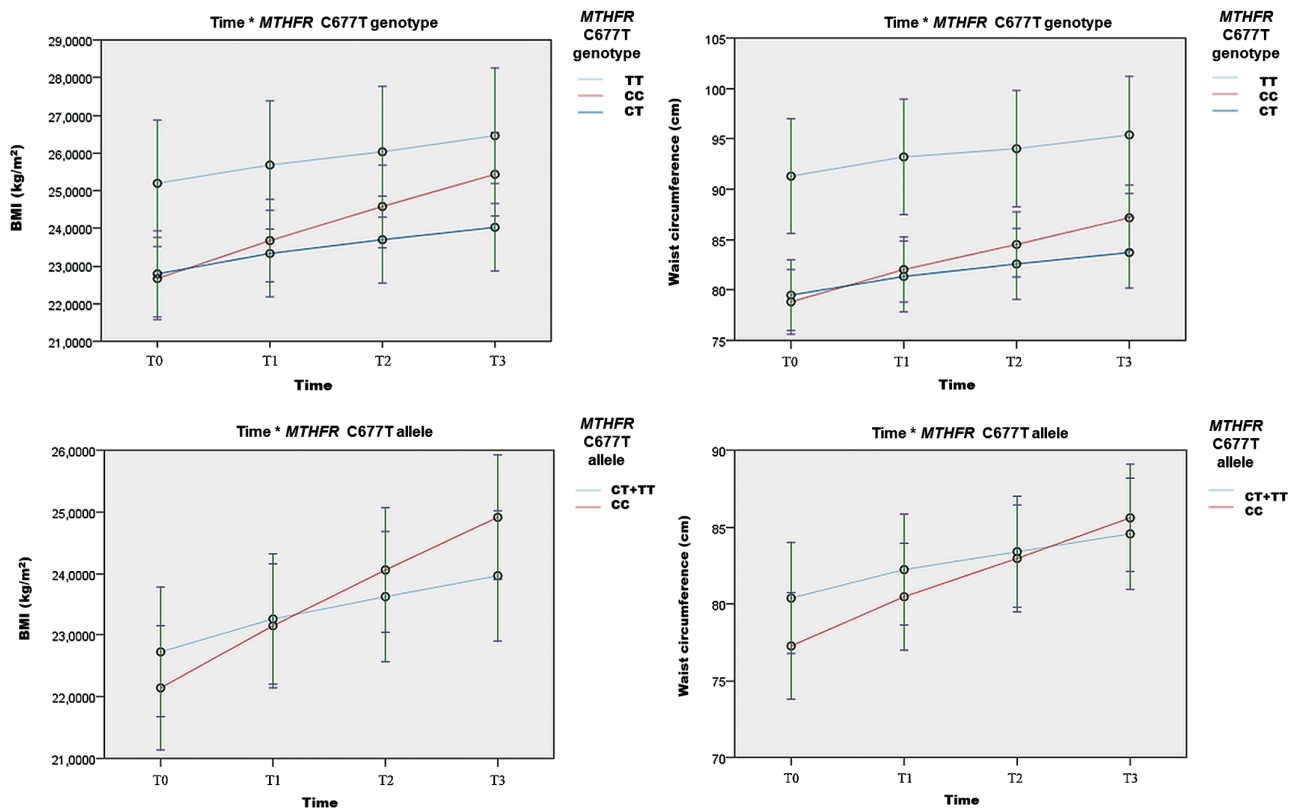
**Table 3.** Effects of Treatment Group on Metabolic Parameters

	Time	Treatment Group <sup>a</sup>	Treatment Group <sup>a</sup> × Time
BMI, kg/m <sup>2</sup>	<b>F = 155.58, P &lt; .001</b>	F = 0.25, P = .614	F = 1.06, P = .366
WC, cm	<b>F = 197.52, P &lt; .001</b>	F = 1.41, P = .236	F = 0.45, P = .717
Glucose, mg/dL	F = 1.64, P = .201	F = 0.001, P = .979	F = 0.19, P = .667
TC, mg/dL	<b>F = 72.22, P &lt; .001</b>	F = 0.538, P = .465	F = 2.10, P = .150
TG, mg/dL	<b>F = 75.44, P &lt; .001</b>	F = 0.79, P = .374	F = 2.35, P = .128
LDL, mg/dL	<b>F = 28.81, P &lt; .001</b>	F = 2.35, P = .128	F = 0.002, P = .965
HDL, mg/dL	F = 0.05, P = .827	F = 0.327, P = .569	F = 3.744, P = .055
Hcy, μmol/L	F = 0.50, P = .481	F = 0.478, P = .491	F = 0.554, P = .458
Vitamin B12, pg/mL	F = 1.90, P = .171	F = 0.13, P = .722	F = 0.14, P = .708
Folate, ng/mL	<b>F = 16.06, P &lt; .001</b>	F = 1.43, P = .234	F = 0.73, P = .395

Abbreviations: BMI, body mass index; Hcy, homocysteine; HDL, high density lipoproteins; LDL, low density lipoproteins; TC, total cholesterol; TG, triglycerides; WC, waist circumference.

Significant effects ( $P < .05$ ) marked in bold characters.

<sup>a</sup>Patients were divided into 2 treatment groups: patients receiving olanzapine ( $n = 87$ ) and those receiving other antipsychotics (amisulpride,  $n = 2$ ; aripiprazole,  $n = 1$ ; quetiapine,  $n = 1$  and risperidone,  $n = 44$ ) after recruitment.



**Figure 1.** Effects of the MTHFR C677T polymorphism on anthropometric measures.

assessment of possible confounding factors might provide the explanation of differential effects of this polymorphism in initial and long-term phase of antipsychotic treatment. Moreover, the inclusion of DNA methylation measures together with the MTHFR polymorphisms in studies exploring predictors of antipsychotic-induced metabolic complications might provide further insight into mechanisms explaining the interaction between variation in the MTHFR gene and antipsychotic-induced weight gain.

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## Statement of Interest

None.

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