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Cardiometabolic risk and the *MTHFR* C677T variant in children treated with second-generation antipsychotics

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Second-generation antipsychotics (SGAs) are increasingly being used to treat children with a variety of psychiatric illnesses. Metabolic syndrome (MetS), a risk factor for cardiovascular disease, is a side-effect of SGA-treatment. We conducted a cross-sectional study and assessed the association of the methylenetetrahydrofolate reductase (*MTHFR*) C677T variant with features of MetS in SGA-treated ($n=105$) and SGA–naïve ($n=112$) children. We targeted the *MTHFR* C677T variant, because it is associated with risk for cardiovascular disease, and features of MetS in adults without psychiatric illness. MetS in children is based on the presence of any three of the following: waist circumference $\geqslant$90th percentile for age and sex; plasma triglyceride $\geqslant 1.24\,\text{mmol}\,l^{-1}$; plasma high-density lipoprotein-cholesterol $\leqslant 1.03\,\text{mmol}\,l^{-1}$; systolic or diastolic blood pressure $\geqslant$90th percentile for age, sex, and height; and fasting glucose $\geqslant 5.6\,\text{mmol}\,l^{-1}$. We found that 15% of SGA-treated children had MetS compared with 2% of SGA-naïve children (OR 8.113, $P<0.05$). No effect of the *MTHFR* C677T variant on psychiatric diagnosis was observed. The *MTHFR* 677T allele was associated ($P<0.05$) with MetS (OR 5.75, 95% CI = 1.18–28.12) in SGA-treated children. Models adjusted for duration of SGA treatment, ethnicity, sex, age and use of other medications revealed a positive relationship between the *MTHFR* 677T allele and diastolic blood pressure *Z*-scores ($P=0.001$) and fasting plasma glucose ($P<0.05$) in SGA-treated children. These findings illustrate the high prevalence of MetS in SGA-treated children and suggest metabolic alterations associated with the *MTHFR* C677T variant may have a role in the development of MetS features in SGA-treated children.

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Introduction

Metabolic syndrome (MetS) is a side-effect associated with use of second-generation antipsychotic (SGA) medications in adults.[1] SGAs, also known as atypical antipsychotics, are increasingly being used to treat children with a wide range of psychiatric illnesses, including anxiety disorder, attention deficit hyperactivity disorder, bipolar disorder, depressive disorder, pervasive developmental disorder, psychotic disorder and disruptive behavior disorder. Recent estimates have shown that SGA prescriptions for children (14 years and younger) in British Columbia increased 10-fold from 1997–2007.[2] Concern is now being raised as to whether MetS develops in children treated with SGAs and the potential long-term consequence of this side-effect.

One classification of MetS in children is based on the National Cholesterol Education Program-Adult Treatment Panel definition[3] and modified to adhere to the American Diabetes Association definition of impaired fasting glucose of $\geqslant 5.6\,\text{mmol}\,l^{-1}$.[4] This definition includes the presence of any three of the following features: waist circumference $\geqslant$90th percentile for age and sex; plasma triglyceride (TG) $\geqslant 1.24\,\text{mmol}\,l^{-1}$; plasma high-density lipoprotein (HDL) $\leqslant 1.03\,\text{mmol}\,l^{-1}$; systolic or diastolic blood pressure $\geqslant$90th percentile for age, sex, and height; and fasting glucose $\geqslant 5.6\,\text{mmol}\,l^{-1}$. We recently conducted a cross-sectional study and found that 19% of SGA-treated children ($n=84$) had MetS compared with 0.8% of SGA-naïve children ($n=127$) ($P<0.001$).[5] Furthermore, a prospective study showed that SGA-naïve children initiated on SGA treatment and followed for a mean duration of 10.8 weeks had significant increases in weight, body mass index (BMI), waist circumference, and plasma TGs relative to untreated children.[6] This study further showed that the effect of SGA treatment on other cardiometabolic risk factors was SGA-specific with elevated fasting blood glucose levels observed only in children treated with olanzapine. The means by which to distinguish children at risk for developing MetS from those that do not and the mechanism by which MetS develops in SGA-treated children are not known.

Methylenetetrahydrofolate reductase (encoded by *MTHFR*), is an enzyme that coverts folate, a B-vitamin, to the metabolically active form of 5-methyltetrahydrofolate. Homozygosity for a common variant in *MTHFR*, C677T, occurs in ~10% of individuals of European descent[7] and is associated with increased risk of schizophrenia,[8] major

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depressive disorder and bipolar disorder in adults.[8,9] Interestingly, homozygosity for this variant is also associated with increased risk of coronary heart disease,[10] and a recent cross-sectional study found that 53% of SGA-treated adult schizophrenia patients that carried the T-allele had MetS compared with 23% of SGA-treated adult schizophrenia patients with the CC genotype.[11]

As a first step towards delineating the mechanism by which MetS develops in SGA-treated children, we assessed the association of the *MTHFR* C677T variant with features of MetS in a cross-sectional population of SGA-treated and SGA-naïve children with mental illness. Furthermore, given the association of the *MTHFR* C677T variant with psychiatric illness in adults,[8,9] we also assessed the association of the *MTHFR* C677T variant with psychiatric diagnoses in these children.

Materials and methods

Study design and patient recruitment. A cross-sectional study design was used to examine the relationship between the *MTHFR* C677T variant and the metabolic consequences of SGA-treatment in children. Study participants were inpatients of either the emergency or long-stay units of the Child and Adolescent Psychiatry Department at British Columbia Children's Hospital. Patients were assessed between April 2008 and June 2011. Inclusion criteria included: either currently receiving SGA-treatment (SGA-treated patients) or not currently being treated with an SGA on admission or not previously treated with an SGA (SGA-naïve patients), and $\leqslant$18 years of age. Exclusion criteria included: known cardiometabolic or endocrine disease (including pre-existing type 1 and type 2 diabetes mellitus), diagnosed eating disorder or current treatment with medications known to affect metabolism (e.g., glucocorticoids). On admission, parents or legal guardians provided written informed consent to participate in the study. Children provided written assent where capable. All protocols described were approved by the University of British Columbia Clinical Research Ethics Board and the Children's and Women's Health Centre Research Ethics Board.

Clinical, biochemical and anthropometric measurements. The medical records of patients were reviewed over the course of admission and the following data were collected: socio-demographic information (sex, age, self-reported ethnicity); medical history, including psychiatric diagnoses, and medications (including dose and duration); smoking habits, family medical and psychiatric illness history. The Global Assessment of Functioning score (range: 1–100), designed to reflect overall psychological, social and occupational functioning, and not functioning based upon physical impairments, was determined by the attending psychiatrist using a Multiaxial evaluation as described in the DSM-IV-TR.[12] Psychiatric diagnoses were based on independent assessments by two Royal College of Physicians and Surgeons (Canada)-certified psychiatrists.

Anthropometric measures included height, weight and waist circumference. Weight was assessed in patients wearing light clothing, pockets emptied, without shoes or socks, and recorded to the nearest 0.1 kg (Tronix Scale model 5002, White Plains, NY, USA). Height was assessed in patients to the nearest 0.1 cm (Seca 240 Stadiometer, Hamburg, Germany). Waist circumference was assessed using a non-elastic flexible tape measure at the level of the umbilicus (two measurements averaged).[13] BMI was calculated (weight (kg)/height^2 (m^2)) and then standardized for sex and age using data from the US Centers for Disease Control growth charts.[14] Overweight was defined as BMI $\geqslant$85th and $<$95th percentile, and obesity as BMI $\geqslant$95th percentile for age and sex.[15]

Diastolic and systolic blood pressure measures (average of three readings) were collected after the child had settled into the unit (at least 24 h post admission), using a Dinamap automated monitor and an appropriately sized cuff.[16] A blood sample was collected by venipuncture after an overnight fast of $\geqslant$8 h. The following biochemical analyses were conducted in the clinical laboratory at British Columbia Children's Hospital: plasma glucose, insulin, total cholesterol, low-density lipoprotein cholesterol, HDL, TG, alanine aminotransferase and aspartate aminotransferase.

MetS was diagnosed based on the National Cholesterol Education Program-Adult Treatment Panel definition[3] and modified to adhere to the American Diabetes Association definition of impaired fasting glucose.[4] A diagnosis was made based on the presence of any three of the following features: waist circumference $\geqslant$90th percentile for age and sex;[17] plasma TG $\geqslant$1.24 mmol l^{-1}; plasma HDL $\leqslant$1.03 mmol l^{-1}; systolic or diastolic blood pressure $\geqslant$90th percentile for age, sex, and height;[16] and fasting plasma glucose $\geqslant$5.6 mmol l^{-1}.

Genomic DNA isolation and genotyping. Buccal epithelial cells were collected by scraping the inner cheek of patients with collection swabs (Puritan Medical Products, Guilford, ME, USA). Genomic DNA was isolated using the QIAamp DNA Mini Kit (Qiagen, Mississauga, Canada) and quantified using a NanoVue Spectrophotometer (GE Healthcare, Montreal, Canada). Genotyping of the *MTHFR* C677T variant (rs1801133) was accomplished using Taqman SNP Genotyping reagents and a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA).

Statistical analyses. Subjects were categorized as SGA-treated and SGA-naïve. Pearson's χ^2-tests were used to compare categorical variables between SGA-treated and SGA-naïve patients. General linear models were used to compare continuous variables between SGA-treated and SGA-naïve patients. Logistic and linear regression models were used to assess the effect of the *MTHFR* C677T genotype on MetS diagnosis and characteristics of MetS. Models were adjusted for age, sex, duration of SGA treatment, treatment with medications known to promote weight gain (mood stabilizers, antidepressants and first-generation antipsychotics), treatment with medications known to promote weight loss (psychostimulants and Atomoxetine) and ethnicity. Analyses were conducted using SPSS software version 18.0 (SPSS, Chicago, IL, USA).

Results

Socio-demographic, anthropometric and metabolic characteristics of study population. In total, 261 subjects consented to the study; 44 subjects were excluded due to pre-existing cardiometabolic disease, endocrine abnormalities, diagnosed eating disorders and/or treatment with glucocorticoids; and 8 subjects did not have DNA collected. The socio-demographic and characteristics of the subjects are given in Table 1. There were no differences in age, sex, ethnicity, smoking or global assessment of functioning scores between SGA-treated and SGA-naïve children. The majority of our study population was of European-descent (74% SGA-treated and 71% SGA-naïve). The median duration of SGA-treatment was 6 months (ranging from 0.25 to 76 months). The majority of SGA-treated children were receiving quetiapine (46.7%) or risperidone (43.8 %) (Table 1). Children were also often treated with other medications including antidepressants, psychostimulants, mood stabilizers, atomoxetine and first generation antipsychotics, which were controlled for in our logistic and linear regression models.

Similar to our previous findings, SGA-treated children had higher ($P<0.05$) zBMI with 30.1% of SGA-treated patients having a BMI $\geqslant$95th percentile compared with 17% of SGA-naïve patients (Table 2). SGA-treated children had a higher ($P=0.001$) prevalence of MetS (15.6% of patients) than SGA-naïve children (2.1% of patients), with an odds ratio of 8.11 (95% CI = 1.41–46.77) (Table 2). We further assessed characteristics of the MetS and found that SGA-treated children had higher ($P<0.05$) systolic blood pressure *Z*-scores; a higher ($P<0.05$) prevalence of children with blood pressure $\geqslant$90th percentile for age, sex and height; and a higher ($P<0.05$) prevalence of children with waist circumference $\geqslant$90th percentile for age and sex than SGA-naïve children. SGA-treated children also had higher fasting plasma glucose ($P=$ 0.005), insulin ($P<0.043$), total cholesterol ($P<0.01$) and low-density lipoprotein ($P<0.05$) levels compared with the SGA-naïve patients (Table 2).

Table 1 Characteristics of the study population

Characteristic	*SGA-treated* (n = *105*)	*SGA-naïve* (n = *112*)	P *value*
Age (years), mean (s.d.)	12.58 (3.14)	13.19 (2.86)	0.133
Male sex, *n* (%)	70 (66.7)	64 (57.1)	0.096
Ethnicity, *n* (%)			0.890
European	77 (74.0)	79 (70.5)	
Asian	9 (8.7)	9 (8.0)	
Aboriginal	3 (2.9)	4 (3.6)	
South Asian	3 (2.9)	3 (2.7)	
African/Caribbean	7 (10.7)	12 (10.6)	
Hispanic	5 (4.8)	4 (3.6)	
Other	0 (0)	1 (0.9)	
Smoker, *n* (%)	15 (15.0)	21 (19.1)	0.274
GAF score, mean (s.d.)	54.05 (10.83)	55.13 (8.68)	0.436
SGA median duration in months (range)	6.00 (0.25-76.0)	N/A	
SGA			
Quetiapine, *n* (%)	49 (46.7)		
Risperidone, *n* (%)	46 (43.8)		
Aripiprazole, *n* (%)	5 (4.8)		
Olanzapine,*n* (%)	4 (3.8)		
Ziprasidone, n (%)	1 (1.0)		

Abbreviations: GAF, global assessment of functioning; NA, not applicable; SGA, second-generation antipsychotic.
Differences between SGA-treated and SGA-naïve patients were assessed by unadjusted Pearson's χ^2-tests for categorical variables and by general linear models for continuous variables.

Table 2 Cardiometabolic characteristics of the study population

Characteristic	*SGA-treated* (n = *105*)	*SGA-naïve* (n = *112*)	P *value*
MetS,*n* (%)	15 (15.6)	2 (2.1)	0.001
zBMI, mean (s.d.)	1.013 (0.979)	0.731 (0.973)	0.038
[a]obese, *n* (%)	31 (30.1)	18 (17.0)	
overweight,*n* (%)	23 (22.3)	24 (22.6)	
normal, *n* (%)	49 (47.6)	64 (60.4)	
WC $\geqslant$90thpercentile, *n* (%)	39 (37.1)	28 (25.0)	0.037
WC (cm), mean (s.d.)	78.9 (15.0)	77.3 (17.5)	0.488
BP $\geqslant$90th percentile, *n* (%)	25 (25.0)	15 (14.4)	0.042
SBP *Z*-score, mean (s.d.)	0.445 (1.07)	0.112 (0.90)	0.016
DBP *Z*-score, mean (s.d.)	0.409 (0.72)	0.412 (0.67)	0.976
Impaired fasting glucose ($\geqslant$5.6 mmol l^{-1}), *n*(%)	6 (5.7)	3 (2.7)	0.218
Fasting glucose (mmol l^{-1}), mean (s.d.)	4.92 (0.34)	4.78 (0.34)	0.005
Fasting insulin (pmol l^{-1}), mean (s.d.)	61.31 (40.76)	50.94 (26.02)	0.043
Elevated TG ($\geqslant$1.24 mmol l^{-1}), *n* (%)	24 (22.9)	19 (17.0)	0.179
TG (mmol l^{-1}), mean (s.d.)	1.09 (0.67)	0.96 (0.41)	0.100
Low HDL ($\leqslant$1.03 mmol l^{-1}), *n* (%)	23 (21.9)	15 (13.4)	0.071
HDL (mmol l^{-1}), mean (s.d.)	1.32 (0.39)	1.30 (0.30)	0.716
LDL (mmol l^{-1}), mean (s.d.)	2.76 (0.96)	2.45 (0.72)	0.017
Total cholesterol (mmol l^{-1}), mean (s.d.)	4.54 (1.05)	4.19 (0.84)	0.009
AST (U/L), mean (s.d.)	32.57 (12.24)	30.12 (10.22)	0.138
ALT (U/L), mean (s.d.)	22.82 (16.12)	20.74 (13.66)	0.339

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; MetS, metabolic syndrome; SBP, systolic blood pressure; SGA, second-generation antipsychotic; TG, triglyceride; WC, waist circumference.
The effect of SGA-treatment was analyzed by unadjusted Pearson's χ^2-tests for categorical variables and unadjusted general linear models for continuous variables.
[a]Obese = BMI $\geqslant$95th percentile; overweight = BMI $\geqslant$85th percentile; and normal = BMI $<$85th percentile.

Association of the *MTHFR* C677T variant with psychiatric diagnosis. The *MTHFR* 677TT genotype occurs at a frequency of ~10–12% in populations of European-decent.[7,18] In this study, we found 17 out of 209 (8.1%) of our study patients (8 out of 99 SGA-treated and 9 out of 110 SGA-naïve) had the *MTHFR* 677TT genotype with a 27% T-allele frequency. Given the association of the *MTHFR* C677T variant with psychiatric illness in adults,[8,9] we assessed the association of the *MTHFR* C677T variant with psychiatric diagnoses in our pediatric study patients. The patients in our study had a wide-range of psychiatric diagnoses. As such, patients with multiple DSM-IV Axis I diagnoses were assessed under multiple diagnostic categories. Interestingly, we found no association between the *MTHFR* C677T variant and psychiatric diagnoses in our patient population (Table 3).

Association of the *MTHFR* C677T variant and MetS characteristics. A higher prevalence of MetS has been previously reported in SGA-treated adults with schizophrenia carrying the T-allele of the *MTHFR* C677T variant than those with the CC genotype.[11] As such, we investigated the association of the *MTHFR* C677T variant with MetS and MetS components in the SGA-treated children in our study population. The SGA-naïve patients were not included in this analysis, because of the low prevalence of MetS diagnosis (2%) in this group. We found that SGA-treated patients carrying the T-allele had a higher prevalence ($P<0.05$) of MetS, higher ($P=0.005$) diastolic blood pressure *Z*-scores, and higher ($P<0.05$) fasting plasma glucose compared with patients with the CC genotype (Table 4). Further analyses using logistic regression models adjusted for duration of SGA-treatment, ethnicity, sex, age and use of other medications revealed that the T-allele of the *MTHFR* C677T variant was associated with a greater ($P=0.03$) chance of having MetS (odds ratio = 5.75, 95% CI = 1.18–28.12).

Table 3 *MTHFR* C677T variant and pediatric psychiatric diagnoses

Diagnosis	***MTHFR C677T Genotype***		
	***CC* (n = *113*)**	***CT and TT* (n = *96*)**	**P *value***
Psychotic disorder, *n* (%)	11 (9.7)	8 (8.3)	0.812
Depressive disorder, *n* (%)	29 (25.7)	22 (22.9)	0.448
Bipolar disorder, *n* (%)	55 (48.7)	49 (51.0)	0.174
Anxiety disorder, *n* (%)	14 (12.4)	16 (16.7)	0.884
ADHD, *n* (%)	36 (31.9)	26 (27.1)	0.707
Disruptive behavior disorder, *n* (%)	14 (12.4)	11 (11.5)	0.672
Pervasive developmental disorder, *n* (%)	7 (6.2)	14 (14.6)	0.056
Substance-related disorder, *n* (%)	11 (9.7)	9 (9.4)	0.930

Abbreviations: ADHD, attention deficit hyperactivity disorder; *MTHFR,* methylenetetrahydrofolate reductase.
The effect of the *MTHFR* C677T variant on pediatric psychiatric diagnoses was analyzed by Pearson's χ^2-tests.

Table 4 Cardiometabolic characteristics of SGA-treated patients and *MTHFR* C677T genotype

Characteristic	***MTHFR C677T genotype***		
	***CC* (n = *54*)**	***CT and TT* (n = *45*)**	**P *value***
MetS,*n* (%)	4 (7.8)	10 (25.0)	0.039
WC $\geqslant$90thpercentile, *n* (%)	16 (29.6)	20 (44.4)	0.146
WC (cm), mean (s.d.)	76.3 (13.4)	80.5 (15.2)	0.170
BMI *Z*-score, mean (s.d.)	0.897 (0.885)	1.111 (1.084)	0.285
BP $\geqslant$90th percentile, *n* (%)	9 (17.3)	13 (31.0)	0.145
SBP *Z*-score, mean (s.d.)	0.278 (1.08)	0.562 (1.07)	0.205
DBP *Z*-score, mean (s.d.)	0.195 (0.755)	0.604 (0.596)	0.005
Fasting glucose $\geqslant$5.6 mmol l, *n* (%)	2 (3.7)	3 (6.7)	0.657
Fasting glucose (mmol l^{-1}), mean (s.d.)	4.85 (0.30)	5.01 (0.37)	0.028
Fasting insulin (pmol l^{-1}), mean (s.d.)	56.27 (37.96)	65.00 (43.06)	0.318
TG $\geqslant$1.24 mmol l^{-1}, *n* (%)	10 (18.5)	13 (28.9)	0.242
TG (mmol l^{-1}), mean (s.d.)	1.10 (0.71)	1.09 (0.57)	0.896
HDL $\leqslant$1.03 mmol l^{-1}, *n* (%)	12 (22.2)	10 (22.2)	1.000
HDL(mmol l^{-1}), mean (s.d.)	1.35 (0.36)	1.27 (0.42)	0.304
LDL (mmol l^{-1}), mean (s.d.)	2.71 (0.92)	2.91 (1.00)	0.331
Total cholesterol (mmol l^{-1}), mean (s.d.)	4.58 (1.03)	4.62 (1.06)	0.850

Abbreviations: BMI, body mass index; BP, blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; MetS, metabolic syndrome; *MTHFR,* methylenetetrahydrofolate reductase; SBP, systolic blood pressure; SGA, second-generation antipsychotic; TG, triglyceride; WC, waist circumference.
The effect of the *MTHFR* C677T variant on cardiometabolic characteristics was analyzed by unadjusted Pearson's χ^2-tests for categorical variables and unadjusted general linear models for continuous variables.

Table 5 Relationship between the *MTHFR* C677T variant and cardiometabolic characteristics in SGA-treated children

Characteristic	*Standardized β-coefficient*	P *value*
BMI *Z*-score	0.095	0.357
WC	0.150	0.090
SBP *Z*-score	0.172	0.068
DBP *Z*-score	0.313	0.001
Fasting glucose	0.256	0.022
Fasting insulin	0.120	0.259
Triglyceride	0.003	0.978
HDL	−0.123	0.249

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein cholesterol; SBP, systolic blood pressure; SGA, second-generation antipsychotic; WC, waist circumference.
Adjusted for duration of SGA treatment, ethnicity, sex, age and use of other medications.

Linear regression models adjusted for duration of SGA-treatment, ethnicity, sex, age and use of other medications were used to assess the relationship of the *MTHFR* C677T genotype with individual characteristics of MetS in SGA-treated children. The T-allele of the *MTHFR* C677T variant was positively associated with diastolic blood pressure *Z*-scores (β-coefficient = 0.313, $P = 0.001$) and fasting glucose levels (β-coefficient = 0.256, $P < 0.05$) (Table 5). No relationships were observed between anthropometric measures (waist circumference, BMI *Z*-scores) and plasma HDL and TG levels (Table 5).

Discussion

The goal of this study was to determine if the *MTHFR* C677T variant is associated with psychiatric diagnoses and characteristics of the MetS in a cross-sectional population of SGA-treated children and SGA-naïve children recruited through our inpatient psychiatric units. There are three main findings of this study. As we predicted, we found that MetS is more prevalent in SGA-treated children compared with SGA-naïve children. Elevated blood pressure and elevated fasting glucose, both components of the MetS, are also more prevalent in SGA-treated children compared with SGA-naïve children. Interestingly, we found that the *MTHFR* C677T variant was associated with increased prevalence of MetS in SGA-treated children, with the T-allele associated with significantly higher blood pressure and higher fasting plasma glucose levels than children with the *MTHFR* 677CC genotype.

This current study confirms the higher prevalence of MetS in a second, distinct population of SGA-treated children and is similar to the findings of our initial study that showed MetS is more prevalent in SGA-treated children than in SGA-naïve children.[5] Studies in adults have reported MetS in 37.3% of SGA-treated patients,[1] which is higher than the prevalence rates of 15.6% in SGA-treated children (median duration 6 months) in the current study and 19% in SGA-treated children (median treatment duration of 14 months) in our prior study.[5] The difference in prevalence rates between adults and children is likely because MetS is not as common in children as it is in adults.

When we further investigated differences in the components of the MetS between SGA-treated and SGA-naive children, we found higher waist circumference, blood pressure, and fasting plasma glucose levels but no differences in plasma TGs or HDL in the SGA-treated children compared with SGA-naïve children. A study in children treated with risperidone and followed for 8 weeks found significant increases in body weight, BMI *Z*-scores and waist circumference, but no differences in plasma lipids or glucose.[19] Another study showed that children treated with olanzapine and risperidone and followed for 4 weeks had significant increases in BMI, but only those treated with olanzapine had elevations in blood pressure and diagnosis of MetS from baseline[20] suggesting the type of SGA-treatment may influence the development of MetS characteristics. However, in our current study only 3.8% of the children were treated with olanzapine, with the majority treated with either risperidone (43.8%) or quetiapine (46.7%), similar to our prior study.[5] Furthermore, a prospective study showed significant increases in BMI *Z*-scores, waist circumference, fat mass and TGs in children initiated on treatment with olanzapine, risperidone or quetiapine and followed for a mean duration of 10.8 weeks.[6] However, only olanzapine treatment in this study was associated with significant elevations in fasting blood glucose. Taken together, these studies suggest that both the type of SGA treatment and duration of treatment may influence the development of MetS characteristics.

Surprisingly, we found no association of the *MTHFR* 677T allele with psychiatric diagnoses. Studies in adults have reported an association between the *MTHFR* 677TT genotype and bipolar disorder, major depressive disorder and schizophrenia.[8,9] Furthermore, we recently found greater depressed mood in pregnant women that were homozygous for the *MTHFR* C677T variant, an effect that was not influenced by serotonin reuptake inhibitor treatment.[21] In the current study we did not assess the effects of the *MTHFR* 677TT genotype on psychiatric diagnosis because of the low frequency of the genotype in our population. Further larger studies are required to definitely assess the effects of the *MTHFR* 677TT genotype on psychiatric diagnosis in children.

Similar to what has been reported in studies in SGA-treated adults with schizophrenia,[11] we found a significant association of the T-allele of the *MTHFR* C677T variant with MetS and also observed a positive association of the T-allele with diastolic blood pressure *Z*-scores and fasting plasma glucose levels. However, others have reported no association of the *MTHFR* C677T variant with MetS in SGA-treated adults, but did report an association between the *MTHFR* A1298C variant and MetS.[22] Further, this same group investigated the influence of the *MTHFR* C677T and A1298C variants on the development of MetS characteristics within the first 3 months of SGA-treatment and reported that only the *MTHFR* 1298CC genotype was associated with greater weight gain, fasting plasma glucose and plasma glucose concentrations at 120 min during an oral glucose tolerance test.[23] However, another study reported no effect of the *MTHFR* A1298C variant on MetS in SGA-treated adults.[11] We also assessed the effect of the *MTHFR* A1298C variant in our population, but found no association with MetS or its components. This may in part be because of the low frequency of the *MTHFR*

1298CC genotype (7.1%) in our patients (unpublished data).

Little is known regarding the mechanisms by which the *MTHFR* C677T variant may be associated with MetS in SGA-treated children. The MTHFR enzyme is required for the metabolism of folate and the production of 5-methyltetrahydrofolate, which is used in the remethylation of homocysteine to methionine, an important step in the generation of methyl groups. Homozygosity for the *MTHFR* C677T variant is associated with a thermolabile form of the enzyme,[7] elevated plasma total homocysteine levels,[7,18] and global changes in DNA methylation.[24–26] Given the metabolic tie of MTHFR to DNA methylation, an epigenetic process important for the regulation of gene expression, the mechanism by which the *MTHFR* 677T allele is associated with development of MetS in SGA-treated children may be related to changes in DNA methylation and a gene expression profile that favours development of MetS characteristics.

Prior studies in adult populations, with no underlying psychiatric diagnoses or SGA-treatment, have shown the *MTHFR* C677T variant is associated with an increased risk for cardiovascular disease,[10] an effect attributed to elevations in plasma total homocysteine[27] and the vascular metabolism of folates.[28] In the current study, we were not able to quantify plasma total homocysteine levels. We also found significant associations of the T-allele of the *MTHFR* C677T variant with diastolic blood pressure *Z*-scores and fasting plasma glucose levels in SGA-treated children. Interestingly several studies in adult populations have reported associations of the *MTHFR* C677T variant with blood pressure.[29–31] A meta-analysis showed that the *MTHFR* C677T variant is associated with increased risk for hypertension.[29] Furthermore, recent genome-wide association studies have identified the *MTHFR* loci to be associated with systolic and diastolic blood pressure.[30,31] Therefore, the *MTHFR* C677T variant may have a strong influence on blood pressure in SGA-treated children and contribute to the association of the T-allele with MetS diagnosis.

Overall, this study is the first to demonstrate that the T-allele of the *MTHFR* C677T variant is associated with MetS and its characteristics of elevated blood pressure and higher fasting plasma glucose levels in SGA-treated children. The mechanism by which the *MTHFR* 677T allele may contribute to the development of MetS and its components is not known. Further studies are required to investigate potential mechanisms and to determine whether SGA-treatment produces disturbances in methyl metabolites and epigenetic changes in gene expression, especially those regulated by DNA methylation.

Conflict of interest

The authors declare no conflict of interest.

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