Contribution of Methylenetetrahydrofolate Reductase (*MTHFR*) Polymorphisms to Negative Symptoms in Schizophrenia

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Background: Folate deficiency may contribute to negative symptoms in schizophrenia, but the underlying mechanism remains uncertain. We examined whether the methylenetetrahydrofolate reductase (*MTHFR*) C677T and A1298C functional polymorphisms contribute to negative symptoms.

Methods: Outpatients with schizophrenia ($n = 200$) were evaluated with the Positive and Negative Syndrome Scale (PANSS). Subjects also provided a blood sample for *MTHFR* genotype and serum chemistries. Comparisons of PANSS symptoms, folate, and homocysteine status were conducted based on genotype.

Results: The 677T allele load was associated with negative symptom severity. Contrary to our expectations, the T allele was also found to be protective against positive symptoms. The A1298C polymorphism did not contribute to negative symptoms, and only weakly to positive symptoms. The specific effects of the C677T polymorphism were confirmed with haplotype analysis. Among patients homozygous for the 667T allele, serum folate levels correlated with negative symptom severity.

Conclusions: Increased *MTHFR* 677T allele load confers risk for negative symptoms in schizophrenia, while reducing severity of positive symptoms. Further, the biochemical interaction of low serum folate with 677T-variant MTHFR may induce downstream effects salient to the expression of negative symptoms.

Key Words: Folate, genetics, homocysteine, MTHFR, negative symptoms, schizophrenia

Folic acid is essential to human biochemistry, serving roles in homocysteine metabolism, neurotransmitter synthesis, and gene expression. Successful processing of folate relies on proper dietary intake and on functional intracellular machinery for folate absorption and metabolism. Deficits in either area can lead to functional folate deficiency, which has been associated with several neuropsychiatric conditions (Alpert *et al.* 2000; Clarke *et al.* 1998) including schizophrenia (Godfrey *et al.* 1990; Goff *et al.* 2004; Muntjewerff *et al.* 2003). Prior work by our group suggests that individuals with schizophrenia and low serum folate levels are especially vulnerable to negative symptoms (Goff *et al.* 2004).

There are several potential explanations for the relationship between folate deficiency and schizophrenia. On one hand, folate deficiency may not be problematic itself, but rather could represent an epiphenomenon of other problems associated with schizophrenia (e.g., negative symptoms resulting in poor dietary intake). The success of two folate supplementation trials in patients with schizophrenia (Godfrey *et al.* 1990; Levine *et al.* 2006) argues against this notion: supplementation isolates folate intake from other variables that might mediate negative symptoms (such as general nutrition), and its effectiveness as a controlled intervention suggests that folate levels determine negative symptom severity, rather than vice versa. Alternatively, low folate intake, impaired absorption, altered folate metabolism, or some combination of these deficits could induce downstream effects relevant to the pathophysiology of schizophrenia.

Evidence implicating methylenetetrahydrofolate reductase (MTHFR), an enzyme in the folate metabolic pathway, has been gradually accumulating over the last 30 years of schizophrenia research. MTHFR catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (Figure 1). This reaction product serves as a carbon donor for homocysteine metabolism and other intracellular methylation processes. A dramatic case of folate-responsive psychotic symptoms in an individual with 82% reduced MTHFR activity was reported in 1975 (Freeman *et al.* 1975). A subsequent study of brain tissue indicated 18–42% reductions in MTHFR activity across brain regions in 10 patients with schizophrenia (Elliott *et al.* 1978). More recently, gene association studies have generated renewed interest in MTHFR. These studies have examined whether heritable variations in MTHFR activity, attributable to the *MTHFR* C677T and A1298C single nucleotide polymorphisms (SNPs), contribute to schizophrenia.

Under physiologic conditions, each copy of the 677T allele causes a 35% reduction in MTHFR function (Frosst *et al.* 1995). Thus, individuals carrying T alleles have reduced availability of 5-methylenetetrahydrofolate for the conversion of homocysteine to methionine and s-adenosylmethionine. Several groups have reported increased frequency of the *MTHFR* 677T allele in patients with schizophrenia (Arinami *et al.* 1997; Joober *et al.* 2000; Muntjewerff *et al.* 2005; Sazci *et al.* 2003, 2005). Although other investigators have failed to reproduce this finding (Kunugi *et al.* 1998; Muntjewerff *et al.* 2003; Vilella *et al.* 2005; Virgos *et al.* 1999; Yu *et al.* 2004) a recent meta-analysis (Lewis

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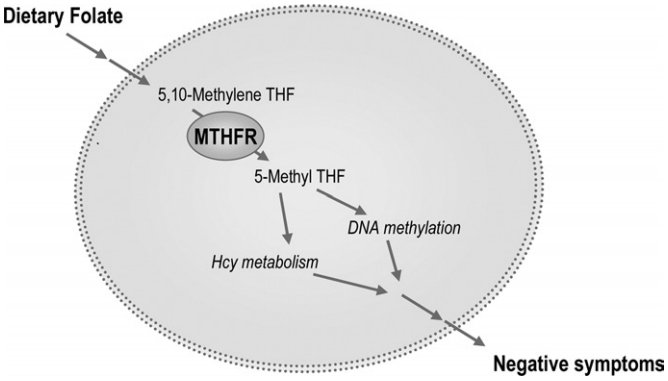

Figure 1. Intracellular effects of MTHFR, and its role as a potential mediator of the serum folate-negative symptoms relationship. MTHFR, methylenetetrahydrofolate reductase; THF, tetrahydrofolate; Hcy, homocysteine.

et al. 2005) supported the relationship between T/T genotype and schizophrenia risk (odds ratio 1.48, 95% confidence interval, 1.18–1.86).

Individuals with the 1298C allele demonstrate more modest reductions in *MTHFR* activity (van der Put *et al.* 1998). Among a Turkish cohort, schizophrenia patients exhibited the C/C genotype more frequently than healthy controls (Sazci *et al.* 2005; Virgos *et al.* 1999). The T677T/A1298A (Sazci *et al.* 2005; Virgos *et al.* 1999) and C677C/C1298C (Sazci *et al.* 2005) compound genotypes were also over-represented in schizophrenia samples. However, a study of Spanish patients and controls found no differences in A1298C genotype (Vilella *et al.* 2005).

We studied a population of 200 schizophrenia outpatients to determine whether the *MTHFR* C677T and A1298C genotypes contribute to our previously reported relationship between folate and negative symptoms (Goff *et al.* 2004). Given the complementary roles of substrate and enzyme in folate metabolism, we also conducted a secondary analysis to determine whether *MTHFR* genotype interacts with serum folate concentrations to influence negative symptoms.

Methods and Materials

Clinical Ratings

Study procedures were approved by the Partners HealthCare and Massachusetts Department of Mental Health institutional review boards (Boston, Massachusetts). After providing written informed consent, 200 outpatients with chronic schizophrenia who receive treatment at an urban community mental health center in Boston were enrolled consecutively. Subjects were confirmed to meet DSM-IV criteria for schizophrenia by a consensus diagnostic conference based on results from a clinical diagnostic interview, chart review, and review of clinical history with treating clinicians. All subjects were clinically stable, with global assessment of function (GAF) scores ≥30. Patients who were screened but found to have significant alcohol abuse (n = 5) or renal insufficiency (n = 1) were not enrolled. Of enrolled subjects, 157 were Caucasian (78.5%), 40 were of African descent (20%), one was of East/Southeast Asian descent (.5%), and one was of Latino descent (.5%).

On the day of testing, patients were rated with the Positive and Negative Syndrome Scale (PANSS) (Kay *et al.* 1987), and phlebotomy was performed for genotype assays. Subjects provided medication and smoking histories and completed the Fagerstrom Test for Nicotine Addiction (Fagerstrom 1978), obtained because tobacco use (Piyathilake *et al.* 1994) and anticonvulsant medications (Froscher *et al.* 1995) may influence serum folate and homocysteine levels. They were also assessed with the Hamilton Depression Rating Scale (HDRS) (Hamilton 1960) and Simpson-Angus Scale (Simpson and Angus 1970) to rule out contributions of depression and akinesia to negative symptoms. PANSS ratings were administered by one of four trained raters who were blind to genotype and serum assays; inter-rater reliability was maintained by use of videotaped interviews every six months.

MTHFR Genotype

Genotyping was performed with allele-specific fluorescent probes in an assay combining polymerase chain reaction (PCR) and the 5' nuclease (Taqman) technique using the GeneAmp PCR System 9700 or ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, California). The specific primers and probes were obtained on the basis of published data (Keku *et al.* 2002; Skibola *et al.* 2004) and synthesized by Applied Biosystems. Allelic discrimination was accomplished when fluorogenic probes with either a 6FAM or VIC reporter dye attached to the 5' end of the oligonucleotide were cleaved by the 5' nuclease activity of Taq DNA polymerase. PCR reactions were carried out in a total volume of 10 μL containing 900 nM of each primer, 200 nM each probe, 25 m M each dNTP, 1 M Tris-HCL (pH 8.4), 1 M $MgCl_2$, 300 mM KCl, ROX reference dye, 100% glycerol, 1 U Taq DNA polymerase (Invitrogen, Carlsbad, California), and 50 ng genomic DNA. The thermocycling condition consisted of 95°C for 10 min (Taq activation), followed by 40 cycles at 92°C for 15 sec (denature), and at 60°C for 1 min (anneal/extend). A postPCR plate read on the ABI 7900HT Sequence Detection System detected polymorphisms at the *MTHFR* C677T and A1298C loci. Negative and positive controls were included in each run. Samples that could not be determined were repeated. Unreadable results on the second run were scored as missing.

Folate and Homocysteine Levels

Serum folate and homocysteine concentrations (nonfasting) were available from the subset of patients (n = 85) described in our previous report (Goff *et al.* 2004). Biochemical measurements were taken on the day of clinical evaluation. Folate concentrations were determined using cloned enzyme donor immunoassay kits (BioRad, Hercules, California) according to the manufacturer's instructions (between-day coefficient of variation 6.8%). Serum homocysteine was measured by a fluorescence polarization immunoassay method (coefficient of variation 3.7%–5.2%).

Statistical Analysis

Primary statistical analysis was conducted with the program Whap (URL: http://pngu.mgh.harvard.edu/~purcell/whap/) (Curran *et al.* 2005; Purcell *et al.* 2007), which was used to perform simple regression analyses of PANSS symptoms based on allele dosage (0, 1, or 2 copies), assuming an additive model of gene action. Because the C677T and A1298C SNPs are in linkage disequilibrium (Vilella *et al.* 2005), we also performed a two-marker haplotype-based test of association to evaluate the possibility of interactive effects of the two polymorphisms on negative symptoms. The association of phenotype on haplotype was based on a mixture of regression models that account for the potential ambiguity in individuals' statistically-inferred haplotypes. Chi-square or analysis of variance (ANOVA) was applied

as appropriate to observe whether *MTHFR* genotype groups differed on demographic measures or medication use (SPSS for Windows, version 13.0, SPSS, Inc., Chicago, Illinois). ANOVA determined the effect of each *MTHFR* SNP on folate and homocysteine concentrations and clinical ratings. Given the a priori hypothesis of negative symptom-specific effects [based on our previous work (Goff *et al.* 2004)], alpha was set at .05 for the PANSS analyses.

A final, exploratory analysis was conducted to clarify the specific relationship among serum folate and homocysteine levels, *MTHFR* genotype, and negative symptoms. Other groups have found that significant downstream sequelae of 677T-variant MTHFR (e.g., elevated homocysteine, reduced DNA methylation) occur only in subjects with serum folate levels less than reported median values of 12 nmol/L (Friso *et al.* 2002), 15.4 nmol/L (Jacques *et al.* 1996) or 19.9 nmol/L (Papoutsakis *et al.* 2005); in a larger study (n = 1,042) (Devlin *et al.* 2006), T/T-related homocysteine elevations were present only in subjects in the lowest folate quintile (< 6.4 nmol/L). These findings suggest that T/T effects become rate limiting only at low folate concentrations. Therefore, to assess for folate-genotype interactions on negative symptoms, we performed a median split of subjects based on serum folate levels, and then used two-way ANOVA. A similar analysis assessed genotype-homocysteine interactions on negative symptoms. For subjects found to be at higher risk for negative symptoms due to *MTHFR* genotype, we also used Pearson correlations to assess the relationship of serum folate and homocysteine levels to negative symptom severity.

Results

MTHFR C677T

Of 200 subjects, 97 (49%) were homozygous for the *MTHFR* C allele, 82 (41%) were heterozygotes, and 21 (10%) were homozygous for the T allele (Table 1), consistent with Hardy Weinberg equilibrium (p = .664). Genotype groups did not differ on gender, duration of illness, Global Assessment of Function (GAF) score, or smoking measures. However, C/C subjects were somewhat younger (F = 2.73, df = 2, 200, p = .067; post hoc Tukey LSD: C/C < C/T, p = .041), and T allele carriers were more likely to be Caucasian (χ^2 = 25.74, df = 6, p < .001). Neither medication use nor serum folate or homocysteine concentrations differed among genotype groups. Co-variance analysis indicated that race and age, the two demographic variables that differed among groups, did not influence folate or homocysteine results. One subject agreed to genotyping but did not continue with the PANSS assessment. PANSS scores did not differ between Caucasian and non-Caucasian subjects (Table 2) and did not correlate with age (R^2 < .01).

T allele load effects on PANSS symptom scales are given in Table 3. Negative symptom scores were significantly related to T allele dose, with T/T subjects exhibiting the most pronounced symptoms [Likelihood Ratio Test (LRT) = 4.18, p = .041]. Unexpectedly, positive symptom scores were also influenced by T allele load; however, in this case, the T allele appeared to be protective (LRT = 5.07, p = .024). There were no effects of C677T genotype on PANSS general psychopathology or total scores. Because of the differences in race distribution and age among

Table 1. Effects of *MTHFR* Genotype on Demographic and Clinical Measures

	C677T					A1298C				
	C/C (n = 97)	C/T (n = 82)	T/T (n = 21)	Statistics		A/A (n = 99)	A/C (n = 83)	C/C (n = 18)	Statistics	
	%	%	%	X^2	p	%	%	%	X^2	p
Demographics										
Gender										
Female	35	26	33	1.91	NS	30	34	22	.96	NS
Race										
Caucasian	64	90	100	25.74	.001	70	83	100	12.50	.052
Smoking Status										
Current Smoker[a]	64	71	62	.82	NS	69	64	67	.33	NS
	Mean (SD)	Mean (SD)	Mean (SD)	F	p	Mean (SD)	Mean (SD)	Mean (SD)	F	p
Age (y)	41.8 (11.0)	44.8 (8.8)	45.7 (7.7)	2.73	.067[b]	43.1 (9.9)	43.4 (8.9)	45.6 (14.0)	.45	NS
Duration of Illness (y)	19.3 (11.2)	21.2 (10.3)	17.7 (11.7)	1.05	NS	19.7 (9.8)	19.8 (10.9)	22.2 (15.7)	.42	NS
Fagerstrom Score[a]	65.6 (13.0)	69.4 (16.6)	72.7 (26.8)	.79	NS	66.8 (17.6)	68.5 (14.4)	70.9 (14.3)	.27	NS
HDRS[a]	12.1 (4.2)	11.2 (6.2)	10.6 (2.6)	.62	NS	11.7 (5.1)	11.6 (5.3)	10.6 (4.3)	.26	NS
Simpson-Angus[a]	4.1 (4.4)	3.5 (3.4)	4.1 (2.7)	.36	NS	3.1 (3.3)	4.1 (3.9)	6.3 (5.2)	3.56	.032[c]
GAF	52.7 (9.7)	52.2 (10.0)	52.0 (10.2)	.07	NS	53.4 (9.2)	50.3 (10.6)	52.4 (9.6)	1.75	NS
	%	%	%	X^2	p	%	%	%	X^2	p
Medications										
Antipsychotics										
Typical	25	25	10	2.44	NS	21	25	22	.39	NS
Atypical	73	67	81	1.98	NS	71	70	78	.45	NS
Antidepressants	26	30	28	.32	NS	27	27	33	.27	NS
Anticonvulsants	31	20	16	2.95	NS	21	29	27	1.16	NS

HDRS, Hamilton Depression Rating Scale; PANSS, Positive and Negative Symptoms Scale; NS, Not significant at level of alpha (p < .05) or trend (p < 10).
[a]Data for this variable were not available for all subjects.
[b]Post hoc test results: C/C < C/T, p = .041.
[c]Post hoc test results: C/C > A/A, p = .011; C/C > A/C, p = .084.

Table 2. Effect of Race on PANSS Measures

	Caucasian (n = 157)		Non-Caucasian (n = 42)	
	Mean	SD	Mean	SD
Positive Symptoms	15.7	6.0	15.5	5.7
Negative Symptoms	18.1	5.0	18.0	4.3
General Psychopathology	31.7	7.9	30.1	7.2
Total	65.6	14.5	63.2	14.1

Omnibus analysis of variance (ANOVA) indicates no between-group measures on PANSS symptoms (F = .94, df = 4, p = .44). Non-Caucasian group includes African American (n = 40), East/Southeast Asian (n = 1), and Latino (n = 1) subjects. PANSS, Positive and Negative Syndrome Scale.

genotype groups, the analysis was repeated, co-varying by these variables. The effects of the T allele remained significant (negative symptoms: LRT = 4.04, p = .044; positive symptoms: LRT = 6.88, p = .009).

MTHFR A1298C

Among 200 subjects, 99 (49.5%) were homozygous for the A allele (A/A), 83 (41.5%) were heterozygous (A/C) and 18 (9%) were homozygous for the C allele (C/C), consistent with Hardy Weinberg equilibrium (p = 1.0). Genotype groups differed by race due to the predominance of Caucasian subjects in the C/C cohort (χ^2 = 12.50, df = 6, p = .052). Genotype groups did not differ on medication use, nor on folate or homocysteine levels. C/C subjects had significantly higher Simpson-Angus scores (F = 3.56, df = 2, 200, p = 032; post hoc Tukey LSD: CC > A/A, p = .011; CC > A/C, p = .084). No significant differences were observed among A1298C genotype groups on any PANSS rating, although the C allele increased positive symptoms at the trend level (LRT = 3.39, p = .065). Using race and Simpson-Angus scores as covariates did not influence any of the results.

Haplotype Analysis

Estimated haplotype frequencies were as follows: 677C/1298A, 40%; 677T/1298A, 30%; 677C/1298C, 30%. Linkage disequilibrium between the two SNPs was strong (D' = .95, r^2 = .17). The 677T/1298A (χ^2 = 28.27, p < .001) and 677C/1298C (χ^2 = 10.30, p = .001) haplotypes occurred more frequently in Caucasian subjects, and 677C/1298A (χ^2 = 55.32, p < .001) occurred more frequently in non-Caucasians. Individuals with the 677C/1298A haplotype were younger than those without it (χ^2 = 8.06, p = .005) and those with the 677T/1298A haplotype were older than those without it (χ^2 = 4.35, p = .037).

A joint test of all three haplotypes, co-varying by age and race, indicated significant overall effects of haplotype on negative symptoms (LRT = 6.15, df = 2, p = .046) and positive symptoms (LRT = 7.31, p = .026). Testing each haplotype versus the others on negative symptoms indicated significant protective effects of 677C/1298A (β = −1.32, χ^2 = 5.22, p = .022) and detrimental effects of 677T/1298A (β = 1.13, χ^2 = 3.93, p = .048). For positive symptoms, protective effects were seen with the 677T/1298A haplotype (β = −1.73, χ^2 = 6.71, p = .010), and detrimental effects were seen with 677C/1298C (β = 1.33, χ^2 = 3.97, p = .046). No significant trans interactions were observed.

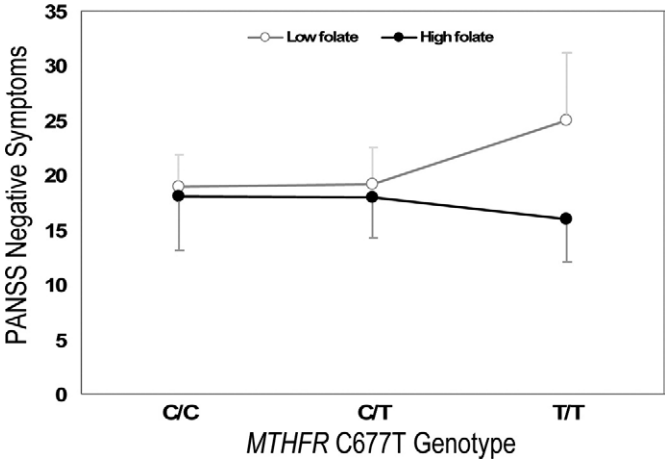

Figure 2. Interaction of *MTHFR* C677T genotype and serum folate level on negative symptoms. Patients with T/T genotype and low serum folate exhibited significantly worse negative symptoms than those with high serum folate (genotype x folate interaction F = 3.30, df = 2, 85, p = .042). MTHFR, methylenetetrahydrofolate reductase; PANSS, Positive and Negative Symptoms Scale. Error bars indicate standard deviation.

Interaction of Risk Genotype with Folate and Homocysteine

All subjects for whom both genotype and folate data were available were split into high and low folate groups, relative to the median folate concentration of 11.2 nmol-L. Within the folate group, there were no genotype-based differences in anticonvulsant use or Fagerstrom scores.

Folate and homocysteine concentrations were available for 8 subjects homozygous for the 677T allele. Despite exhibiting the at-risk genotype, T/T subjects in the high folate group appeared to be protected against negative symptoms relative to those in the low folate group (genotype x folate interaction F = 3.30, df = 2, 85, p = .042; Figure 2). Among T/T subjects, negative symptoms correlated significantly with serum folate level (r = −.710, p = .048), but not with serum homocysteine level (r = .035, p = .934).

Discussion

The present findings suggest that the *MTHFR* C677T polymorphism contributes to variation in negative symptom severity in schizophrenia, with T/T subjects at greatest risk. Further, *MTHFR*

Table 3. Effects of *MTHFR* C677T Genotype on PANSS Measures

	C/C (n = 96)		C/T (n = 82)		T/T (n = 21)		Effect of T Allele Load	
	Mean	SD	Mean	SD	Mean	SD	LRT	p
Positive Symptoms	16.4	5.7	15.4	6.3	13.2	5.2	5.07	024
Negative Symptoms	17.5	4.7	18.5	4.7	19.7	5.6	4.18	.041
General Psychopathology	32.1	8.0	30.7	7.8	30.3	6.8	1.76	NS
Total	65.8	15.2	64.8	14.0	63.1	11.7	.67	NS

PANSS, Positive and Negative Syndrome Scale; LRT, Likelihood Ratio Test; NS, not significant at level of alpha (p < .05) or trend (p < .10).

C677T genotype may play a significant role in the previously described relationship between serum folate levels and negative symptoms. We did not find a significant role for the A1298C polymorphism in schizophrenia symptoms.

C677T and *A1298C* Effects

Individuals homozygous for the 677T allele exhibited negative symptoms that were more severe than those observed in C/T and C/C subjects. This pattern supports previous results indicating a relationship between negative symptoms and serum folate levels (Goff *et al.* 2004). Negative symptoms have been described as heterogeneous, reflecting both symptoms intrinsic to schizophrenia as well as secondary symptoms attributable to concomitant depression, distraction due to active positive symptoms, or medication-induced akinesia (Sommers 1985). However, the fact that genotype groups did not differ on medication use, nor on HDRS or Simpson-Angus scores suggests that primary rather than secondary negative symptoms are most affected by *MTHFR* genotype.

The 677T allele was also associated with *less* pronounced positive symptoms. This finding was unexpected, given that serum folate levels were only associated with negative symptoms in our previous report (Goff *et al.* 2004). As such, the biochemical mechanism by which *MTHFR* genotype would influence positive symptoms remains unclear, although presumably it would reflect differing downstream effects of *MTHFR* on the neural circuitries underlying positive and negative symptoms. In the absence of an a priori hypothesis, the validity of this finding should remain in doubt until it is replicated. At present, though, it emphasizes the importance of studying how genetic variation influences symptom clusters *within* schizophrenia, a markedly heterogeneous disorder (Potash 2006) – for in this case, the T allele appears advantageous for one set of symptoms and disadvantageous for another.

The C677T polymorphism accounted for 2.1% of the total variance in negative symptoms, and 2.4% of total variance in positive symptoms. These modest effects are comparable to other phenotypic investigations of schizophrenia risk genes; for example, the *COMT* Val158Met polymorphism has been found to account for 4.1% of the variance in working memory performance (Egan *et al.* 2001). Studying cumulative effects of risk alleles, on clinical phenotypes as well as brain activity, will likely yield a greater understanding of symptom variance in schizophrenia [e.g., (Bertolino *et al.* 2006)].

The A1298C polymorphism was not significantly associated with symptom severity. Moreover, haplotype analysis reinforced that the salient effects of *MTHFR* on negative symptoms are driven by the C677T polymorphism. The previously reported association of A1298C with schizophrenia risk (Sazci *et al.* 2005; Virgos *et al.* 1999) appears less robust than for C677T, and has not been replicated by other investigators. Furthermore, downstream alterations in *MTHFR* activity appear less pronounced for A1298C than for C677T (van der Put *et al.* 1998). Although A1298C is transmitted in disequilibrium with C677T, the r^2 value for the two polymorphisms is relatively low, consistent with their differing clinical effects. In sum, these findings suggest that C677T effects in schizophrenia substantially outweigh any salient effects of A1298C.

Folic Acid and its Interaction with *MTHFR* Genotype

Serum folate levels did not differ significantly among genotype groups. This is somewhat surprising, given that the product of MTHFR, 5-methyltetrahydrofolate, comprises the predominant form of circulating folate, and is included (along with other folate species) in the folate concentration measured by the assay. Variation in dietary intake or other relevant environmental factors might obscure *MTHFR*-related effects on serum folate. Other investigations have strongly linked nutritional folate intake with serum folate levels in healthy subjects (Iso *et al.* 2003; Pufulete *et al.* 2002), a similar correlation is also present among patients with schizophrenia ($r = .41$, $p = .01$) (Borba *et al.* 2005). We found no genotype-related differences in smoking or anticonvulsant use, which can also influence folate levels (Froscher *et al.* 1995; Piyathilake *et al.* 1994).

Folate supplies the substrate for the MTHFR reaction; thus, in the presence of reduced serum folate, individuals with dysfunctional MTHFR would be expected to show especially profound deficits in physiologic processes dependent on MTHFR activity. In previous investigations, subjects homozygous for the 677T allele exhibited more pronounced deficits in intracellular methylation, and greater serum homocysteine levels, during conditions of low serum folate (Devlin *et al.* 2006; Friso *et al.* 2002; Jacques *et al.* 1996; Papoutsakis *et al.* 2005). The present study suggests that adverse effects of the T/T genotype on negative symptoms are also significantly influenced by serum folate levels (Figure 2). The small number of T/T subjects in this exploratory analysis precludes any firm conclusion. However, given the presence of a plausible biochemical mechanism, and the possibility of folate supplementation as an effective treatment (Godfrey *et al.* 1990; Levine *et al.* 2006), this preliminary data warrants additional investigations of folate-MTHFR interactions on negative symptoms.

Potential Downstream Effects of *MTHFR* Dysfunction

Homocysteine levels are inversely related to folate levels by virtue of MTHFR activity. In the present study, homocysteine levels were greater, but not significantly so, in T/T subjects. T/T individuals did not differ from those in other groups on confounding measures such as tobacco or anticonvulsant use. Rather, the lack of a significant difference likely reflects the relatively small sample size: among analogous studies of healthy subjects, 677 T/T effects on homocysteine also failed to reach significance in an investigation smaller than ours ($n = 41$) (Shelnutt *et al.* 2003), but were significant in several larger studies ($n \geq 186$) (Devlin *et al.* 2006; Friso *et al.* 2002; Jacques *et al.* 1996; Papoutsakis *et al.* 2005). Further, the 38% elevation in homocysteine we observed in T/T subjects is consistent with the larger studies (range = 16% to 44%) (Devlin *et al.* 2006; Friso *et al.* 2002; Jacques *et al.* 1996; Papoutsakis *et al.* 2005).

Prior studies have implicated homocysteine as a risk factor in schizophrenia (Muntjewerff *et al.* 2006; Muntjewerff *et al.* 2003). Homocysteine may also be toxic to dopaminergic neurons and has been shown to adversely affect dopamine turnover in the striatum (Lee *et al.* 2005). However, other results have argued against a role for homocysteine in the pathophysiology of schizophrenia (Levine *et al.* 2005) or suggested that folate itself may be more directly relevant to schizophrenia symptoms (Goff *et al.* 2004; Muntjewerff *et al.* 2003). Some of the inconsistencies among investigations, which were conducted in different countries, may reflect variation in government-mandated folate supplementation of grain products. Our negative symptom correlation findings suggest a stronger role for folate than homocysteine in influencing this measure; however, a larger study would be required to address this question more definitively.

However, if downstream effects of the *MTHFR* 677T allele on negative symptoms are not primarily mediated through homocysteine, they may be driven by altered DNA methylation (Figure 1) or

disruption of other vital intracellular methylation processes. In addition to playing a critical role during development of the central nervous system, DNA methylation patterns have been proposed to underlie important gene-environment interactions in schizophrenia (Abdolmaleky *et al.* 2004). The effects of *MTHFR* genotype on DNA methylation have been observed by Friso *et al.* (2002), who found that MTHFR isolated from 677 T/T subjects induced significantly less DNA methylation than did MTHFR from C/C subjects. However, among T/T subjects with serum folate levels greater than 13.8 nmol/L, DNA methylation was similar to that of C/C subjects. Thus, as in the present study, the presence of increased substrate (reflected by serum folate) appeared to counterbalance MTHFR dysfunction due to the 677T allele.

The 677T allele may also affect dopamine metabolism, and thus induce downstream effects salient to negative symptoms, through potential effects on catechol-o-methyltransferase (COMT). Abnormalities in prefrontal dopamine signaling have long been speculated to contribute to schizophrenia (Laruelle *et al.* 2003). COMT inactivates prefrontal dopamine through s-adenosylmethionine (SAM)-dependent methylation of aromatic hydroxyl groups, and thus plays a key role in the regulation of dopamine transmission. Genetic variation in COMT has been associated with deficits in executive function that contribute to schizophrenia (Tunbridge *et al.* 2006) and with differential response of negative symptoms to antipsychotic treatment (Bertolino *et al.* 2004). Reduced availability of SAM due to 677T-variant MTHFR could therefore disrupt normal dopamine signaling, although this hypothesis has not yet been evaluated directly.

Population Stratification Effects

This investigation was limited by relatively small sample size, especially for folate and homocysteine analysis. Because the subject group was racially heterogeneous, the possibility of confounds due to population stratification requires consideration. Both the 677T allele and the 1298C allele were significantly over-represented in Caucasian subjects. The C677T genotype distribution was highly congruent with other studies, which have reported T/T frequencies of approximately 10% in Caucasian subjects, and approximately 1% in subjects of African descent (Ueland *et al.* 2001). The distribution of A1298C was also consistent with prior reports (Botto and Yang 2000). However, we found no significant effects of race on negative symptoms, and co-varying by race did not affect the significance of the main genotype effects. These analyses suggest that the observed effects of *MTHFR* genotype do not reflect stratification artifact, although replication using larger and more homogenous samples, other racial or ethnic groups, or a genomic control panel would further support this notion.

Conclusion

Negative symptoms remain incompletely treated in many patients with schizophrenia, despite the advent of atypical antipsychotic medications. A better understanding of the genetic underpinnings of negative symptoms could lead to more effective treatment. The present study provides preliminary evidence that the *MTHFR* C677T polymorphism contributes both to variance in schizophrenia symptom expression, and to the previously reported relationship between negative symptoms and serum folate concentrations.

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