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Effects of the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism on executive function in schizophrenia[☆]

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Abstract

Background: The methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism has been associated with both overall schizophrenia risk and severity of negative symptoms. This study examined whether schizophrenia patients homozygous for the risk allele (T/T) exhibit greater impairment in executive function, and determined the extent to which MTHFR's effects on negative symptoms underlie this relationship.
Methods: 200 outpatients with chronic schizophrenia were evaluated with the Verbal Fluency Test (VFT), Wisconsin Card Sort Test (WCST), and California Verbal Learning Test (CVLT). Performance was stratified by MTHFR C667T genotype. Path analysis determined the extent to which MTHFR effects on negative symptoms mediated the relationship between genotype and cognitive measures.
Results: T/T subjects exhibited significantly greater deficits on the VFT and had more difficulty achieving the first category on the WCST. Genotype groups did not differ in CVLT performance. C677T effects on negative symptoms contributed to, but did not fully account for, genotype effects on VFT. Negative symptoms did not mediate WCST performance.
Conclusions: MTHFR C677T genotype contributes to certain executive function deficits in schizophrenia. These deficits remained significant when taking into account mediating effects of negative symptoms. Although the intermediate mechanisms for C677T effects remain uncertain, these results suggest that MTHFR-related cognitive impairment and negative symptoms reflect differing neural substrates.
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Keywords: Schizophrenia; MTHFR; Executive function; Negative symptoms; Path analysis

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1. Introduction

Schizophrenia is characterized by significant, treatment–refractory cognitive deficits, including impaired executive function (Heinrichs and Zakzanis, 1998; Sharma and Antonova, 2003). A growing number of

genetic polymorphisms have been associated with cognitive impairment in schizophrenia (Harrison and Weinberger, 2005), suggesting that these deficits are heritable (Egan et al., 2000; Appels et al., 2003). Although negative symptoms and cognitive impairment have distinct features, they are closely related (Harvey et al., 2006), and it is possible that the same risk genes contribute to both symptom clusters. This investigation focuses on whether the methylenetetrahydrofolate (MTHFR) C677T polymorphism, previously associated with both overall schizophrenia risk (Lewis et al., 2005) and risk for negative symptoms (Roffman et al., in press), also influences executive function.

An enzyme in the folate metabolic pathway, MTHFR provides methyl moieties for such vital intracellular processes as gene transcription regulation and homocysteine metabolism. Reduced MTHFR activity has been found in brain tissue of schizophrenia patients (Elliott et al., 1978) and implicated in folate–responsive psychosis (Freeman et al., 1975). Each copy of the 677T allele causes a 35% reduction in MTHFR activity under physiologic conditions (Frosst et al., 1995). The 677T allele is overrepresented in patients with schizophrenia [see (Lewis et al., 2005) for a recent meta analysis].

Homozygosity for the T allele also confers increased risk for negative symptoms in schizophrenia. A previous report by our group (Roffman et al., in press) indicated that among 200 outpatients with schizophrenia, individuals with the 677T allele exhibited significantly greater negative symptom scores than their C/C counterparts. Similar patterns were not observed for positive symptoms or general psychopathology, and neither race nor ethnicity influenced the results.

The present investigation examined whether T/T genotype also augments risk for executive dysfunction in schizophrenia. We chose three tests of executive function, each associated with impaired performance in schizophrenia: the Verbal Fluency Test (VFT) (Benton and Hamsher, 1989; Henry and Crawford, 2005), Wisconsin Card Sort Test (WCST) (Berg, 1948; Laws, 1999), and California Verbal Learning Test (CVLT) (Delis et al., 1987; Paulsen et al., 1995; Hill et al., 2004). A secondary path analysis explored whether MTHFR's effects on executive function are mediated through its influence on negative symptoms. This pattern would argue that negative symptoms and executive dysfunction reflect a unified core pathology, from the level of genotype to that of behavior, as several investigators have speculated [see (Harvey et al., 2006) for a review]. However, if MTHFR's effects on executive function were found not to rely upon the gene's contributions to negative symptoms, this would suggest that despite some shared genetic risk, negative symptoms and executive dysfunction reflect differing intermediate biology.

2. Materials and Methods

2.1. Subjects

Study procedures were approved by the institutional review boards of Partners HealthCare and the Massachusetts Department of Mental Health. Outpatients with schizophrenia ($n=200$) were recruited from an urban community mental health center in Boston, as described elsewhere (Roffman et al., in press), and provided written informed consent. Patients with a history of significant alcohol abuse ($n=5$) or renal insufficiency ($n=1$) were excluded. Based on DSM-IV criteria, a diagnosis of schizophrenia was confirmed in each case by a research psychiatrist. Of enrolled subjects, 157 (78.5%) were Caucasian, 40 (20%) were African–American, two (1%) were of East/Southeast Asian descent, and one (0.5%) was of Latino descent.

2.2. Neurocognitive battery

Patients participated in a neurocognitive battery that included the CVLT (Benton and Hamsher, 1989), VFT (Delis et al., 1987), and WCST (Heaton et al., 1993). The Wechsler Adult Intelligence Scale—Third Edition (WAIS—III) (Wechsler, 1997) was conducted to measure global intelligence. Subjects were also rated with the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987), Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960) and Simpson–Angus Scale (Simpson and Angus, 1970), a measure of extrapyramidal symptoms. Tests were administered by one of four trained and certified raters who were blind to genotype results.

To minimize the number of comparisons, we used selected measures from each test that best represent executive function. These included: VFT, total score; WCST, percent perseverative errors and ability to generate at least one category; and CVLT, overall strategy (serial versus semantic clustering). These measures are supported by a substantial literature showing impaired performance in schizophrenia (Paulsen et al., 1995; Laws, 1999; Hill et al., 2004; McGurk et al., 2004; Henry and Crawford, 2005). Data were excluded from those subjects who were unable to engage in the tasks (VFT: $n=4$; WCST: $n=11$; CVLT: $n=5$) or who had incomplete data (VFT: $n=1$).
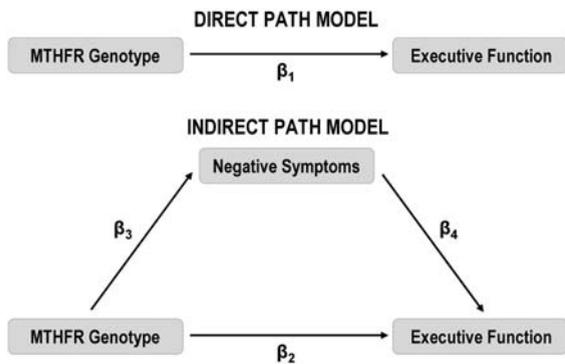
Fig. 1. Path analysis models. (a) The direct path measures strength of genotype effect on executive function regardless of negative symptoms (β_1). (b) The indirect path measures strength of genotype effect on executive function (β_2) taking into account genotype effects on negative symptoms (β_3), and effects of negative symptoms on executive function (β_4).

2.3. MTHFR genotype

Blood obtained on the day of testing was genotyped for the MTHFR C677T polymorphism using allele-specific probes in an assay combining PCR and the 5′ nuclease (Taqman) technique. The specific primers and probes were obtained on the basis of published data (Keku et al., 2002) and synthesized by Applied
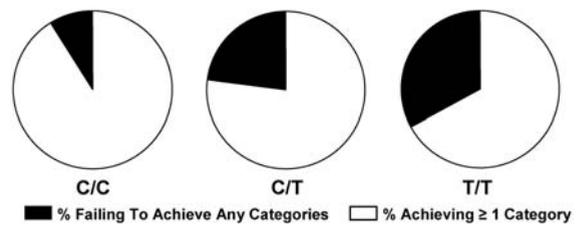

Fig. 2. Percent of subjects in each genotype group able to complete at least one category on the Wisconsin Card Sort Test ($\chi^2 = 10.125$, $df = 2$, $p = .006$).

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 mM 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, CA), 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 s (denature), and at 60 °C for 1 min (anneal/ extend). A post-PCR plate read on the ABI 7900HT Sequence Detection System detected polymorphisms at

Table 1
Effects of MTHFR C677T genotype on demographic and clinical measures

	C/C (N=97)		C/T (N=82)		T/T (N=21)		Statistics	
	%		%		%		X^2	P
Demographics								
Gender (% female)	35		26		33		1.91	NS
Race (% Caucasian)	64		90		100		25.74	0.001
	Mean	SD	Mean	SD	Mean	SD	F	P
Age (years)	41.8	11.0	44.8	8.8	45.7	7.7	2.73	0.067 [a]
Duration of illness (years)	19.3	11.2	21.2	10.3	17.7	11.7	1.05	NS
HDRS (total) [b]	12.1	4.2	11.2	6.2	10.6	2.6	0.62	NS
Simpson–Angus (total) [b]	4.1	4.4	3.5	3.4	4.1	2.7	0.36	NS
WAIS full scale IQ	85.2	13.8	87.1	16.4	80.0	12.5	1.86	NS
	%		%		%		X^2	P
Medications								
Typical antipsychotics	25		25		10		2.44	NS
Atypical antipsychotics	73		67		81		1.98	NS
Antidepressants	26		30		28		0.32	NS
Anticonvulsants	31		20		16		2.95	NS

SD = Standard deviation; HDRS = Hamilton Depression Rating Scale; WAIS = Wechsler Adult Intelligence Scale; NS = Not significant at level of alpha ($p < .05$) or trend ($p < .10$).
[a] Post hoc test results: CC < CT, $p = 0.041$.
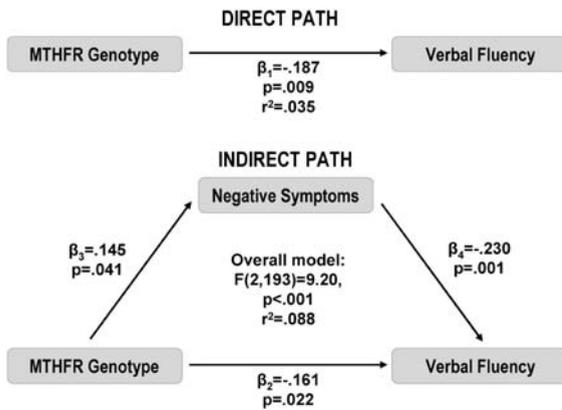[b] Data for this variable were not available for all subjects.

Fig. 3. Effect of negative symptoms on MTHFR-verbal fluency relationship. (a) The direct path indicates linear regression of verbal fluency score across MTHFR genotype (T/T vs other, $p = .009$). (b) The indirect path takes into account negative symptoms via stepwise regression. The influence of genotype on verbal fluency score remained significant ($p = .022$) even when factoring in the significant effects of negative symptom scores on verbal fluency ($p = .001$), indicating a partial mediation.

the MTHFR C677T locus. Negative and positive controls were included in each run.

2.4. Statistical analysis

Statistical analysis was conducted with SPSS for Windows, version 13.0, using chi-square or one-way analysis of variance (ANOVA) as appropriate to assess MTHFR genotype effects on cognitive measures. For ANOVA, genotype (C/C, C/T, or T/T) was used as the between subjects factor, and demographic variables that differed among genotype groups were entered as covariates in a subsequent analysis of covariance (ANCOVA). Because of the a priori hypothesis that T/T

subjects would underperform relative to C/C and C/T subjects, and the likely overlap in performance among executive function measures, a two-tailed uncorrected alpha of 0.05 was used for all analyses.

A series of path analyses (Baron and Kenny, 1986; Kraemer et al., 2001) was conducted to explore whether the effects of genotype on cognitive impairment were expressed through negative symptoms. For each cognitive variable that differed among genotype groups, the relationship between negative symptom scores and cognitive impairment (regardless of genotype) was determined with a Pearson correlation. Effects of genotype on cognitive impairment were then evaluated using simple and multiple regression, comparing two path models (Fig. 1). In the direct path model, group differences in cognitive impairment are expressed directly without mediation by negative symptoms. In the indirect path model, genotype differences in cognitive impairment are expressed indirectly ("mediated") through negative symptoms. A multiple regression solution was calculated for each model. For the direct path model (β_1), genotype was the independent variable, and cognitive performance (e.g. verbal fluency score) was the dependent variable. For the indirect path model, genotype (β_2) and PANSS Negative Symptoms Scale scores (β_4) were simultaneously entered as independent variables into a multiple regression equation as cognitive predictors. Simple regression determined the beta coefficient from genotype (independent) to PANSS Negative Symptoms score (dependent) in the indirect path model (β_3). Full (or total) mediation occurred if genotype significantly predicted cognitive performance only through negative symptoms (i.e., β_3 and β_4 were statistically significant, but β_2 was not). In contrast, significant direct (β_2) and indirect (β_3 and β_4) pathways indicated partial mediation.

Table 2
Effects of MTHFR C677T genotype on executive function measures (continuous variables)

	C/C ($N=97$)		C/T ($N=82$)		T/T ($N=21$)		Statistics	
	Mean	SD	Mean	SD	Mean	SD	F	P
Verbal Fluency Test								
Total score	30.8	11.1	31.3	13.1	23.6	11.4	3.52	0.031 [a]
Wisconsin Card Sort Test								
Percent perseverative errors	21.3	11.9	23.0	14.6	28.1	17.9	1.90	NS
California Verbal Learning Test								
Semantic clustering	1.50	0.70	1.57	0.80	1.24	0.64	1.56	NS
Serial clustering	2.33	1.79	3.02	2.12	2.53	2.59	2.52	0.083 [b]

SD = Standard deviation; NS = Not significant at level of alpha ($p < .05$) or trend ($p < .10$).
[a] Post hoc test results: T/T < C/C, $p = .015$; T/T < C/T, $p = .011$.
[b] Post hoc test results: C/T > C/C, $p = .027$; difference drops below trend level after co-varying for age and race, $F(2,195) = 1.60$, $p = .205$.

3. Results

3.1. Genotype groups

Genotype results from this cohort have been described elsewhere (Roffman et al., in press) and are briefly summarized here (Table 1). Of the 200 subjects, 97 (48.5%) were C/C, 82 (41%) were C/T, and 21 (10.5%) were T/T, consistent with Hardy Weinberg equilibrium ($p=.664$). Genotype groups did not differ in gender or duration of illness. C/C subjects were 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 ($\chi^2=25.74$, $df=6$, $p=.001$); therefore, neurocognitive analyses were co-varied by age and race. Aside from negative symptoms, groups did not differ among measures that could reflect secondary causes of cognitive impairment, including medication use, HDRS scores, or Simpson–Angus scores, nor in full scale IQ.

3.2. Neurocognitive testing

Primary neurocognitive analyses are given in Table 2 and Fig. 2. For the VFT, T/T subjects generated fewer total words than C/C or C/T subjects [omnibus ANOVA: F (2195)$=3.523$, $p=.031$; post hoc Tukey LSD: T/T<C/C, $p=.015$, T/T<C/T, $p=.011$]. For the WCST, only 67% of T/T subjects completed at least one category, compared to 77% of C/T and 91% of C/C subjects ($\chi^2=10.125$, $df=2$, $p=.006$) (Fig. 2). T/T subjects also tended to make more frequent perseverative errors, but this pattern did not reach significance. For the CVLT, genotype groups did not differ in serial or semantic clustering scores, although there was a trend for C/T subjects to exhibit increased serial clustering [omnibus ANOVA: $F(2195=2.52$, $p=.083$; post hoc Tukey LSD: C/T>C/C, $p=.027$]. A subsequent ANCOVA including age and race as covariates did not affect the significance level of the VFT, WCST, and CVLT results, with the exception of serial clustering, which fell below trend level [$F(2195)=1.60$, $p=.205$].

3.3. Path analysis

Path analysis was conducted to clarify the causal relationships between negative symptoms and cognitive impairment among genotype groups. T/T subjects were compared to other genotype groups for total VFT score and the ability to generate at least one category on the WCST, reflecting the significant findings in the primary analysis. Negative symptoms correlated significantly with VFT scores ($r=-.249$, $p<.001$), and partially mediated the effect of MTHFR genotype on VFT performance ($\beta_4=-.230$, $p=.001$; Fig. 3). Specifically, while taking negative symptoms into account, the direct pathway between genotype and VFT remained significant (β changed from $-.187$ to $-.161$, p remained <.05). In contrast, negative symptoms did not correlate significantly with WCST performance ($r=-.128$ $p=.079$), precluding negative symptoms as a mediator of genotype effects on the WCST.

4. Discussion

In this investigation of 200 schizophrenia outpatients, individuals homozygous for the MTHFR 677T allele performed significantly more poorly than C/C or C/T subjects in two tests of executive dysfunction, VFT and WCST. No significant effects of genotype on CVLT measures were observed.

4.1. MTHFR effects on executive function measures

Verbal fluency and WCST performance are consistently impaired in schizophrenia. A meta analysis of 84 studies comparing schizophrenia patients with healthy controls (Henry and Crawford, 2005) reported significant reductions in semantic fluency in the schizophrenia group, with an overall moderate effect size (Cohen's $d=.49$). A review of 29 studies comparing WCST performance in schizophrenia patients and healthy individuals (Laws, 1999) found an even larger effect size in categories achieved ($d=.91$) and absolute number of perseverative errors ($d=.53$), and a more modest effect size in percent perseverative errors ($d=.18$) In each case, the authors argue that these differences are difficult to interpret in light of significant between-group differences in IQ. However, in both papers, the authors cite the need to explore other variables, besides general intelligence, that may underlie the heterogeneity of results *within* the schizophrenia samples.

In this study, T/T subjects exhibited a 24% reduction in verbal fluency output compared to other genotype groups. On the WCST, although genotype groups did not differ significantly in percent perseverative errors, there was a 24% differential in the ability of C/C versus T/T subjects to successfully formulate the central concept of the task, i.e., complete at least one category. Genotype groups differed neither in full scale IQ nor in other measures that could potentially confound cognitive performance (including medication use, duration of illness, depression, or extrapyramidal symptoms).

No significant differences in CVLT performance were observed among genotype groups, although C/C

subjects appeared to make greater use of semantic clustering. Semantic clustering is considered a more sophisticated strategy than serial clustering, as the former relies on an individual's previous experience with organizing words into categories (Stricker et al., 2002). One group (Paulsen et al., 1995) found that schizophrenia patients were less likely to employ semantic clustering. While a second group (Hill et al., 2004) failed to replicate this finding, they did report a significant correlation between semantic clustering and verbal memory performance (Trial 1–5 recall score) that was more pronounced in schizophrenia subjects than healthy controls. This finding suggests that the ability of schizophrenia patients to use the strategy of semantic clustering may represent an important determinant in verbal memory performance. It is possible that MTHFR genotype may influence the strength of this correlation in schizophrenia patients, a hypothesis that might be tested in a larger cohort of subjects.

4.2. The role of negative symptoms

There is substantial clinical overlap between negative symptoms and cognitive impairment in schizophrenia patients. Several models could account for how a risk allele that has been associated with two phenotypic measures influences the relationship between them: genotype effects on both measures could be independent, or genotype effects on one measure may partially or wholly account for its effects on the other (Kraemer et al., 2001; Harvey et al., 2006). Here, we used path modeling to weigh these possible relationships.

As suggested in Fig. 3, MTHFR effects on VFT performance reflect, in part, its effects on negative symptoms (i.e., negative symptoms serve as a partial mediator). It is also possible that the reverse is true: MTHFR effects on negative symptoms reflect, in part, its effects on the VFT. With the current study design, it is not possible to disambiguate the direction of this relationship. However, the current data support the notion that there is some overlap in contributory biological underpinnings of negative symptoms and verbal fluency, especially those occurring downstream of MTHFR effects. It is also noteworthy that together, negative symptoms and MTHFR genotype accounted for 8.8% of the total variance in VFT scores. This is somewhat higher than studies examining effects of genotype alone on executive function variation [e.g., COMT Val108/158Met genotype contributes to 4.1% of the variance in working memory performance (Egan et al., 2001)].

Negative symptoms did not correlate with WCST performance, precluding a path analysis of negative symptoms as a mediator of genotype effects on WCST. The correlation approached significance, though, suggesting that it might emerge in a larger study with greater power. However, within this cohort, it appears that the effects of T/T genotype on negative symptoms and WCST performance are independent. This pattern suggests that MTHFR genotype ultimately affects these two symptom clusters via disparate mechanisms, and supports the idea that negative symptoms and certain variants of cognitive impairment reflect discrete neural processes.

4.3. Limitations and future directions

The current investigation included only patients with schizophrenia. However, it would be valuable to determine whether MTHFR genotype predisposes healthy individuals to subtle, but similar differences in executive function performance. This pattern has been demonstrated among other polymorphisms thought to confer risk for cognitive impairment in schizophrenia: BDNF Val66Met (Hariri et al., 2003), DISC1 Cys704-Ser (Callicott et al., 2005), and COMT Val108/158Met (Egan et al., 2001) have each been associated with variation in working memory, although not always consistently, e.g. (Ho et al., 2005). Healthy subject studies are also free of such potential confounds as chronic illness and medication use; although in the present study these measures did not differ significantly among genotype groups, they may still have influenced performance.

Another potential confound is the possibility of a population stratification artifact. The T/T allele was significantly over-represented in Caucasian subjects, consistent with prior reports. However, co-varying by race did not significantly affect the pattern of results. Reanalysis using only Caucasian subjects ($n=153$) indicated analogous effects of MTHFR genotype on the VFT (omnibus $F=4.702$, $df=2,153$, $p=.010$). For the WCST, the percentage of subjects in each genotype group who successfully achieved greater than one category was similar between Caucasian subjects (C/C: 92%, C/T: 79%, T/T: 67%) and all subjects (C/C: 91%, C/T: 77%, T/T: 67%), and genotype effects remained significant within the Caucasian group ($\chi^2=7.139$, $df=2$, $p=.028$). It therefore seems unlikely that MTHFR effects on cognition reflect stratification artifact, although replication using more homogenous samples would be confirmatory.

Additional studies are also needed to understand the intermediate biochemical and neural systems-level steps through which MTHFR influences cognition and

negative symptoms. MTHFR provides methyl moieties for a variety of intracellular processes, including conversion of homocysteine to methionine and regulation of gene transcription. Previous studies have documented significant reductions in homocysteine metabolism (Jacques et al., 1996) and DNA methylation (Friso et al., 2002) in cell culture samples obtained from T/T individuals. As reported elsewhere (Roffman et al., in press), MTHFR genotype did not significantly influence serum folate or homocysteine levels in this study cohort, although T/T subjects tended to have higher homocysteine concentrations. Future studies may reveal whether MTHFR-related effects on homocysteine, DNA methylation, or other intracellular methylation processes mediate cognitive effects of the C677T polymorphism. Moreover, while the pattern of executive dysfunction and negative symptoms seen in T/T individuals strongly implicates frontal lobe impairment, the neuroanatomical basis of MTHFR effects on cognition could be clarified with functional neuroimaging studies. Finally, given the relevance of psychomotor and processing speed to cognitive functions, it would be worthwhile to determine whether MTHFR-related differences in executive function reflect, in part, upstream effects of genotype on motor speed.

Cognitive impairment has long been theorized to reflect heritable abnormalities of brain function in schizophrenia. However, marked clinical heterogeneity has complicated the search for contributory neural substrates, as negative or active positive symptoms, medication use, and co-morbid conditions such as depression can all contribute significantly to cognitive dysfunction. Although it is unlikely that specific genes map uniquely to specific symptom clusters (Harrison and Weinberger, 2005), gene association studies can provide a relatively unambiguous starting point from which to disentangle the discrete biologic substrates of heritable schizophrenia symptoms, including cognitive impairment. This investigation suggests that the MTHFR C677T polymorphism contributes to executive function variance in schizophrenia patients, and that it does so somewhat independently of its effects on negative symptoms.

Acknowledgements

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