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Brief Research Communication

Interactive Effects of COMT Val108/158Met and MTHFR C677T on Executive Function in Schizophrenia

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Schizophrenia is characterized by heritable deficits in executive function. Two common, functional polymorphisms, catechol-*O*-methyltransferase (COMT) Val108/158Met and methylenetetrahydrofolate reductase (MTHFR) C677T, have separately been associated with executive function performance in schizophrenia. Given the closely related biochemistry of MTHFR and COMT, it is plausible that the T and Val alleles act synergistically to impair executive function. This investigation of 185 outpatients with schizophrenia examined the interactive effects of these two polymorphisms on Wisconsin Card Sorting Task (WCST) performance. Two WCST measures consistently associated with schizophrenia, perseverative errors and inability to generate categories, were contrasted among compound COMT-MTHFR genotype groups. Individuals homozygous for the COMT Val allele who also carried at least one copy of the MTHFR T allele exhibited a significantly higher percentage of perseverative errors than patients in the other genotype groups. While the T allele also exerted a negative effect on category generation, COMT genotype did not contribute to category performance. It is plausible that cumulative effects of the MTHFR T and COMT Val alleles on intracellular methylation profiles and prefrontal dopamine transmission underlie their interactive effect on perseverative errors. © 2008 Wiley-Liss, Inc.

KEY WORDS: schizophrenia; executive function; COMT; MTHFR; methylation

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INTRODUCTION

Heritable deficits in executive function contribute to cognitive impairment in schizophrenia, and are a substantial and often intractable source of morbidity [Snitz et al., 2006; Trandafir et al., 2006; Gur et al., 2007]. Effects of the catechol-*O*-methyltransferase (COMT) G452/675A polymorphism (rs4680, usually referred to as Val108/158Met) on executive function have been studied extensively in healthy individuals and those with schizophrenia [Tunbridge et al., 2006; Barnett et al., 2007]. COMT catabolizes prefrontal dopamine, which facilitates executive function by optimizing or "tuning" pyramidal cell firing [Williams and Castner, 2006]. The 108/158Met variant is thermolabile under physiologic conditions, leading to significantly reduced COMT function (and hence increased synaptic dopamine concentrations) [Chen et al., 2004]. Several investigators have reported increased risk for schizophrenia among individuals who carry the hyperfunctional Val allele, and even among healthy individuals, Val homozygotes have performed less well on working memory tasks than Met allele carriers [Egan et al., 2001; Joober et al., 2002]. However, others have failed to replicate the association between COMT genotype and schizophrenia risk [Rosa et al., 2004; Rybakowski et al., 2006], including two meta-analyses [Fan et al., 2005; Munafo et al., 2005]. Another recent meta-analysis revealed detrimental Val allele effects on executive function in healthy subjects, but not in schizophrenia patients [Barnett et al., 2007].

This inconsistency may reflect, in part, the role of unrecognized variation in other genes that interact epistatically with COMT [Nicodemus et al., 2007] or that otherwise affect COMT function. A common variant in methylenetetrahydrofolate reductase (MTHFR), C677T (rs1801133), may play such a role. A key enzyme in the folate metabolic pathway, MTHFR supplies single carbon moieties for intracellular methylation reactions. There has been longstanding interest in whether abnormal folate metabolism [Kreisler et al., 1948], and specifically MTHFR deficiency [Freeman et al., 1975; Elliott et al., 1978], influence symptom severity in schizophrenia. It has been suggested that abnormal methylation contributes to executive dysfunction in schizophrenia and other psychiatric disorders through downstream effects on dopamine signaling [Deth, 2003].

Each copy of the 677T variant imparts a 35% reduction in MTHFR activity [Frosst et al., 1995], compromising downstream biochemical processes such as DNA methylation and homocysteine metabolism [Friso et al., 2002]. The T allele has also been associated with schizophrenia risk, described in a recent meta-analysis [Gilbody et al., 2007], as well as allele dose-dependent risk for negative symptoms [Roffman et al., 2007b] and executive dysfunction [Roffman et al., 2007a].

Genetic variation in MTHFR could influence COMT function, and hence prefrontal dopamine concentrations, through at least one potential mechanism (Fig. 1). Methylation of the COMT promoter decreases COMT expression [Sasaki et al.,

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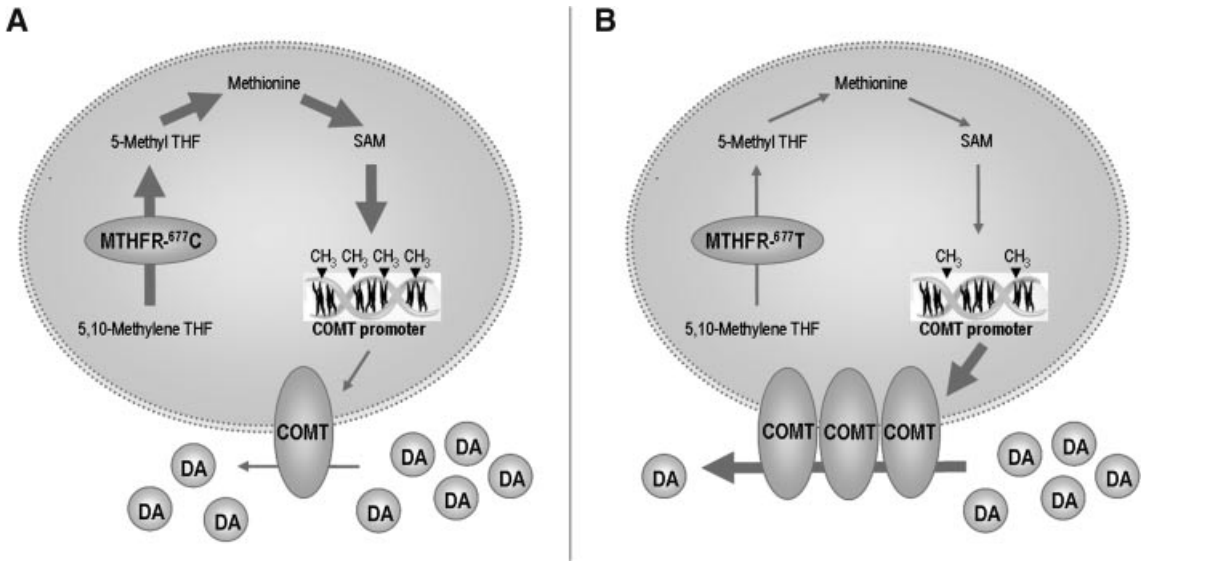

Fig. 1. Potential epigenetic interaction of MTHFR and COMT. **A**: In the presence of the MTHFR 677C allele, abundant methyl moieties are available for COMT promoter methylation. As a result, COMT expression is diminished, and synaptic dopamine is preserved. **B**: In the presence of the MTHFR 677T allele, a paucity of available methyl moieties results in hypomethylation of the COMT promoter, increased COMT expression, and increased synaptic dopamine breakdown. Dopamine catabolism would be further augmented in the presence of the fully functional COMT 108/158Val allele. THF: tetrahydrofolate; SAM: *S*-adenosylmethionine; DA: dopamine.

2003]. COMT promoter methylation deficits have been described in schizophrenia, with concordant increases in COMT expression [Abdolmaleky et al., 2006]. Further, methylation of CpG sites in the COMT promoter is strongly heritable [Mill et al., 2006]. MTHFR C677T may contribute to this pattern, wherein T allele carriers would exhibit diminished promoter methylation, increased COMT expression, and reduced dopamine signaling. The MTHFR T allele would also magnify COMT Val effects on prefrontal dopamine signaling by virtue of increased expression of the hyperfunctional Val variant.

This investigation examined whether COMT Val108/158Met and MTHFR C677T interactively affect executive function performance in schizophrenia. Given the previously reported detrimental effects of the Val and T alleles (individually) on executive function, as well as their putative epigenetic interaction, we hypothesized that the T allele would augment the detrimental effect of the Val allele on executive function in schizophrenia. We examined specifically perseverative errors and impaired category generation, two aspects of executive dysfunction consistently observed in schizophrenia [Laws, 1999] and for which performance has been linked to COMT Val108/158Met [Egan et al., 2001] or MTHFR C677T [Roffman et al., 2007a] genotype.

MATERIALS AND METHODS

All study procedures were approved by the Partners HealthCare and Massachusetts Department of Mental Health Human Research Committees. Following informed consent procedures, 185 individuals with chronic schizophrenia (previously described in Roffman et al. [2007a]) were enrolled consecutively in a genotype-phenotype study at an urban community mental health care clinic. Schizophrenia diagnosis was confirmed by a research psychiatrist using DSM-IV criteria, and was reviewed in each case by a consensus diagnostic conference. The population reflected a convenience sample, with subjects of Caucasian (n = 143, 77.3%), African (n = 39, 21.1%), East/Southeast Asian (n = 2, 1.1%), and Latino (n = 1, 0.5%) descent represented. The sample was 68.6% male (n = 127), with a mean age of 43.2 years and duration of illness of 19.5 years.

Subjects were rated for core schizophrenia symptoms with the Positive and Negative Syndrome Scale (PANSS) [Kay et al., 1987] as well as for depression (Hamilton Depression Rating Scale) [Hamilton, 1960], extrapyramidal symptoms (Simpson–Angus Scale score) [Simpson and Angus, 1970], and general intelligence (Wechsler Adult Intelligence Scale full scale IQ) [Wechsler, 1997]. They also underwent a neurocognitive battery including the Wisconsin Card Sorting Task (WCST) [Heaton et al., 1993]. Two WCST scores that measure differing aspects of executive function were used as outcome measures: percent perseverative errors, and ability to achieve ≥ 1 category. Category achievement reflects subjects' deducing the organizing principle of the task, while perseveration indicates a failure to recognize when those principles change (or in the case of the first category, failure to learn from previous mistakes). These are the two WCST measures that are most frequently abnormal in schizophrenia [Laws 1999], and in the case of category generation, impairment is so substantial among T allele carriers that they often cannot achieve even one category [Roffman et al., 2007a]. Therefore, as in our previous investigation, category generation was treated as a dichotomous variable. Genotyping for the COMT Val108/158Met and MTHFR C677T polymorphisms was performed using the Taqman platform (Applied Biosystems, Foster City, CA) using allele-specific probes [Egan et al., 2001; Roffman et al., 2007a].

SPSS Version 13.0 (Chicago, IL) was used for statistical analysis. Demographic and clinical characteristics among the four groups were compared with analysis of variance (ANOVA) or χ^2 as appropriate. For percent perseverative errors, a 2×2 factorial analysis of covariance (ANCOVA) was used, with COMT and MTHFR genotypes as factors, and presence (≥ 1) or absence (0) of hypofunctional alleles as levels. Demographic factors which differed significantly among genotype groups were entered as covariates. For significant gene interactions, post hoc *t*-tests were used to localize differences among the four compound genotype groups. The approach of combining

subjects heterozygous and homozygous for a dysfunctional allele has been deployed in numerous other association studies of co-dominant functional polymorphisms [including COMT [Barnett et al., 2007] and MTHFR [Hong et al., 2007]], and provided greater power to contrast individuals with full (Val/Val or C/C) versus reduced ($\geq$1 Met or $\geq$1 T) enzyme function in a relatively small sample. For ability to generate $\geq$1 category, an omnibus χ^2 assessed any differences among the four compound genotype groups; subsequent pairwise χ^2 tests were used to localize main effects of COMT or MTHFR, or any COMT $\times$ MTHFR interactions. Alpha (2-tailed) was set at 0.05.

RESULTS

Distribution and characterization of the compound genotype groups is given in Table I. Met carrier + T carrier subjects were significantly older, and consistent with previous reports [Ueland et al., 2001], Caucasians were more highly represented in T allele groups. Therefore, three steps were taken to address the possibility of age or population stratification artifact influencing the results: (1) age and race were entered as covariates in the ANCOVA; (2) WCST measures were compared between Caucasian and non-Caucasian subjects; and (3) analyses were repeated using Caucasian subjects only (n = 143). Positive and negative symptoms, as measured with the appropriate subscales of the PANSS, did not differ by compound genotype group ($P > 0.10$), and did not correlate with WCST measures ($P \geq 0.10$). No other clinical or demographic variables differed among compound genotype groups.

For WCST perseverative errors (Fig. 2), although there was no main effect of COMT genotype ($F = 2.26$, df = 1,185, $P = 0.135$), performance was significantly poorer among carriers of the MTHFR T allele ($F = 3.97$, df = 1,185, $P = 0.048$). A significant COMT $\times$ MTHFR interaction was found, with Val/Val + T carriers performing worse than the other compound genotype groups ($F = 4.33$, df = 1,185, $P = 0.039$; post hoc *t*-tests: Val/Val + T > all other compound genotype groups, $P < 0.05$). Race ($F = 0.34$, df = 1,185, $P = 0.559$) and age ($F = 1.93$, df = 1,185, $P = 0.166$) did not covary with performance. Furthermore, performance did not differ between Caucasian and non-Caucasian subjects ($F = 0.176$, df = 1,185, $P = 0.675$), and among Caucasian subjects, genotype group performance patterns were comparable to the entire cohort (MTHFR main effect $F = 9.25$, df = 1,143, $P = 0.011$; COMT main effect $F = 2.79$, df = 1,143, $P = 0.097$; interaction $F = 4.45$, df = 1,143, $P = 0.037$).

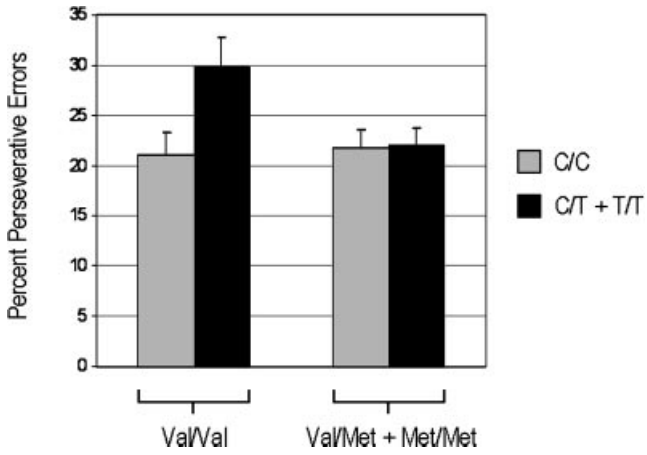

Fig. 2. Effect of compound COMT-MTHFR genotype on percent perseverative errors. There was a significant main effect of MTHFR genotype ($F = 3.97$, df = 1,185 $P = 0.048$) and a significant MTHFR $\times$ COMT interaction ($F = 4.33$, df = 1,185, $P = 0.039$). Group sizes were as follows: Val/Val + C/C, N = 35; Val/Val + T carrier, N = 21; Met carrier + C/C, N = 57; Met carrier + T carrier, N = 72.

For ability to generate $\geq$1 category (Fig. 3), an omnibus test of the four genotype groups indicated significant between-group differences ($\chi^2 = 9.54$, df = 3, $P = 0.023$). As previously described in this cohort [Roffman et al., 2007a], there was a significant main effect of MTHFR genotype, where T allele carriers exhibited poorer performance than C/C patients

TABLE I. Characterization of Compound Genotype Groups

	Val/Val + C/C, N = 35 (%)	Val/Val + T carrier, N = 21 (%)	Met carrier + C/C, N = 57 (%)	Met carrier + T, carrier N = 72 (%)	Statistics χ^2	Statistics *P*
Gender (% female)	37.1	28.6	31.6	29.2	0.78	NS
Race (% Caucasian)	45.7	81.0	73.7	94.4	32.32	<.001
Typical antipsychotics	22.9	14.3	28.1	23.9	1.63	NS
Atypical antipsychotics	68.6	66.7	77.2	74.6	1.38	NS
Antidepressants	16.1	15.0	32.6	32.3	4.87	NS
Anticonvulsants	22.6	15.0	39.5	22.6	5.87	NS

	Val/Val + C/C, N = 35		Val/Val + T, carrier N = 21		Met carrier + C/C, N = 57		Met carrier + T carrier, N = 72		Statistics	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*F*	*P*
Age	41.6	11.7	41.8	7.3	41.3	10.4	46.0	8.3	3.27	0.023[b]
Duration of illness	19.3	12.8	18.4	9.6	18.3	9.7	20.8	10.5	0.60	NS
Positive symptoms	15.9	5.1	14.6	6.5	16.4	6.2	15.0	6.2	0.83	NS
Negative symptoms	17.6	4.5	19.0	3.6	17.6	4.7	18.9	5.2	1.07	NS
HAM-D[a]	13.5	4.0	9.3	6.3	11.3	4.1	11.5	5.7	1.76	NS
Simpson-Angus[a]	3.5	3.7	4.9	3.7	4.7	4.9	3.3	3.2	1.16	NS
WAIS full scale IQ	88.1	13.7	84.5	15.0	82.1	13.9	83.6	16.6	1.20	NS

SD: standard deviation; HAM-D: Hamilton Depression Rating Scale; WAIS: Wechsler Adult Intelligence Scale; NS: not significant at level of alpha ($P < 0.05$) or trend ($P < 0.10$).
[a]Data for this variable were not available for all subjects.
[b]Post hoc test results: Met carrier + T carrier > Val/Val + C/C ($P = 0.027$), Val/Val + T carrier ($P = 0.082$), and Met carrier + T/T ($P = 0.006$).

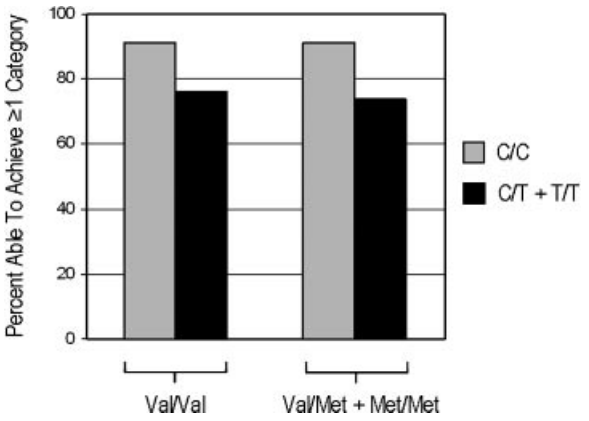

Fig. 3. Effect of compound COMT-MTHFR genotype on category generation. There was a significant main effect of MTHFR genotype ($\chi^2 = 9.47$, $P = 0.002$) but no effects related to COMT genotype. Group sizes were as follows: Val/Val + C/C, N = 35; Val/Val + T carrier, N = 21; Met carrier + C/C, N = 57; Met carrier + T carrier, N = 72.

($\chi^2 = 9.47$, $P = 0.002$). No main or interactive effects of COMT genotype were found ($\chi^2 = 0.51$, df = 1, $P = 0.475$). Caucasian and non-Caucasian subjects performed equivalently ($\chi^2 = 0.34$, df = 1, $P = 0.557$) and MTHFR effects persisted within the Caucasian sub-sample ($\chi^2 = 6.00$, df = 1, $P = 0.014$).

DISCUSSION

This investigation provides initial evidence that the COMT Val108/158Met and MTHFR C677T polymorphisms contribute interactively to executive dysfunction in schizophrenia. In previous studies, Val/Val genotype has been weakly and inconsistently associated with poorer executive function [Barnett et al., 2007]. Here, while the Val/Val + C/C group did not differ from the Met allele carrier groups, Val/Val + T allele carrying subjects committed perseverative errors more frequently (relative increase of 37%) than did subjects in all other compound genotype groups. Furthermore, while COMT and MTHFR genotype each accounted for 1.6% of the variance in performance, together they accounted for 4.7%, suggesting an effect that is more than additive. These results, although preliminary, may suggest a partial explanation for why COMT analyses have been inconsistent in previously described schizophrenia cohorts, which presumably contained a mixture of C/C and T carrier subjects.

Perseverative errors have long been associated with dysfunction of the dorsolateral prefrontal cortex, both in schizophrenia [Sullivan et al., 1993; Goldberg et al., 1994] and other neuropsychiatric illnesses [Lombardi et al., 1999]. COMT 108/158Val has been reliably linked to inefficient prefrontal activation during executive function tasks, as measured with functional neuroimaging [Egan et al., 2001; Bertolino et al., 2004; Ho et al., 2005]. However, Val allele effects appear less hardy further downstream, at the level of executive function *performance*. To the extent that such performance relies on prefrontal dopamine signaling [Goldman-Rakic, 1996], it is likely that additional genetic or epigenetic influences on COMT function, besides Val108/158Met, account more fully for variation in executive function [Tunbridge et al., 2006]. In fact, other variants in the COMT gene, as well as functional polymorphisms in the dopamine transporter (DAT), have been found to interact with Val108/158Met on working memory performance and prefrontal activation [Bertolino et al., 2006; Caldu et al., 2007; Diaz-Asper et al., 2007].

Additive effects of the COMT Val and MTHFR T alleles on prefrontal dopamine turnover can be inferred based on previous work examining methylation of the COMT promoter (Fig. 1). In a study of monozygotic twin pairs, concordance of CpG methylation in the COMT promoter region was robust ($r = 0.87$, $P < 0.001$) [Mill et al., 2006]. This result suggests that COMT promoter methylation is strongly heritable, although the specific role of MTHFR was not examined. Moreover, in a recent study comparing brain tissue from 115 schizophrenia patients and controls [Abdolmaleky et al., 2006], methylation of the COMT promoter was lower in patients by a factor of two; the difference was more pronounced in the left dorsolateral prefrontal cortex (Brodmann area 46). Promoter hypomethylation was also associated with increased COMT expression (2.7 times higher in patients). Presumably, the MTHFR T allele would contribute to hypomethylation of the COMT promoter, and subsequently to reduced dopamine signaling (Fig. 1). This deficit would be compounded in individuals who are already at risk for low synaptic dopamine due to homozygosity for the hyperfunctional Val allele.

Of note, a paucity of available methyl moieties might also compromise the ability of COMT to deactivate synaptic dopamine through transmethylation, with the net effect of *increasing* synaptic dopamine. In fact, the activity of blood COMT has been significantly correlated with blood concentrations of *S*-adenosylmethionine (SAM), which is generated downstream of the MTHFR reaction [Matthysse and Baldessarini, 1972]. However, given the detrimental effects of the T allele on executive function measures, this second putative interaction may be less salient to perseveration. It is also possible that the statistical interaction between COMT and MTHFR does not reflect a synergistic biochemical effect per se, but rather independent cumulative effects on prefrontal function (e.g., COMT via its impact on dopamine, and MTHFR via an effect on homocysteine, which has also been negatively associated with cognitive function) [Teunissen et al., 2005]. Additional work examining biochemical and neural interactions of the COMT and MTHFR polymorphisms will be necessary to validate their joint effects on frontal lobe physiology.

Category generation in the WCST is thought to reflect another aspect of executive function: the ability to deduce a rule set based on trial and error. Although deficits in category generation have been frequently described in the schizophrenia literature [Laws, 1999], category effects have not been as widely described in COMT studies as have perseverative errors [Barnett et al., 2007]. Our previous work suggests that the MTHFR T allele undermines category generation in schizophrenia, with 33% of T/T patients unable to complete a single category [Roffman et al., 2007a]. The present analysis suggests that COMT Val108/158Met does not significantly influence this aspect of executive function, by itself or in interaction with MTHFR C677T. Rather, other downstream methylation-dependent processes that have been implicated in schizophrenia (e.g., expression of other genes such as DRD2 and HTR2A, and metabolism of homocysteine) [Abdolmaleky et al., 2004] may play a more direct role.

Several limitations of the current study are important to recognize. The number of subjects was relatively small, especially for a gene interaction study, and the results require replication in other samples. With a convenience sample, the possibility of stratification artifact requires careful consideration, especially given that MTHFR C677T and COMT Val108/158Met allele distributions vary among differing racial groups. However, performance did not differ between racial groups, and post hoc analysis selecting for patients of one geographic ancestry (Caucasians) produced results that were analogous to those observed in the entire sample. Finally, although COMT effects on executive function have been studied extensively in healthy subjects [Barnett et al., 2007], MTHFR effects in healthy individuals have not yet been characterized.

Confounders related to chronic illness might have influenced the results, although medication use and duration of illness did not differ among genotype groups in the current investigation. Still, it would be useful and informative to examine whether similar gene interactions affect executive function performance among individuals free of psychiatric illness or psychotropic (especially dopaminergic) medications.

In summary, an epigenetic interaction of MTHFR C677T and COMT Val108/158Met genotype may contribute to executive function deficits in schizophrenia, wherein the T and Val alleles cumulatively increase perseverative errors. Additional work may verify whether MTHFR genotype-dependent methylation of the COMT promoter region, with consequent alteration in prefrontal dopamine transmission, underlies this effect.

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