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ORIGINAL ARTICLE

Epistasis between *COMT* Val^{158}Met and *DRD3* Ser^{9}Gly polymorphisms and cognitive function in schizophrenia: genetic influence on dopamine transmission

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Objective: To assess the relationship between cognitive function, a proposed schizophrenia endophenotype, and two genetic polymorphisms related to dopamine function, catechol-*O*-methyl transferase (*COMT*) Val^{158}Met and dopamine receptor 3 (*DRD3*) Ser^{9}Gly.
Methods: Fifty-eight outpatients with schizophrenia/schizoaffective disorder and 88 healthy controls underwent neurocognitive testing and genotyping. Analyses of covariance (ANCOVAs) using age, sex, and years of education as covariates compared cognitive performance for the proposed genotypes in patients and controls. ANCOVAs also tested for the epistatic effect of *COMT* and *DRD3* genotype combinations on cognitive performance.
Results: For executive functioning, *COMT* Val/Val patients performed in a similar range as controls (30.70-33.26 vs. 35.53-35.67), but as *COMT* Met allele frequency increased, executive functioning worsened. *COMT* Met/Met patients carrying the *DRD3* Ser/Ser genotype performed poorest (16.184 vs. 27.388-31.824). Scores of carriers of this *COMT*/*DRD3* combination significantly differed from all *DRD3* Gly/Gly combinations ($p < 0.05$), from *COMT* Val/Met *DRD3* Ser/Gly ($p = 0.02$), and from *COMT* Val/Val *DRD3* Ser/Ser ($p = 0.01$) in patients. It also differed significantly from all control scores ($p < 0.001$).
Conclusion: Combined genetic polymorphisms related to dopamine neurotransmission might influence executive function in schizophrenia. Looking at the effects of multiple genes on a single disease trait (epistasis) provides a comprehensive and more reliable way to determine genetic effects on endophenotypes.

Keywords: Endophenotype; psychosis; genetics; dopamine; executive function

Introduction

The intricate etiopathogenetic mechanisms underlying the clinical manifestations of schizophrenia greatly hamper the search for gene-illness associations.[1] Since psychotic symptoms represent an array of biological, psychological, and behavioral phenomena,[2] an alternative to understanding this complex array has been to study gene-environment interactions.[3] However, such studies must evaluate large samples and control for a multitude of confounding factors. Consequently, an operative definition of the exact role of genes in schizophrenia has yet to be achieved.[4] One strategy to overcome these difficulties relies on the identification of endophenotypes, which are stable across the life span, usually related to fewer but more specific genes, and less susceptible to the effects of environmental factors.[5] The occurrence of cognitive impairment in schizophrenia has been widely studied, and there is robust evidence supporting it as one of the better-described endophenotypes of the disorder.[5]

Cognitive impairment has been suggested by some as one of the core manifestations of schizophrenia.[6] Cognitive deficits are pervasive, usually starting years before the onset of psychosis[7] and progressing with the disease course.[8] Cognitive deficits are also present among non-affected relatives of persons with schizophrenia, further strengthening the role of these deficits as endophenotypes.[9] Moreover, the dopaminergic system – which plays a central role in the pathophysiology of psychotic symptoms[10] – has been implicated in the workings of several cognitive functions.[11]

Thus, researchers have focused their efforts in determining the link between genes related to dopamine function and cognitive endophenotypes. One important target is the gene that encodes the catechol-*O*-methyl

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transferase (*COMT*) enzyme. *COMT* degrades catecholamines, including dopamine, in the synaptic cleft; it is expressed throughout the brain, but especially in the prefrontal cortex and striatum.[12] A functional polymorphism at codon 158 (*COMT* Val^{158}Met) results in its loss of function: Met/Met carriers hold 25% of Val/Val wild-type activity, while heterozygotes display intermediate enzymatic activity. Several studies have implicated *COMT* polymorphisms in both cognitive function[13] and schizophrenia.[14]

Dopamine receptors are another potential target for the investigation of genetic determinants of cognitive endophenotypes. Five different dopamine receptors have been well studied so far. They are classified into two groups according to structural similarity: D1/D5 receptors and D2/D3/D4 receptors. D1 and D2 receptors have been most extensively researched, with several studies supporting their association with cognition.[15-18] Regarding the D3 receptor, a genetic polymorphism characterized by a substitution of serine by glycine at codon 9 (*DRD3* Ser^{9}Gly) has been hypothesized to be associated with susceptibility to development of schizophrenia.[19] However, few studies have addressed the association of this polymorphism with cognitive deficits in schizophrenia.[20,21]

The aim of the present study was to investigate the influence of the *COMT* Val^{158}Met and *DRD3* Ser^{9}Gly polymorphisms on various neurocognitive functions of individuals with schizophrenia and healthy controls. Since both polymorphisms affect dopamine neurotransmission, and since recent studies have addressed the effect of genotype $\times$ genotype interactions,[22] we also tested for a possible epistatic effect of these genes on cognition.

Method

Participants

Outpatients with schizophrenia or schizoaffective disorder (diagnosed per DSM-IV criteria) were recruited from the Institute of Psychiatry, Universidade de São Paulo, SP, Brazil. Neurological disorders, mental retardation, and unstable medical conditions affecting brain functioning constituted our exclusion criteria. Controls had no current or past history of psychiatric disorder according to an evaluation conducted by trained psychiatrists using the Mini International Neuropsychiatric Interview (MINI),[23] and no family history (first-degree relatives) of mood or psychotic disorders.

The study was approved by the local ethics committee, and all subjects provided written consent to participate in the study after being fully oriented about its procedures.

Clinical and neurocognitive assessments

All clinical assessments were conducted by experienced and trained psychiatrists (AAL, MTB, RTS, LTV, MVZ) and a psychologist (DSB). Psychiatric diagnosis was established with the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I). Symptom severity was assessed using the Positive and Negative Symptom Scale (PANSS).

Neurocognitive assessment was carried out by one of the investigators (DSB), an experienced psychologist trained in standardized neuropsychological testing. A 2-hour test battery was administered in fixed order, assessing the following domains: a) attention: Wechsler Adult Intelligence Scale III (WAIS-III) subtest Digit Span (WAIS-DS), Trail Making Test – Part A (TMT-A), Stroop Color-Word Test (SCWT); b) verbal memory: Wechsler Memory Scale subtest – Logical Memory (WMS-LM), immediate (1) and delayed (2); c) visual memory: Rey-Osterrieth Complex Figure Test (RCFT) delayed recall; d) visuospatial function: WAIS-III – Block Design (WAIS-BD), RCFT copy; e) language: Controlled Oral Word Association Test (FAS), WAIS-III Vocabulary subtest (WAIS-V); f) psychomotor speed: TMT-A; g) executive function: WAIS-III Letter-Number Sequence (WAIS-LNS), WAIS-DS, SCWT, TMT-B, WAIS Similarities (WAIS-S), Matrix Reasoning (WAIS-MR), RCFT copy, Wisconsin Card Sorting Test (WCST) Conceptual Level Responses (WCST-CONC), Perseverative Responses (WCST-PR), Failure to Maintain Set (WCST-FMS), Corrected Categories (WCST-CC), Errors (WCST-E), Non-Perseverative Errors (WCST-NP), Perseverative Errors (WCST-P); h) intelligence: WAIS – Total Intelligence Quotient (IQ), Estimated IQ (EIQ), Execution IQ (EXIQ), and Verbal IQ (VIQ). In the majority of these tests, better performance is indicated by higher scores, with the exception of the SCWT and TMT (scores measured in seconds).

Genotyping

DNA was extracted from peripheral blood according to the salting-out protocol[24] and then genotyped for *COMT* rs4680 (Val^{158}Met) and *DRD3* rs6280 (Ser^{9}Gly). Polymorphisms were determined by real-time polymerase chain reaction (PCR) allelic discrimination with TaqMan® SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Amplification and allelic discrimination were carried out using a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Thermal cycling consisted of initial denaturation for 10 min at 95 °C, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing at 60 °C for 1 min. The allele-detection process and allelic discrimination were performed for 1 min at 60 °C.

Statistical analysis

The allele frequencies of both patients and healthy controls were tested for Hardy-Weinberg equilibrium (HWE). Estimation of allele and genotype frequencies was performed by the gene counting method. Afterwards, chi-square statistics were calculated to compare allele and genotype frequencies between patient and control groups.

Since neurocognitive measures were the dependent variables, the data was tested for normality using a Q-Q plot with their transformed residuals and the standard normal distribution.

After observing a normal distribution, analyses of covariance (ANCOVAs) were used to compare neuropsychological performance between the various genotypes of

COMT rs4680 and of *DRD3* rs6280. To test for the differential influence of age, sex, and years of education, these three sociodemographic variables were used as covariates in the ANCOVA models. A type IV sum of squares model was used. To identify a possible epistatic interaction between *COMT* rs4680 and *DRD3* polymorphisms, further ANCOVAs were conducted. Pairwise analysis for the correction of multiple comparisons, with Fisher's least significant difference test, further analyzed possible significant differences between each *DRD3*/*COMT* combination in patients and controls. Since only one subject had the combination of *DRD3* Gly/Gly and *COMT* Val/Val, this individual's performance was pooled with *DRD3* Ser/Ser *COMT* Val/Val for the pairwise comparison. The results of this single subject on the RCFT (copy and recall) were very similar to those of *DRD3* Ser/Ser *COMT* Val/Val subjects, allowing us to proceed with this merger and thus enabling statistical analyses. SPSS version 18.0 was used for all analyses.

Results

The sample comprised 58 patients who agreed to participate in the study and completed the study protocol (40 men, 18 women; mean age 37.2±10.6 years). Four participants (7%) had received a diagnosis of schizoaffective disorder, and the remaining patients were diagnosed as having schizophrenia (93%). Overall, 40 patients (70.2%) were white, and the mean educational attainment was 10.2±2.6 years. At the time of evaluation, 51 (88%) were on atypical antipsychotics, 9 (16%) were on typical antipsychotics, 12 (21%) were on a mood stabilizer, 24 (41%) were on antidepressants, and 12 (21%) were on benzodiazepines. Ethnicity was self-reported according to the Brazilian Institute of Geography and Statistics (IBGE) classification. Our control sample comprised 88 healthy individuals recruited through local advertisements from the Universidade de São Paulo. The mean age of controls was 24±3.95 years; 43 controls (48.9%) were men, 67 (76%) were white, and the mean educational attainment was 13.8±2.4 years.

Allele frequencies in patients and healthy controls showed that both samples were in HWE (data not shown). Table 1 shows *DRD3* Ser^{9}Gly and *COMT* Val^{158}Met genotype frequencies in patients and healthy controls. Regarding *DRD3* Ser^{9}Gly, while Ser/Gly variant frequencies were nearly identical between both groups (48.2 vs. 50.6% for patients and controls, respectively), patients had significantly more Gly/Gly (26.8 vs. 8.0%) and less Ser/Ser (25.0 vs. 41.4%) relative to healthy controls (chi-square = 10.43, p = 0.005). For *COMT* Val^{158}Met polymorphisms, heterozygosity was significantly more frequent in patients than in healthy controls (Val/Met = 57.1 vs. 26.1%; chi-square = 14.14, p = 0.001).

Table 2 shows cognitive performance according to *DRD3* Ser^{9}Gly and *COMT* Val^{158}Met genotypes for patients and healthy controls. Concerning *DRD3* Ser^{9}Gly genotypes, while there was no effect of genotype on any of the cognitive measures in patients, the Ser/Gly genotype was associated with poorer executive function performance in healthy controls, as measured by TMT-B (p = 0.04). For *COMT* Val^{158}Met genotypes, only in the patient group was the Val/Val variant significantly related to poorer performance on attention measures (WAIS-DS; p = 0.02). The Met/Met genotype was associated with poorer performance on executive function tests in both patients and healthy controls. However, this was detected by means of the WCST-FMS in patients (p = 0.02), whereas in controls, the TMT-B showed a statistically significant difference (p = 0.01). Results from the other cognitive tests are provided in supplementary tables.

In healthy controls, all combinations between the two genotypes had the same effect on executive functioning as measured by the RCFT copy (all $p > 0.05$) (Figure 1). In the patient group, *COMT* Val/Val individuals also performed equally to controls (30.70-33.26 vs. 35.53-35.67), regardless of *DRD3* genotype. However, as *COMT* Met allele frequency increased, cognitive functioning worsened for patients. For the *COMT* Val/Met genotype, this effect was most pronounced for *DRD3* Ser/Ser subjects (25.903 for Ser/Ser vs. 29.642-30.304 for other *DRD3* genotypes), whereas for *COMT* Met/Met genotypes, the *DRD3* Ser/Ser genotype was associated with the poorest performance on executive functioning (16.184 vs. 27.388-31.824).

Pairwise comparisons showed that this specific *COMT*/*DRD3* combination significantly differed from all *DRD3* Gly/Gly combinations ($p < 0.05$), from *COMT* Val/Met *DRD3* Ser/Gly (p = 0.02), and from *COMT* Val/Val *DRD3* Ser/Ser (p = 0.01) in patients. It also significantly differed from all control scores ($p < 0.001$). Patients' scores significantly differed from those of controls, except for those with the *COMT* Val/Val and *COMT* Met/Met *DRD3* Gly/Gly genotypes.

When executive functioning and visual memory were assessed with the RCFT recall (Figure 2), all combinations

Table 1 *DRD3* Ser^{9}Gly and *COMT* Val^{158}Met genotype frequencies among patients and controls

	Patients	Controls	Pearson chi-square	p-value
DRD3 Ser^{9}Gly				
Gly/Gly	15 (26.8)	7 (8.0)		
Ser/Gly	27 (48.2)	44 (50.6)		
Ser/Ser	14 (25.0)	36 (41.4)	10.43	0.005
COMT Val^{158}Met				
Met/Met	11 (19.6)	26 (29.5)		
Val/Met	32 (57.1)	23 (26.1)		
Val/Val	13 (23.2)	39 (44.3)	14.14	0.001

Data expressed as n (%).
COMT = catechol-*O*-methyl transferase; *DRD3* = dopamine receptor 3.

Table 2 Analysis of variance (ANOVA) between neurocognitive performance, *DRD3* Ser[9]Gly and *COMT* Val[158]Met genotypes

Polymorphism/ cognitive domain/test	Patients				Controls			
	Gly/Gly	Ser/Gly	Ser/Ser	p-value	Gly/Gly	Ser/Gly	Ser/Ser	p-value
DRD3 Ser[9]Gly								
Executive function								
TMT-B	144.88±96.98	135.65±55.58	149.00±96.47	0.92	48.00±29.58	**66.15±41.45**	50.05±20.99	**0.04**
	Met/Met	Val/Met	Val/Val	p-value	Met/Met	Val/Met	Val/Val	p-value
COMT Val[158]Met								
Attention								
WAIS-DS	7.27±1.55	7.39±1.98	**6.67±1.72**	**0.04**	10.83±2.95	10.62±2.21	10.25±2.06	0.25
Executive function								
WCST-FMS	**1.18±0.87**	0.48±0.71	0.42±0.67	**0.02**	0.21±0.77	0.42±1.10	0.33±0.73	0.66
TMT-B	137.18±62.42	154.48±93.11	110.33±78.76	0.68	**72.34±47.11**	53.31±24.55	53.08±25.95	**0.01**

Data expressed as mean ± standard deviation.
COMT = catechol-*O*-methyl transferase; *DRD3* = dopamine receptor 3; TMT-B = Trail Making Test – Part B; WAIS-DS = Wechsler Adult Intelligence Scale – Digit Span subtest; WCST-FMS = Wisconsin Card Sorting Test – Failure to Maintain Set.
Data in bold indicate poorest performance.

between the two genotypes had the same effect on cognitive functioning in controls and in patients. The only exceptions were *COMT* Val/Val carriers heterozygous for *DRD3* Ser[9]Gly; their scores were significantly different from all other patient scores (19.739 vs. 1.28-10.833, $p < 0.01$), and this was the only genotype combination that showed performance comparable to that of controls (19.739 for patients and 18.943-28.77 for controls, $p > 0.05$).

Discussion

To our knowledge, this is the first study to evaluate the effects of epistasis between *COMT* Val[158]Met and *DRD3* Ser[9]Gly polymorphisms on the cognitive functioning of patients with schizophrenia and healthy controls.

Our results showed that the *DRD3* Ser[9]Gly Gly/Gly and *COMT* Val[158]Met heterozygote genotypes were significantly more frequent in patients than controls. Control heterozygotes for *DRD3* Ser[9]Gly and both patients and controls carrying *COMT* Val[158]Met Met/Met showed poorer performance on executive function tests compared to the other groups. Patients carrying *COMT* Val[158]Met Val/Val performed poorly on attention tasks. Epistasis between *DRD3* Ser[9]Gly Ser/Ser and *COMT* Val[158]Met Met/Met significantly worsened executive functioning in patients relative to healthy controls.

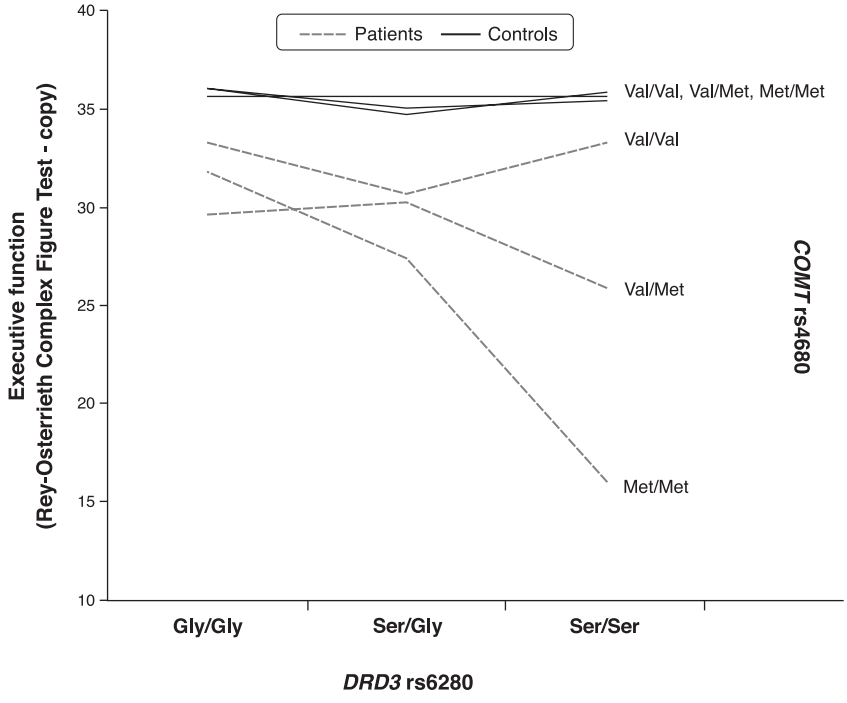

Figure 1 Analysis of covariance (ANCOVA) between executive function performance (Rey-Osterrieth Complex Figure Test – copy) and combinations of *COMT* rs4680 and *DRD3* Ser[9]Gly genotypes. *COMT* = catechol-*O*-methyl transferase; *DRD3* = dopamine receptor 3.

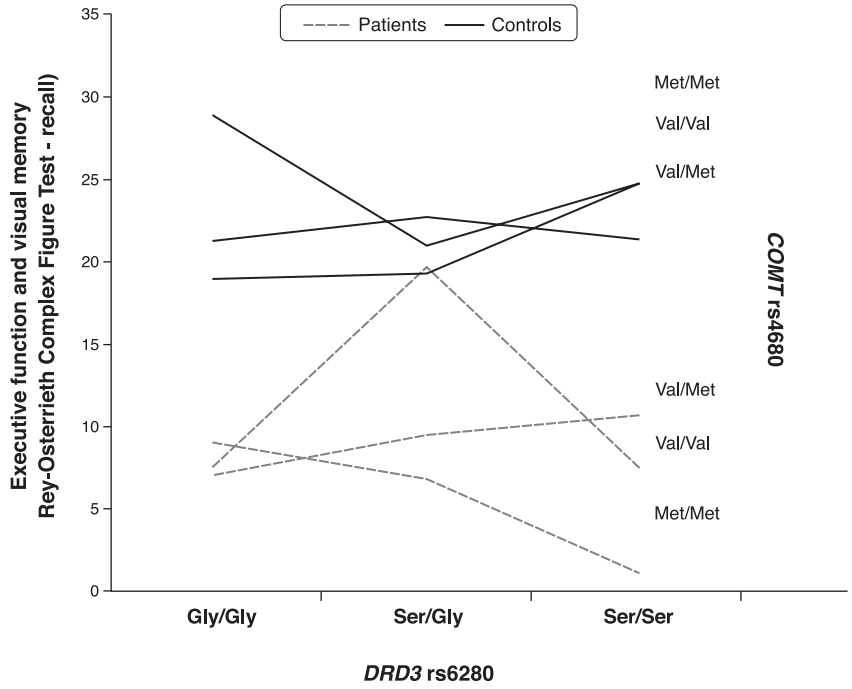

Figure 2 Analysis of covariance (ANCOVA; age, sex, and years of education as covariates) between executive function/visual memory performance (Rey-Osterrieth Complex Figure Test – recall) and combinations of *COMT* rs4680 and *DRD3* Ser[9]Gly genotypes. *COMT* = catechol-*O*-methyl transferase; *DRD3* = dopamine receptor 3.

Genotype frequencies in patients with schizophrenia versus healthy controls

Differing from our finding of a higher frequency of *DRD3* Ser[9]Gly Gly/Gly in patients, a number of previous studies reported no association between *DRD3* Ser[9]Gly polymorphisms and schizophrenia.[25] The synergistic effects of the *DRD3* gene on susceptibility to schizophrenia and the possibility that this association might only be found in certain racial or clinical subgroups have been proposed as possible explanations for this absence of a relationship. Nevertheless, consistent with our results, more recent findings suggest an association between the Gly/Gly variant and schizophrenia.[26-28] The Gly allele frequency reported herein is similar to that described in these studies, which provides further evidence supporting the hypothesis that this allele is under the influence of natural selection, being more frequent in Asian and Latin American regions.[28]

Regarding the relationship between the *COMT* Val[158]Met polymorphism and schizophrenia, although we found a higher frequency of heterozygosity among patients, there is no consensus in the literature about this issue. A meta-analysis conducted by Glatt et al.[29] indicated that the Val[158] allele might be a weak risk factor for the development of schizophrenia. However Costas et al.[14] suggested that heterozygosity possesses a small but significant protective effect for the development of the disorder. Given the variety of findings,[30,31] it is increasingly clear that such relationships are more complex than simple allele-disorder associations.[32]

Interactions between genotypes and cognitive measures

Few studies have evaluated the impact of *DRD3* Ser[9]Gly polymorphisms on cognition. Our results corroborate the findings of Lane et al.,[33] who observed poorer executive functioning performance in healthy volunteers heterozygous for the *DRD3* Ser[9]Gly polymorphism. We also found that this polymorphism had no effect on the executive functioning of schizophrenic patients, similarly to the findings of Rybakowski et al.[16] One group has reported results contrary to these findings,[2] but the authors acknowledged that the different cognitive paradigm they studied possibly accounted for this contrary effect.

Regarding the effect of *COMT* Val[158]Met polymorphisms on cognition, our patients with schizophrenia carrying the Val/Val allele performed poorly in attention tests, a finding similar to those reported by previous studies[34] on a normal population. However, when executive function was tested, Met/Met patients performed poorly compared to healthy controls. The effects of different *COMT* genotypes on cognition have been extensively studied, but great heterogeneity of results has been observed.[35] Some studies found individuals with schizophrenia carrying the Val[158] allele to present better cognitive performance,[36] whereas others reported better performance of Met[158] allele carriers[37] or negative findings.[38] Savitz et al.[39] argued that differences in the specific cognitive domain assessed might, at least partly, account for these discrepancies. They hypothesized that hyperdopaminergic states would be associated with perseverative errors and thus with executive functioning deficits, while hypodopaminergic states would be more related to attention deficits. The fact

that Met^{158} carriers display lower enzymatic activity, thus contributing to a hyperdopaminergic state, while the presence of a Val^{158} allele produces the opposite effect, corroborates our findings. We can thus hypothesize that patients with schizophrenia can be classified into two major groups according to the *COMT* genotype versus cognition interaction: individuals with schizophrenia carrying *COMT* Val/Val will have more genetically determined dopaminergic hypoactivity and attention deficits, while *COMT* Met/Met carriers will have more genetically determined dopaminergic hyperactivity and executive dysfunction. This could possibly explain, at least in part, why results simply relating *COMT* polymorphisms to the disorder fail to lead to a consensus.

Epistatic genetic interactions on cognition

Combining the effects of *DRD3* Ser^{9}Gly and *COMT* Val^{158}Met genotypes, schizophrenia patients with a *COMT* Val/Val genotype performed similarly to healthy controls in executive functioning. Interestingly, schizophrenia patients carrying the *COMT* Met^{158} allele showed poorer executive function performance relative to controls. Resuming what was stated previously and further discussing the issue, poor performance on cognitive tasks with a greater cognitive integration requirement, such as executive function tests, have been associated with hyperdopaminergic states and with the presence of the Met^{158} allele.[35] According to Howes & Kapur,[10] cognitive dysfunction (and other schizophrenia symptoms) would be a result of a dopamine imbalance, mainly due to depletion of dopamine in the prefrontal cortex, leading to uncontrolled dopaminergic firing in other brain areas.[40] As with executive functioning, demand for cognitive integration would also decrease in this case, and the lack of dopaminergic control inherent to schizophrenia would make Met^{158} carriers more vulnerable to poorer cognitive performance. Resembling Savitz's theory, this might result from disordered and increased firing of dopaminergic neurons.[41]

According to our results, if considered separately, *DRD3* genotypes had no effect on patients' cognition. However, when epistatic effects under high dopamine availability (*COMT* Met/Met) were weighed in the analyses, we found results similar to those of Szekeres et al.,[20] who also reported an association between *DRD3* Ser/Ser genotype and poorer executive function performance in schizophrenic individuals.

Thus, the effect of the *DRD3* polymorphism on cognition could only be unveiled when it was analyzed in combination with the *COMT* polymorphism; *DRD3* Ser/Ser potentiated the poor cognitive performance effect of the *COMT* Met^{158} allele. Similar findings were observed by Lee et al.[42] when studying the same epistatic interaction in patients with bipolar disorder: the authors found that the same combination of polymorphisms was associated with type I bipolar disorder.[42]

When visual memory was added to the cognitive paradigm through the RCFT recall subtest, the epistatic effects of the *DRD3* Ser^{9}Gly and *COMT* Val^{158}Met polymorphisms on cognitive performance were amplified. Among patients with *COMT* Val^{158}Met Val/Val, only those heterozygous for *DRD3* rs6280 persisted, performing similar to controls. For all other genotype combinations, patients performed comparably worse than healthy controls. This suggests that, under a greater cognitive load,[43] deficits in patients became more apparent, and that the above combination was the only one to confer "protection" against low cognitive performance.

Several methodological limitations should be considered in the interpretation of our results. First, the relatively modest sample size increases the risk of both type I and type II statistical errors. For instance, we contextualized our results with the findings of Lee et al.,[42] who investigated the same genotype combination and found similar data. However, their paradigm related diagnosis to genotype, while our paradigm related genotype to cognition within the diagnosis. Taking into account that Bombin et al.[2] also found positive results for *DRD3* but in the other direction (though pooling patients and controls in their results), our results should be approached with caution due to the possibility of type I error. Furthermore, sample size did not allow us to control our results for current use of medication, and obligated us to combine two genetic polymorphisms for pairwise comparisons. Even though these limitations were present, as cited above, our data are consistent with the international literature and provide some important contributions for future research. Thus, replication of the present findings in larger samples is warranted.

In conclusion, it is important to consider the epistasis of multiple genetic polymorphisms related to a neurotransmitter system.[44] Our study confirmed the importance of dopaminergic neurotransmission in the pathophysiology of psychosis, especially in determining endophenotypic characteristics such as cognitive performance. More studies with larger samples should further analyze epistatic phenomena in an attempt to replicate these findings. Finally, this genetic paradigm could be tested in individuals at risk for psychosis, to determine whether it influences conversion rates or predicts a clinical course with poor cognitive performance.

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Disclosure

The authors report no conflicts of interest.

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