Association study between the rs165599 catechol-O-methyltransferase genetic polymorphism and schizophrenia in a Brazilian sample

Estudo de associação entre o polimorfismo genético rs165599 da catecol-O-metiltransferase e esquizofrenia em uma amostra brasileira

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ABSTRACT

Schizophrenia is a severe psychiatric disorder with frequent recurrent psychotic relapses and progressive functional impairment. It results from a poorly understood gene-environment interaction. The gene encoding catechol-O-methyltransferase (COMT) is a likely candidate for schizophrenia. Its rs165599 (A/G) polymorphism has been shown to be associated with alteration of COMT gene expression. Therefore, the present study aimed to investigate a possible association between schizophrenia and this polymorphism. The distribution of the alleles and genotypes of this polymorphism was investigated in a Brazilian sample of 245 patients and 834 controls. The genotypic frequencies were in Hardy-Weinberg equilibrium and no statistically significant differences were found between cases and controls when analyzed according to gender or schizophrenia subtypes. There was also no difference in homozygosis between cases and controls. Thus, in the sample studied, there was no evidence of any association between schizophrenia and rs165599 (A/G) polymorphism in the non-coding region 3' of the COMT gene.

Key words: enzyme, genetics, psychosis, schizophrenia.

RESUMO

A esquizofrenia é um grave transtorno psiquiátrico que apresenta freqüentes recorrências psicóticas e incapacitação progressiva. Resulta de uma interação gene-ambiente ainda pouco compreendida. O gene da catecol-O-metiltransferase (COMT) é considerado um possível candidato para esquizofrenia. O polimorfismo genético rs165599 (A/G) da COMT foi associado com alteração da expressão do seu gene. Assim, o presente trabalho tem como objetivo investigar a possível associação de tal polimorfismo com esquizofrenia. A distribuição de seus alelos e genótipos foi investigada em uma amostra brasileira composta de 245 pacientes e 834 controles. As frequências genotípicas estavam em equilíbrio de Hardy-Weinberg e não se encontrou diferença estatisticamente significante entre casos e controles, quando analisados por gênero e subtipos da esquizofrenia. Não houve também diferença de homozigosidade entre casos e controles. Desse modo, na amostra estudada, não houve evidência de associação entre esquizofrenia e o polimorfismo rs165599 (A/G) localizado na região 3' não codificadora do gene da COMT.

Palavras-Chave: enzima, genética, psicose, esquizofrenia.

Schizophrenia is a severe psychiatric disorder characterized by psychotic symptoms and functional impairment, with recurrent relapses and continuing disability. The risk factors for schizophrenia are indicators currently perceived as epiphenomena of pathophysiological processes resulting from gene-environment interactions that remain poorly understood[1].

Widespread impairments in brain function and structure have been shown in schizophrenia cases. It is also clear that, to a lesser degree, unaffected family members share many of the neurobiological abnormalities found in affected individuals, which suggests that the alleles that underlie the genetic risk of schizophrenia may primarily exert their effects on intermediate traits such as cerebral structure or function[1].

Although the etiology of schizophrenia is not well established yet, data from many studies have shown that genetic load alone cannot determine development of schizophrenia, thus favoring an additive model of interactive genetic and environmental risk factors. This gene-environment interaction

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might occur at specific stages of development of occurrences of this disorder[1].

Genetic epidemiological investigations have suggested that there is a genetic component with significant participation in the etiology of schizophrenia, and heritability estimates as high as 80% have been reported[1]. The mode of inheritance is complex, with polygenic environmental interaction. The role of single relevant genes must be small, and therefore association studies involving case-control approaches have been conducted to evaluate the allelic variations of specific candidate genes that may be related to the etiopathology of the disorder[1].

Some of the most investigated genes in studies on vulnerability to schizophrenia are those that code for proteins of the dopaminergic system, because of evidence of the role of central dopamine pathways in the pathophysiology of the disorder. Therefore, the genes involved in the dopaminergic system are potential targets for genetic association investigations in schizophrenia. Polymorphisms in dopamine transporter and receptors genes have been widely investigated as risk factors for schizophrenia development, but the results have been inconclusive[2-5]. Thus, other possible candidates for such investigations are the enzymes that metabolize dopamine, such as catechol-O-methyltransferase (COMT).

The gene encoding the enzyme COMT has been considered to be a likely candidate for an association with schizophrenia. Most COMT genetic association studies have focused on a particular single nucleotide polymorphism (SNP) that results in a change from valine to methionine at codon 158/108 (rs4680). It has been suggested that this may contribute towards increased prefrontal catabolism and impaired prefrontal cortex function[6].

Another interesting polymorphism of the COMT gene is rs165599 (A/G), which is located in the 3' untranslated region of the COMT gene, close to exon 1. Its G allele was first shown to be highly associated with schizophrenia in Ashkenazi Jews by Shifman et al., as a component of a haplotype (G-G-G haplotype of three SNPs: rs737865, rs4680 and rs165599) of the COMT gene[7]. This haplotype has also been reported to be linked with lower expression of COMT mRNA in the human brain[7]. In a postmortem study, Bray et al. demonstrated that this polymorphism altered COMT gene expression, which was less expressed when the G allele was present[8].

On the other hand, Okochi et al. conducted a meta-analysis study in which no clear association between COMT genotypes and schizophrenia was found[9]. However, some studies have found associations between rs165599 (A/G) COMT genetic polymorphism and different clinical aspects of schizophrenia. Gu et al. showed an association between the polymorphism investigated and violent behavior in a sample of Chinese male schizophrenic patients[10].

In a study conducted on a Taiwanese sample, the A allele of rs165599 COMT polymorphism was transmitted preferentially to the affected individuals, and was significantly associated with later age of onset, greater severity of the delusion/hallucination symptom dimension and poorer performance of neurocognitive functioning[11]. Studying tardive dyskinesia, in a sample of schizophrenia/schizoaffective disorder patients, Zai et al. found a significant association between the marker rs165599 in the 3' untranslated region of COMT and this clinical condition[12].

Thus, since schizophrenia is a mental disorder with different clinical phenotypes, and despite the negative findings from meta-analyses that investigated the association between rs165599 catechol-O-methyltransferase genetic polymorphism and schizophrenia, several studies have found associations of this polymorphism with different clinical features of the disorder.

Taking this into account, we conducted a study with the aims of investigating a possible association between A/G COMT polymorphism in the non-coding region 3' (rs165599) and schizophrenia, and also investigating different aspects of the disorder, such as schizophrenia subtypes and gender. We also examined whether there was any association between homozygosity of the polymorphism investigated and schizophrenia.

METHODS

Sample

The sample was composed of 245 Brazilian patients diagnosed with schizophrenia and 834 sex and age-matched control subjects recruited at the Institute of Psychiatry, University of São Paulo Medical School. Schizophrenia was diagnosed in accordance with the DSM-IV criteria, based on clinical interviews conducted by psychiatrists. The 834 healthy control subjects were selected at the Blood Donation Unit at the University of São Paulo Medical School.

All the patients and control subjects provided written informed consent for taking blood samples. Approval for the study was obtained from the local Ethics Committee.

DNA extraction

A blood sample of 20 ml was drawn from each participant in the study, and DNA was extracted from leukocytes using the "salting out" protocol[13].

Genotyping

Genotyping was performed under contract by Prevention Genetics (USA) (www.preventiongenetics.com). The polymorphism investigated was genotyped blind to the individuals' clinical status.

Statistical analysis

The statistical power of the sample was evaluated using the CaTS software (Center for Statistical Genetics, University of Michigan; http://www.sph.umich.edu/csg/abecasis/CaTS/index.html). A test for deviations from Hardy-Weinberg equilibrium was performed using the HWE program[14].

The chi-square test was used to investigate possible associations shown by genotypes and alleles with schizophrenia and to assess differences between gender and schizophrenia subtype distributions in both patient and control groups. The analyses were performed using Epi-Info version 6.0.

For all statistical tests, the significance level was set at $\alpha<0.05$ or 5%.

RESULTS

The sample size power was calculated to be 80% under the following conditions: 200 patients, 400 control subjects, disorder prevalence of 1%, average allelic frequency of 30%, significance level of 0.05 and increased susceptibility towards developing the disorder, of 1.5 for the allele (OR=1.5). If the numbers of patients and control subjects became 245 and 829 respectively, i.e. the number of individuals in the present study, the sample size power would rise to 91%.

For Hardy-Weinberg equilibrium analysis, the actual genotype frequencies were compared with Hardy-Weinberg-based expected genotype frequencies. In both groups (cases: p=0.94; controls: p=0.43), the genotype frequencies were in Hardy-Weinberg equilibrium.

According to our analysis, no statistically significant differences in the distribution of allelic and genotypic frequencies between the groups of patients and controls were observed (Table 1). Taking into consideration the data suggesting that schizophrenia is a heterogeneous disorder, we also repeated our analyses with division of our sample according to the current most important schizophrenia subtypes (paranoid and disorganized) (Tables 2 and 3). Furthermore, the patients and controls were also investigated according to gender, since some studies have suggested that there are differences in the clinical manifestation of schizophrenia according to the patients' gender (Table 4). Likewise, the samples were also studied in order to ascertain whether homozygosity of the polymorphism investigated could be associated with schizophrenia in our sample (Table 5). However, we did not find any significant associations when the analysis was conducted using the phenotypic variables described above.

Table 1. Distribution of the genotypes and alleles of rs165599 genetic polymorphism of catechol-O-methyltransferase between patients and controls.

Polymorphism	Cases (%)	Controls (%)	χ^2	d.f.	p-value
GENOTYPE					
A/A	73 (29.79)	257 (31)			
A/G	122 (49.8)	400 (48.25)	0.22	2	0.89
G/G	50 (20.41)	172 (20.75)			
Total	245 (100)	829 (100)			
ALLELE					
A	268 (54.69)	914 (55.13)	0.02	1	0.88
G	222 (45.31)	744 (44.87)			
Total	490 (100)	1658 (100)			

d.f: degree of freedom

Table 2. Distribution of the genotypes and alleles of rs165599 genetic polymorphism of catechol-O-methyltransferase, according to schizophrenia subtype (paranoid subtype).

Polymorphism	Cases (%)	Controls (%)	χ^2	d.f.	p-value
GENOTYPE					
A/A	49 (28.99)	257 (31)			
A/G	84 (49.71)	399 (48.13)	0.27	2	0.87
G/G	36 (21.3)	173 (20.87)			
Total	169 (100)	829 (100)			
ALLELES					
A	182 (53.85)	913 (55.07)	0.17	1	0.68
G	156 (46.15)	745 (44.93)			
Total	338 (100)	1658 (100)			

d.f: degree of freedom

Table 3. Distribution of the genotypes and alleles of rs165599 genetic polymorphism of catechol-O-methyltransferase, according to schizophrenia subtype (disorganized subtype).

Polymorphism	Cases (%)	Controls (%)	χ^2	d.f.	p-value
GENOTYPES					
A/A	15 (28.3)	257 (31)			
A/G	27 (50.95)	399 (48.13)	0.2	2	0.9
G/G	11 (20.75)	173 (20.87)			
Total	53 (100)	829 (100)			
ALLELES					
A	57 (53.77)	913 (55.07)	0.07	1	0.79
G	49 (46.23)	745 (44.93)			
Total	106 (100)	1658 (100)			

d.f: degree of freedom

Table 4. Distribution of the genotypes and alleles of rs165599 genetic polymorphism of catechol-O-methyltransferase between patients and controls, according to gender.

Male					
Polymorphism	Cases (%)	Controls (%)	χ^2	d.f	p-value
GENOTYPE					
A/A	49 (30.82)	161 (31.14)			
A/G	75 (47.17)	245 (47.39)	0.03	2	0.98
G/G	35 (22.01)	111 (21.47)			
Total	159 (100)	517 (100)			
ALLELE					
A	173 (54.4)	567 (54.84)			
G	145 (45.6)	467 (45.16)	0.02	1	0.88
Total	318 (100)	1034 (100)			
Female					
Polymorphism	Cases (%)	Controls (%)	χ^2	d.f	p-value
GENOTYPE					
A/A	24 (27.91)	96 (30.77)			
A/G	47 (54.65)	155 (49.68)	0.66	2	0.71
G/G	15 (17.44)	61 (19.55)			
Total	86 (100)	312 (100)			
ALLELE					
A	95 (55,23)	347 (55.61)			
G	77 (44,77)	277 (44.39)	0.00	1	0.95
Total	172 (100)	624 (100)			

d.f: degree of freedom

Table 5. Distribution of the genotypes and alleles of rs165599 genetic polymorphism of catechol-O-methyltransferase for homozygosis.

Polymorphism	Cases (%)	Controls (%)	χ^2	d.f.	p-value
GENOTYPES					
AA/+G/G	123 (50.2)	430 (51.87)	0.21	1	0.64
A/G	122 (49.8)	399 (48.13)			
Total	245 (100)	829 (100)			

d.f: degree of freedom

DISCUSSION

In the present investigation, we tested the association between rs165599 A/G COMT genetic polymorphism and schizophrenia in a Brazilian sample of 245 patients (159 males and 86 females) and 829 healthy controls (565 males and 264 females). Despite previous investigations, our study is, to the best of our knowledge, the first association study analyzing this polymorphism in a Brazilian sample of patients with schizophrenia. However, we did not find any association between the polymorphism investigated and schizophrenia.

As mentioned above, we also conducted analyses dividing the sample according to the current most important schizophrenia subtypes: paranoid and disorganized. However, we did not find any significant association when the analysis was conducted using the phenotypic variables described above.

The patients and controls were also investigated according to gender, since some studies have suggested that there may be differences in age of onset and clinical prognosis according to the patients' gender[15]. No difference whatsoever was found.

We have tested whether there was any association between homozygosity and schizophrenia. There have been some reports showing associations between homozygosity of other genetic polymorphisms, like Ser9Gly of the dopamine receptor subtype 3 (DRD3), and schizophrenia[16]. This type of association may represent an advantage for heterozygosity, probably because the presence of two different molecular forms of the neurotransmitter receptor, thereby resulting in an increased ability to respond adaptively to variations in the environment, happening either during neural development or in adulthood. However, in our sample, we did not find any such association.

According to our analysis, no statistically significant differences in the distribution of allelic and genotypic frequencies between the groups of patients and controls were observed, even when we stratified the sample according to gender and schizophrenia subtype. Furthermore, no significant association was found when we investigated a possible association between homozygosity of the polymorphism investigated and schizophrenia.

One possible limitation of the present study may relate to population stratification, especially because the Brazilian population is not ethnically homogeneous, which may lead to lower power to detect associations. In populations with highly admixed ethnicity like in Brazil, problems regarding ethnic stratification may be faced. Since physical phenotype in Brazil is not an adequate predictor of genomic ancestry, ethnical matching in case-control studies is rendered difficult[17,18,19]. However, the present sample (patients and controls) was in Hardy-Weinberg equilibrium, which indicates that our sample may not have had any important problems regarding population stratification[20].

In conclusion, the results from the present study do not provide evidence for any association between A/G COMT polymorphism in the non-coding region 3' (165599) and schizophrenia, in the sample investigated. This finding is in accordance with a recent published meta-analysis. However, further studies on this particular polymorphism in relation to schizophrenia are still needed, especially with regard to investigating the different clinical features of the disorder. Investigations in samples with different ethnic backgrounds must be carried out as well.

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