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ARTICLE

Association study between the *Taq*1A (rs1800497) polymorphism and schizophrenia in a Brazilian sample

Estudo de associação entre o polimorfismo genético *Taq*1A (rs1800497) e esquizofrenia em uma amostra brasileira

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ABSTRACT

Schizophrenia is a severe psychotic disorder with recurrent relapse and functional impairment. It results from a poorly understood gene-environment interaction. The *Taq*1A polymorphism (located in the gene cluster NTAD) is a likely candidate for schizophrenia. Its rs1800497 polymorphism was shown to be associated with DRD2 gene expression. Therefore the present work aims to investigate a possible association between schizophrenia and such polymorphism. The compared distribution of the alleles and genotypes of the studied polymorphism was investigated in a Brazilian sample of 235 patients and 834 controls. Genotypic frequencies were in Hardy-Weinberg equilibrium. There was a trend of allelic association between the *Taq*1A polymorphism (rs1800497) with schizophrenia in the studied sample. However no statistically differences were found between cases and controls when analyzed by gender or schizophrenia subtypes.

Keywords: association study, dopamine, genetics, psychosis.

RESUMO

A esquizofrenia é um grave transtorno psicótico que apresenta frequentes recaídas e incapacitação progressiva. Resulta de uma interação gene-ambiente ainda pouco compreendida. O polimorfismo *Taq*1A (localizado no grupamento genético NTAD) é considerado um possível candidato para esquizofrenia. O polimorfismo genético rs1800497 foi associado com alteração da expressão do gene do DRD2. Assim, o presente trabalho objetivou investigar a possível associação de tal polimorfismo com esquizofrenia. A distribuição de seus alelos e genótipos foi investigada em uma amostra brasileira composta de 235 pacientes e 834 controles. As frequências genotípicas estavam em equilíbrio de Hardy-Weinberg. Houve uma tendência de associação alélica entre o polimorfismo *Taq*1A (rs1800497) e esquizofrenia na amostra estudada. No entanto, não houve diferenças estatisticamente significantes entre os grupos de casos e controles, quando analisados por gênero e subtipos da esquizofrenia.

Palavras-chave: estudo de associação, dopamina, genética, psicose.

Schizophrenia is a severe psychiatric disorder characterized by psychotic symptoms, alterations of thought, affect, volition, behavior, with recurrent relapses and continuing disability. The risk factors for schizophrenia are epiphenomena of pathophysiological processes resulting from gene-environment interactions. Genetic epidemiological investigations have suggested that there is an important participation of a genetic component on the etiology of schizophrenia and heritability estimates as high as 80% have been reported[1]. As the role of a single relevant gene must be small, association studies, involving case-control approaches, have been performed to evaluate the allelic variations at specific candidate genes which may be related to the etiopathology of the disorder[2].

Some of the most investigated genes in studies of vulnerability to schizophrenia are those that code for proteins of the dopaminergic system because the evidences of the role of central dopamine pathways in the pathophysiology of the disorder[3,4,5,6,7]. Central nervous stimulant drugs, that block reuptake of dopamine or facilitate its release on neuronal synapses, may cause psychotic symptoms[8]. L-DOPA has also been related to psychotic symptoms, releasing dopamine into the synapses[8]. On the other hand, some antipsychotic drugs correlate their efficacy with their action at dopaminergic

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receptors, especially blocking the subtype receptor D2 (DRD2)[9]. The DRD2 is a seven transmembrane G protein linked receptor that binds dopamine and inhibits adenylate cyclase, acting as an autoreceptor on dopaminergic cell bodies and as a postsynaptic receptor on dopaminergic targets[9]. There has been postulated that DRD2-binding density is increased in the brains of patients with schizophrenia[10]. Therefore the DRD2 seems to play an important role in the expression of schizophrenia and consequently its gene has been considered a promising candidate risk gene for the disorder. DRD2 gene is localized to chromosome 11q22-q23[11], a region of the genome that was reported as highly suggestive of linkage to schizophrenia in a meta-analyses study[12]. Thus, there are converging biological, clinical, and genetic evidences implicating DRD2 as a viable candidate gene for genetic susceptibility for schizophrenia, especially its neighboring single-nucleotide polymorphism (SNP) rs1800497 (*Taq*1A). The rs1800497 SNP was considered a silent mutation located 10 kb from DRD2 gene, in the 3' untranslated region. However recently the identification of a novel gene in the neighboring forward-strand region of DRD2 gene, named ANKK1 gene, showed that the rs1800497 SNP is located in exon 8 of the ANKK1 gene[13]. This polymorphism actually causes an amino acid change (Glu713Lys) in its 11th ankyrin repeat which has been suggested to alter substrate-binding specificity[14]. Although the rs1800497 polymorphism is localized in ANKK1 gene, it seems to be in linkage disequilibrium with several DRD2 genetic variants and, therefore, may be important for the understanding of the dopaminergic role in the etiopathogenesis of schizophrenia[14].

Taking that into account, we have performed an association study in order to investigate a possible association between the *Taq*1A polymorphism (rs1800497) with schizophrenia, also investigating different aspects of the disorder, such as schizophrenia subtypes and gender. We have also studied if there was an association between homozygosity of the investigated polymorphism and schizophrenia.

METHOD

Sample

The sample consisted of 235 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 according to DSM-IV criteria, based on clinical interviews conducted by psychiatrists. The 834 healthy control subjects were selected at the Blood Donation Unity at the University of São Paulo Medical School.

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

Dna extraction

A blood sample of 20 ml was drawn from each participant of the study, and DNA was extracted from leukocytes using the "salting out" protocol[15].

Genotyping

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

Statistical analysis

The statistical power of the sample was evaluated using the CaTS software (Center for Statistical Genetics – The 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[16].

Chi-square test was used to investigate possible association between genotypes and alleles with schizophrenia and to assess difference between gender and schizophrenia subtypes distributions in both patient and control groups. The analyses were performed by EpiInfo version 6.0.

For all statistic tests the adopted significance level was $\alpha<0.05$ or 5%.

RESULTS

The sample size power should 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 to develop the disorder of 1.5 for the allele (OR=1.5). If the number of patients and control subjects is changed for 235 and 834 respectively – the number of individuals in the present study – the sample size power rises up to 91%.

For Hardy-Weinberg equilibrium analysis, the actual genotype frequencies were compared to Hardy-Weinberg-based expected genotype frequencies. In both groups (cases: p=0.62; controls: p=0.79), the genotype frequencies were in Hardy-Weinberg equilibrium.

There was trend of allelic association between the *Taq*1A polymorphism (rs1800497) with schizophrenia in the studied sample (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). 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). The samples were also investigated in order to verify whether homozygosity of the

Table 1. Distribution of the genotypes and alleles of the *Taq*1A polymorphism between patients and controls.

Polymorphism	Cases (%)	Controls (%)	χ^2	d.f.	p
Genotype			3.59	2	0.16
A1/A1	29 (12.35)	138 (16.55)			
A1/A2	112 (47.65)	407 (48.8)			
A2/A2	94 (40)	289 (34.65)			
Total	235 (100)	834 (100)			
Allele			3.49	1	0.06
A1	170 (36.17)	683 (40.95)			
A2	300 (63.82)	985 (59.05)			
Total	470 (100)	1,668 (100)			

Table 2. Distribution of the genotypes and alleles of the *Taq*1A polymorphism between patients and controls, according to schizophrenia subtype (paranoid).

Polymorphism	Cases (%)	Controls (%)	χ^2	d.f.	p
Genotype			2.04	2	0.36
A1/A1	21 (12.96)	138 (16.55)			
A1/A2	77 (47.53)	407 (48.8)			
A2/A2	64 (39.51)	289 (34.65)			
Total	162 (100)	834 (100)			
Allele			2.01	1	0.15
A1	119 (36.73)	683 (40.95)			
A2	205 (63.27)	985 (59.05)			
Total	324 (100)	1,668 (100)			

polymorphism investigated could be associated with schizophrenia in the studied sample (Table 5). However, there were no significant associations when the analysis was conducted using the phenotypic variables described above.

DISCUSSION

As far as we know, this is the first association study analyzing *Taq*1A polymorphism (rs1800497) in a Brazilian sample of patients with schizophrenia. The patients and controls were also investigated according to differences in clinical aspects of the disorder, such as schizophrenia subtypes and gender. The analysis found a trend of allelic (A2 allele) association between the *Taq*1A polymorphism (rs1800497) with schizophrenia in the studied sample. However no statistically differences were found between cases and controls when analyzed by gender or schizophrenia subtypes.

Table 3. Distribution of the genotypes and alleles of the *Taq*1A polymorphism between patients and controls, according to schizophrenia subtype (disorganized subtype).

Polymorphism	Cases (%)	Controls (%)	χ^2	d.f.	p
Genotype			0.23	2	0.89
A1/A1	7 (14)	138 (16.55)			
A1/A2	25 (50)	407 (48.8)			
A2/A2	18 (36)	289 (34.65)			
Total	50 (100)	834 (100)			
Allele			0.15	1	0.7
A1	39 (39)	683 (40.95)			
A2	61 (61)	985 (59.05)			
Total	100 (100)	1,668 (100)			

Table 4. Distribution of the genotypes and alleles of the *Taq*1A polymorphism between patients and controls, according to gender.

Polymorphism	Cases (%)	Controls (%)	χ^2	d.f.	p
Male					
Genotype			2.3	2	0.32
A1/A1	19 (12.42)	86 (16.54)			
A1/A2	73 (47.71)	254 (48.85)			
A2/A2	61 (39.87)	180 (36.16)			
Total	153 (100)	520 (100)			
Allele			2.17	1	0.14
A1	111 (36.27)	426 (40.96)			
A2	195 (63.73)	614 (59.04)			
Total	306 (100)	1,040 (100)			
Female					
Genotype			1.36	2	0.5
A1/A1	10 (12.2)	52 (16.56)			
A1/A2	39 (47.56)	153 (48.73)			
A2/A2	33 (40.24)	109 (34.71)			
Total	82 (100)	314 (100)			
Allele			1.33	1	0.24
A1	59 (35.98)	257 (40.92)			
A2	105 (64.02)	371 (59.08)			
Total	164 (100)	628 (100)			

Table 5. Distribution of the genotypes and alleles of the *Taq*1A polymorphism genetic polymorphism for homozygosis.

Polymorphism	Cases (%)	Controls (%)	χ^2	d.f.	p
Genotype			0.1	1	0.75
A1/A1+A2/A2	123 (52.34)	427 (51.2)			
A1/A2	112 (47.66)	407 (48.8)			
Total	235 (100)	834 (100)			

The *Taq*1A SNP (rs1800497) is the most studied genetic variation in different psychiatric disorders and personality traits. The *Taq*1A A1 allele is mainly associated with addictions, antisocial disorders, eating disorders, and attention-deficit/hyperactivity disorders, while the A2 allele occurs more frequently in schizophrenic and obsessive-compulsive patients[17].

Evidences coming from neuroimaging studies have supported the *Taq*1A SNP (rs1800497) as a functional polymorphism, influencing DRD2 gene expression. A positron emission tomography (PET) study revealed significant inter-individual variation in dopamine D2 receptor density in human striatum[18]. Thus association between the A1 allele and low D2 receptor availability in healthy subjects indicates that the A1 allele of the *Taq*1A polymorphism might be in linkage disequilibrium with a mutation in the promoter/regulatory gene element that affects dopamine D2 receptor

expression[18]. Another study that searched for relationships between DRD2 gene polymorphisms and striatal dopamine D2 receptor density in vivo, as measured by PET in 56 healthy subjects, evidenced that the presence of the DRD2 *Taq*1A A1 allele was associated with measures of low striatum dopamine receptor density in healthy volunteers[19].

However despite the evidences that point the association between *Taq*1A SNP (rs1800497) with dopamine receptor D2 binding/expressing density, it is important to note that the *Taq*1A cut site is located within exon 8 of the adjacent gene: ankyrin repeat and protein kinase domain-containing protein 1 (ANKK1) on chromosome 11. Thus, it could be difficult to disentangle the direct contribution of DRD2-*Taq*1A or nearby genes such as TTC12 gene (tetratricopeptide repeat protein 12) and ANKK1 gene[20]. Another important question is that these different SNPs influence quantity of DRD2 mRNA, different isoforms, as well as presynaptic relative to postsynaptic DRD2 in the brain and can influence biological susceptibility for schizophrenia[20].

All these genes are located on chromosome 11 (11q22-23 region) and actually form a 521 kb gene cluster that comprises the NCAM1, TTC12, ANKK1 and DRD2 genes, known as the NTAD gene cluster[14]. The genes that form the NTAD (NCAM1-TTC12-ANKK1-DRD2) cluster act on the central nervous system. The neural cell adhesion molecule 1 (NCAM1) has an important role in neurogenesis, TTC12 encodes the tetratricopeptide repeat domain 12 protein, which is involved in dopaminergic transmission and neurodevelopment, the ankyrin repeat and kinase domain containing 1 (ANKK1) gene encodes a signaling protein which participates in the modulation of the expression of DRD2, constituting the currently evidence of co-regulation in the NTAD cluster[14]. One hypothesis to explain such genetic cluster is that neurogenesis and dopaminergic neurotransmission may be related in the course of the evolution of the complex vertebrate neural system via a common functional genomic architecture, comprising the NTAD cluster as a candidate functional unit, rather than its genes separately[14]. In the case of schizophrenia NTAD gene cluster is particularly interesting because schizophrenia is a mental disorder caused by problems in neurodevelopment that causes dopaminergic neurotransmission imbalance[1].

A possible limitation of the present study may be related to population stratification, especially because the Brazilian population is not ethnically homogeneous, so the power to detect association may be reduced. In populations of highly admixed ethnicity like the Brazilian one, we may face problems about ethnical stratification[21]. Since physical phenotype in Brazil is not an adequate predictor of genomic ancestry, ethnical matching in case-controls studies is rendered difficult[22,23]. The present sample (patients and controls), however, is in Hardy-Weinberg equilibrium, which indicates that our sample may not have important problems regarding population stratification[24].

In conclusion, the results of the present study evidenced a trend of allelic association between the *Taq*1A polymorphism (rs1800497) with schizophrenia in the Brazilian studied sample, showing that patients presented a higher prevalence of A2 allele compared to controls. This finding is in accordance with recent evidences that have shown A2 allele occurring more frequently in patients with schizophrenia[17]. So further studies of this particular polymorphism, that is part of a gene cluster (NTAD) located on chromosome 11, with schizophrenic patients are still needed.

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