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LACK OF ASSOCIATION BETWEEN VNTR POLYMORPHISM OF DOPAMINE TRANSPORTER GENE (*SLC6A3*) AND SCHIZOPHRENIA IN A BRAZILIAN SAMPLE

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ABSTRACT - A role of dopaminergic dysfunction has been postulated in the aetiology of schizophrenia. We hypothesized that variations in the dopamine transporter gene (*SLC6A3*) may be associated with schizophrenia. We conducted case-control and family based analysis on the polymorphic *SLC6A3* variable number tandem repeat (VNTR) in a sample of 220 schizophrenic patients, 226 gender and ethnic matched controls, and 49 additional case-parent trios. No differences were found in allelic or genotypic distributions between cases and controls and no significant transmission distortions from heterozygous parents to schizophrenic offspring were detected. Thus, our results do not support an association of the *SLC6A3* VNTR with schizophrenia in our sample.

KEY WORDS: *DAT1*, *SLC6A3*, schizophrenia, genetic polymorphism, genetic association, trios.

Ausência de associação entre o polimorfismo VNTR do gene do transportador de dopamina (*SLC6A3*) e esquizofrenia em uma população brasileira

RESUMO - Genes do sistema dopaminérgico são de escolha para a pesquisa de susceptibilidade para a esquizofrenia. Desse modo, possível contribuição do polimorfismo do gene do transportador de dopamina *(SLC6A3)* no aumento da vulnerabilidade para a esquizofrenia foi investigada no presente estudo. Analisou-se a distribuição do sítio polimórfico do gene do transportador de dopamina (VNTR) em uma população de 220 pacientes com esquizofrenia (critério diagnóstico: DSM-IV) e comparou-se com a distribuição em uma população controle de 226 indivíduos pareados para sexo e etnia. Nenhuma diferença foi observada na distribuição dos alelos entre casos e controles. O mesmo polimorfismo também foi investigado em uma segunda amostra composta por 49 trios (pais e probando). O resultado também foi negativo. Tais dados não dão suporte para a participação do polimorfismo do gene do transportador de dopamina no aumento de susceptibilidade para esquizofrenia na amostra estudada.

PALAVRAS-CHAVE: *DAT1, SLC6A3*, esquizofrenia, polimorfismo genético, associação genética, trios.

Schizophrenia (SCZ) affects some 1% of the general population. Epidemiological studies have indicated a strong genetic component in the pathogenesis of SCZ and heritability estimates as high as 80% have been reported[1]. Pharmacological evidence suggests an involvement of the dopaminergic system as many antipsychotic drugs block dopamine receptors in the brain and are highly effective in treating symptoms of SCZ[2]. Further, amphetamines and cocaine tend to provoke or exarcebate psychotic symptoms in susceptible individuals by preventing dopamine re-uptake[3]. L-DOPA has also been implicated in psychotic symptoms through variable release of dopamine into the synapse[4]. Therefore, genes involved in the dopaminergic system are potential targets for genetic association studies with SCZ[5].

Polymorphisms in dopamine receptors have been widely examined, but the results have been inconclusive[6-12]. Another possible candidate is the dopamine transporter gene (*SLC6A3 or DAT1*). The dopamine transporter (*SLC6A3*) plays an important role in the regulation of dopamine levels and neurotransmission by mediating the active re-uptake of synaptic dopamine back into the neurons[13]. Two post-mortem studies on *SLC6A3* binding and SCZ showed decreased striatal *SLC6A3* density in chronic SCZ[14,15]. A recent study using positron emission tomography found lower *SLC6A3* density in sites

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in the basal ganglia, particularly in the middle third of putamen, in chronic SCZ patients than controls. This may suggest a decreased expression of *SLC6A3* in a subset of chronic SCZ patients[16].

The *SLC6A3* has been cloned and mapped to human chromosome 5 (5p15.3)[17]. A 40-bp variable number tandem repeat polymorphism (VNTR) has been reported in the 3'-untranslated region (3'-UTR) of *SLC6A3*, ranging from 3 to 11 copies of the repeated sequence in a non-coding region[15]. Linkage studies in SCZ pedigrees from Utah[18,19], Italy[20], Rouen, France, the Island of La Reunion[21], Germany[22], and India[23] have all failed to demonstrate positive linkage of this VNTR to SCZ. Most of the past association studies have also reported no significant evidence for association between this *SLC6A3* polymorphism and SCZ[22-30]. However, Persico and Macciardi (1997) showed that the *SLC6A3* genotypes in SCZ patients displayed significantly enhanced homozygote (genotypes 9/9 and 10/10) and reduced heterozygote (genotype 9/10) frequencies of the most common genotypes when contrasted with controls[31]. An association between the10 allele and 10/10 genotype of *SLC6A3* and schizoid/avoidant personality disorder has been reported in a sample of patients with distinct diagnoses, not specifically with SCZ[32].

Thus, we report here the results of case-control and family based analyses of the *SLC6A3* VNTR polymorphism in an ethnically diverse SCZ Brazilian sample.

METHOD

Sample

All controls, parents and schizophrenic patients provided written informed consent. The ethical approval for the study was obtained from the Ethics Committee at the Hospital das Clínicas, University of São Paulo Medical School (CAPPesq).

Case-Control Study – A) Patients sample: 220 patients were recruited from inpatient and outpatient services at the Institute of Psychiatry of the Hospital das Clínicas, University of São Paulo Medical School and diagnosed according to DSM-IV[33] criteria for SCZ. B) Controls sample: 226 sex and ethnic matched healthy controls were recruited from the Blood Donation Service at the Hospital das Clínicas, University of São Paulo Medical School.

Parents-Offspring Trios – A separate sample of SCZ patients (n = 49) with both parents available for study comprised the case-parent trios. These families were recruited from inpatient and outpatient services at the Institute of Psychiatry of the Hospital das Clínicas, University of São Paulo Medical School and diagnosed according to DSM-IV[33] criteria for SCZ.

Genotyping

Genomic DNA was obtained from venous blood from each subject using the phenol chloroform method. Standard polymerase chain reaction (PCR) was performed in a reaction volume of 12 *M*l which included 20 ng genomic DNA, STS reaction buffer 10x concentrated (160 mM $(NH_4)_2SO_4$, 500 mM Tris-HCl, pH 9.2, 0.1 mM EDTA, 20 $ DMSO, 1% Tween 20), 5 pmol of each primer, 200 mM d NTP's, 1 unit of TaqDNA polymerase (STS), 1.5 mM $MgCl_2$. Primer sequences were: 5'TGTGGTGTAGGGA CGGCCTGAG-3' (forward) and 5'CTTCCTGGAGGTCACG-GCTCAAGC-3' (reverse). Amplification consisted of 94°C for 3 min, touchdown PCR conditions using primer annealing temperatures of 66°C and 64°C at two cycles each, followed by 30 cycles of 94°C for 30 s, 62°C for 45 s and 72°C for 45 s and a final extension step at 72°C for 10 min. The fragment sizes were: 280 bp (five repeats), 320 bp (six repeats), 360 bp (seven repeats), 400 bp (eight repeats), 440 bp (nine repeats), 480 bp (10 repeats), 520 bp (11 repeats), 600 bp (13 repeats). The amplification products were resolved on 1% agarose/1.5% Metaphore gels and visualized by ethidium bromide transillumination. Alleles ranging from 3 to 11 repeats were observed.

Statistical analysis

In the case-control design, the allelic and genotypic distribution in SCZ patients and health controls were contrasted using the CLUMP Program[34]. For family based analysis of case-parent trios, the preferential transmission of alleles from parents to affected offspring was analysed by the Extended Transmission Disequilibrium Test (ETDT), which performs the test for markers with multiple alleles[35]. The *p* values were corrected for multiple testing using Monte Carlo ETDT (MCETDT)[36]. Hardy- Weinberg equilibrium was calculated using the STATA Program[37].

RESULTS

There were no significant deviations from the Hardy-Weinberg equilibrium in any of the populations for the polymorphism studied. Case-control analysis provided no evidence for allelic or genotypic association of *SLC6A3* VNTR polymorphism and SCZ (Table 1). Family based analyses revealed no significant preferential transmission for any of the alleles (allele-wise TDT, χ^2=5.36; 4df; p=0.25) or genotypes (genotype-wise TDT, χ^2=5.39; 4df; p=0.24). When correcting our results for multiple testing we used MCETDT program and obtained values of p=0.43 for both allelic and genotypic transmission (Table 2).

DISCUSSION

Past findings of post-mortem and neuroimaging studies have suggested that *SLC6A3* may play a role in the pathophysiology of SCZ[14,15,16]. However, most genetic studies investigating differences in SCZ cas-

Table 1. Distributions of SLC6A3 VNTR common alleles and genotypes in SCZ patients and controls.

	SCZ	Controls	χ^2	*p* value
ALLELES				
9	109 (24.77)	111 (24.55)		
10	314 (71.36)	328 (72.56)	3.9	0.45
11	8 (1.81)	6 (1.32)		
Other	9 (2.04)	7 (1.54)		
Total	440 (100)	452 (100)		
GENOTYPES				
10/10	112 (50.90)	124 (54.86)		
10/9	78 (35.45)	70 (30.97)	5.1	0.68
9/9	15 (6.81)	19 (8.40)		
11/10	5 (2.27)	4 (1.76)		
Other	10 (4.54)	9 (3.98)		
Total	220 (100)	226 (100)		

Table 2. Alleles of SLC6A3 VNTR polymorphism transmission from parents to offsprings.

Alleles	Transmitted	Untransmitted	χ^2	*p* value
3	1	1		
8	0	1	5.3	0.43
9	22	30		
10	32	25		
11	2	0		

es and controls of this VNTR polymorphism at *SLC6A3* have failed to find an association with the disorder[18-30]. However, one previous study has reported that the *SLC6A3* genotypes in SCZ patients displayed significantly enhanced homozygote (genotypes 9/9 and 10/10) and reduced heterozygote (genotype 9/10) frequencies of the most common genotypes, maybe representing stigmata of assortative mating[31].

Thus, we conducted both case-control and family based analyses of the VNTR polymorphism in a large sample of SCZ cases as well as a smaller sample of case-parent trios. We compared the frequencies of alleles and genotypes between 220 cases and 226 controls and did not find significant differences. Our family based analysis also failed to detect linkage or association of this polymorphism with SCZ.

Distinct racial and ethnic groups display significant differences in *SLC6A3* marker distributions[38]. Thus, case-control association studies can potentially be underpowered to detect association in a sample of this size, particularly in the presence of ethnic admixture. This potential confound may be critical in populations of high ethnic admixture such as a Brazilian population[8,39]. Therefore, we subsequently performed family based analyses of transmission distortions from heterozygous parents to SCZ offspring. This type of analysis avoids stratification biases[40]. Thus, our family based analysis may provide more conclusive evidence of a lack of association at this locus with SCZ.

This finding is in accordance with most of the studies conducted to date on this polymorphism in the dopamine transporter gene. However, the *SLC6A3* VNTR polymorphism may be involved in the susceptibility for other psychotic disorders, such as bipolar disorder[41-43]. Further, cocaine acts on *SLC6A3*, enhancing the dopaminergic transmission and making this gene a strong candidate in cocaine-induced paranoia[44]. Future analysis on larger populations are also required to determine if other variations in the dopamine transporter provide evidence for association to SCZ.

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