Arq Neuropsiquiatr 2005;63(2-B):380-382

LACK OF ASSOCIATION BETWEEN THE G681C POLYMORPHISM IN THE 5-HT1Dβ AUTORECEPTOR GENE AND SCHIZOPHRENIA

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ABSTRACT - A major role of the serotonergic system has been hypothesized in the pathogenesis of schizophrenia, mostly based on the evidence of action of atypical antipsychotics. Disturbances of serotonergic pathways have been implicated in the etiology of schizophrenia. The aim of this study was to investigate the association between schizophrenia and the G861C polymorphism in the 5-HT1Dβ autoreceptor gene. There was conducted a case-control analysis in a sample of 196 schizophrenic patients and 143 gender, age and ethnic matched controls. No statistically differences were found in allelic or genotypic distributions between cases and controls. Thus, the results do not support an association of the G861C polymorphism in the 5-HT1Dβ autoreceptor gene with schizophrenia in the studied sample.

KEY WORDS: schizophrenia, 5-HT1Dβ, G861C polymorphism, genetic association

Ausência de associação entre o polimorfismo G861C do gene do auto-receptor 5-HT1Dβ e esquizofrenia

RESUMO - O sistema serotonérgico tem sido implicado na fisiopatologia da esquizofrenia, principalmente por conta dos mecanismos de ação dos antipsicóticos atípicos. Alterações nas vias cerebrais serotonérgicas têm sido relacionadas com a etiologia desse transtorno psiquiátrico. Assim, o objetivo do presente estudo foi investigar uma possível associação entre esquizofrenia e o polimorfismo G861C no gene do auto-receptor serotonérgico 5-HT1Dβ. Para tanto, conduziu-se estudo do tipo caso-controle em amostra de 196 pacientes com esquizofrenia e 143 indivíduos pareados para sexo, idade e etnia. Não se evidenciaram diferenças estatísticas nas distribuições alélicas e genotípicas entre as populações de pacientes e controles. Desse modo, os resultados dessa investigação não correlacionaram o polimorfismo G861C no gene do auto-receptor serotonérgico 5-HT1Dβ como fator de susceptibilidade genética para o desenvolvimento de esquizofrenia na amostra estudada.

PALAVRAS-CHAVE: esquizofrenia, 5-HT1Dβ, polimorfismo G861C, associação genética.

Schizophrenia (SCZ) is a psychiatric disorder that affects some 1% of the general population, being associated with disturbances of thought, affect, sensoperception and behavior. Data from twin, adoption and family studies have demonstrated that genetics plays an important role in the etiology of this disorder, and heritability as high as 80% has been reported[1]. The most common mode of inheritance of SCZ involves multiple susceptibility loci[2]. Some evidences suggest that serotonergic dysfunctions may be related to the susceptibility for SCZ. The serotonin hypothesis emerged following observations of psychotic features related to LSD, a serotonergic drug. Later, reports showing that serotonergic-depleting drugs, such as reserpine could alleviate some symptoms of SCZ helped to build the theory that an increase in serotonin may be related to the disorder[3]. More evidences of the interest of serotonin in SCZ come from the finding of Joyce et al.[4] that this neurotransmitter uptake sites and receptors are altered in the limbic system of patients with the disorder, and the observation that the new generation antipsychotics have potent serotonin-related activities[5].

Serotonin is involved in a variety of sensory, motor and cortical functions, being particularly relevant in modulating the effects of dopamine, the first neurotransmitter supposed to be implicated in SCZ. Serotonin receptors are classified into seven main classes which can be further divided into different subclasses[6]. Among many serotonin receptors subtypes, 5-HT1D receptors are important for SCZ because their major function is to control serotonin release from serotonergic neuron terminals

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Received 13 September 2004, received in final form 29 November 2004. Accepted 26 January 2005.

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(autoreceptors) in the brain[6]. Cloning studies found two 5-HT1D receptors subtypes, termed 5-HT1Dα and 5-HT1Dβ[7-9]. The gene for 5-HT1D receptor has been localized to chromosome 6p13 and it is widely expressed in the human brain[10], with the highest levels of receptor expression observed in limbic regions and basal ganglia[9]. Considering the important role of 5-HT1Dβ receptor in the control of serotonin release and its location of preferential expression[6], mutations occurring in this serotonin autoreceptor gene may contribute to the susceptibility for SCZ by causing altered function of serotonergic neurons. Some studies have reported genetic linkage between SCZ and regions on chromosome 6[11-13]. These linkage studies have been performed to localize major effect susceptibility genes for SCZ, although, since its genetic etiology may be multifactorial and polygenic, association study design is more appropriated. Any single susceptibility gene contributes only a small fraction to the overall risk for SCZ and can be directly evaluated as susceptibility factors using candidate gene association studies[14,15].

Serotonergic systems have been implicated in the pathophysiology of SCZ and the genetic association study is one of the most important approaches to detect genes for susceptibility to a complex disorder, such as SCZ. Thus in the present study, the silent polymorphism G681C, which is located in the coding region of 5-HT1Dβ autoreceptor gene, will be investigated as a possible risk factor for SCZ.

METHOD

Sample – All controls and SCZ 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).

A) Patients sample: 196 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[16] criteria for SCZ.

B) Controls sample: 143 sex, age and ethnic matched healthy controls were recruited from the Blood Donation Service at the Hospital das Clínicas, University of São Paulo Medical School.

Genotyping – We collected ten milli-liters of venous blood from each subject and genomic DNA was extracted using the phenol chloroformmethod. The G861C genotypes (GG, GC, CC) were determined by the polymerase chain reaction method[17]. Determination of genotypes was performed by raters blind to the clinical status of the individuals.

Table Distributions of the G861C alleles and genotypes frequencies in SCZ patients and controls samples

	SCZ	Controls	χ^2	p value
Alleles				
G	270 (68.87)	212 (74.12)	2.22	0.13
C	122 (31.12)	74 (25.87)		
Total	392 (100)	286 (100)		
Genotypes				
G/G	96 (48.97)	77 (53.84)		
G/C	78 (39.79)	58 (40.55)	3.36	0.18
C/C	22 (11.22)	8 (5.59)		
Total	196 (100)	143 (100)		

Statistical analysis – For comparisons between cases and controls regarding frequencies of alleles and genotypes, we utilized the chi-square test. Hardy-Weinberg equilibrium was calculated using the STATA Program[18].

RESULTS

There were no significant deviations from the Hardy-Weinberg equilibrium in any of the samples for the polymorphism studied (SCZ: p=0.31; controls: p=0.49). Case-control analysis provided no difference in allelic (p=0.13, χ^2=2.22, OR=0.77 0.54<OR<1.10) and genotypic (p=0.18, χ^2=3.36, 2d.f.) distributions between patients and controls samples (Table).

DISCUSSION

The results of the present study found no evidence for an association between the polymorphism and SCZ. These findings are in agreement with a linkage study in a Canadian population[19], a case-control association study in a Portuguese population[20], and a trio-based (father, mother and proband) study also in a Portuguese sample[21]. However, these results do not supportsome linkage studies that found evidence of linkage in regions of chromosome 6 and SCZ. Such phenomenon may reflect the possibility that there might be other mutations in this gene that play a role in SCZ, or that there are other genes related to the susceptibility for SCZ in chromosome 6.

A possible limitation of the present investigation is related to the population stratification. Association studies using case-control approach can generate false association because it is difficult to precisely match patients and controls for ethnicity because physical characteristics are not always related to genetic background, especially in Brazil where the population is so mixed[22]. So differences in allele frequencies between populations could generate false results. Even trying to match the populations for ethnicity,

as done in this work, this is an issue that has to be considered in case-control investigations.

In conclusion, the results of this work do not provide evidence of association between SCZ and alleles and genotypes of the G861C polymorphism in the 5-HT1Dβ autoreceptor gene with SCZ in the studied sample.

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