Lack of Association or Linkage Disequilibrium Between Schizophrenia and Polymorphisms in the 5-HT1Dα and 5-HT1Dβ Autoreceptor Genes: Family-Based Association Study

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Genetic factors play a major role in the etiology of schizophrenia and disturbances of serotonergic pathways have been implicated in this disorder. The aim of the present study was to examine genetic association between schizophrenia and polymorphisms in the 5-HT1Dα (TaqI) and 5-HT1Dβ (T261G and G861C) autoreceptor genes in ninety trios from Portugal. No association or linkage disequilibrium was obtained between schizophrenia and 5-HT1Dα and 5-HT1Dβ autoreceptor genes with both haplotype relative risk (HRR) and transmission disequilibrium test (TDT). Concerning 5-HT1Dβ autoreceptor gene, also negative results was obtained in the analysis of the haplotypes with transmit. Thus, our data provide no support for the hypothesis that polymorphisms at 5-HT1Dα (TaqI) and 5-HT1Dβ (T261G and G861C) genes contributes to susceptibility to schizophrenia in the Portuguese population.

KEY WORDS: candidate genes; serotonergic system; linkage disequilibrium; haplotypes; Introduction

INTRODUCTION

Schizophrenia is a major psychiatric disorder. Data from twin, adoption and family studies have clearly demonstrated that genetic factors play an important role in this disease [Riley and McGuffin, 2000]. The most common mode of transmission of schizophrenia probably involves multiple susceptibility loci [Baron, 2001; Prasad et al., 2002], although their number and contribution to develop the disease, and the degree of interaction between them remain unanswered.

Several lines of evidence suggest that a serotonergic dysfunction is involved in the susceptibility to schizophrenia [Kunzle and Stahl, 1985]. Serotonin (5-HT) is involved in a wide variety of sensory, motor, and cortical functions, being particularly relevant in modulating the effects of dopamine [Kapur and Remington, 1996]. The first neurotransmitter supposed to be implicated in schizophrenia [Meltzer, 1987; Bleich et al., 1988]. To date, 5-HT receptors are classified into seven main classes, 5-HT1-7, which can be further divided into different subclasses [see Barnes and Sharp, 1999]. The manner in which 5-HT exerts its regulatory influence on physiological and pathological functions of the central nervous system not only through multiple interactions with other systems, but also through a multiplicity of 5-HT receptor subtypes [see Barnes and Sharp, 1999]. Among many 5-HT receptors subtypes, for example, 5-HT1D receptors are important for schizophrenia because their major function is to control 5-HT release from serotonergic neuron terminals (autoreceptors) in the brain [Barnes and Sharp, 1999]. Cloning studies have revealed two 5-HT1D receptor subtypes, termed 5-HT1Dα and 5-HT1Dβ [Hamblin and Metcalf, 1991; Demchyshyn et al., 1992; Weinschank et al., 1992]. The genes for these receptors have been localized to chromosomes 1p36.3-p34.3 and 6q13, respectively [Libert et al., 1991; Jin et al., 1992], and are widely expressed in the human brain [Domenech et al., 1997; Gaster et al., 1998], with the highest levels of receptor expression observed in limbic regions and basal ganglia [Hamblin and Metcalf, 1991; Demchyshyn et al., 1992; Weinschank et al., 1992]. Considering the important role of 5-HT1Dα and 5-HT1Dβ receptors in the control of serotonin release [Barnes and Sharp, 1999], mutations occurring in the serotonin autoreceptor genes may contribute to the development of schizophrenia by causing altered function of serotonergic neurons. Therefore, polymorphisms such as the silent TaqI polymorphism in the coding region of 5-HT1Dα, the T261G polymorphism situated in the 5'-untranslated region and the G861C polymorphism situated in the coding region of 5-HT1Dβ gene may be implicated directly or indirectly through dopaminergic or other systems. In addition, genetic variation might account for interindividual differences in response to drugs acting via serotonergic pathways. In this way, alterations in genes coding for receptor proteins may affect their binding affinities for neuroleptics, the efficiency of signal transduction, or their levels of expression, which may in turn alter the drug’s therapeutic action.

Several studies have reported genetic linkage between schizophrenia and markers located on chromosomes 1 and 6 [Hovatta et al., 1999; Hwu et al., 2000; Baron, 2001; Blackwood et al., 2001; Gurling et al., 2001]. Also, support for linkage on
chromosome 1q21-q22 was obtained with a genome-wide scan for schizophrenia susceptibility loci in 22 affected Canadian families [Brzustowicz et al., 2000]. Although several linkage studies have been performed to localize major effect susceptibility genes for schizophrenia; association studies based on linkage disequilibrium are suitable, since the genetic etiology may be multifactorial or polygenic. Any single susceptibility gene contributes only a small fraction to the overall risk, and allelic variation at such genes must be high, and can be directly evaluated as susceptibility factors using candidate gene association studies [Lander, 1996; Risch and Merikangas, 1996; Collins et al., 1997]. Thus, the best approach to detect genes for susceptibility to schizophrenia is to study homogenous populations by using methods based on linkage disequilibrium, which is a potentially powerful alternative to linkage analysis for detection of minor effect genes possibly implicated in this disorder.

In the present study, we investigated polymorphisms of 5-HT1D and 5-HT1Dβ autoreceptor genes for susceptibility to schizophrenia in a Portuguese population which is considered highly homogeneous [Schindler et al., 1999].

**MATERIALS AND METHODS**

**Sample**

Ninety probands with diagnosis of schizophrenia (58 males and 32 females) and their parents of Portuguese-Caucasian origin were recruited from Azores and Mainland, Portugal. The ascertainment criteria for the study included only one proband with schizophrenia in each family. The age of onset ranged between 15 and 50 years. Local ethical committee approval and written consent from each subject were obtained. Ascertainment and diagnostic methods for the families have been described elsewhere [Pato et al., 2000; Xu et al., 2001]. The sample used in the present study has been partially included in the analyses of other candidate genes [Schindler et al., 2002]. All probands were administered the Diagnostic Interview for Genetics Studies (DIGS) (National Institute of Mental Health-Molecular Genetic Initiative, 1992) [Nurnberger et al., 1984], Portuguese version [Azevedo et al., 1993] by a clinician with an extensive training in this interview. Data from the DIGS for each subject was compared with medical records and information from close relatives. For each proband, the Operational Criteria (OPCRIT) [McGuffin et al., 1991] checklist was completed, and all were diagnosed according to DSM-IV.

**Laboratory Procedures**

Blood samples were collected with EDTA anticoagulant, and genomic DNA was obtained using the standard method [Miller et al., 1988], with slight modifications.

**5-HT1Dα Gene Polymorphism**

The silent polymorphism in the coding region of the 5-HT1Dα autoreceptor gene was determined by PCR, using the same conditions described by Ozaki et al. [1995]. Amplification was performed in a final volume of 25 μl, containing 150 ng genomic DNA as template, 200 μM dNTPs, 0.6 μM of each primer, 10 mM Tris-HCl (pH = 8.3), 1.5 mM MgCl₂, and 1 U of Taq polymerase (Gibco, BRL). PCR amplification was initiated at 95°C for 5 min and performed for 30 cycles each consisting of 95°C for 30 sec, 57°C for 30 sec, and 72°C for 40 sec. PCR products were digested using enzyme HincII (Biolabs). The digested fragments were separated in 3.0% agarose gel and were visualized by ethidium bromide staining. Allele frequencies for G861 and C861 were 0.799 and 0.201, respectively.

**5-HT1Dβ Gene Polymorphisms**

Two polymorphisms in 5-HT1Dβ gene were examined, one polymorphism situated in the 5′-untranslated region (T261G) and other polymorphism situated in the coding sequence region (G861C). The polymorphism situated in the 5′-translated region was carried out by using a PCR-based restriction analysis according to the method described by Nothen et al. [1994], with slight modifications. Standard PCR was carried out in a volume of 25 μl, containing 150 ng genomic DNA as template, 200 μM dNTPs, 10 pmol of each primer, 10 mM Tris-HCl (pH = 8.3), 1.5 mM MgCl₂, 0.01% gelatine and 1 U of Taq polymerase (Perkin Elmer Cetus, Toronto). After initial denaturation at 94°C for 5 min, 35 cycles of PCR reaction were performed under conditions of denaturation at 94°C, for 20 sec, annealing at 61°C, for 20 sec, and extension at 72°C, for 30 sec. Amplification products were digested with BsmAI restriction enzyme (Biolabs), and separated by electrophoresis in a 3.5% agarose gel, and visualized with ethidium bromide staining under ultraviolet light. Allele frequencies for G861 and C861 were 0.788 and 0.212, respectively.

The polymorphism situated in the coding sequence region was genotyped using a modified protocol of the Lappalainen et al. [1995]. Amplification was performed in a final volume of 25 μl, containing 150 ng genomic DNA as template, 200 μM dNTPs, 0.6 μM of each primer, 10 mM Tris-HCl (pH = 8.3), 1.5 mM MgCl₂, and 1 U of Taq polymerase (Gibco, BRL). PCR amplification was initiated at 95°C for 5 min and performed for 30 cycles each consisting of 95°C for 30 sec, 57°C for 30 sec, and 72°C for 40 sec. PCR products were digested using enzyme HincII (Biolabs). The digested fragments were separated in 3.0% agarose gel and were visualized by ethidium bromide staining. Allele frequencies for G861 and C861 were 0.799 and 0.201, respectively.

**Statistical Analysis**

The association studies were performed with ninety nuclear families with one affected (trios), and two strategies were used, the haplotype relative risk (HRR) [Terwilliger and Ott, 1992], and the transmission disequilibrium test (TDT) [Spielman et al., 1993; Spielman and Ewens, 1998], that are robust to population stratification. We used the HRR and TDT to test if the marker locus and the hypothetical disease locus were linked or in linkage disequilibrium. The TDT-SDTDT programe v 1.1 uses data from heterogeneous parents only, and tests for individual markers. Furthermore, multiple marker haplotype transmission was performed with program TRANSMIT v 2.5 [Clayton, 1999]. The program TRANSMIT tests for association between markers and disease, examining the transmission of multilocus haplotypes.

**RESULTS**

In this study, ninety nuclear families (90 Portuguese schizophrenic patients and their parents) were used. Association studies using case-control approach can generate false positives as a result of population stratification. Indeed, it is difficult to precisely match patients and controls for ethnicity, and differences in allele frequencies between populations could be generating false association. Thus, an elegant and powerful strategy for overcoming this problem is to use the parents of probands and we used the HRR and the TDT methods to test if the marker locus and the hypothetical disease locus were linked or in linkage disequilibrium. The results of HRR and TDT designs of the TaqI polymorphism of the HT1Dα gene in the schizophrenic trios are given in Table I. Using these strategies, we found no evidence for association or linkage disequilibrium between the 5-HT1Dα gene (HRR, $\chi^2 = 0.988$,}
df = 1, \( P = 0.320 \); TDT, \( \chi^2 = 1.923, df = 1, P = 0.166 \) and schizophrenia.

The T261G and G861C polymorphisms in the 5'-untranslated and coding regions, respectively, at the 5-HT1D\( b \) were investigated and the results of HRR and TDT analysis for the RFLP's markers individually are shown in Table II. Using the HRR design, no significant differences were observed between passed and non-passed alleles for both polymorphisms of the 5-HT1D\( b \) gene (HRR of the T261G variant, \( \chi^2 = 0.000, df = 1, P = 1.000 \); HRR of G861C variant, \( \chi^2 = 0.264, df = 1, P = 0.607 \). Similarly, TDT analysis yielded non-significant \( P \)-values for association of 5-HT1D\( b \) polymorphisms and schizophrenia (TDT of the T261G variant, \( \chi^2 = 0.333, df = 1, P = 0.56 \); TDT of G861C variant, \( \chi^2 = 0.040, df = 1, P = 0.841 \); see Table II). In addition with TRANSMIT, we estimated the haplotype frequencies of the two markers in the 5-HT1D\( b \) gene, and a total of four haplotypes were detected, with one common haplotype of 65.5% (Table III). Also with TRANSMIT, we analyzed haplotypes of paired markers for transmission disequilibrium, and the results indicated no evidence for linkage disequilibrium (Table III).

DISCUSSION

The serotonergic system, which is thought to mediate various physiological and psychological conditions related to certain disease state, including eating disorders, obsessive compulsive disorders, bipolar disorder, and schizophrenia, has been the subject of much research in psychiatric genetics. Because for schizophrenia, 5-HT function could be particularly important in modulating the effects of dopamine, we have chosen a candidate gene from serotonergic system for search schizophrenia susceptibility genes. Using association studies, we have found no evidence for association or linkage disequilibrium of the polymorphism at the 5-HT1D\( b \) to schizophrenia. These results obtained with two powerful strategies of association (HRR and TDT) indicate that a TaqI polymorphism in the 5-HT1D\( b \) gene is unlikely to be involved in schizophrenia, in our sample, although association in case-controls from Portuguese population has been reported [Coelho et al., 1997]. However, association studies with case-controls can generate false positives as a result of population stratification. This problem can be addressed by the use of nuclear families (transmission disequilibrium test [Ott, 1992; Terwilliger and Ott, 1992; Schaid and Sommer, 1994]), which we used in the present work. Similarly, no evidence for association or linkage disequilibrium was found at the individual alleles or their haplotypes of the polymorphisms in 5'-untranslated and coding regions of the 5-HT1D\( b \) gene and schizophrenia. Our findings are in agreement with case-controls association study in the Portuguese population [Coelho et al., 1997] and linkage study in the Canadian population [Siderynberg et al., 1993], and/or are of pharmacogenetic relevance. The ability to subdivide the syndrome into more homogenous clinical subtypes will help in identifying etiological factors. DNA typing together

<table>
<thead>
<tr>
<th>5-HT1D( b ) gene</th>
<th>Passed</th>
<th>Not passed</th>
<th>( \chi^2 ) (df = 1), P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRR (1) G261</td>
<td>142</td>
<td>143</td>
<td>( \chi^2 = 0.000, P = 1.000 )</td>
</tr>
<tr>
<td>T261</td>
<td>38</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>(2) G861</td>
<td>139</td>
<td>139</td>
<td>( \chi^2 = 0.264, P = 0.607 )</td>
</tr>
<tr>
<td>C861</td>
<td>36</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>TDT (1) G261</td>
<td>5</td>
<td>7</td>
<td>( \chi^2 = 0.333, P = 0.563 )</td>
</tr>
<tr>
<td>T261</td>
<td>12</td>
<td>7</td>
<td>( \chi^2 = 0.264, P = 0.607 )</td>
</tr>
<tr>
<td>(2) G861</td>
<td>12</td>
<td>13</td>
<td>( \chi^2 = 0.040, P = 0.841 )</td>
</tr>
<tr>
<td>C861</td>
<td>12</td>
<td>13</td>
<td></td>
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</tbody>
</table>

TABLE III. Estimated Haplotype Probabilities and Transmission of Multi-Marker Haplotypes at the 5-HT1D\( b \) Gene Using TRANSMIT

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>Obs</th>
<th>Exp</th>
<th>O-E</th>
<th>Frequency</th>
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<td>118</td>
<td>14</td>
<td>0.655</td>
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<tr>
<td>2-1</td>
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<td>24.5</td>
<td>10.75</td>
<td>0.136</td>
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<td>1-2</td>
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<tr>
<td>2-2</td>
<td>15</td>
<td>14</td>
<td>5.5</td>
<td>0.077</td>
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</table>

5-HT1D\( a \) and 5-HT1D\( b \) Genes and Schizophrenia

<table>
<thead>
<tr>
<th>Gene</th>
<th>Obs</th>
<th>Exp</th>
<th>O-E</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT1D( b )</td>
<td>121</td>
<td>118</td>
<td>14</td>
<td>0.655</td>
</tr>
<tr>
<td>5-HT1D( b )</td>
<td>21</td>
<td>24.5</td>
<td>10.75</td>
<td>0.136</td>
</tr>
</tbody>
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REFERENCES


