Serotonin Genes and Attention Deficit/Hyperactivity Disorder in a Brazilian Sample: Preferential Transmission of the HTR2A 452His Allele to Affected Boys

Ana Paula M. Guimarães, Cristian Zeni, Guilherme V. Polanczyk, Julia P. Genro, Tatiana Roman, Luis A. Rohde, and Mara H. Hutz

INTRODUCTION

Attention-deficit hyperactivity disorder (ADHD) is one of the most common psychiatric disorders of childhood. The role of genetic factors in its etiology is strongly supported by family, adoption, and twin studies. Low serotonin activity has been associated in both animal and human studies with measures of impulsivity, aggression, and disinhibited behaviors, which make genes from the serotonin system reasonable candidates for ADHD susceptibility. In the present study, we investigated a polymorphism in the promoter region of the serotonin transporter (SLC6A4) and two polymorphisms (−1438 A>G and His452-Tyr) in the serotonin 5-HT2A receptor gene using family based association analyses in a sample of 243 Brazilian ADHD children and adolescents and their parents. No linkage disequilibrium between the two HTR2A polymorphisms was detected in this sample (P = 0.76). Considering several evidences from animal models for sexual dimorphism in serotonin genes expression, analyses were performed separately for the whole sample and for male probands. No evidences for biased transmissions of both HTR2A −1438 A>G and SLC6A4 polymorphisms to ADHD youths were observed. Preferential transmission of the HTR2A His452 allele was observed only in families with affected boys (P = 0.04). Our results suggest that findings from ADHD association studies for serotonin genes might be understood in the context of a gender effect, which may help to explain conflicting results in these association studies.

KEY WORDS: ADHD; 5-HTT; 5-HT2A receptor; gender susceptibility; sexual dimorphism

serotonergic system is a very promising one. Thus, the receptors and the serotonin transporter are potential candidate genes for ADHD. The human serotonin transporter is the major regulator of synaptic serotonin concentration and actively transports this neurotransmitter, by a high affinity of Na ÷ and Cl - into the presynaptic terminal [Lesch et al., 1994]. The gene encoding the human serotonin transporter (SLC6A4) is located at 17q11.2. One common polymorphism in the promoter region of the serotonin transporter gene has been identified which is defined by a 22 bp VNTR with two alleles, termed long (L with 16 repeats) and short (S with 14 repeats) [Lesch et al., 1994; Ogilvie et al., 1996]. There are some studies demonstrating that the long variant is associated with greater transcriptional efficiency than the short variant. Therefore, this polymorphism might influence ADHD symptoms [Heils et al., 1996; Lesch et al., 1996]. The serotonin transporter has been investigated in several studies on ADHD with both positive [Manor et al., 2001; Seeger et al., 2001; Kent et al., 2002; Cadoret et al., 2003] and negative reports [Langley et al., 2002; Cadoret et al., 2003] and negative reports [Langley et al., 2002; Cadoret et al., 2003] and negative reports [Langley et al., 2002; Cadoret et al., 2003] and negative reports [Langley et al., 2002; Cadoret et al., 2003] and negative reports [Langley et al., 2002; Cadoret et al., 2003] and negative reports [Langley et al., 2002; Cadoret et al., 2003]. The serotonin transporter has been shown high levels of 5-HT2A binding sites in many forebrain regions, particularly in cortical areas [Pazos and Palacios, 1985; Pazos et al., 1987; López-Giménez et al., 1997]. Evidence from pharmacological studies have suggested that striatal 5-HT2A receptors regulate stimulant-induced dopamine release and hyperactivity, providing support for the interaction of serotonergic and dopaminergic systems in mediating hyperactivity behavior [O’Neill et al., 1999].

Two polymorphisms of the HTR2A gene are focused in the present study: The His452Tyr and –1438 A > G variants [Erdmann et al., 1996; Ozaki et al., 1996; Spurlock et al., 1998]. The –1438 A > G polymorphism in the HTR2A promoter region might be a functional SNP that affects promoter activity, having functional effects on 5-HT2A receptor expression in the brain and might be responsible for associations with many neuropsychiatric phenotypes [Parsons et al., 2004]. Only one association study with this polymorphism and ADHD with negative results has been reported so far [Zoroglou et al., 2003].

The second polymorphism investigated in the 5-HT2A receptor gene is a His452Tyr substitution that might alter the 5-HT2A function and consequently contribute to the development of psychiatric disorders [Ozaki et al., 1997]. Two association studies between the His452Tyr polymorphism and ADHD have been reported with conflicting results [Ques et al., 2000; Hawi et al., 2002].

In the current study, we investigated the possible role of the SLC6A4 gene (22 bp VNTR) and the HTR2A (His452Tyr and –1438 A > G) polymorphisms in a Brazilian sample of families with ADHD probands.

Materials and Methods

Families of children with diagnosed ADHD were recruited from the Child and Adolescent Psychiatric Division of the Hospital de Clínicas de Porto Alegre (HCPA). A consensus diagnosis of ADHD based on DSM-IV criteria was achieved through a three-stage process, described in detail previously [Roman et al., 2001; Rohde, 2002].

The sample included 243 families comprising 186 parent proband trios and 57 parent-child duos. Table I presents demographic and diagnostic characteristics of proband subjects. The Ethical committee of HCPA and the Coordinating Committee of the Graduate Program in Genetics and Molecular Biology of the Federal University of Rio Grande do Sul approved the study protocol. Parents provided written informed consent and probands provided verbal assent to participate.

| TABLE I. Demographic and Diagnostic Characteristics of the Sample* |
|-----------------------------|-----------------------------|
| Age (years)                | 10.32 (3.13)                |
| Sex (males)                | 199 (82.6)                  |
| Ethnicity (European-Brazilian) | 209 (92.1)               |
| DSM-IV ADHD types          |                             |
| Predominantly hyperactive  | 15 (6.2)                    |
| Predominantly inattentive   | 54 (22.4)                   |
| Combined                   | 158 (65.6)                  |
| Patients with no comorbidity | 76 (21.5)               |
| Main comorbid conditions*  |                             |
| Oppositional defiant disorder | 113 (47.1)            |
| Conduct disorder           | 37 (15.4)                   |
| Anxiety disorder           | 51 (21.5)                   |
| Mood disorder              | 33 (13.8)                   |
| Other comorbidities        | 48 (20.1)                   |

*Mean and standard deviations (in parentheses) are reported for continuous variables and percent (in parentheses) are reported for categorical variables.

**Multiple comorbid conditions may be presented.

Genotyping

DNA was extracted from whole blood lymphocytes by a salting out procedure as already described [Lahiri and Nurnberger, 1991]. The polymorphism at the SLC6A4 promoter was amplified using the polymerase chain reaction (PCR) adapted from Seeger et al. [2001] and Manor et al. [2001]. The HTR2A –1438 A > G and His452Tyr polymorphisms were carried out using the primers and methods described, respectively, by Arranz et al. [1998] and Ozaki et al. [1996].

Statistical Analyses

Allele frequencies were estimated by counting. Haplotypes and linkage disequilibrium (D) were estimated using the ARLEQUIN software (version 2.000) [Schneider et al., 2000]. Dmax (D theoretical maximum) and D* (the relative magnitude of D as compared to its theoretical maximum, calculated as (D/Dmax) values were calculated as described by Lewontin [1988]. For family based association analyses we used the haplotype relative risk (HRR) statistics [Terwilliger and Ott, 1992]. Both trios composed of father, mother, and affected child and parent/proband pairs were included in the HRR analyses. Heterozygous parent/proband pairs with the same genotype were excluded since the transmission status of parental alleles could not be determined [Curtis and Sham, 1995]. The statistical package Transmit [Clayton, 1999] was also used to test for biased transmission of alleles as well as to test for evidence of association with haplotypes at the HTR2A (~1438 A > G and His452Tyr polymorphisms) gene. Odds ratio estimates were performed with the PEPI statistical program Version 4.0 (Abramson and Gahlinger, 2001). A significance level of 5% was accepted in all comparisons.

Results

The ADHD patients were predominantly males (82.6%), from European descent (92.1%), and their mean age was 10.3 years. Most of them presented the combined type (65.6%), and oppositional defiant disorder was the most common comorbid condition (Table I).

Allele frequencies for all markers did not show any significant deviation from those expected according to Hardy–Weinberg equilibrium. The HRR analyses for SLC6A4 and HTR2A –1438 A > G and His452Tyr polymorphisms are presented in Table II. No evidences for biased transmission of alleles for both HTR2A –1438 A > G and SLC6A4
TABLE II. HRR Analysis of the Genes SLC6A4, HTR2A (−1438A > G), and HTR2A (His452Tyr)

<table>
<thead>
<tr>
<th>ADHD sample</th>
<th>Allele</th>
<th>T</th>
<th>NT</th>
<th>$X^2$</th>
<th>$P$</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLC6A4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (n = 231)</td>
<td>L</td>
<td>237</td>
<td>228</td>
<td>0.40</td>
<td>0.53</td>
<td>1.10 (0.82–1.45)</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>177</td>
<td>186</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys (n = 192)</td>
<td>L</td>
<td>205</td>
<td>186</td>
<td>2.13</td>
<td>0.14</td>
<td>1.25 (0.92–1.71)</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>140</td>
<td>159</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HTR2A (−1438A > G)

| Boys (n = 178)    | G      | 186 | 189 | 0.06  | 0.81  | 0.96 (0.70–1.33) |
|                   | A      | 140 | 137 |       |       |             |

HTR2A (His452Tyr)

| Boys (n = 187)    | His    | 313 | 297 | 4.18  | 0.04  | 1.70 (0.99–2.97) |
|                   | Tyr    | 32  | 47  |       |       |             |

T, transmitted; NT, not transmitted; OR, odds ratio.

polymorphisms to the ADHD probands were observed. However, a trend for association between the HTR2A His452Tyr polymorphism and ADHD was detected ($P = 0.07$; Table II). As in animal models differential expression of this receptor between males and females has been described, this analysis was also performed with male probands only. Preferential transmission of the His452 allele (OR = 1.70; $P = 0.04$) was observed in families with affected boys (Table II). In order to confirm these findings we also estimated biased transmission of this polymorphism in female probands only. The values obtained in these analyses were different from those observed in boys. No effect of the His452 allele was detected in the sample of girls ($P = 0.75$; OR = 0.82; CI 95% = 0.19–3.41).

In this sample, no linkage disequilibrium between the two HTR2A markers was detected ($P = 0.76$), indicating that −1438A > G and His452Tyr loci are essentially independent. Therefore, no specific haplotype was preferentially transmitted to affected children (data not shown).

**DISCUSSION**

In the present study, we conducted HRR analyses on three markers in two serotonergic genes, which have been previously analyzed, for association with ADHD. A significant overtransmission of the His452 of HTR2A gene to cases was observed in boys ($P = 0.04$). No preferential transmission of the SLC6A4 and HTR2A promoter (−1438A > G) polymorphisms was observed in Brazilian ADHD probands and their families.

Four independent case-control studies reported associations between the serotonin transporter gene polymorphisms and ADHD [Manor et al., 2001; Seeger et al., 2001; Kent et al., 2002; Cadoret et al., 2003]. However, three further studies found no evidence for this association [Langley et al., 2003; Kim et al., 2005; Xu et al., 2005]. Our results are in agreement with those investigators. Several evidences suggest that gender should be taken into account in research evaluating the effects of serotonin gene polymorphisms on central nervous functions or diseases [Williams et al., 2003]. Sexual dimorphisms in neuroanatomy and neurochemistry including higher serotonin concentrations in female rats, have been described [Zhang et al., 1999]. Sumner and Fink [1998] and Zhang et al. [1999] have shown that these differences are in part due to the presence of sexual dimorphism in 5-HT2A mRNA levels and receptor density. Additionally, these authors have also demonstrated that the transcriptional regulation of 5-HT2A receptors in the rat brain is sex-steroid dependent. Recently, Robichaud and Debonnel [2005] reported an increase in 5-HT neuronal firing activity induced by testosterone and 17β-estradiol in both male and female rats.

Taken together with previous findings, these data suggest that potential interactions between gender and candidate genes could be important in understanding gender differences in various psychiatric disorders. Preliminary evidences for sexual dimorphism of HTR2A in obsessive-compulsive disorder and anorexia nervosa have also been suggested [Norton and Owen, 2005]. If the sexual dimorphism of HTR2A expression were not been considered, the association between this polymorphism at the receptor and ADHD would not have been disclosed in the present study.

Sex differences also extend to cognitive functions such as memory, attention, and perception. These differences may also be protective in girls, who have much lower rates than boys of childhood developmental and mental disorders like ADHD [Holden, 2005].

Investigations of sex differences in the neurobiology of ADHD are just beginning. Hermens et al. [2005] investigated sex differences in adolescent ADHD using concurrently recorded electroencephalography (EEG) and electrodermal activity (EDA). These investigators concluded that different psychophysiological processes might underlie ADHD in each sex.
sex. The profile of theta enhancement in ADHD males is consistent with a developmental deviation model of ADHD, whereas ADHD in females may be better understood within an arousal model, which emphasizes both central and autonomic functions. In complex traits like ADHD, where the phenotype is dependent of multiple genes interacting both one to each other and with multiple aspects of the environment over time, it is not surprising that factors like gender are associated with different effects of polymorphisms at serotonin-related genes. The present findings also suggest that samples with different proportions of boys and girls might be one source of conflicting results in ADHD association studies.

In conclusion, we did not find evidence for association between ADHD and serotonin genes investigated herein, but additional analyses performed suggest a possible role for gender in the genetic susceptibility of a candidate gene in ADHD. The results of this study should be interpreted cautiously due to both the significance level found and the absence of adjustment for multiple testing. More studies replicating our findings are clearly needed.

ACKNOWLEDGMENTS
The authors thank the financial support provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) to L.A.R. and M.H.H.

REFERENCES
Serotonin Genes and ADHD


