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ADRA2A polymorphisms and ADHD in adults: Possible mediating effect of personality

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ABSTRACT

Several studies have tested for the association between polymorphisms in the *ADRA2A* gene and childhood ADHD. A meta-analysis of these results, however, has pointed towards a significant heterogeneity, raising the need for explanatory studies. As the effect of other relevant clinical characteristics could be a possible source, we studied three polymorphisms in the *ADRA2A* gene (−1291 C>G–*MspI* or rs1800544; −262 G>A–*HhaI* or rs1800544; 1780 C>T–*DraI* or rs553668) in 403 adult patients with ADHD assessed in relation to comorbidity and personality characteristics, as well as in 232 controls. The diagnosis followed DSM-IV criteria, and personality dimensions were evaluated with the Temperament and Character Inventory (TCI). There were no significant differences in allele and genotype frequencies between cases and controls. Patients carrying the G allele of rs1800544 presented lower scores in harm avoidance, and carriers of the T allele of rs553668 had more novelty seeking and less harm avoidance and persistence. Additionally, the haplotype carrying the G-G-T alleles (rs1800544–rs1800545–rs553668) was associated with lower scores in harm avoidance and persistence, and higher scores in novelty seeking compared to other haplotypes. These findings suggest that the conflicting findings obtained in association studies between *ADRA2A* polymorphisms and ADHD might be related to temperament profiles, and support additional studies addressing these effects in larger samples.

1. Introduction

The worldwide prevalence estimation of Attention Deficit Hyperactivity Disorder (ADHD) is 5.29% among children (Polanczyk et al., 2007) and 2.5% among adults (Simon et al., 2009). While the heritability of ADHD was estimated at approximately 76% when considering studies on children (Faraone et al., 2005), Boomsma et al. (2010) estimated this heritability in adults at approximately 30%. However, a low level of heritability does not exclude the possibility of significant genetic effects on specific aspects or subtypes of the disorder. There is probably much to improve in the characterization of the phenotype, including intermediate phenotypes, the role of the environment, as well as gene–gene and gene–environment interactions. For example, a promising approach in the genetics of mental disorders is the investigation of gene–environment interactions, which may even explain the inconsistent findings of association studies with ADHD. Unfortunately, such studies have been restricted to a limited number of genes such as *DAT1* (Kahn et al., 2003; Brookes et al., 2006a; Laucht et al., 2007) and *DRD4* (Seeger et al., 2004; Froehlich et al., 2007).

Most genetic research on ADHD consists of candidate gene-based association studies. These investigations have suggested the role of several genes with relatively limited effects (Brookes et al., 2006b; Gizer et al., 2009). A few genome-wide association studies have been performed on different aspects of ADHD (Anney et al., 2008; Lasky-Su et al., 2008; Lesch et al., 2008; Neale et al., 2008; Sonuga-Barke et al., 2008). However, none of these studies found associations that retained significance after correction for multiple comparisons. A recent meta-analytic review assessed all polymorphisms analyzed in at least four studies on childhood ADHD (Gizer et al., 2009). Polymorphisms in *DAT1*, *DRD4*, *DRD5*, *5HTT*, *HTR1B*, and *SNAP25* presented significant associations. A number of other genes revealed more complex findings, with significant heterogeneity among studies, thereby indicating the need for further investigation. One such gene is the Alpha-2A adrenergic receptor gene (*ADRA2A*), located on chromosome 10q24–q26 (Lario et al., 1997). This gene has served as a candidate in ADHD studies because it is the most prevalent noradrenergic

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receptor in the prefrontal cortex (Arnsten et al., 1996; Park et al., 2005). There is strong evidence that adequate noradrenergic function is required for optimal function of the prefrontal cortex, which is important for attention control (Arnsten, 1999; Arnsten and Li, 2005).

The meta-analysis of Gizer et al. (2009) included three *ADRA2A* polymorphisms that have been the focus of most association studies. The most intriguing findings are related to the rs553668 polymorphism (1780 C>T −*DraI*). Park et al. (2005) verified preferential transmission of the T allele towards children with ADHD. Nonetheless, Cho et al. (2008b) found preferential transmission of the C allele, while two other studies (Deupree et al., 2006; Wang et al., 2006) found no association at all. The meta-analysis of Gizer et al. (2009) revealed a significant heterogeneity in these findings.

The rs1800544 (−1291 C>G–*MspI*) has been associated with ADHD, as well as with subtypes or dimensions of this disorder (Roman et al., 2003; Park et al., 2005; Stevenson et al., 2005; Roman et al., 2006; Schmitz et al., 2006). In contrast, other studies did not sustain this association (Xu et al., 2001; Deupree et al., 2006; Wang et al., 2006; Cho et al., 2008b). The meta-analysis point towards a non-significant result for allele G of rs1800544 when pooling eleven studies. Finally, there is no evidence of association with the rs1800545 polymorphism (−262 G>A −*HhaI*) (Park et al., 2005; Deupree et al., 2006).

Considering that there is no compelling evidence regarding functional effects of these polymorphisms, haplotype analyses may provide additional information. Only a limited number of haplotype studies, however, have been performed until now. Previous research considered the haplotype G-G-T (rs1800544–rs1800545–rs553668) as a risk for ADHD and the haplotype C-G-C as a factor of reduced risk (Park et al., 2005). Opposite results, however, were also observed (Deupree et al., 2006). It is noteworthy that all mentioned studies were performed on children. To the best of our knowledge, among samples of adults there is no information available on the possible association between these polymorphisms and ADHD. In addition, there is a lack of knowledge on the possible sources of the diversity of results generated by different studies, such as the putative role of comorbidities and personality.

Characteristic TCI profiles have been associated to many psychiatric disorders (Cloninger et al., 2006). ADHD has been associated with higher scores in the temperament dimension of novelty seeking among both adults (Downey et al., 1997; Lynn et al., 2005; Anckarsäter et al., 2006; Salgado et al., 2009; Sizoo et al., 2009) and children (Tillman et al., 2003). Another dimension, harm avoidance, has also been positively associated with adult ADHD (Lynn et al., 2005; Anckarsäter et al., 2006; Salgado et al., 2009; Sizoo et al., 2009). With regard to comorbidities, the evidence suggests that a large proportion of adults with ADHD (~80%) presents at least one lifetime psychiatric comorbidity (Biederman et al., 1993; Murphy and Barkley, 1996; Kooij et al., 2001; McGough et al., 2005; Grevet et al., 2006). There is also evidence that comorbidities have a substantial impact on the clinical presentation of ADHD (Fischer et al., 2007).

In this context, the present study investigated the effects of these three polymorphisms in the *ADRA2A* gene (rs1800544, rs1800545 and rs553668) on ADHD. In addition, it assessed the effects of comorbidity and personality dimensions in a search for characteristics that could explain the heterogeneity observed in the meta-analysis of Gizer et al. (2009).

2. Methods

2.1. Recruitment and diagnosis

The study recruited patients from the adult ADHD outpatient clinic of the Hospital de Clínicas de Porto Alegre (a major teaching hospital in Southern Brazil). Subjects included in this research were Brazilians of European descent, 18 years or older, fulfilling diagnostic criteria for DSM-IV ADHD (American Psychiatric Association, APA, 1994). Exclusion criteria consisted of the presence of significant neurological diseases that might affect cognition, such as epilepsy, sequelae of cerebrovascular accidents and degenerative disorders, a current or past history of psychosis or dementia and an estimated IQ of ≤70 (Kaplan et al., 1991).

The diagnostic procedures for ADHD followed the DSM-IV criteria (American Psychiatric Association, APA, 1994), using the Portuguese version of the "Schedule for Affective Disorders and Schizophrenia, Epidemiological Version for school age Children" (K-SADS-E) (Mercadante et al., 1995) assessing present and worst lifetime episodes of DSM-IV psychiatric disorders in children (Ambrosini, 2000). It was adapted for adulthood symptoms (Grevet et al., 2005) with the adjustment of symptom onset to age 12 or earlier—instead of 7 as reported by others—because of the operational advantages and diagnostic reliability this offers (Applegate et al., 1997; Barkley and Biederman, 1997; Rohde et al., 2000). The severity of current ADHD and oppositional defiant symptoms was assessed by the Swanson, Nolan, and Pelham scale-version IV (SNAP-IV) (Swanson, 1992). Axis I psychiatric comorbidities were evaluated using the SCID-IV-R structured interview system (First et al., 1998). The diagnoses of conduct and anti-social personality disorder were obtained using the appropriate sections of the Mini-International Neuropsychiatric Interview (M.I.N.I.) (Sheehan et al., 1998).

Temperament and character dimension scores were assessed by the Temperament and Character Inventory (TCI) (Cloninger et al., 1993), validated to Portuguese (Fuentes et al., 1999). TCI employs a list of 240 sentences to be read and rated as true/false. The instrument comprises 4 temperament dimensions (novelty seeking, harm avoidance, reward dependence and persistence) and 3 character dimensions (self-directedness, cooperativeness and self-transcendence) (Cloninger et al., 1993). Several studies have supported a high test–retest reliability of TCI (e.g. Brändström et al., 1998). In addition, the validity of TCI was evaluated by the comparison of TCI with other personality models (e.g. Bayon et al., 1996). In a previous study with part of the sample included here, the combined subtype of ADHD among adults was associated with higher scores in novelty seeking and lower scores in cooperativeness than the inattentive subtype. Higher inattention scores were associated with decreased self-directedness and increased harm avoidance, whereas higher hyperactivity/impulsivity scores correlated positively with novelty seeking and persistence (Salgado et al., 2009).

Previous studies have already provided a more detailed phenotypic characterization of the sample, in terms of comorbidity (Grevet et al., 2006; Fischer et al., 2007), cognition (Kalil et al., 2008), and personality (Salgado et al., 2009).

2.2. Participants and study samples

The patient sample included 403 adult Brazilians of European descent, from the ADHD adult outpatient clinic of Hospital de Clínicas of Porto Alegre. The average age was 34 years, with 48.2% of females and 51.8% of males. These patients were classified in the combined subtype (54.8%), inattentive subtype (39.4%) or hyperactive/impulsive subtype (5.8%).

The control sample ($n=232$) was recruited from a blood bank in the vicinity of the hospital. Ethnicity in the control sample was deduced exactly in the same way as in the patient's sample. The degree of African admixture in the European-derived population of Porto Alegre has been estimated at approximately 6% (Zembrzuski et al., 2006). A review of ethnic admixture in Brazilian and other Latin American populations has been published by Salzano and Bortolini (2002). Our control group was designed to be non-screened and representative of the average gene frequencies in Porto Alegre. All subjects (patients and controls) signed an informed consent approved by the Ethics Committees of the Hospital and the Federal University of Rio Grande do Sul.

2.3. Laboratory methods

DNA was extracted by a salting out method (Lahiri and Nurnberger, 1991). The polymorphism rs1800544 in the *ADRA2A* gene was amplified with PCR conditions adapted from Lario et al. (1997) and Lima et al. (2007), followed by digestion with *MspI* and genotyping in a 10% polyacrylamide gel stained with ethidium bromide. The other two polymorphisms (rs1800545 and rs553668) were amplified using the primers and conditions suggested for the TaqMan allelic discrimination system (Applied Biosystems 7500 Real Time PCR System). The rate of successful genotyping is 97% for rs1800544 and rs553668 and 96% for rs1800545. The ADHD patients and controls included in the analyses correspond to those successfully genotyped for the three polymorphisms.

2.4. Statistical analysis

The genotype, allele and haplotype frequency comparisons between ADHD patients and controls, as well as analysis of other categorical variables were performed with the chi-square test. Continuous variables were analyzed by ANOVA. Linkage disequilibrium and haplotype estimation comprising the rs1800544, rs1800545 and rs553668 polymorphisms were performed with the MLOCUS program (Long et al., 1995; Long, 1999). Potential confounders (demographic characteristics, IQ, ADHD subtype, comorbidities and temperament profiles) were included as covariates using a statistical definition (association with both the study factor and outcome for a $p\leq 0.20$) (Maldonado and Greenland, 1993). No multiple comparison correction was applied to this study since both dependent variables (temperament dimensions and ADHD itself) and the polymorphisms, which are in linkage disequilibrium, are moderately correlated and independence between variables is an assumption for such corrections.