Gene 528 (2013) 320–327

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Short Communication

The relationship between the presence of ADHD and certain candidate gene polymorphisms in a Turkish sample

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ARTICLE INFO

Article history:
Accepted 2 July 2013
Available online 18 July 2013

Keywords:
Genetics of ADHD
SNAP-25 gene
NET1 gene
5-HT2A gene

ABSTRACT

Due to the high heritability of attention-deficit hyperactivity disorder (ADHD), parents of children with ADHD appear to represent a good sample group for investigating the genetics of the disorder. The aim of this study was to investigate the association between ADHD and six polymorphisms in five candidate genes [*5-HT2A* (rs6311), *NET1* (rs2242447), *COMT* (rs4818), *NTF3* (rs6332), *SNAP-25* (rs3746544) and (rs1051312)]. We included 228 parents of children diagnosed with ADHD and 109 healthy parents as the control group. The polymorphisms were genotyped using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) assays and analyzed using the chi-square test and the multinomial logit model. *SNAP-25* (rs3746544) polymorphism was associated with loading for ADHD, while *5-HT2A* (rs6311) and *NET1* (rs2242447) polymorphisms were associated with ADHD. On the other hand, there was no significant association between the *SNAP-25* (rs1051312), *NTF3* (rs6332), or *COMT* (rs4818) gene polymorphisms and ADHD.

In addition, we found that even if variation in the *SNAP-25* gene alone does not affect the phenotype, it may nevertheless lead to the emergence of a clinical ADHD picture in the presence of other genetic factors. Our findings suggest that a combination of *NET1* (rs2242447) and *SNAP-25* (rs3746544) is a risk factor for ADHD. Problems associated with the noradrenergic and serotonergic systems and *SNAP-25* may play a role, both alone and in interaction with one another, in the pathophysiological mechanisms of ADHD.

1. Introduction

Attention-deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder that starts in childhood. It can persist into adulthood and cause numerous significant difficulties in various areas of life (social, occupational, academic) in both children and adults (Faraone et al., 2005).

The role of genetics in the etiology of the disease has been revealed by family, twin and adoption studies (Kuntsi et al., 2005; Sprich et al., 2000). The heritability of the disease is approximately 76% (Chang et al., 2013; Faraone et al., 2005). The inherited aspect of ADHD has led researchers to focus on genetic research into its etiology. The relationship between genes potentially associated with the pathophysiology of the disorder and ADHD has been investigated in numerous association studies in both children and adults (Akutagava-Martins et al., 2013; Faraone and Mick, 2010; Franke et al., 2012; Gizer et al., 2009; Thapar et al., 2012).

Norepinephrine transporter (*NET1*), *selective serotonin 2A receptor* (*5-HT2A*), and *catechol-O-methyl-transferase* (*COMT*) are relevant genes due to the importance of the monoaminergic system in the etiology of ADHD and have been investigated by several groups of researchers (Bobb et al., 2005; Halleland et al., 2009; Müller et al., 2008; Retz et al., 2008; Ribasés et al., 2009; Xu et al., 2008). Since ADHD is a neurodevelopmental disorder, genes encoding neurotrophic factors (NTFs), which play important roles in the differentiation and survival of the nerve cells, have emerged as candidate genes in association studies (Conner et al., 2008; Ribasés et al., 2008). *Synaptosomal-associated protein-25* (SNAP-25), another investigated candidate gene, plays a central role

Abbreviations: ADHD, attention-deficit hyperactivity disorder; NE, norepinephrine; 5-HT2, serotonin; COMT, catechol-O-methyl-transferase; SNAP-25, synaptosomal-associated protein-25; NTF, neurotrophic factor; NET1 gene, norepinephrine transporter gene; 5-HT2A gene, selective serotonin 2A receptor gene; COMT gene, catechol-O-methyl-transferase gene; SNAP-25 gene, synaptosomal-associated protein-25 gene; NTF3 gene, neurotrophic factor-3 gene; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; DSM-IV, the Diagnostic and Statistical Manual of Mental Disorders, fourth edition; SCID, Structured Clinical Interview for the DSM-IV; WURS-25, the Wender-Utah Rating Scale-25; CSS, the Current Symptom Scale; ASRS, the Adult ADHD Self-Report Scale; SNP, single nucleotide polymorphism; NCBI, National Center for Biotechnology Information.

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http://dx.doi.org/10.1016/j.gene.2013.07.004

in synaptic transmission and plasticity mechanisms (Forero et al., 2009; Kustanovich et al., 2003; Mill et al., 2004; Sarkar et al., 2012). Although most of these studies reported no association between the candidate genes and ADHD, a few have described an association between certain candidate genes and the disease. However, these associations were either weak or moderate, and the results were inconsistent (Faraone and Mick, 2010; Franke et al., 2012; Gizer et al., 2009). One genome-wide association study reported no significant association in German patients with ADHD compared to the control group (Hinney et al., 2011). It has therefore been suggested that gene-associated variations may affect ADHD, but that this effect is minor and may have a multigenic or multifactorial genetic pattern (Elia et al., 2012; Thapar et al., 2013; Wallis et al., 2008).

Although the incidence of ADHD among the general adult population is approximately 2.5–4.9% (Simon et al., 2009), it is reported to be 2–8 times higher in the parents of children with ADHD (Biederman et al., 1990; Chronis et al., 2003; Faraone and Biederman, 2000; Mahomedy et al., 2007). There is known to be an additive genetic effect for ADHD in families with more than one case (familial ADHD) (Franke et al., 2012). Parents of children with ADHD may have a greater genetic effect associated with this disorder than the normal population. They therefore appear to represent a good sample group for examining the relationship between ADHD and candidate gene polymorphisms. To the best of our knowledge, no previous case–control studies have investigated the relationship between ADHD and candidate gene polymorphisms in this population.

Our hypothesis is that persons with ADHD have more polymorphisms of certain candidate genes compared to control groups.

This study aimed to investigate the relationship between polymorphisms of certain candidate genes and ADHD.

2. Materials and methods

2.1. Participants

Biological parents of children diagnosed with ADHD, treated and followed up during the 1-year study period at the Department of Child and Adolescent Psychiatry, Faculty of Medicine, Ondokuz Mayis University, comprised the study sample. The Department of Child and Adolescent Psychiatry referred 335 parents for screening. Exclusion criteria included other active Axis I psychiatric diseases, mental retardation, history of psychotic disorders, bipolar disorder or substance abuse disorder, neurological disease, or any known genetically transmitted disease. The parents underwent a psychiatric assessment using the Turkish version of the Structured Clinical Interview for the DSM-IV-TR (SCID-I) (Çorapçıoğlu, 1999) conducted by a psychiatrist and lasting approximately 45 min. Forty-two parents were excluded from the study. Reasons for exclusion included current major depressive disorder ($n = 11$), current anxiety disorder ($n = 15$), history of bipolar disorder ($n = 6$) or substance use disorder ($n = 8$), and a history of neurosurgery ($n = 2$). Sixty-five parents refused to participate, the most common reason given being insufficient time to complete the study procedures. Ultimately, 228 parents of 146 children with ADHD were enrolled. Both parents were available for 78 families, and mothers or fathers alone in 50 and 22 families, respectively. A healthy control group ($n = 109$) was established consisting of one participant only from families of hospital staff and their relatives who were informed about the study and agreed to participate. Inclusion criteria for individuals in the healthy control group were having at least one child above six years of age and having no children diagnosed with ADHD. The purpose of this last criterion was to reduce the possibility of ADHD-associated genetic effect. The exclusion criteria for the healthy control group were the same as those of the study group, except for diagnosis of ADHD.

All subjects were of Turkish origin and from the Black Sea coastal region. All were informed about the purpose and design of the study before taking part and gave written informed consent. The study was conducted in accordance with the Helsinki Declaration and with the approval of the Ethics Committee of Ondokuz Mayis University.

2.2. ADHD diagnosis

A diagnostic assessment based on DSM-IV which included the adult ADHD diagnostic criteria recommended by Barkley et al. for DSM-V was performed by a psychiatrist (Barkley et al., 2008). Retrospective data were largely obtained from participants, and also from their first-degree relatives where possible. Additionally, we used three self-report measures to assess ADHD symptoms. The Turkish version (Öncu et al., 2005) of the Wender-Utah Rating Scale-25 (WURS-25), a 5-point Likert scale based on Utah criteria (Ward et al., 1993), was used to evaluate childhood ADHD symptoms retrospectively. The Turkish versions (Aycicegi et al., 2003; Doğan et al., 2009) of the Current Symptom Scale (CSS) (Barkley and Murphy, 1998) and Adult ADHD Self-Report Scale (ASRS) (Kessler et al., 2005) were used to evaluate current adulthood ADHD symptoms. These two self-report scales each contain 18 items based on DSM-IV diagnostic criteria. Higher scores on these scales represent greater ADHD symptoms. These measurements were used solely for support purposes during the assessments, not for diagnosis. This phase lasted approximately 90 min (30 min to complete the forms and 60 min of clinical assessment). One hundred and twenty ($n = 120$) parents of children with ADHD had received a diagnosis of ADHD at some time in their lives (childhood or adulthood) (defined as the ADHD group). Sixty-one of these received a diagnosis of childhood ADHD and 59 of adulthood ADHD. One hundred and eight parents had never received a diagnosis of ADHD at any time in their lives (defined as the Non-ADHD group). The HC group underwent the diagnostic procedures described for the study groups; five individuals met the study criteria for ADHD (childhood or adulthood), and 14 had a current psychiatric diagnosis (8 major depressive disorders, 3 obsessive compulsive disorder, 2 panic disorders, and 1 dysthymia).

2.3. Single nucleotide polymorphism (SNP) selection

The SNPs were selected from the National Center for Biotechnology Information (NCBI) public database (http://www.ncbi.nlm.nih.gov/SNP). We selected SNPs either found in both databases or provided by independent submitters to NCBI. We thus selected 6 SNPs: *5-HT2A* (rs6311), *NET1* (rs2242447), *COMT* (rs4818), *NTF3* (rs6332), and *SNAP-25* (rs3746544) and (rs1051312) polymorphisms. These SNPs had previously been reported to be associated with ADHD (Brophy et al., 2002; Halleland et al., 2009; Kustanovich et al., 2003; Retz et al., 2008; Ribasés et al., 2008).

2.4. DNA extraction and identification of genotypes

Blood samples were obtained from subjects, and genomic DNA was isolated using the “salting out” technique from peripheral leukocytes (Miller et al., 1988). The polymorphisms were determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis using methods previously described elsewhere (Ho et al., 2008; Musil et al., 2008; Retz et al., 2008; Syed et al., 2007; Tander et al., 2008). To test the accuracy of our RFLP results, 3% of samples were repeated.

2.5. Statistical analyses

Diagnostic groups and demographic and psychometric test variables were analyzed using one-way ANOVA. Significant differences between groups were analyzed using Tukey's post-hoc test. Associations between study groups and gene polymorphisms were first examined using the chi-square analysis. Significant differences between groups were analyzed using the Bonferroni correction method for post-hoc testing.