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ScienceDirect
Journal of Psychiatric Research 42 (2008) 117–124
JOURNAL OF PSYCHIATRIC RESEARCH
www.elsevier.com/locate/jpsychires

Investigation of the G protein subunit $G\alpha_{olf}$ gene (*GNAL*) in attention deficit/hyperactivity disorder

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Received 1 June 2006; received in revised form 22 October 2006; accepted 24 October 2006

Abstract

The dopamine system plays an important role in the regulation of attention and motor behavior, subsequently, several dopamine-related genes have been associated with Attention Deficit/Hyperactivity Disorder (ADHD). Among them are the dopamine receptors D1 and D5 that mediate adenylyl cyclase activation through coupling with G_s-like proteins. We thus hypothesized that the G_s-like subunit $G\alpha_{olf}$, expressed in D1-rich areas of the brain, contributes to the genetic susceptibility of ADHD. To evaluate the involvement of the $G\alpha_{olf}$ gene, *GNAL*, in ADHD, we examined the inheritance pattern of 12 *GNAL* polymorphisms in 258 nuclear families ascertained through a proband with ADHD (311 affected children) using the transmission/disequilibrium test (TDT). Categorical analysis of individual marker alleles demonstrated biased transmission of one polymorphism in *GNAL* intron 3 (rs2161961; $P = 0.011$). We also observed significant relationships between rs2161961 and dimensional symptoms of inattention and hyperactivity/impulsivity ($P = 0.003$ and $P = 0.008$). In addition, because of recent evidence of imprinting at the *GNAL* locus, secondary analyses were split into maternal and paternal transmissions to assess a contribution of parental effects. We found evidence of strong maternal effect, with preferential transmission of maternal alleles for rs2161961A ($P = 0.005$) and rs8098539A ($P = 0.035$). These preliminary findings suggest a possible contribution of *GNAL* in the susceptibility to ADHD, with possible involvement of parent-of-origin effects.

Keywords: Attention deficit/hyperactivity disorder; Genetics; G protein; $G\alpha_{olf}$; Transmission/disequilibrium test; Dopamine receptor D1

1. Introduction

Attention Deficit/Hyperactivity Disorder (ADHD) is a common neurodevelopmental condition characterized by a pattern of inattention, hyperactivity and impulsivity. Current hypotheses on the biological basis of ADHD have centered on the dysregulation of fronto-striatal circuits and the neurotransmitters involved in these pathways. In particular, accumulating evidence implicate altered dopamine signalling in the disorder (Davids et al., 2003; Durston, 2003; Seeman and Madras, 1998; Viggiano et al., 2003) and genetic association of several genes engaged in dopamine signalling is supported by meta-analysis of pooled data (e.g. *DRD4*, *DRD5*, *DAT1*, and *SNAP25*) (Thapar et al., 2005).

We previously reported the association between the dopamine receptor D1 gene (*DRD1*) and ADHD (Misener et al., 2004), particularly between one haplotype and inattention symptoms ($P = 0.008$). Recently, we replicated the association between this haplotype and inattentive behaviors in children selected for reading difficulties ($P = 0.004$) (Luca et al., submitted for publication).

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0022-3956/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jpsychires.2006.10.010

Positive findings were also found for one *DRD1* marker in an ADHD case-control sample (Bobb et al., 2005), although negative results were also obtained with smaller family-based samples for single markers (Bobb et al., 2005; Kirley et al., 2002). Our findings for *DRD1* in ADHD symptoms are suggestive of a potential role of the D1/D5 signalling pathways in genetic susceptibility of this disorder. This is further supported by a large combined analysis of 14 independent samples of 1980 probands ($P = 0.00005$), odds ratio 1.24 (Lowe et al., 2004) for *DRD5* (a D1-like receptor). In the same vein, we have recently reported evidence of association between ADHD and the calcyon gene, a D1-interacting protein (Laurin et al., 2005).

D1/D5 signalling mediates executive abilities including working memory (Goldman-Rakic et al., 2000), attention (Bayer et al., 2000; Granon et al., 2000), motor control (Dreher and Jackson, 1989; Meyer, 1993), and reward and reinforcement mechanisms (Beninger and Miller, 1998). Impairment of those functions is often observed in individuals with ADHD (Arnsten and Li, 2005b; Lijffijt et al., 2005; Luman et al., 2005; Martinussen et al., 2005; Willcutt et al., 2005). Moreover, a recent study in rodents suggested that D1 stimulation contributes to cognitive-enhancing effects of methylphenidate, a leading treatment for ADHD (Arnsten and Dudley, 2005a).

D1 signalling is mediated in the brain by the heterotrimeric G proteins G_s and G_{olf} (Corvol et al., 2001; Zhuang et al., 2000), which cause activation of adenylyl cyclase, cAMP-dependant protein kinase, and DARPP32. D1 receptors also signal via phospholipase C-dependent mobilization of intracellular calcium (Undie and Friedman, 1990; Wang et al., 1995), likely involving calcyon (Lezcano et al., 2000). Lesion experiments and knockout studies have indicated that the coupling of D1 receptors to adenylyl cyclase is mostly provided by $G\alpha_{olf}$ in the striatal neurons, and that $G\alpha_{olf}$ is required for D1-mediated behaviour and biochemical effects in the striatum (Corvol et al., 2001; Herve et al., 1993; Zhuang et al., 2000). $G\alpha_{olf}$ appears to be highly regulated by receptor usage and availability of interacting/effector proteins (Corvol et al., 2004, 2001; Herve et al., 2001, 1993; Iwamoto et al., 2004; Schwindinger et al., 2003; Zhuang et al., 2000), suggesting that it represents a limiting factor in the coupling efficiency of D1 receptors.

Based on our previous finding for *DRD1* in ADHD symptoms and the regulatory role played by $G\alpha_{olf}$ in D1 signalling, we believe that the $G\alpha_{olf}$ gene, *GNAL*, is a reasonable candidate for involvement in ADHD susceptibility. This is further supported by the locomotor behaviour of the mice deficient for $G\alpha_{olf}$. When tested in open field exercises, the *GNAL*+/− mice exhibit a slight decrease in basal locomotor activity, while the −/− mice display locomotor hyperactivity (Belluscio et al., 1998; Schwindinger et al., 2003) similar to a D1 knockout (Xu et al., 1994a,b).

The *GNAL* gene is located on the short arm of chromosome 18 in a region that has been linked to bipolar disorder and schizophrenia (Berrettini, 2000; Schwab et al., 2000; Segurado et al., 2003), with some evidence of parent-of-origin effects (Gershon et al., 1996; Nothen et al., 1999; Stine et al., 1995). However, replication studies have led to conflicting results (Van Broeckhoven and Verheyen, 1998; Zill et al., 2003).

In the present study, we sought evidence for association between *GNAL* and ADHD in a sample of clinically ascertained nuclear families. We tested for the non-random transmission of alleles of 12 single nucleotide polymorphisms (SNPs) using the transmission/disequilibrium test (TDT) statistic (Spielman and Ewens, 1996). Given previous findings suggesting parent-of-origin effects at 18p and evidence of epigenetic modification of *GNAL* (Corradi et al., 2005), we also assessed transmissions from mothers and fathers separately. Finally, we performed quantitative analysis using ADHD inattentive and hyperactive/impulsive symptom counts.

2. Materials and methods

2.1. Study sample and diagnostic assessment

The methods of assessment, characteristics of the subjects, and inclusion/exclusion criteria have been described previously, including the instruments used to collect information for the diagnosis of ADHD and co-morbid conditions (Barr et al., 1999; Laurin et al., 2005; Quist et al., 2000). Briefly, probands and their siblings between 7 and 16 years old were included if they met DSM-IV criteria for one of the three ADHD subtypes. The study sample was comprised of 258 nuclear families from the Toronto area, including 53 affected siblings. This gave a total of 311 affected children (251 boys and 60 girls) that were genotyped along with 209 fathers and 243 mothers. The sample consists of 194 parents-child trios and 64 families in which a single parent was genotyped. The distribution of the affected children among the DSM-IV ADHD subtypes was 14% of the predominantly hyperactive/impulsive subtype, 24% of the predominantly inattentive subtype and 62% of the combined subtype. All children were free of medication for 24 h before assessment. This protocol was approved by the Hospital for Sick Children's Research Ethics Board and informed written consent or verbal assent (children) was obtained for all participants.

Information on ADHD symptoms was obtained using semi-structured interviews for parents (Parent Interview for Child Symptoms: PICS-IV; Ickowicz et al., 2006) and teachers (Teacher Telephone Interview: TTI-IV; Tannock et al., 2002). These instruments were used to determine symptom scores based on the nine DSM-IV criteria for both inattention and hyperactivity/impulsivity dimensions. In our study sample, parent-reported symptom scores range from 0 to 9 for both hyperactive/impulsive (mean $= 5.54 \pm 2.34$) or inattentive (mean $= 5.85 \pm 1.99$) behavior. The corresponding teacher-reported scores also range from 0 to 9 (mean $= 4.17 \pm 2.78$ and 5.22 ± 2.21, respectively).