

Analysis of *GNAL* Polymorphisms in Attention Deficit Hyperactivity Disorder

Hande Ayraler Taner¹, Sahnur Sener², Sezen Güntekin Ergün³, Mehmet Ali Ergün^{3*} and Esra Güney^{2*}

¹Kastamonu Dr. Münif Islamoglu State Hospital, Kastamonu, Turkey, 37100

²Gazi University Medical Faculty, Child and Adolescent Psychiatry Department, Ankara, Turkey, 06500

³Gazi University Medical Faculty, Department Of Medical Genetics, Ankara, Turkey, 06500

E-mail: ¹<h_ayraler@hotmail.com>, ²<sahnur@gazi.edu.tr>, ³<nsezen@yandex.com>, ^{3*}<mali@yandex.com>, ^{2*}<dresraguney@gmail.com>

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ABSTRACT Attention deficit hyperactivity disorder (ADHD) is a childhood-onset neuropsychiatric disorder. Dopamine related genes have been reported to be associated with ADHD. Dopamine 1 and 5 receptors together with the olfactory alpha subunit of the GTP-binding protein (G_{olf}) in the striatum, mediate adenylyl cyclase activation. The aim of the paper was to investigate the correlation between ADHD, subtypes, family history of ADHD in rs8095592, and rs3892113 in *GNAL* (the gene that codes G_{olf}). 100 children with ADHD and 81 healthy controls were recruited for the study. Genetic evaluation was performed with venous blood samples. The frequency of the genotypes and alleles in rs8095592 and rs3892113 was not significantly different between the patient and control groups. The GG genotype in rs8095592 was significantly more common in the patients who had a family history of ADHD. In conclusion, the presence of the allele A in rs8095592 could be preventive from ADHD in those with family history.

INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is a neuropsychiatric disorder with early childhood onset that is characterized by attention deficit, over activity, and impulsivity. Polanczyk et al. (2007) reported that the worldwide prevalence of ADHD was 5.29 percent and Ramtekkar et al. (2010) observed the male/female ratio of ADHD as 2.28:1. It's a non-Mendelian dominant genetic aspect which has variable phenotypic expression. ADHD shows a high heritability (Schachar 2014). Some studies suggested that genes involved in dopamine metab-

olism, especially the dopamine receptor D4 (*DRD4*) gene, dopamine transport gene, and *DRD5* gene, as well as genes that influence serotonin and noradrenaline pathways, play various roles in the etiology of ADHD (Caylak 2012; Wu et al. 2012; Akutagawa-Martins et al. 2013). Dopamine receptor 1 (*DRD1*) and Dopamine receptor 5 (*DRD5*) belong to the family of D1-like receptors. Some studies have reported an association between the *DRD1* gene and ADHD (Misener et al. 2004; Bobb et al. 2005; Ribases et al. 2012). Trampush et al. (2014) reported that there was an association between improved higher order working memory and manipulation skills in ADHD as well as with SNPs rs4532 and rs265978 in the *DRD1* gene.

The activation of cyclic adenosine monophosphate (cAMP) in the *DRD1* signaling pathway occurs via G proteins G_s and G_{olf} . G_{olf} plays a more important role than G_s in the regulation of striatal *DRD1* activities (Corvol et al. 2001). Research has shown that the frontostriatal circuitry of children with ADHD differs from that of children without the condition (Cortese 2012). Striatal neuroanatomical differences have also been observed between patients who respond well to ADHD medication and those who do not

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Address for correspondence:

Dr. Hande Ayraler Taner
Kastamonu Dr. Münif Islamoglu Devlet
Hastanesi Ek Hizmet Binası
Candaroğulları mah. Alparslan
Türkeç Bulvarı No:19 37100
Merkez/Kastamonu, Turkey
Telephone: +90 366 214 1053
Fax: +90366 2120366
E-mail: h_ayraler@hotmail.com

(Moreno et al. 2014). The function of the striatal dopamine 1 receptor depends on G_{olf} and the researchers showed that locomotor and rearing activity increased significantly in G_{olf} knockout mice and that they did not respond to dopamine 1 receptor agonists (Zhuang et al. 2000). Many studies have investigated the role of the *GNAL* gene, which codes the G_{olf} protein, in the etiology of psychiatric disorders; including bipolar disorder and major depression (Berrettini et al. 1998; Zill et al. 2002; Zill et al. 2003) and recently in a neurological disorder, dystonia (Fuchs et al. 2013). Laurin et al. (2008) reported that there may be an association between ADHD and *GNAL*, and Das Banerjee et al. (2008) observed altered expression of the gene in genetic and environmental models of ADHD.

This study aimed to detect the relationship between the single nucleotide polymorphisms (SNPs) rs8095592 and rs3892113 found in intron 3 and intron 10, in the *GNAL* gene and ADHD respectively. It also aimed to investigate the relationship of these SNPs with ADHD subtypes and a family history of ADHD.

METHODOLOGY

The paper included 100 patients within the range of 6–18 years who were diagnosed with ADHD according to Diagnostic and Statistical Manual of Mental Disorders (DSM IV) ADHD criteria (APA, 2000) at Gazi University, Pediatric Psychiatry Department between May 2010 and June 2012 and 81 healthy controls. Patients diagnosed with additional psychiatric diseases by clinical examination and psychometric assessments, a family history of bipolar disorder or schizophrenia, an IQ <70, and learning disability were excluded. A detailed psychiatric and developmental history was obtained from each patient. Blood residues of routine blood tests were drawn into EDTA-containing tubes. The control group included 81 subjects of the above department who fall within the age bracket of 6–18 years and without psychiatric disease. All the children and families included in the study gave written informed consent. The study was approved by Gazi University Institutional Research Rating Commission.

Genetic Analysis

Peripheral venous blood samples containing 5 ml of blood were drawn into EDTA-containing tubes and stored at -20° C until the isolation stage.

DNA isolation was carried out with the spin column method (MN Macherey-Nagel, Germany) using blood samples of patients and controls. The isolated DNA samples were measured in a spectrophotometer (NanoDrop, ND 1000, USA). The polymerase chain reaction (PCR) method was used to magnify genetic material.

A forward primer, 5'-ATTCTGGACATCCG-GAAAAA3', and backward primer, 5'-CAGTTT-TAGAATGAAGAGCACTgG-3', pair was used for the SNP rs8095592, which contains an A/G missense mutation 35 bp away from the third intron of the 3' region of the *GNAL* gene. A forward primer, 5'-CCTGGGCAAGAGAGTGAGAC-3', and backward primer, 5'-TTGGGATCTTCTC-CTGCATC-3', pair was used for the SNP rs3892113, which contains a T/G missense mutation 7 bp away from the 10th intron of the 3' region of the *GNAL* gene.

A total of 100 and 214 bp products were obtained for the region in the third intron and the region in the 10th intron of the *GNAL* gene, respectively. The PCR product was cut with an *Ava*II restriction enzyme in order to assess the polymorphism in the third intron of the *GNAL* gene. It was tagged a G allele when two bands of 74 and 26 bp were observed and as an A allele when no DNA was cut (100 bp). To assess polymorphism in the 10th intron of the *GNAL* gene, the PCR product was cut with a *Bsr*DI restriction enzyme. It was tagged as a G allele when two bands of 175 and 39 bp were observed and as a T allele when no band was cut (214 bp). For both polymorphisms, the genotypes were determined according to bands obtained in agarose gel.

Statistical Analysis

Statistical analysis of the data was performed using Windows SPSS 15.0 software package. In categorical variables percentages and numbers and in continuous variables mean and standard deviation were used. Categorical variables were compared using a Chi-square test and Fisher's exact test. A logistic regression analysis was carried out to detect risks. A *p* value less than 0.05 was considered statistically significant.

RESULTS

This study included 88 males and 12 females with a diagnosis of ADHD and 48 males and 33 females with no psychiatric disease as the con-

trol group. The mean age of the patient group was 9.74 $\pm$ 2.4 years, while the mean age of the control group was 14.3 $\pm$ 3.6 years.

The frequency of the A and G alleles in the healthy controls with the rs8095592 SNP was 21.6 percent and 78.4 percent, respectively, and the frequency of the T and G alleles in those with the rs3892113 SNP was 92 percent and 8 percent, respectively. A Chi-square analysis of the frequency of three different genotypes (AA, AG, and GG) formed by A/G transitions in intron 3 in the patient and control groups revealed no significant differences ($\chi^2=1.51$, $p=0.46$). The Chi-square test of the A and G allele frequency in the ADHD and control groups showed no statistically significant difference between the groups ($\chi^2=0.42$, $p=0.51$). The results are presented in Table 1.

Table 1: The distribution of rs8095592 genotypes and frequency of alleles in ADHD and control groups

	Genotype	ADHD N (%)	Healthy N (%)	<i>p</i>
	AA	10 (10)	9 (11.1)	0.46
	AG	61 (61)	55 (67.9)	
	GG	29 (29)	17 (21)	
Allelic frequency	A	0.245	0.216	0.51
	G	0.755	0.784	

A Chi-square analysis of the frequency of three different genotypes (TT, TG, and GG) formed by a T/G transversion in intron 10 showed that the frequency of the three genotypes was similar in both groups ($\chi^2=0.855$, $p=0.652$). There was also no significant difference in the frequency of the T and G alleles in the ADHD and control groups ($\chi^2=0.5$, $p=0.47$). The results are shown in Table 2.

The distribution of the ADHD sub-types in the 100 patients was as follows: mixed type 81 percent ($n=81$), attention deficit dominant type

Table 2: The distribution of rs3892113 genotypes and frequency of alleles in ADHD and control groups

	Genotype	ADHD N (%)	Healthy N (%)	<i>p</i>
	TT	82 (82)	68 (84)	0.65
	TG	17 (17)	13 (16)	
	GG	1 (1)	0 (0)	
Allelic frequency	T	0.905	0.92	0.47
	G	0.095	0.08	

18 percent ($n=18$), and hyperactivity/impulsivity dominant type 1 percent ($n=1$). In the ADHD subtypes with the rs8095592 SNP, the percentage of AA and AG genotypes was 71.6 ($n=58$) in mixed type ADHD, 66.7 ($n=12$) in attention deficit dominant type, and 79 ($n=64$) in the control group. The percentage of GG genotypes in the mixed type was 28.4 ($n=23$), whereas it was 33.3 ($n=6$) in the attention deficit dominant type and 21 ($n=17$) in the control group. Although the GG genotype was relatively common in the attention deficit dominant type, yet it was not statistically significant ($\chi^2=1.8$, $p=0.40$). In the ADHD subtypes with the rs3892113 SNP, 84 percent ($n=68$) of patients diagnosed with mixed-type ADHD had the TT genotype, 14.8 percent ($n=12$) had the TG genotype, and 1.2 percent ($n=1$) had the GG genotype. Of the patients with attention deficit dominant-type disorder, 72.2 percent ($n=13$) had the TT genotype, and 28.7 percent ($n=5$) had the TG genotype. Since only one patient had a hyperactivity/impulsivity dominant-type disorder, it was not included in the analysis. Eighty-four percent ($n=68$) of those in the control group had the TT genotype, and 16 percent ($n=13$) had the TG genotype. Compared to the control group, there was a relatively high rate of the TG genotype, albeit statistically non-significant, in those with the attention deficit dominant-type disorder ($\chi^2=30$, $p=0.55$).

Comparison between the family history of ADHD in the patient and control groups revealed a significantly higher rate of ADHD in the former ($\chi^2=8.68$, $p=0.03$). From the patient group, comparison of subjects with or without a family history of ADHD with the control group for rs8095592 polymorphism revealed that the GG genotype was significantly more common in patients with a family history of ADHD compared to the other two groups ($\chi^2=6.38$, $p=0.04$). The results are presented in Table 3.

Table 3: The comparison of rs809592 genotypes between the ADHD patients with the family history of ADHD, ADHD patients without the family history of ADHD and healthy control groups

Geno- type	ADHD F.H.A. N (%)	ADHD- F.H.A. N (%)	Healthy N (%)	<i>p</i>
AA+AG	6 (46.2)	65 (74.6)	64 (74)	0.04
GG	7 (53.8)	22 (25.3)	17 (21)	

ADHD+F.H.A.: Patients with Family History of ADHD
ADHD-F.H.A.: Patients without Family History of ADHD

The risk of having the GG genotype increased 4.3 fold as compared with the controls when there was a familial history of ADHD ($p=0.01$, OR: 4.39 95% CI: 1.3–14.7).

Evaluation of the rs3892113 genotypes in terms of the presence of a family history of ADHD yielded no significant difference between the patient ($\chi^2=0.52$, $p=0.77$) and control ($\chi^2=0.19$, $p=0.66$) groups with respect to the genotypes.

DISCUSSION

This paper examined the potential association between the rs8095592 polymorphism formed by the A/G transition in intron 3 of the *GNAL* gene and the rs3892113 polymorphism formed by the T/G transversion in intron 10 of this gene. The allelic frequencies of A and G were 21.6 percent and 78.4 percent, respectively, in controls with the rs8095592 polymorphism. The frequency of the T and G alleles was 92 percent and 8 percent, respectively, in those with the rs3892113 SNP. In a study of these two polymorphisms in German and Greek samples, Zill et al. (2002, 2003) reported that the allelic frequency of A and G was 28–31 percent and 69–72 percent, respectively, in a control group with the rs8095592 SNP. They reported that the allelic frequency of T and G was 88–94 percent and 6–12 percent, respectively, in those with the rs3892113 SNP. In a study of an American sample, the allelic frequency of A and G was 69 percent and 31 percent, respectively, in controls with the rs8095592 SNP, while that of T and G was 84 percent and 16 percent, respectively, in those with the rs3892113 SNP (Berrettini et al. 1998). The results of the present paper are similar to those of the European sample. As this study is the first to investigate rs8095592 and rs3892113 polymorphisms in a Turkish population, the allelic frequencies in the control group can guide future studies.

In the present paper, similar to that reported by Laurin et al. (2008), there was no significant difference in the frequencies of the rs8095592 and rs3892113 polymorphisms between the patient and control groups. Moreover, the paper revealed no association between the rs8095592 and rs3892113 polymorphisms and ADHD subtypes. However, the presence of the GG genotype in those with the rs8095592 SNP and the TG genotype in those with the rs3892113 SNP increased the tendency of the attention deficit dominant-type disorder, although this tenden-

cy was statistically non-significant. Attentional deficits in ADHD are related to nigrostriatal dopaminergic mechanisms (del Campo et al. 2013). Dopamine 1 like receptors in the dorsal striatum plays an important role in attention processes (Agnoli et al. 2013). Reward-based learning systems are supported by D1 receptors in the basal ganglia (Cools 2008). Based on the aforementioned cases, it has been suggested that SNP's may alter the amount and function of $G_{\alpha i}$, thereby impairing attention-related pathways and giving rise to the attention deficit dominant-type disorder.

Family history of ADHD is greater in ADHD patients than controls (Biederman et al. 2010). The current paper demonstrated that ADHD patients with a family history of ADHD had a significantly higher possibility of having the GG genotype in rs8095592, which suggests that having an A allele may be a protective factor against ADHD. On the other hand, having the GG genotype may trigger the emergence of ADHD among those at risk of the disorder (for example, those with a family history of ADHD).

CONCLUSION

ADHD is a multifactorial disorder which has genetic factors. Although many papers have investigated the genetic mechanisms of ADHD, data are lacking on the role of dopamine D1 receptors in this disorder. In this paper, the frequency of the GG genotype in rs8095592 SNP of *GNAL* was increased in patients diagnosed with ADHD who had a family history of the disorder.

RECOMMENDATIONS

The findings of this paper and those of similar papers of candidate genes may be used to screen individuals who are at risk (for example, those with a family history of ADHD) for polymorphisms and will also help to diagnose the disorder at an early stage. The information can be used to provide genetic counseling, refer patients for appropriate therapy, and eliminate preventable risk factors.

LIMITATIONS

This paper has some limitations. The control group and patient group weren't matched in sex and age. Also, this paper lacked a structured/semi structured interview scale. Further studies

with age and sex matched patient-control groups, using a structured/ semi structured interview scale is needed.

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