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Association of 5-HT1A and 5-HT1B Gene Polymorphisms with Obsessive-Compulsive Disorder in a Turkish Population

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ABSTRACT:

Association of 5-HT1A and 5-HT1B gene polymorphisms with obsessive-compulsive disorder in a Turkish population

Objective: Obsessive-compulsive disorder (OCD) is a frequent neuropsychiatric disorder, in which genetic factors play important causative roles. We investigated the roles of the (-1019 C/G) promoter region polymorphism of 5-HTR1A and the G861C coding region polymorphism of 5-HTR1B genes in susceptibility to OCD in a Turkish population.

Methods: Two single nucleotide polymorphisms, 5-HTR1A (rs6296) and 5-HTR1B (rs6295) genes were genotyped in 76 OCD patients and 57 healthy controls that were unrelated, using PCR-RFLP method.

Results: We did not observe any difference in the genotype distributions of rs6296 and rs6295 between the OCD patient and control groups.

Conclusions: As far as we know, our study is the first to establish the association of genetic variants 5-HTR1A (rs6296) and 5-HTR1B (rs6295) with OCD in a Turkish population. Based on our results, the relationship between polymorphisms of 5-HTR1A (rs6296) and 5-HTR1B (rs6295) with OCD do not seem.

Keywords: genetics, OCD, 5HTT gene

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INTRODUCTION

Obsessive-compulsive disorder (OCD) is a psychological disorder characterized by obsession and/or compulsion[1]. Obsession or compulsion is defined as repetitive ideas, urges, or images that enter the unconscious mind and are known by the person with OCD to be irrational. Compulsions are generally motor or mental actions performed to prevent an obsession with significant rules[2].

OCD is a defect that is considered neurobiological. Family, twins, and adoption studies have suggested roles for hereditary genetic factors in OCD etiology. In twins studied for OCD, the concordance rate in monozygotic twins was higher than in dizygotic twins (75% and 30%, respectively)[1,2]. In family studies, it was found that 35% of first-degree relatives of OCD patients were

affected by this disorder[1,2]. Besides these, first-degree relatives of patients with OCD have been reported to have 5 times more OCD than the first-degree relatives of healthy people, and it was also demonstrated that compulsions have a lesser probability of passing to the next generations than obsessions[3]. However, studies performed to date have not demonstrated Mendelian models of transmission. For this reason, inheritance patterns of OCD are believed to be complex and irregular[2-4]. In recent years, genetic studies have explored the therapeutic and protective roles of drugs and chemical agents, as well as the molecular basis of neuropsychologics, such as OCD. These studies increase our understanding of the molecular basis of OCD.

Possible associations have been suggested between serotonin (5-hydroxytryptamine, 5-HT), dopamine (DA), and several physiological and pathological conditions. Different subtypes of serotonin receptors including the 5-hydroxytryptamine 1A (HTR1A), 5-hydroxytryptamine 1B (HTR1B), and 5-hydroxytryptamine 2A (HTR2A) receptors have been shown to mediate the regulation of 5-HT over DA neurotransmission. Previous studies have also demonstrated an important role of 5-HT in OCD pathophysiology[5,6]. Since SSRIs show their action on the serotonin transporter (5HTT), it has been suggested that the 5HTT gene (SCL6A4), located on chromosome 17q11.2, could be a good candidate for OCD[7,8]. This gene encodes a G-protein-coupled receptor for 5-HT which belongs to the 5-HT receptor subfamily. Inactivation of this gene in mice was found to be associated with a behavior consistent with an increased anxiety and stress response[9,10].

In this study, we aimed to examine the effect of (-1019 C/G) promoter polymorphisms of the 5-HTR1A gene and G861C coding region polymorphism of the 5-HTR1B gene on the susceptibility to OCD in a Turkish population. To our knowledge, this is the first report of the relationship between the variants 5-HTR1A (rs6296) and 5-HTR1B (rs6295) and OCD in a Turkish population.

METHODS

Study Sample

This study was approved by the local Institutional Ethics Review Committee. Study groups were chosen from patients that applied to the Psychiatry Outpatient Clinic of Turgut Ozal University Hospital. Patient and control groups were not gender-matched since OCD has no known associations with gender. This study included 76 OCD patients (22 male and 54 female) and 57 control subjects (32 male and 25 female). Patient group consisted of those patients with OCD that consequtively admitted to the outpatient clinics and whose disease started at least one year ago. Control subjects were selected from healthy controls without any history of psychiatric disorder. Informed consent was obtained from all subjects that participated in the study. The OCD patients and healthy controls were chosen from the same geographic region and were of identical ethnicity.

Clinical Evaluation Interview

All OCD patients were diagnosed by experienced psychiatrists after a clinical interview. This interview included Yale-Brown Obsessive-Compulsive Scale (YBOCS) ratings and incorporated mood, anxiety, and psychotic disorder questions based on the Structured Clinical Interview for DSM-IV (SCID-I)[11]. SCID-I is a semi-structured evaluation scale developed by First et al.[12] which systematically reviews the DSM-IV diagnostic categories in clinical samples and is used for diagnostic purposes. Validity and reliability of the Turkish version of this scale was performed by Çorapcioglu et al.[13].

The patients were unrelated. Patients with neurological, metabolic, or psychiatric diseases, mental retardation, and alcohol and/or drug dependence were excluded. Subjects were questioned about a history of suffering from vocal and/or motor tics at any time in their life. Family history was obtained either by a direct interview

Table 1: Primer sequences of each single-nucleotide polymorphism (SNP)

SNP	Polymerase chain reaction primers	Denaturation temperature	Products length	Restriction enzyme
HTR1A C1019G; rs6295	F5'GAGGGAGTAAGGCTGGACTGT3' R5'TGGAAGAAGACCGAGTGTGTCTAC3'	61°C 56°C	176bp 548bp	HpyCH4IV HincII
HTR1B G861C; rs6296	F5' GAAACAGACGCCCAACAGGAC 3' R 5' TA GAAGAAAGCGCCAAAGACC 3'			

with the subject or through indirect interviews with close relatives. Physical examinations were performed and blood samples were obtained for complete blood count and blood chemistry, as well as for the molecular analysis of 5- HTR1A and 5-HTR1B polymorphisms.

Instruments

The Sociodemographic Data Form was prepared to register sociodemographic properties and disorder-related information of the patients.

Yale-Brown Obsessive Compulsive Scale (Y-BOCS): Y-BOCS, as improved by Goodman in 1989, was used to measure rage, type, and changes in clinical processes with prognostic treatment of obsessive-compulsive behavior. The first section of YBOCS is a symptom and control list that includes 74 questions. The second section comprises 19 items. The first 5 questions test obsession and the next 5 test rage compulsions. All symptoms were estimated between 0 to 4 points according to patient visits, effects on the life of these patients, the disorder, resistance rate, and level of control. Next, the subtotal of obsession and compulsion and the grand total were estimated[14]. Validity and reliability of the Turkish version of Y-BOCS had been demonstated by Tek et al. (1995)[15].

HTR1A and HTR1B Single-Nucleotide Polymorphism Genotyping: Genomic DNA was extracted from peripheral blood leukocytes using a DNA extraction Kit (Qiagen: Puregene Blood Core Kit B). The primers of these two single nucleotide polymorphisms (SNPs) were designed using Primer Premier 5.0. Determinations of the 5-HTR1A and 5-HTR1B polymorphisms were performed by using the Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR-RFLP) method according to the manufacturer's instructions. The primer sequences, annealing temperatures, and restriction enzymes used for detecting each SNP were presented in Table 1.

The PCR conditions were as follows: an initial denaturation for 5 min at 94°C followed by 35 cycles of denaturation for 30 sec at 94°C, annealing for 1 min at 61°C (5-HTR1A) and 30 sec at 56°C (5-HTR1B), and extension for 45 sec at 72°C, and a final extension for 5 min at 72°C. A 5-µl aliquot of the PCR products were electrophoresed on a 2% agarose gel at 90 V for 30 min. The fragments were visualized using ethidium bromide under a UV transilluminator. The resulting PCR products were 176 and 548 bp specific for the (-1019 C/G) and G861C alleles, respectively.

The PCR products of the HTR1A gene were also digested with HpyCH4IV (New England Biolabs) enzyme. The restrictions were performed using 15 µl of PCR product, 3 µl of NE Buffer (10x concentrate), 0.1 of µl of enzyme (HpyCH4IV), and 31.9 µl of ddH2O. In a final volume of 50 µl, samples were incubated at 37°C for 25 hours and then incubated a further 15 minutes at 65°C. PCR products were digested with HincII Fast Digest (Thermo Scientific) enzyme. Restrictions were performed using 10 µl of PCR product, 2 µl of 10× Fast DigestBuffer (Thermo Scientific), 1 µl of enzyme (HincII), and 17 µl of ddH2O. In a final volume of 30 µl, samples were incubated at 37°C for 1 hour and then incubated for a further 5 minutes at 65°C for the HTR1B gene. PCR-RFLP products were electrophoresed on a 3% agarose gel at 90 V for 50 min. The fragments were visualized

by ethidium bromide under a UV transilluminator.

The homozygous G/G genotype produced a 176-bp fragment, and the homozygous C/C genotype produced two fragments of 27 and 149 bp, whereas the heterozygous genotype G/C produced three fragments of 27, 149, and 176 bp for the HTR1A (-1019 C/G) polymorphism. The intact DNA fragment was 548 bp; after digestion, the G allele yielded two fragments (452 and 96 bp) and the C allele yielded 3 products (142, 310, and 96 bp).

Statistical Analysis

SPSS 16.0 (SPSS Inc., Chicago, IL, USA) was used for performing the statistical analyses. The distribution of 5-HTR1A and 5-HTR1B gene polymorphisms between OCD patients and controls were compared by using the χ^2 test. Results with p values <0.05 were considered to be statistically significant. Median age of the participants in the two groups was compared by using the Mann Whitney U Test. Comparison of the categorical variables between the groups was performed by using the chi-square test.

RESULTS

The study group consisted of 76 patients with OCD (22 male and 54 female; median age (min-max):31 (15-60) and 57 healthy subjects (32 male and 25 female; median age (min-max):29 (19-39) who had participated in our previous study. The size of sample required for the study is calculated via (or with the help of) a free computer based software, G*Power (G*Power Ver. 3.1.9.2, Franz Faul, Universität Dusseldorf, Germany, http://www.gpower.hhu.de/). The minimum size of the sample required for our primary outcome variable 5HT1b α=0.05 Type I error, β=0.10 Type II error and impact width with 90% power w=0.33 (to determine the CC gene incidence of 5HT1b between the groups with at least 15% difference) in the study was determined as 99 volunteers. As long as we have two groups and at least a total of 99 volunteers are required for the study, it was determined to include at least 50 volunteers to each group, a total of 100 volunteers to the study. As a precaution for possible data loss, we took more than 50 volunteers to each group, thus the study is completed with a

Table 2: The demographic characteristics of patients with OCD and healthy controls

Demographic features	OCD patients n=76	Healthy controls n=57	Test	p
Age (years) median (min-max)	31(15-60)	29(19-39)	z=-1.917	0.055
Gender (female/male)	54/22	25/32	χ^2=9.98	0.002

χ^2: Ki kare test, z: Mann Whitney U test, SD: Standart Deviation

Table 3: Frequencies of the genotypes and alleles of C/G and G/C polymorphism of 5HTR1 gene of turkish patients with OCD and healthy subjects

		Genotype distrubition (%)		Genotype association p-value
		OKB n=76	Control n=57	
HTR1A	CC	24 (31.6)	10 (17.5)	0.066
C1019G;rs6295	CG+GG	52 (68.4)	47 (82.5)	
HTR1B G861C;rs6296	GG	35 (46.1)	28 (49.1)	0.726
	CG+CC	41 (53.9)	29 (50.9)	
		Allele distrubition (%)		Allele association p-value
HTR1A C1019G; rs6295	C	87 (57.2)	56 (49.1)	0.189
	G	65 (42.8)	58 (50.9)	
HTR1B G861C; rs6296	G	106 (69.7)	83 (72.8)	0.585
	C	46 (30.3)	31 (27.2)	

G allel carriers (GG, GC), C allel carriers (CC)

total of 133 volunteers, 57 in control group and 76 in OKB group.

The demographic characteristics of OCD patients and healthy controls are shown in Table 2. Mean Y-BOCS scores of the patients was 19.47±6.20 and that of the controls was 1.63±2.48 ($p<0.001$). There were no statistically significant differences between the two groups in terms of median age ($p>0.05$). The gender distribution in the two groups was statistically different ($p<0.01$). After the clinical interviews, we did not detect any tic disorder or Tourette disorder among patients or their first-degree relatives. All of the polymorphisms were in Hardy-Weinberg equilibrium. The genotype distribution and allelic frequencies of the 5-HTR1A (-1019 C/G) and 5-HTR1B G861C polymorphisms in patients with OCD and healthy controls are shown in Table 3.

There were no statistically significant differences in genotypes ($p=0.066$; $p=0.726$ respectively) and allele frequencies ($p=0.189$; $p=0.585$ respectively) in OCD patients and healthy controls for 5-HTR1A C-1019G and 5-HTR1B G861C polymorphisms. There were no statistically significant correlations between OCD and these polymorphisms. Since our sample size was small, statistical analyses with sufficient power could not be performed between 5-HTR1A and 5-HTR1B polymorphisms in our study.

DISCUSSION

OCD is a severe multifactorial psychiatric condition affecting up to 3% of the general population[16,17]. Despite similar clinical results, the underlying etiological mechanisms show differences between societal groups depending on the genetic foundation. Recently, more than 200 functional and positional candidate gene studies of OCD have been reported, predominantly for variants within genes in the dopamine, serotonin, and glutamate pathways. Serotonergic system-related genes are likely involved in mechanisms underlying the OCD.

The 5-HT1A receptors are found in different regions of the brain. These receptors seem to have roles in the regulation of body temperature, feeding behavior, depression and anxiety disorders[9]. G861C variations in 5-HT1B receptors have been extensively analyzed in OCD patients. Especially, the G allele, which was shown to be associated with a lower expression profile in the prefrontal cortex of subjects, was found to be risk factor for obsessive symptoms or to be associated with a worse presentation of symptoms[19]. There are conflicting results because they may be associated with underpowered samples or different ethnic groups[19,20].

We examined whether (-1019 C/G) polymorphisms in 5-HT1A and G861C polymorphisms in 5-HT1B receptors are associated with OCD in a Turkish population. We observed that neither of the polymorphisms are associated with OCD in the study population. The same receptor polymorphisms have been studied in panic disorder which is a different form of neuropsychiatric disorder. Rothe et al.[20] found no association between the 5-HTR1A C(-1019)G polymorphism and panic disorder. However, in a subsequent study a significant association between the G allele and panic disorder with agoraphobia was reported, also an association between the C allele and panic disorder was also detected[21]. A recent study demonstrated that the G allele is significantly associated with reduced anxiety-related amygdala reactivity[22]. Our findings suggest that the 5-HTR1A (-1019 C/G) polymorphism may contribute to the relationship between the 5-HT1A receptor gene and OCD.

A significant association was reported by Mundo and colleagues between OCD and the G861C polymorphism in a large Canadian family-based sample. There was an association between the G-allele and OCD, OC severity. Although the association between severity of symptoms and the G-allele was confirmed in a subsequent study, other studies have not reported such association[1]. As our OCD study sample was small, or because of the ethnic differences our results could be misleading and inconclusive. Thus studies of larger groups of patients are required to clarify the roles of the 5-HTR1A (-1019 C/G) and

5-HTR1B G861C polymorphisms as a risk factor for OCD. Another important limitation of this study is that the present study was designed according to DSM-IV. Following studies with larger sample sizes may be designed according to DSM-V. Further studies are required to evaluate other SNPs in different ethnic groups of patients to be able to determine the role of the serotonergic receptor gene variants more clearly as risk factors for OCD.

Since some allele and genotypes reach significance when only the haplotype or diplotype are related to the low incidence of patients other polymorphisms are required to show necessary penetrance. This relationship can be further explored based on studies involving a greater number of patients. Over time, treatment strategies progress to target specific modalities. If the underlying etiological mechanisms are known, treatment is organized according to this information. Based on our results, genetic and molecular studies applicable to other societal groups are required.

CONCLUSIONS

Based on our results, an allelic variation in the 5-HTR1A (rs6296) and 5-HTR1B (rs6295) do not seem to increase risk of OCD. However, our study was performed on a small group of patients and results of much larger studies are needed to make definitive conclusions. We consider that our study may contribute to initiation and performance of other studies related to this issue.

References:

1. Mattheisen M, Samuels JF, Wang Y, Greenberg BD, Fyer AJ, McCracken JT, et al. Genome-wide association study in obsessive-compulsive disorder: results from the OCGAS. Mol Psychiatry 2015;20(3):337-44. [CrossRef]
2. Demet MM. Genetic studies in obsessive-compulsive disorder. Klinik Psikofarmakoloji Bulteni-Bulletin of Clinical Psychopharmacology 2005;15(1):45-52. (Turkish)
3. Scherrer JF, Xian H, Slutske WS, Eisen SA, Potenza MN. Associations between obsessive-compulsive classes and pathological gambling in a national cohort of male twins. JAMA Psychiatry 2015;72(4):342-9. [CrossRef]
4. Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, et al. International union of pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). Pharmacol Rev 1994;46(2):157-203.
5. Vardar E. Genetics of obsessive compulsive disorder. Klinik Psikofarmakoloji Bulteni-Bulletin of Clinical Psychopharmacology 2000;10(3):153-159. (Turkish)
6. Atmaca M, Onalan E, Yildirim H, Yuce H, Koc M, Korkmaz S, et al. Serotonin 5-HT1DB gene's interaction with key brain regions in obsessive-compulsive disorder Klinik Psikofarmakoloji Bulteni - Bulletin of Clinical Psychopharmacology 2010;20(1):23-8.
7. Goddard AW, Shekhar A, Whiteman AF, McDougle CJ. Serotoninergic mechanisms in the treatment of obsessive-compulsive disorder. Drug Discov Today 2008;13(7-8):325-32. [CrossRef]
8. Lesch KP, Balling U, Gross J, Strauss K, Wolozin BL, Murphy DL, et al. Organization of the human serotonin transporter gene. J Neural Transm Gen Sect 1994;95(2):157-62. [CrossRef]
9. Lesch KP, Gutknecht L. Focus on the 5-HT1A receptor: emerging role of a gene regulatory variant in psychopathology and pharmacogenetics. Int J Neuropsychopharmacol 2004;7(4):381-5. [CrossRef]
10. Ansorge MS, Zhou M, Lira A, Hen R, Gingrich JA. Early-life blockade of the 5-HT transporter alters emotional behavior in adult mice. Science 2004;306(5697):879-81. [CrossRef]
11. American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). Washington DC: American Psychiatric Association, 1994.
12. First MB, Spitzer RL, Gibbon M ve ark. Structured Clinical Interview for DSM-IV Axis I Disorders, Clinician Version (SCID-CV). Washington DC: American Psychiatric Press, Inc, 1996.
13. Corapcioglu A, Aydemir O, Yildiz M, Esen Danaci A, Koroglu E. Clinical version of clinical discussion that was structered for DSM-IV axis I disorders (SCID-I). Ankara, Association of Medical Doctors Edition, 1999.
14. Goodman WK, Price LH, Rasmussen SA, Mazure C, Fleismann RL, Hill CL, et al. The yale-brown obsessive compulsive scale: I-Development, use and reliability. Arch Gen Psychiatry 1989;46(11):1006-11. [CrossRef]
15. Tek C, Ulug B, Rezaki BG, Tanriverdi N, Mercan S, Demir B, et al. Yale-brown obsessive compulsive scale and US national institute of mental health global obsessive compulsive scale in Turkish: reliability and validity. Acta Psychiatr Scandinavica 1995;91(6):410-3. [CrossRef]
16. Shoja-Shafti S, Kaviani H. Aripiprazole versus quetiapine in treatment-resistant obsessive-compulsive disorder: a double-blind clinical trial. Ther Adv Psychopharmacol 2015;5(1):32-7. [CrossRef]

17. Ruscio AM, Stein DJ, Chiu WT, Kessler RC. The epidemiology of obsessive compulsive disorder in the national comorbidity survey replication. Mol Psychiatry 2010;15(1):53-63. [CrossRef]

18. Taylor S. Molecular genetics of obsessive-compulsive disorder: a comprehensive meta-analysis of genetic association studies. Mol Psychiatry 2013;18(7):799-805. [CrossRef]

19. Hemmings SM, Kinnear CJ, Niehaus DJ, Moolman-Smook JC, Lochner C, Knowles JA, et al. Investigating the role of dopaminergic and serotonergic candidate genes in obsessive-compulsive disorder. Eur Neuropsychopharmacol 2003;13(2):93-8. [CrossRef]

20. Rothe C, Gutknecht L, Freitag C, Tauber R, Mössner R, Franke P, et al. Association of a functional -1019C/G 5-HT1A receptor gene polymorphism with panic disorder with agoraphobia. Int J Neuropsychopharmacol 2004;7(2):189-92. [CrossRef]

21. Maron E, Nikopensius T, Kõks S, Altmäe S, Heinaste E, Vabrit K, et al. Association study of 90 candidate gene polymorphisms in panic disorder. Psychiatr Genet 2005;15(1):17-24. [CrossRef]

22. Strobel A, Gutknecht L, Rothe C, Reif A, Mössner R, Zeng Y, et al. Allelic variation in 5-HT(1A) receptor expression is associated with anxiety- and depression-related personality traits. J Neural Transm (Vienna) 2003;110(12):1445-53. [CrossRef]