Lack of Evidence For Association Between Serotonin Transporter Gene Polymorphism and Obsessive Compulsive Disorder

Şenel Tot Acar*, M. Emin Erdal**, Kemal Yazıcı***, Aylin Ertekin Yazıcı****, Ayşe Devrim Baştürzi*****

* Department of Psychiatry Mersin University, Mersin, Turkey
** Department of Medical Biology and Genetics, Mersin University, Mersin, Turkey
*** Department of Psychiatry Mersin University, Mersin, Turkey
**** Department of Psychiatry Mersin University, Mersin, Turkey
***** Department of Psychiatry Mersin University, Mersin, Turkey

Correspondence:
Dr. Şenel Tot Acar
Department of Psychiatry Mersin University
Zeytinlibahçe Cad. 33070
Mersin, Turkey
Tel: +903243374300 (1139)
Fax: +903243374305
E-mail: seneltot@mersin.edu.tr

ABSTRACT

Objective: Although it is believed that obsessive compulsive disorder (OCD) has a genetic component, the type and number of genes involved in this disorder have not been understood entirely. The serotonin transporter (5-HTT) gene has been considered a candidate gene for OCD. The aim of this study was to determine the association of the 5-HTT gene with OCD by examining the polymorphisms in the promoter region (5-HTTLPR) and in the second intron (VNTR) in a Turkish sample.

Method: Sixty OCD patients and 85 control subjects were included in the study. All subjects were unrelated Turkish people.

Findings: The genotypic pattern of distribution of 5-HTTLP or 5-HTT VNTR was not different between the OCD patients and controls. There were no significant differences among patients with positive family history for OCD, those with negative family history for OCD and controls with respect to allele frequencies of 5-HTTLPR or 5-HTT VNTR polymorphisms. Allele frequencies were not statistically different in patients having tics themselves or in family compared to patients without such history. No significant genetic differences were observed between serotonin reuptake inhibitor responders and non-responders.

Discussion: The 5-HTT gene has been considered a candidate gene that could affect the risk of OCD. Literature about this issue is quite controversial. Some studies reported an association of -HTTLP or 5-HTT VNTR polymorphisms with OCD, whereas others including our study did not. However, this does not exclude the possibility that there may be other mechanisms affecting serotonergic systems thought to be involved in OCD or polymorphisms of other genomic regions of 5-HTT may play role in the pathogenesis of the disorder.

Conclusion: We could not find any evidence for the association of 5-HTTLP or 5-HTT VNTR polymorphisms with OCD, response to treatment with serotonin reuptake inhibitors, positive family history for OCD and presence of tics.

Keywords: genetics, OCD, 5-HTTLP, 5-HTT VNTR
INTRODUCTION

Obsessive compulsive disorder [OCD] is a relatively common illness characterized by recurrent and disturbing thoughts, [obsessions] and/or repetitive, stereotyped behaviors that the person feels driven to perform [compulsions] but recognizes as irrational or excessive. OCD is now known to be among the most common of psychiatric disorders, with lifetime prevalence estimates of 2-3% of the population (Hollander 1993). Etiology of the disorder is not clearly understood. Recent findings in neuroimaging and treatment studies indicate that some cases of OCD may be associated with specific autoimmune, receptor or neuroanatomical abnormalities. These data suggest etiologic heterogeneity within this diagnostic category; they also increased interest in and provided some clues for understanding the possible genetic underpinnings of obsessive-compulsive disorder.

Results of family and twin studies have led to consideration of a genetic factor in the etiology of OCD (Rasmussen 1993, Pauls et al 1995). Genetic studies on this field are new and limited in number. Although it is believed that OCD has a strong genetic component, the type and number of genes involved in this disorder have not been understood entirely.

It is known that serotonin reuptake inhibitors [SRIs] are beneficial in OCD patients and serotonergic dysfunction has been thought to play role in the pathophysiology of OCD (Sullivan and Coplan 2000, Papp 2000). It was suggested that the 5-hydroxytryptamine [serotonin, 5-HT] related genes may be involved in the pathogenesis of OCD. SRIs block the reuptake of 5-HT into the presynaptic neuron, a process mediated by the serotonin-transporter [5-HTT] gene, which plays a critical role in the termination of serotonergic neurotransmission by sodium-dependent uptake of 5-HT into the presynaptic neuron (Amara and Kuhar 1993, Lesch and Bengel 1995). Changes in 5-HTT gene expression have been noted after chronic treatment with SRIs (Lesch et al 1993). The 5-HTT gene has been considered a candidate gene that could affect the risk of OCD in the last few years. The 5-HTT gene is located on chromosome 17q 11.2 (Ramamoorthy et al 1993, Gelertner et al 1995). Two polymorphic sites in 5-HTT gene expression have been noted after chronic treatment with SRIs (Lesch et al 1996, Heils et al 1996, Collier et al 1996).
It has been reported that those two polymorphisms of the 5-HTT gene play role in the etiology of psychiatric disorders and display ethnic variation also (Murasaki et al 1999, Yilmaz et al 2001).

Studies conducted thus far have yielded controversial results concerning the association between 5-HTTLPR polymorphism and OCD. Two studies reported an association between 5-HTT polymorphism and OCD (McDougle et al 1998, Bengel et al 1999).

Altemus et al were unable to find a support for the role of a change in the primary structure of the coding region of the 5-HTT gene in OCD pathogenesis (Altemus et al 1996). Billet et al found no association of 5-HTT promoter region polymorphism and OCD; however, they observed a trend towards increased homozygosity in OCD patients (Billett 1997). Besides, they did not find a relationship between response to SRIs and polymorphism. Cavallini et al. found that only the factor containing counting and repeating rituals was associated with the homozygous long genotype, raising the possibility that some symptoms are associated with distinct genotypes (Cavallini et al 2002). Likewise, some recent studies also reported that no association was detected between the 5-HTTLPR polymorphism and OCD (Chabane et al 2004, Meira-Lima at al 2004, Saiz at al 2008).

VNTR element of 17 bp has been identified in the second intron of the 5-HTT gene (Lesch et al 1994). There have been some studies investigating the association of this polymorphism with major depressive disorder, bipolar disorder, anxiety disorder (Collier et al 1996, Ogilvie et al 1996, Evans et al 1997, Kunugi et al 1997). Ohara et al. (1998) found that the frequency of the allele containing 12 copies of the VNTR element [STin2.12] was significantly higher in the combined patient group, and among patients with OCD and GAD compared to the controls.

The aim of this study was to determine the association of the 5-HTT gene with OCD by examining the polymorphisms in the promoter region [5-HTTLPR] and in the second intron [VNTR] in a Turkish sample of OCD patients. In addition, this study evaluates the relationship between clinical features, treatment response and possible subtypes of OCD and 5-HTT gene polymorphism.

METHODS
Subjects
Sixty OCD patients and 85 control subjects were included in the study. Control subjects were selected among healthy volunteers and none had a history of any psychiatric disorder. Informed consent was obtained from all subjects participated in the study. The OCD patients and healthy controls were from the same geographic region and of the same ethnic origin. All subjects in the study gave informed consent.

All patients were diagnosed by experienced psychiatrists following a clinical interview which included Yale-Brown Obsessive-Compulsive Scale [Y-BOCS] ratings, and incorporated mood, anxiety, and psychotic disorder questions based on the Structured Clinical Interview for DSM-IV [SCID-I] (APA 1994). The patients were unrelated. Those who had mental retardation, drug and/or alcohol dependence, metabolic, psychiatric or neurological diseases were excluded. Subjects were asked to inform if they had suffered from vocal and/or motor tics at some time in their life. All OCD patients and controls were Turkish people. Information about family history was obtained either by direct interviews with the person or through indirect interviews with close relatives.

Physical examinations were carried out and blood samples were taken for complete blood count and blood chemistry as well as for the molecular analysis of 5-HT2A receptor polymorphism.

Y-BOCS was administered to assess severity of obsessive compulsive symptoms (Goodman et al 1989a, Goodman et al 1989b). Validity and reliability of the Turkish version of Y-BOCS has been done by Tek et al (1995).

After clinical evaluation, patients were started on pharmacotherapy [29 patients on fluvoxamine 100-300 mg/day; 5 on fluoxetine 20-80 mg/day and 10 on sertraline 100-200 mg/day]. Dosages were adjusted according to patients’ clinical condition and adverse effect status. Treatment response was evaluated with Clinical Global Impression Scale [CGI] at the end of 3rd month (Guy 1976). Responders were defined as very much improved or much improved, and non-responders as those who showed minimal or no change on CGI.

Molecular analysis:
DNA was extracted from whole blood by standard techniques. A functional 44-bp insertion/deletion polymorphism in the promoter region of the 5-HTT gene was typed by PCR amplification of DNA using flanking primers 5’-CTTGTGAGGTCTCCCGCCTGGCTTT-3’ (forward) and 5’-TGAACGCTGACAGTCTGAGGGACTGGG-3’ [reversed]. PCR was performed with GC Rich PCR system [Roche Molecular Biochemical] in a 25 µl reaction mixture containing 100 ng DNA, 100 µl dNTPs, 20 pmol of each
primer, 1.5 mM MgCl2. DNA was denatured at 96°C for 2 minutes and 35 cycles at 96°C for 1 minute for denaturation, 1 minute at 60°C for annealing and 1 minute at 72°C for extension, followed by 7 minutes at 72°C for final extension. Amplification products were resolved by electrophoresis on 2% agarose gels next to a DNA molecular weight standard and visualized with [UV] ethidium bromide staining. Alleles were designated S [484 bp] and L [528 bp] as described previously (Lesch et al 1996).

The 17-bp VNTR polymorphism in the second intron of the 5-HTT gene was typed by PCR using primers 5’-TGGATTTCCTTCTCTCAGTGATTGG-3’ [forward] and 5’-TCATGTTCCTAGTCTTACGCCAGTG-3’ (reversed). PCR was performed in a 25 µl volume with 100 ng DNA, 100 µm dNTPs, 20 pmol of each primer, 1.5 mM MgCl2, 1x PCR buffer with (NH4)2SO4 [Fermentas, Vilnius, Lithuania] and 2U Taq DNA polymerase [Fermentas, Vilnius, Lithuania]. PCR conditions were 2 minutes for initial denaturation at 95°C, 35 cycles at 95°C for 1 minute for denaturation, 1 minute at 57°C for annealing and 2 minutes at 72°C for extension, followed by 10 minutes at 72°C for final extension. Amplification products were resolved by electrophoresis on 2% agarose gels next to a DNA molecular weight standard and visualized with UV ethidium bromide staining. Alleles were designated 390 bp [12 copy, STin.2.12] 360 bp [10 copy, STin.2.10] or 345 bp [9 copy, STin.2.9] as described previously (Ogilvie et al 1996).

Statistical analysis:
Statistical analyses were performed using SPSS for Windows [version 9.0, Chicago, IL]. Mann-Whitney U test, t test, chi square test and one way ANOVA were used for the statistical analyses of data. A p value less than 0.05 was considered statistically significant.

FINDINGS
The patient group consisted of 21 [35%] male and 39 [65%] female OCD patients with ages ranging from 17 to 56 years [mean=29±9 years]. The control group was composed of 39 male [45%] and 46 female [55%] subjects with ages ranging from 23 to 45 years [mean=27±5]. The patients and the controls were not significantly different with respect to age [student’s t test; t=−1.86, df=158, p=0.065] and sex distribution [chi-square test; ?2=0.07, Df=2, P=0.81].

The mean age of onset of OCD was 21±7 years [range=7-41] and the mean duration of disorder was 87±5 years [range=1-43]. All patients had at least 1 year duration of the disorder.

The mean total score of Y-BOCS was 20.6±7.6 [range 19-38]. The mean score of obsession subscale was 12.5±3.6 [range 3-20] and that of compulsion subscale was 8.4±5.6 [range 0-19].

The genotypic pattern of distribution of 5-HTTLPR
Eight patients had current or past history of tic disorder [13.3%], and none had a history of Tourette’s syndrome. Twenty eight patients had at least 1 first degree relative with OCD, 8 patients had tic disorder among relatives and 1 patient had Tourette’s syndrome in a first degree relative. There were no significant differences among the patients with positive family history for OCD, those with negative family history for OCD and the controls with respect to allele frequenc- es of 5-HTTLPR or 5-HTT VNTR polymorphisms. Al- lele frequencies were not statistically different in the patients having tics themselves or in the family compared to the patients without such history [Table 1 and 2].

Thirty-five patients were responders and 19 were non-responders according to evaluation by CGI scale. Six patients discontinued drug therapy and were excluded from the treatment response analysis. No significant genetic differences of 5-HTTLPR or 5-HTT VNTR were observed between SRI responders and SRI non-responders [Table 1 and 2].

**DISCUSSION**

During the past years, polymorphisms of the 5-HTT gene have been defined in various psychiatric disorders (Collier et al 1996, Evans et al 1997, Kunugi et al 1997, Ogilvie et al 1998). The proved efficacy of SRIs in the treatment of OCD has led to the notion that serotonergic dysfunction plays a pivotal role in the etiology of this disorder (Sullivan and Coplan 2000, Papp 2000). Departing from this point, some studies investigating the association of OCD and 5-HTT polymorphisms have been conducted. The L allele of 5-HTT produces expression levels three times greater than the S allele (Heils et al 1996). The S allele is associated with decreased 5-HT reuptake, leading to a longer duration of serotonergic activity. On the other hand, the L allele is associated with a more rapid reuptake of 5-HT causing shorter serotonergic activity. Within this frame, OCD patients and healthy subjects might be expected to differ in this respect. We did not find any significant differences between the OCD patients and the controls with respect to S and L alleles of 5-HTTLPR or 10 and 12 repeat alleles of 5-HTT VNTR. Altemus et al (1996) reported that no variations in amino acid sequence of the primary structure of the 5-HTT coding region were identified among OCD patients or healthy controls. However the 5-HTT promoter region was not examined in that study. In a study of 72 OCD patients and matched controls, Billet et al. (1997) reported that there was no association between OCD and promoter region polymorphism of the 5-HTT gene, but found a trend towards increased homozygosity [both L/L and S/S] in the patients. More
recent studies also reported no association between the 5-HTTLPR polymorphism and OCD (Chabane et al. 2004, Meira-Lima et al. 2004, Saiz et al. 2008). However, in a recent meta-analysis by Lin OCD was found to be associated with the S/S homozygous genotype, but was inversely associated with the LS heterozygous genotype (Lin 2007). No association with the L/L homozygous genotype or the allelic distribution was found in that study. Yet, in an even more recent meta-analysis study, Bloch et al. found no evidence of association between genetic variation at the 5-HTTLPR locus and OCD (Bloch et al. 2008).

On the other hand, Bengel et al. (1999) found an association between a functional polymorphism in 5’ regulatory region of the 5-HT and OCD. Their results suggested that patients with OCD were more likely to carry two copies of the L allele as compared to controls. Consistent with the above study, Mc Dougle et al. (1998) reported an association and linkage disequilibrium between the L allele of the HTTLPR polymorphism and OCD, using a within-family, transmission disequilibrium test design.

Lesch et al. (1996) reported that individuals with the S allele had greater anxiety-related personality characteristics in healthy Caucasian subjects. Murakami et al. (1999) reported that populations with the S/S genotype of HTTLPR have stronger anxiety-related personality traits than those with the L allele in healthy Japanese subjects. Comparison of these two studies showed that the distribution of alleles were significantly different between Japanese and Caucasian subjects and suggested that allele frequencies might be different among various ethnic groups. Osher et al. (2000) also reported findings in support of the above studies. However, several other studies found no association of the 5-HTTLPR with various personality measures (Ball et al. 1997, Jorm et al. 1998). Anxiety-related personality characteristics are encountered quite commonly in OCD patients. Therefore, one may expect to see higher frequency of the S allele in OCD patients, but we have not observed such an increased rate of the S allele in our patients.

Mc Dougle et al. (1998) found that of the 22 SRI treatment responders, 14 transmitted the L and 8 transmitted the S allele (not significant), whereas of the 13 SRI non-responders, 10 transmitted the S and 3 transmitted the S allele [p=0.052]. They suggested that the L allele may be associated with poorer response to SRIs. We found no association of response to SRIs with 5-HTTLPR and 5-HTT VNTR polymorphisms. In other words, polymorphisms of the 5-HTT gene did not predict SRI response in our OCD patients. Billet et al. (1997) reported similar findings in a retrospective study. Our patients were receiving fluvoxamine, fluoxetine and sertraline as pharmacotherapy which constitutes a more homogenous group of drugs than in the above two studies. In addition, clomipramine used in both Mc Dougle et al. (1998) and Billet et al. (1997) studies are not a selective serotonergic agent and this may be a potentially confounding variable. Overall, it seems that response to SRIs is not associated with polymorphisms of the 5-HTT gene. However, ethnic differences have been demonstrated to play a role in the association between 5-HTTLPR and SSRI response in depression in a meta-analysis (Smith et al. 2004). It is possible that ethnic differences may exert a similar influence on treatment response in OCD also.

Polymorphisms of the 5-HTT VNTR were found to be associated with major depressive disorder, bipolar disorder and anxiety disorder, schizophrenia (Collier et al. 1996, Ogilvie et al. 1996, Evans et al. 1997, Kunugi et al. 1997). Ohara et al. (1998) found that the frequency of the allele containing 12 copies of the VNTR element [STin2.12] was significantly higher in the combined patient group and among patients with OCD and GAD in comparison with controls. Saiz et al. provided supporting evidence of an association between the STin2 VNTR polymorphism of the SLC6A4 gene and OCD (Saiz 2008). Baca-Garcia et al. found a significant excess of 12/12 and 12/10 genotypes in OCD patients compared to psychiatric patients and controls (Baca-Garcia et al. 2007).

The STin 2.12 allele frequency was 85.8% in Japanese controls and about 50-60% in Caucasians (Lesch et al. 1994). This rate was 89.4% in our Turkish sample which is similar to Japanese population. Two other studies of Turkish population have also reported similar ratios (85.4%) (Herken et al. 2001, Yılmaz et al. 2001). This ethnic variation may affect the results regarding the VNTR region of any 5-HTT genotyping study. STin 2.10 and Stin 2.12 existed both in the patients and the controls. However, Stin 2.7 and Stin 2.11 were not detected at all, which means Stin 2.10 and Stin 12 are the polymorphic variants of VNTR which are frequently expressed in Turkish people.

A third variant, the Lg allele, has recently been described (Hu et al. 2006). The Lg allele has a single nucleotide polymorphism compared to the previous identified long form of the allele [LA]. This single nucleotide polymorphism of the Lg allele results in lower transcriptional activity of the gene despite having similar length to the LA allele (Hu et al. 2006). The proportion of L alleles that are of Lg varies from 1-50% in different
ethnic populations (Hu et al. 2006). A meta-analysis study demonstrated a significant association between the L allele and OCD case status among Caucasian subjects, but not in Asian samples (Smits et al. 2004).

Our study was conducted before that new allele was identified. We studied only the L and the S alleles, as in most of the previous studies and all meta-analysis studies to date, have been done using studies of those alleles. This is a considerable limitation for interpretation of our results. However, data of this study can still provide valuable information, as to our knowledge there are no other studies about this polymorphism and OCD conducted in Turkish population.

Twin and family studies provide evidence that some forms of obsessive-compulsive disorder may be etiologically related to Tourette’s syndrome. Family studies have shown that relatives of patients with Tourette’s syndrome had a high rate of OCD and relatives of patients with OCD had a high rate of Tourette’s syndrome and/or tics. It has been reported that a subgroup of OCD patients had a variety of tics (Rasmussen 1986, Pauls et al. 1986, Swedo et al. 1989). We did not find any difference between OCD patients with and without tic disorders with respect to genetic polymorphisms of 5-HTTLPR and 5-HTT VNTR. Various studies have investigated whether OCD patients with tics constituted a specific subgroup and controversial results have emerged from studies of genetic polymorphisms related with various neurotransmitter systems. Cavallini et al. (2000) did not find significant differences with respect to 5-HTTLPR polymorphism in patients with Tourette’s syndrome compared to the controls. Nicolin et al. (1996) reported that homozygosity for the allele dopamine D2 receptor A2 could be associated with the presence of tics in OCD patients and suggested that these patients constituted a genetically different subgroup of patients with tics. Huang et al. (2001) found a significant relationship between Tourette’s syndrome associated with OCD and t102 polymorphism of the 5-HT2A gene. Our study does not bring any evidence that the OCD patients with tics are a different genetic subgroup.

One family-controlled study reported significant evidence of association (McDougle et al. 1998), but other family-based studies found none (Camarena et al. 2001, Chabane et al. 2004, Walitza et al. 2004). We did not find significant differences in the allele distributions of the patients with positive family history for OCD compared to those with negative family history. This could provide additional information about genetic transmission of OCD, if an association was found.

CONCLUSION

As conclusion, we could not find any evidence for the association of 5-HTTLPR and 5-HTT VNTR polymorphisms with OCD, response to SRI treatment, positive family history for OCD, presence of tics. However, this does not exclude the possibility that there may be other mechanisms affecting serotoninergic systems thought to be involved in OCD or polymorphisms of other genomic regions of 5-HTT may play role in the pathogenesis of the disorder. It is highly possible that multiple interactions of several different neurotransmitter systems and signal transduction pathways are involved in OCD.

REFERENCES


