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ORIGINAL ARTICLE

ASSOCIATION BETWEEN OBSESSIVE COMPULSIVE DISORDER AND TUMOR NECROSIS FACTOR-α GENE –308 (G>A) AND –850 (C>T) POLYMORPHISMS IN TURKISH CHILDREN

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ABSTRACT

Obsessive compulsive disorder (OCD) is a neurobio-logical disease characterized with obsessions and compulsions. Obsessive compulsive disorder occurs with an autoimmune mechanism after Group A β hemolytic streptococcus (GABHS) infection. Tumor necrosis factor (TNF) is an important cytokine, as well as having an important role in the apoptosis mechanism of autoimmune diseases. It is expressed by the TNF-α gene. The aim of this study was to examine the relationship between the TNF-α gene promoter region –308 (G>A) and –850 (C>T) polymor-phisms and OCD. In this study, ages of the OCD patients and the control group ranged between 4 and 12 years. We studied two patient groups, one included childhood onset OCD patients (n = 49) and the control group was composed of healthy children (n = 58). Patients were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorder (DSM-IV) criteria and with Kiddie Schedule for Affective Disorders and Schizophrenia-Present and Lifetime (KSAD-S-PL) version. For identifying the poly-morphisms, polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP) and polyacryl-amide gel electrophoresis (PAGE) methods were used.

For the –308 polymorphism, 45 of 49 OCD patients' results were completed, and for the –850 polymorphism, 47 of 49 OCD patients' results were completed. According to our statistical results, there is a positive relationship between OCD and the –308 polymorphism (p <0.001) but no association between OCD and the –850 polymorphism (p = 0.053). There is no positive relationship between antistreptolysin O (ASO) titers and the –308 polymorphism (p = 0.953) but there is an important significance between the –850 polymorphism and ASO (p = 0.010). There is no positive relationship between gender of patients and OCD (p = 0.180) and no positive association between ASO and gender (p = 0.467). According to our results, we hypothesize that we can propose the mutant AA genotype for the –308 polymorphism, and that the mutant CT genotype for the –850 polymorphism may be used as molecular indicators for OCD.

Keywords: Child, Genetic, Obsessive compulsive dis-order (OCD), Polymorphism

INTRODUCTION

Obsessive compulsive disorder (OCD), attention-deficit hyperactivity disorder (ADHD) and tic disorders are prepubertal symptom onset autoimmune neuropsychiatric disorders associated with basal ganglia abnormalities in which antibodies produced against Group A β hemolytic streptococcus (GABHS) infections cross react against neuron epitopes. These disorders are referred to as post

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streptococcal movement disorders [1,2]. Obsessive compulsive disorder may be triggered by stress, anxiety and infections (for example; GABHS infections) [3]. Comor-bidities, GABHS infections, obsessions and compulsions are characteristic features in the etiology of OCD [4,5]. Due to the damage of basal ganglia after a period following a GABHS infection, tics and obsessive compulsive symptoms occur in patients. Exacerbations of OCD are accompanied by unstable emotions, motoric hyperactivity and symptoms of anxiety [4]. The association between Sydenham's chorea and streptococcus infections was first mentioned by Taranta and Stollerman in 1956 [6] but association between OCD and Sydenham's chorea was discovered by Snider and Swedo [4] and Swedo *et al.* [7]. In the late 1980s, the National Institute of Mental Health reported increased obsessive-compulsive symptoms in Sydenham's chorea patients [8]. In the following decades, clinicians and researchers continued to make studies for neuropsychiatric disorders [9]. Recent studies suggest that 50.0-80.0% of OCD cases have a childhood onset [10]. Childhood onset OCD patients usually have a positive history of childhood tonsillitis, tic disorders and more antistrep-tolysin O (ASO) titers than adult onset OCD patients [11]. In addition, obsessions and compulsions are common symptoms for both of them [12,13]. According to Walitza *et al.* [14], 1.0-3.0% of the general population suffers from OCD. Prevalence of OCD in adolescents is 0.4% [7]. Clinical studies indicated that males frequently have earlier age onset of OCD than girls [15]. It was indicated that boys are much more affected than girls (3:2 ratio) [10]. However, epidemiological studies have shown that the incidence of many prepubertal boys diagnosed with OCD is three times that of girls. In addition, it was detected that incidence of OCD in females increases after puberty [15]. The immune system of patients is affected in OCD. In the patient's immune system, the B lymphocyte susceptible to the M group of antigens, kills them. After B lymphocyte activation, some of the susceptible B lymphocytes pass through the blood-brain barrier and associates with the cell membrane of neuron cells because of epitope similarity [16-19]. Neurons are destroyed by the patient's autoimmunity apoptosis mechanism. The loss of neurons, especially in basal ganglia, leads to neuropshychiatric signs such as obsessions, compulsions or tics because the basal ganglia is part of the brain which is responsible for cognition controlling, movement coordination and voluntary movements. Tumor necrosis factor (TNF) is an important cytokine and also has an important role in the apoptosis mechanism of autoimmune diseases. It is expressed by the TNF-α gene. The aim of our study was to identify the influence of the TNF-α gene –308 (G>A) and –850 (C>T) polymorphisms on OCD. Despite other research studies, the relationship between neuropsychiatric disorders and GABHS infections are not yet completely clear. One reason for this uncertainty is due to the triggering of other existing factors [20].

MATERIALS AND METHODS

This study included 49 children with OCD attending the Department of Child and Adolescent Psychiatry, Medical Faculty, Çukurova University, Adana, Turkey, and 58 healthy children attending the Department of Social Pediatrics, Medical Faculty, Çukurova University, Adana, Turkey. Ages of the patients and those of the control group ranged between 4 and 12 years. Psychiatric diagnoses were determined by the specialist doctors from the Department of Child and Adolescent Psychiatry, Medical Faculty, Çukurova University, Adana, Turkey in accordance with the Diagnostic and Statistical Manual of Mental Disorder (DSM-IV) criteria and with Kiddie Schedule for Affective Disorders and Schizophrenia-Present and Lifetime (KSAD-S-PL) version. The Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS) was used to evaluate the severity of OCD symptoms [20,21]. Demographic data and previous infection histories of patients and their families included a positive account of infection: tonsillitis (more than six tonsillitis attacks/year), prophylactic antibiotic/penicillin use, tonsillectomy, ASO titers, as well as symptom aggravation associated with infections. This study was approved by the Ethics Committee of the Medical Faculty of Çukurova University, Adana, Turkey. The patient and control groups were informed and we obtained written parental consent; all consented to be a part of this study before peripheral blood samples were drawn. In order to identify the TNF-α gene polymorphisms, we isolated the DNA from blood employing the salting out method of Miller *et al.* [22] and it was stored at 4°C until use.

Areas with the –308 and –850 polymorphisms were amplified with polymerase chain reaction (PCR). Poly-morphisms were genotyped using avail-

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able restriction enzymes with restriction fragment length polymorphism (RFLP) and polyacrylamide gel electrophoresis (PAGE) methods [23]. For the –308 polymorphism, the 142 bp product was amplified with the following primers: F-5'-GGG ACA CAC AAG CAT CAA GG-3' and R-5'-AAT AGG TTT TGA GGG CCA TG-3'; the 126 bp restricted product was named as the mutant AA genotype; the non restricted 142 bp product was named as the normal GG genotype. This polymorphism includes only one base variation (G>A), is differentiated by the *Nco*I restriction enzyme on an 8.0% polyacrylamide gel. For the –850 polymorphism, the 131 bp product was amplified with primers F-5'-AAG TCG AGT ATG GGG ACC CCC CGT TAA-3' and R-5'-CCC CAG TGT GTG GCC ATA TCT TCT T- 3'; the 106 bp restricted product was named as the normal CC genotype; the non restricted 131 bp product was named as the mutant TT genotype. This polymorphism, which includes only one base variation (C>T) was differentiated by the *Hind*II enzyme on an 8.0% polyacrylamide gel [24,25].

The normal value of ASO is in fact 200, but in our study the cut-off value was taken as 400, considering these children were already in the active infection period. Antristreptolysin O was considered by the TODD (Strepto-lysin O enzyme) unit. Values above 200 were accepted as significant and referred to a new infection as an indicator [26]. Serum ASO titers of patients were determined using the Beckman Delta Nephelometor at the Department of Central Laboratory Biochemistry, Balcalı Hospital, Medical Faculty, Çukurova University, Adana, Turkey. This laboratory is accredited by the International Joint Commission, (IL, USA).

Statistical analyses for comparing the prevalence of mutant genotypes between OCD patients and the control group was carried out using the Chi-square ($\chi 2$) test. To measure the effect of ASO titer values on the mutant genotypes in OCD patients, the Kruskal-Wallis test was used for overall comparison and Bonferroni adjustments (dividing the type I error by three, the number of subgroups, or equivalently, multiplying the p values by three, *i.e.,* $\alpha = \alpha/3 = 0.017$) were used for multiple comparisons. The Mann Whitney U test was applied. Throughout the analysis, the SPSS 15.0 package program was used with the level of statistical significance accepted as $p < 0.05$.

RESULTS

The current study was carried out with the aim of defining the effect of mutant genotypes in the progress of OCD. For the –308 polymorphism, 45 of 49 OCD patients' results were completed. In the OCD patient group, we found four mutant GA genotypes (8.9%) and 41 mutant AA genotypes (91.1%); we did not find any normal GG genotypes. In the control group, five mutant GA genotypes (8.6%) were observed with 53 of genotypes consisting of the normal GG sequence (91.4%) (Table 1).

For the –850 polymorphism, 47 of 49 OCD patients' results were completed. Twenty-five OCD patients had the CT genotype (53.2%), seven pa-

Table 1. Genotypes, numbers and percentages in the obsessive compulsive disease patients and control group.

Polymorphisms	Groups	Genotypes	n (%)	%
–308 (G>A)	Patient group	GG (normal)	–	0.0
		GA (mutant)	4	8.9
		AA (mutant)	41	91.1
	Control group	GG (normal)	53	91.4
		GA (mutant)	5	8.6
		AA (mutant)	–	0.0
–850 (C>T)	Patient group	CC (normal)	15	31.9
		CT (mutant)	25	53.2
		TT (mutant)	7	14.9
	Control group	CC (normal)	32	55.2
		CT (mutant)	19	32.8
		TT (mutant)	7	12.1

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tients had the mutant TT genotype (14.9%) and 15 patients had the normal CC genotype (31.9%). In the control group, 19 mutant CT genotypes (32.8%) and seven mutant TT genotypes (12.1%) were found, but the majority (32) consisted of normal CC genotypes (55.2%). Nineteen of the OCD patients (38.8%) were boys and 30 were girls (61.2%). In the control group, 30 children were boys (51.7%) and 28 were girls (48.3%) (Table 1). According to our statistical results, a positive relationship existed between OCD and the –308 polymorphism (p <0.001) but no association was observed between OCD and the –850 polymorphism (p = 0.053). In addition, we determined the ASO titers of the patients. We compared only OCD patients and ASO titers but we did not compare ASO titers of the control group because we only had the ASO titer values of the patients' group. There exists no positive relationship between ASO titers and the –308 polymorphism (p =0.953) but there was an important significance between the –850 polymorphism and ASO titers (p = 0.010). No positive relationship existed between the genders of the patients and OCD (p =0.180) and no positive association between ASO titers and gender (p = 0.467). For the –850 polymorphism, we made an assumption about the TT genotypes. The TT genotype has a low incidence in the patients' group (15.0%) and it seemed to reduce ASO titer values, however, this cannot be considered to be a valid comment as patients with normal CC genotypes (32.0%) should also have low ASO titers in their blood (Table 1).

DISCUSSION

In our country, GABHS infections in childhood are much more common when compared to the other countries. In addition, some patients cause delays in treatment because they do not seek immediate medical attention or do not pay attention to the disease once diagnosed. Taking these conditions into consideration, we decided to conduct our study. We know that GABHS infections are associated with OCD symptoms and GABHS preceded 31.0% of exacerbations of OCD [21]. We studied two polymor-phisms of the TNF-α gene and ASO titers of patients. According to our statistical results, we have demonstrated a positive association between OCD and the TNF-α gene –308 polymorphism. In different parts of the world, studies are conducted for different OCD patients. The study by Hounie *et al.* [27] is consistent with our results about the –308 polymorphism. According to their report, the –308 polymorphism is strongly associated with OCD [27]. On the other hand, the study conducted by Zai *et al.* [25] indicates that the TNF-α gene –308 polymorphism is not associated with OCD in Canadian patients. In particular, we saw the remarkable effects of the –308 polymorphism on phenotype-genotype relationship because of the increased frequency of mutant genotypes in OCD patients. We did not find a positive correlation between ASO titers and the –308 polymorphism, but there is statistically relevant difference between ASO titers and the –850 polymorphism. In other words, ASO is a determinant for the –850 polymorphism in OCD. The result of another study revealed a positive correlation between ASO titers and the severity of tic disorder for 150 consecutive children presenting with tics [28]. Also, an additional study supporting our results was conducted on 105 patients diagnosed with chronic tic disorder (CTD), OCD or ADHD and ASO titers. They found that ASO titers of ADHD patients were increased significantly [29]. According to the study conducted by Gause *et al.* [5] there is no relationship between ASO titers in children with OCD only, OCD+PANDAS, OCD+ chronic tic disorder patients and the control group. It was verified that boys were affected much more when compared to girls by Lewin *et al.* (3:2 ratio) [9]. We did not find any association between OCD and gender. With this new comprehension, we can say that epigenetic mechanism plays a role for inactivation of some genes, despite the fact that some of the patients had a polymorphism; thus, we have to broaden our perspective of the disease. In a lot of multifactorial and single gene neurodegenerative disorders, the symptoms are similar, but also these are the common characteristics of polygenic traits. In addition, there are symptoms that differ from patient to patient. For that reason, simultaneous expression profiles of immune system genes should be researched as well. The factors or susceptibilities and taking blood from patients on different dates may cause a certain extent of differentiation to our results. In light of our findings, we propose that the mutant AA genotype for the –308 polymorphism and the mutant CT genotype for the –850 polymorphism may be used as molecular indicators of OCD, with verification from future research.

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