The Polymorphisms of Ser49Gly and Gly389Arg in Beta-1-Adrenergic Receptor Gene in Major Depression

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ABSTRACT

Introduction: It was reported that the genetic susceptibility of major depressive disorder (MDD) is related with genetic polymorphisms. The aim of this study was to investigate the possible association of the genotype and allele frequencies of Ser49Gly and Arg389Gly polymorphisms in MDD by comparing them with healthy subjects.

Methods: A total of 144 patients with MDD diagnosed according to Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) criteria and 105 healthy controls were included in the study. Polymerase chain reaction (PCR) with restriction fragment length polymorphism (RFLP) was used for genotyping.

Results: Of the 144 participants in the MDD group, 77 (53.5%) had homozygous wild type (AA), 57 (39.6%) had heterozygous type (AG),

and 10 (6.9%) had mutant (GG) genotype for Ser49Gly, whereas 75 (52.1%) had homozygous wild type (GG), 59 (41.0%) had heterozygous (GC) type, and 10 (6.9%) had mutant homozygous (CC) genotype for Gly389Arg. There were no significant difference in the allele and genotype frequencies of the beta-1-adrenergic receptor (ADRB1) gene for Ser49Gly and Arg389Gly polymorphisms after comparing with healthy controls ($p=0.626$; $p=0.863$ and $p=0.625$; $p=0.914$).

Conclusion: The results of our study did not reveal a major effect of the polymorphism of Ser49Gly and Gly389Arg in the ADRB1 gene in MDD. Further studies with larger sample size are required to elucidate the role of other beta-1 adrenergic gene polymorphisms in MDD.

Keywords: Depression, polymorphism, beta-1-adrenergic receptor, genotype

INTRODUCTION

The beta-1-adrenergic receptor (ADRB1) gene, which is a G-protein coupled postsynaptic receptor, mediates the physiological effects of catecholamines (1). ADRB1 is encoded by a gene composed of 45 amino acids and is located on chromosome 10q24-26, a site where positive linkage to affective disorders is reported (2,3). Many polymorphisms of the ADRB1 gene were studied before, but 13 of them were found to change the amino acids of the ADRB1 protein (4,5). Ser49Gly and Gly389Arg have been identified as two functional polymorphisms among these variants. The change of adenine to guanine at the 145th position results in the change of the amino acid serine to glycine (Ser49Gly), and the substitution of cytosine with guanine results in the change of the amino acid glycine to arginine (Gly389Arg) (6,7). Although the majority of studies for Ser49Gly and Gly389Arg are about cardiovascular disorders, the C allele of the Gly389Arg polymorphism was found to be a functional polymorphism associated with an enhanced coupling to the stimulatory Gs-protein and increased adenylyl cyclase activation, which are often observed in affective disorders (8). Another study about Gly389Arg polymorphism revealed an association of CC homozygosity with antidepressant treatment (9).

It's known that low trait levels of extraversion are linked to increased vulnerability to major depression and anxiety (10), and a research on Ser49Gly functional polymorphism in the beta-1-adrenergic receptor has indicated an association between low extraversion and depression (11). Additionally, another study revealed that mental stress-induced myocardial ischemia is more common among patients homozygous for the Ser49 allele (12).

The relation of increased adenylyl cyclase activation, antidepressant treatment response, low extraversion properties, and mental stress-related cardiovascular disorders reveals the importance of those polymorphisms. However, although there is remarkable evidence about the association of Ser49Gly and Gly389Arg polymorphisms in depression, only a limited number of studies have focused on these. We therefore tested a possible association of Ser49Gly and Gly389Arg polymorphism in the beta-1-adrenergic receptor in a sample of 144 patients diagnosed as having major depression by comparing them with 105 healthy controls.

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METHODS

Subjects

A total of 144 patients with major depression diagnosed according to the DSM-IV criteria and 105 healthy controls between the ages of 18 and 75 were included in the study. The study included patients who were admitted to the outpatient clinic of the psychiatry department of a university hospital between August 2008 and October 2010. The standardized structured interviews were performed by psychiatrists using SCID I and II questionnaires (13,14,15). Patients with any current comorbid psychiatric and personality disorders, alcohol or substance dependence, any other drug use, a history of serious head injury, and those with a psychiatric disorder history in their first-degree relatives were excluded from the study. Among 167 first episode depression subjects, 15 were excluded when recruiting patients because they met the criteria for the family history of a psychiatric disorder, and eight were excluded because of a comorbid psychiatric disorder. The subjects did not receive any pharmacological or non-pharmacological treatment during the sampling. All patients were chosen from among patients who had their first episode. A sample of 105 healthy subjects for the control group was non-related with the patients matched for age and gender. Both groups were from the same region in Turkey.

All patients and controls gave informed, written consent to participate in the study, which was approved by Süleyman Demirel University Ethic Committee.

Genotype Analysis

Deoxyribonucleic acid was extracted from frozen whole blood according to standard methods. The subjects were genotyped for two *ADRB1* polymorphisms that resulted in serine/glycine (Ser49Gly) and arginine/glycine (Arg389Gly) amino acid substitutions (8,16).

Polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) with primers described by Maqbool et al. (16) was used for genotyping. The following selected primary sequences for the analysis of Ser49Gly polymorphism in the *ADRB1* gene were applied. *Forward Primer*: 5' - CCG GGC TTC TGG GGT GTT CC - 3' and *Reverse Primer*: 5' - GGCGAGGTGATGGCGAGGTAGC - 3'.

The selected primary sequences for Gly389Arg polymorphism in the *ADRB1* gene were as follows: *Forward Primer*: 5' CGCTCTGCTGGCTGC-CCTTCTCC 3' and *Reverse Primer*: 5' TGGGCTTCGAGTTCACCTGCTATC 3'.

Ser49Gly polymorphism of the *ADRB1* gene: A 564-bp gene region including the Ser49Gly polymorphism was amplified by PCR. A 25- μ L reaction mixture, which contained sterile distilled water (9.8 μ L), forward primer (0.5 μ L), reverse primer (0.5 μ L), MgCl₂ (1.5 μ L), deoxynucleotide triphosphate (dNTP) mixture (2.5 μ L), genomic DNA (4.0 mL), dimethyl sulfoxide (DMSO) (2.5 μ L), Taq DNA (0.2 μ L), and buffer (2.5 μ L), was prepared for each sample.

Thirty-five PCR cycles were performed for each reaction. The conditions were denaturation for 4 min at 95°C, amplification (denaturation for 30 s at 95°C, annealing for 30 s at 64°C, and extension for 45 s at 72°C), and a final extension at 72°C for 7 min. The analysis of the Ser49Gly polymorphism was performed by EcoO109I (DraI) restriction enzyme, which was obtained from *Escherichia coli*. The amplified 564-bp target region of the *ADRB1* gene did not include any cutting region for the EcoO109I restriction enzyme; however, the substitution of adenine with guanine caused a cutting region. The PCR products were visualized by electrophoresis on 2% agarose gels.

Gly389Arg polymorphism of *ADRB1* gene: A 530-bp gene region including the Gly389Arg polymorphism was amplified by PCR. A 25- μ L reaction mixture, which contained sterile distilled water (9.8 μ L), forward primer (0.5 μ L), reverse primer (0.5 μ L), MgCl₂ (1.5 μ L), dNTP mixture (2.5 μ L), genomic DNA (4.0 mL), DMSO (2.5 μ L), Taq DNA (0.2 μ L), and buffer (2.5 μ L), was prepared for each sample. The PCR conditions were denaturation for 4 min at 95°C, amplification (denaturation for 30 s at 95°C, annealing for 30 s at 64°C, and extension for 45 s at 72°C), and a final extension at 72°C for 7 min. Analysis of the Gly389Arg polymorphism was performed by BcgI, which was obtained from *Bacillus coagulans* where the substitution of cytosine with guanine formed a cutting region on DNA. The PCR products were visualized by electrophoresis on 2% agarose gels.

Statistical Analysis

General statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) software (version 15.0). Polymorphisms of the *ADRB1* gene between the patients and controls were evaluated by using Chi-square test, ANOVA, t-test, and correlation analysis. A p-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 144 major depressive disorder patients and 105 healthy controls were included in the study. Of the 144 major depressive disorder patients, 112 (77.8%) were female and 32 (22.2%) were male. The mean age for the major depressive disorder group was 43.08 $\pm$ 13.59 years. Of the 105 participants in the control group, 77 (73.3%) were female and 28 (26.7%) were male, and the mean age for the control group was 41.43 $\pm$ 15.50 years. There was no difference between the controls and patients in gender and age ($p=0.529$; $p=0.721$).

Results of the *ADRB1* Gene Ser49Gly Polymorphism

The results for the *ADRB1* gene Ser49Gly polymorphism in the major depressive disorder and control groups were evaluated based on the size and number of fragments obtained by RFLP. The substitution of adenine with guanine at the extracellular amino-terminal 145 results in a change of serine to glycine in the 49th codon. According to our results, we observed that the homozygous wild type (AA) genotype had a single 564-bp DNA fragment, homozygous mutant type (GG) had two DNA fragments with 345 bp and 219 bp, and heterozygous type (AG) had three fragments with 564 bp, 345 bp, and 219 bp (Figure 1).

Of the 144 participants in the major depressive disorder group, 77 (53.5%) had homozygous wild type (AA), 57 (39.6%) had heterozygous type (AG), and 10 (6.9%) had mutant (GG) genotype. Of the 105 participants in the control group, 74 (70.5%) had homozygous wild type (AA), 23 (21.9%) had heterozygous type (AG), and 8 (7.6%) had mutant (GG) genotype. As shown in Table 1, there was no significant difference between the major depressive disorder and control groups after the analysis of genotype and allele frequencies of the Ser49Gly polymorphism in the *ADRB1* gene ($p=0.626$; $p=0.863$).

Results of *ADRB1* Gene Gly389Arg Polymorphism

The results for the *ADRB1* gene Gly389Arg polymorphism in the major depressive disorder and control groups were evaluated based on the size and number of fragments obtained by RFLP. The change of cytosine (C) to guanine (G) at the 1165 position resulted in the substitution of glycine to arginine. This polymorphic variant is located in the intracellular cytoplasmic tail portion (16).

In the major depressive disorder group, the homozygous wild type (GG) had a single 530-bp DNA fragment, homozygous mutant type (CC) had 125

Table 1. Genotype distribution and allele frequencies of the Ser49Gly polymorphism in the ADRB1 gene among patients with major depression (MD) and controls

	n	Genotypes			Allele frequencies	
		AA n (%)	AG n (%)	GG n (%)	A n (%)	G n (%)
MD	144	77 (53.5)	57 (39.6)	10 (6.9)	211 (73.2)	77 (26.8)
Control	105	74 (70.5)	23 (21.9)	8 (7.6)	74 (70.5)	23 (21.9)
		p*=0.626			p*=0.863	
*Chi-square test. MD: major depression; AA: homozygous wild type; AG: heterozygous type; GG: homozygous mutant type; A: adenine; G: guanine						

two DNA fragments with sizes of 342 bp and 154 bp (homozygous mutant type had another fragment that could not be observed on the gel), and heterozygous type (GC) had three DNA fragments of 530 bp, 342 bp, and 154bp (Figure 2).

Of the 144 participants in the major depressive disorder group, 75 (52.1%) had homozygous wild type (GG), 59 (41.0%) had heterozygous type (GC), and 10 (6.9%) had homozygous mutant genotype (CC). Of the 105 participants in the control group, 56 (53.3%) had homozygous wild type (GG), 42 (40.0%) had heterozygous type (GC), and 7 (6.7%) had homozygous mutant (CC) genotype. As shown in Table 2, there was no significant difference between the major depressive disorder and control groups after the analysis of genotype and allele frequencies of the Gly389Arg polymorphism in the ADRB1 gene ($p=0.625$; $p=0.914$).

DISCUSSION

Although some studies have investigated the functions of beta-adrenergic receptors in affective disorders, there is little evidence about the polymorphisms of beta-adrenergic receptors in major depressive disorder. It is necessary to compare the difference of polymorphisms between patients and controls to investigate the susceptibility to major depressive disorder.

There are two known functional polymorphisms of the ADRB1 gene: Ser49Gly and Gly389Arg that are located on 10th chromosome (10q24-10q26) (3,9). Zill et al. (9) suggested that there is no relationship between the polymorphism of Gly389Arg and major depressive disorder. However,

Table 2. Genotype distribution and allele frequencies of the Gly389Arg polymorphism in the ADRB1 gene among patients with major depression (MD) and controls

	n	Genotypes			Allele frequencies	
		GG n (%)	GC n (%)	CC n (%)	G n (%)	C n (%)
MD	144	75 (52.1%)	59 (41.0%)	10 (6.9%)	209 (72.5%)	79 (27.5%)
Control	105	56 (53.3%)	42 (40.0%)	7 (6.7%)	154 (73.3%)	56 (26.7%)
		p*=0.625			p*=0.914	
*Chi-square test. MD: major depression; GG: homozygous wild type; GC: heterozygous type; CC: homozygous mutant type; G: guanine; C: cytosine						

the antidepressant treatment response in major depression. Stein et al. (10) showed the polymorphisms of Ser49Gly and Gly389Arg to be associated with introvert personalities, social phobia, and depressive disorders. Additionally, another study reported that dysfunctional beta-1-adrenergic signaling is found in subjects who are carriers of Ser49 and 389Gly, whereas normal function is maintained for subjects who are homozygous for 49Gly/49Gly and 389Arg/389Arg (17).

With this regard, we investigated the polymorphisms and allele frequencies of Ser49Gly and Gly389Arg in a sample of the Turkish population for major depressive disorder. The mutant variants that may cause a conformational and functional change in ADRB1 receptors were compared between major depressive disorder patients and healthy controls. The mutant genotype (GG) for the Ser49Gly polymorphism was found 6.9% for the major depressive group and 7.6% for the control group. Additionally, the mutant genotype (GG) for the Gly389Arg polymorphism was 6.9% and 6.7% in the major depressive disorder and control groups, respectively. Although we found no significant difference between the control and major depressive disorder groups for the Ser49Gly and Gly389Arg polymorphisms, phenotypic complexity in psychiatric disorders should be considered. Conflicting results in literature and our research supports the hypothesis that specific phenotypes can be effective in different depression groups. The heterogeneity of symptoms in major depressive disorder should be an important factor for determining polymorphisms. In a recent study conducted by Veen et al. (18), it was found that many gene sets associate with anhedonic depression. Therefore, we should not generalize our results for all types of depression not to be associated with Ser49Gly

and Gly389Arg polymorphisms. Additionally, all participants in this study were ethnically homogenous, which is an important consideration for generalizing the results.

The patients with a family history of any psychiatric disorder were not enrolled in our study in order to exclude other genetic susceptibilities and to not define the diagnoses according to the statements of the patients. However, this could be another important reason for obtaining negative results. Further longitudinal studies including first-degree relatives with depression would provide more information about Ser49Gly and Gly389Arg polymorphisms.

The limitations of our study are as follows: we did not sample all known variations of the ADRB1 receptor genes that would have a small affect or interactions with other genes. The small number of study subjects, sample size of control subjects, and population stratification may affect the study and cause a negative result. We did not apply another scale for depression. Although we included the first episode and medication naive patients to exclude the role of psychotropics and other long-term environmental factors, some patients with first episode of major depression could include those with bipolar disorders.

Finally, our preliminary findings do not suggest that the polymorphism of Ser49Gly and Gly389Arg in the ADRB1 gene has a major effect on major depressive disorder. Further case control studies by controlling the types of major depressive disorders and including interactions of gene–gene, gene–environment, and other variations with larger sample sizes are required to elucidate the role of beta-1-adrenergic gene polymorphisms in major depressive disorder.

Conflict of Interest: No conflict of interest was declared by the authors.

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