

Paraoxonase (PON1) L55M and Q192R polymorphisms in major depression and bipolar affective disorder

MESUT YILDIZ^{1*}, FERYAL ÇAM ÇELIKEL², ÖMER ATEŞ³, SERAP ERDOĞAN TAYCAN⁴, İSMAIL BENLİ⁵, OSMAN DEMİR⁶

¹ Department of Psychiatry, Faculty of Medicine, Gaziosmanpaşa University, Tokat, Turkey.

² Department of Clinical Psychology, Institute of Social Sciences, Işık University, Istanbul, Turkey.

³ Department of Medical Biology, Faculty of Medicine, Gaziosmanpaşa University, Tokat, Turkey.

⁴ Psychiatry Department, Haydarpaşa Numune Training and Research Hospital, Istanbul, Turkey.

⁵ Department of Biochemistry, Faculty of Medicine, Gaziosmanpaşa University, Tokat, Turkey.

⁶ Department of Biostatistics, School of Medicine, Gaziosmanpaşa University, Tokat, Turkey.

* At Gaziosmanpaşa University when the study was conducted.

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Abstract

Background: Oxidative and nitrosative stress pathways, along with immune-inflammatory response, might play an important role in the pathogenic mechanisms underlying major depression and bipolar disorder. **Objective:** The aim of the present study is to investigate paraoxonase 1 polymorphisms and its correlations with disease parameters in patients with major depression and bipolar affective disorder. **Methods:** PON1 L55M and Q192R single nucleotide polymorphisms were analyzed in a group consisted of 100 patients with major depression, and 100 patients with bipolar affective disorder and 96 healthy controls. Polymorphisms were analyzed by using polymerase chain reaction. **Results:** Our findings reported no association between Q192R and L55M polymorphisms of PON1 and major depression and bipolar disorder. Additionally, there was no association between the PON1 genotypes and disease variables in both depressed and bipolar patients. **Discussion:** Evaluating the different stages of patients with affective disorders and investigating the connection between PON1 polymorphisms and treatment outcomes will help us to clarify the relationship between PON1 and mood disorders.

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Keywords: Major depression, bipolar affective disorder, paraoxonase (PON1), polymorphism, association study.

Introduction

Mood disorders are thought to be caused by a combination of environmental, psychological, biological and genetic factors. It was shown that oxidative and nitrosative stress pathways, along with immune-inflammatory response, might play an important role in the pathogenic mechanisms underlying major depression and bipolar disorder¹. Reactive oxygen species affect both the immune-inflammatory pathways and the expression of key neurotransmitters which are involved in the pathophysiology of depression². Studies demonstrated discrepancies in antioxidant enzyme levels in different stages of bipolar affective disorder^{3,4}. Additionally, adding antioxidant agents to treatment of patients with bipolar affective disorder caused a substantial decrease in depressive symptoms and increase in clinical functioning or quality of life measures⁵.

Paraoxonase is a calcium-dependent esterase that catalyzes the hydrolysis of neurotoxins such as organophosphates and aromatic carboxylic acids⁶. The paraoxonase (PON) gene family has three members, PON1, PON2, and PON3. Human paraoxonase 1 (PON1) is a high-density lipoprotein (HDL)-associated serum enzyme that exhibits a broad substrate specificity⁷. The PON1 enzyme has both paraoxonase and arylesterase activity and show antiinflammatory and antioxidative properties⁸. The PON1 gene contains two common polymorphisms found in the PON1 coding region, leading to a glutamine (Q) → arginine (R) substitution at position 192 (Q192R; rs 662) and to a leucine(L) → methionine (M) substitution at position 55 (L55M; rs 854560)⁹. The L55M polymorphism affects the enzyme concentration and the Q192R polymorphism is responsible for the hydrolytic activity of the enzyme^{10,11}. Studies concerning the relationship between PON1 polymorphism and mood disorders exhibited inconsistent results.

It was reported that PON1 Q192R polymorphism may be associated with symptoms of depression in older women¹². Another study demonstrated that PON1 Q192R-smoking interaction predicted the odds of depression¹³. Contrarily, no associations were detected between mood disorders and any of the Q192R genotypes¹⁴. In a genome-wide association study, the authors reported no associations between major depression and the Q192R polymorphism or any other polymorphism in the PON1 gene¹⁵. Also, no significant association was found between PON1Q192R polymorphism and depression in population-based studies¹⁶. While some studies found no differences in terms of PON1 activity between patients with depression and controls¹⁷; others demonstrated a diminished PON1 activity^{13,18,19}. Furthermore, it was indicated that antidepressant treatment increased the lowered paraoxonase/arylesterase levels¹⁹.

It was demonstrated that carrying homozygote or heterozygote mutated alleles of L55M and Q192R might cause susceptibility to bipolar I disorder⁹. The odds of bipolar disorder were increased by the QQ genotype of Q192R in smokers¹³. In a genome-wide association study, no associations between bipolar disorder and the Q192R polymorphism or any other polymorphism in the PON1 gene was detected. Two studies investigated PON1 activity in bipolar patients. The first one presented normal PON1 activity but the other showed decreased PON1 activity in bipolar patients^{13,20}. In a study consisting of both depressed and bipolar patients; lowered PON1 activity was found to be associated with comorbid mood disorders and tobacco use disorder¹⁴. In terms of polymorphisms, there were no significant associations between the patient groups and any of the three PON1 Q192R genotypes in the same study.

The aim of the present study is to investigate paraoxonase 1 polymorphisms and its correlations with disease parameters in patients with major depression and bipolar affective disorder.

Methods

This study used the DNA specimens of a former study entitled: "Investigation of Dopamine- β -hydroxylase Polymorphism in Patients with Major Depression, Bipolar Affective Disorder and Schizophrenia". The study group consisted of 100 patients with major depression (83 females and 17 males), and 100 patients with bipolar affective disorder (49 females and 51 males) and 96 healthy controls (48 females and 48 males). All depressed and bipolar patients were followed up at the Outpatient Clinic of the Psychiatry Department of Gaziosmanpasa University, School of Medicine. Diagnoses of major depression and bipolar disorder were made according to the DSM-IV (Diagnostic and Statistical Manual-IV) criteria (American Psychiatric Association, 1994)²¹. The control subjects were recruited from hospital staff. The study protocol was approved by the Clinical Researches Ethics Committee, and the written informed consents were obtained from the study participants.

Genotyping

Genomic DNA was extracted from peripheral leukocytes from EDTA-anticoagulated blood using the High Pure Polymerase Chain Reaction Template Preparation Kit (Roche Molecular Biochemicals, Mannheim, Germany) according to the manufacturer's instructions. To identify PON1 L55M and Q192R singlenucleotide polymorphisms (SNPs), genotyping was performed using commercially synthesized primers and fluorescently labeled probes (Metabion, Martinsried, Germany) and the LightCycler 480 II Real-Time Polymerase Chain Reaction System (Roche Diagnostics). The genotyping method was based on methods developed previously for genotyping both PON1 55 and 192 polymorphisms using LightCycler real-time polymerase chain reaction technology, which relies on fluorescence resonance energy transfer²². Target fragments of the human PON1 gene were amplified with specific primers. To detect the PON1 L55M polymorphism, 10 pmol of the forward primer 5'-CCTGCAATAATATGAAACAACCTG-3' and the reverse primer 5'-CTAGAACACAGAAAAGTCAAAGAAAAC-3' and 3 pmol of the sensor probe 5'-CTCTGAAGACATGGAGATACTGCC-fluorescein-3' and the anchor probe 5'-LCRed640-ATGGACTGGCTTTCATTAGCTCTGTGAGT-3' were added to genomic DNA. To detect the PON1 Q192R polymorphism, we also used 10 pmol of the forward primer 5'-ATTGTTGCTGTGGGACCTGAG-3' and the reverse primer 5'-CCTTCTGCCACCACTCGAAC-3' and 3 pmol of the sensor probe 5'-CCCCTACTTACAATCCTGGGAGAT-fluorescein-3' and the anchor probe 5'-LCRed705-ATTTGGGTTTACGCTGGTTCGTATGTTG-3'. Melting curves were transformed to melting peaks by plotting the negative derivative of the fluorescence signal versus the temperature. The genotypes were identified by creating a melting curve with specific melting points.

Statistical analysis

Descriptive analyses were performed to provide information on general characteristics of the study population. One way ANOVA test was used to compare the continuous data among groups. The continuous data were presented as the mean \pm standard deviation. Chi-Square test was used to compare the categorical data between/among groups. Categorical variables were presented as a count and percentage. A p-value < 0.05 was considered significant. Analyses were performed using SPSS 19 (IBM SPSS Statistics 19, SPSS inc., an IBM Co., Somers, NY)²³.

Results

The baseline clinical and demographics features of patients with major depression and bipolar disorder are shown in Table 1 and

Table 2. The mean age of the depressed group, bipolar group and controls were 38.21 ± 12.07 , 41.19 ± 12.25 and 37.34 ± 10.21 respectively. No significant difference in the mean ages and gender was observed between the bipolar patient group and the control group. The mean age was not different between depressed group and controls but the depressed group was different from controls in terms of gender. Namely, the majority of the depressed group consisted of females. All the PON1 gene polymorphisms analyzed were in Hardy-Weinberg equilibrium. Allele and genotype frequencies were not different between patients with depression and controls in with regard to L55M and Q192R polymorphisms of PON1 (Table 3). Likewise, there was not a significant difference of L55M and Q192R polymorphism between patients with bipolar disorder and controls (Table 4). Additionally, there was no association between the PON1 genotypes and disease variables in both depressed and bipolar patients.

Table 1. Baseline clinical and demographics features of the 100 study patients with major depression

Characteristic	Depressed patients group
Gender, no. male/female (male %/female %)	13/87 (13.0/87.0)
Age, mean \pm SD (range) years	38.21 \pm 12.07

Table 2. Baseline clinical and demographics features of the 100 study patients with bipolar disorder

Characteristic	Bipolar patients group
Gender, no. male/female (male %/female %)	49/51 (49.0/51.0)
Age, mean \pm SD (range) years	41.18 \pm 12.27
Age of onset, mean \pm SD (range) years	25.21 \pm 9.86
Bipolar disorder subtype, n (%)	
Type 1	87 (87%)
Type 2	13 (13%)
Rapid cycling, n (%)	17 (17%)
Seasonal pattern, n (%)	59 (59%)
Alcohol/drug use, n (%)	10 (10%)
Suicidal behavior, n (%)	26 (26%)
Psychotic feature, n (%)	55 (55%)
Hospitalization, n (%)	69 (69%)
Family history of BPD, n (%)	33 (33%)
Family history of suicide, n (%)	13 (13%)

Table 3. PON1 polymorphisms in patients with major depression and healthy controls

PON locus	Healthy controls n = 96 (%)	Major depression patients n = 100 (%)	P
PON55L/M			0.56
L/L	40 (41.7)	37 (37.0)	
L/M	42 (43.7)	43 (43.0)	
M/M	14 (14.6)	20 (20.0)	
Alleles			0.15
L	122 (63.5)	117 (58.5)	
M	70 (36.5)	83 (41.5)	
PON192Q/R			0.61
Q/Q	43 (44.8)	47 (47.0)	
Q/R	39 (40.6)	43 (43.0)	
R/R	14 (14.6)	10 (10.0)	
Alleles			0.24
Q	125 (65.0)	137 (68.5)	
R	67 (35.0)	63 (31.5)	

Table 4. PON1 polymorphisms in patients with bipolar affective disorders and healthy controls

PON locus	Healthy controls n = 96 (%)	Bipolar patients n = 100 (%)	P
PON55L/M			0.89
L/L	40 (41.7)	39 (39.0)	
L/M	42 (43.7)	47 (47.0)	
M/M	14 (14.6)	14 (14.0)	
Alleles			0.41
L	122 (63.5)	125 (62.5)	
M	70 (36.5)	75 (37.5)	
PON192Q/R			0.95
Q/Q	43 (44.8)	44 (44.0)	
Q/R	39 (40.6)	40 (40.0)	
R/R	14 (14.6)	16 (16.0)	
Alleles			0.41
Q	125 (65.0)	128 (64.0)	
R	67 (35.0)	72 (36.0)	

Discussion

The present study examined the L55M and Q192R polymorphisms of PON1 gene in patients with depression and bipolar disorder. We did not find any association between the L55M and Q192R polymorphisms and major depression and bipolar disorder. Also, no association was found between the PON1 genotypes and disease variables in both patient groups.

The lack of association between the L55M and Q192R polymorphisms and major depression in the present study is in line with Nunes et al's report¹⁴. Although, they did not distinguish depressed and bipolar patients and they only looked for the Q192R polymorphisms but not for the L55M polymorphisms; but they found no association between patients with mood disorders and any of the three PON1 Q192R genotypes. In a similar manner, no associations between major depression and the Q192R polymorphism or any other polymorphism in the PON1 gene have been found in a genome-wide association study¹⁵. However, there are some contradictory findings. In a study exploring the PON Q192R polymorphism in major depression in relation to nicotine dependence, it was demonstrated that PON Q192R-smoking interaction might play a role in depression¹³. A different study including British women aged 60–79 years, R allele of PON1 Q192R was found to be associated with increased odds of depression¹². Large differences between ethnic populations are known in the PON1 genotype distribution²⁴. So, it may be the reason for differences among studies. The majority of the subjects in the depressed group were female and it was significantly different from controls. As it was shown that gender had no impact on PON1 polymorphism²⁵, it was thought that the gender difference in the present study did not confound the results. The contradictory results are also present for PON1 activity. No differences were found in terms of PON1 activity between patients with depression and controls¹⁷. A diminished PON1 activity was also reported^{13,18,19}. In addition, long-term AD treatment seems to increase the paraoxonase/arylesterase levels in patients with depression¹⁹. In the present study, we did not measure PON1 activity. As in our study, the vast majority of the studies in the literature did not include any measure of PON1 activity.

We did not find any association between any association between the L55M and Q192R polymorphisms and bipolar disorder. Ezzaher et al. demonstrated that carrying homozygote or heterozygote mutated alleles of L55M and Q192R might cause susceptibility to bipolar I disorder⁹. It was indicated that the QQ genotype of Q192R in smokers increased the risk of bipolar disorder¹³. Our findings are consistent with a genome-wide association study reporting no associations between bipolar disorder and the Q192R polymorphism or any other polymorphism in the PON1 gene²⁶. In terms of PON1 activity in bipolar patients; one study demonstrated normal activity¹³

but the other showed decreased PON1 activity²⁰. The results of the present study and the discrepancies between the other studies might be associated with the distinct genotypic distribution of PON1 across different ethnic populations.

We also found no association between clinical variables of depression and bipolar disorder and the L55M and Q192R polymorphisms of PON1. Diminished serum paraoxonase and arylesterase activities and polymorphisms of PON1 in humans have been linked to heightened systemic oxidative stress^{8,27}. So, it could be expected that, the more serious forms of mood disorders might be associated with lower levels or lower activity of antioxidant properties.

Polymorphism regarding PON1 have not been studied too much in other psychiatric disorders. In a study including patients with schizophrenia, their relatives and healthy controls; authors suggested that the subjects carrying R allele or RR genotype of Q192R polymorphism might be susceptible to schizophrenia and subjects with QQ or LL of L55M polymorphism might be protected against schizophrenia²⁸. Another study looked for an association between Q192R and L55M polymorphisms of PON1 and autism spectrum disorders, but they found no association²⁹.

The results of the present study must be interpreted within the limitations of the study. Firstly, larger sample size of groups would be beneficial and the sample of our depressed and bipolar patients may not be representative of the whole patient populations. Secondly, our work is a cross sectional study that does not permit to follow-up of biological parameters. We did not measure PON activity but it is the common limitation of such association studies. Another limitation of the study is that reports from association studies constitute tentative knowledge and must be interpreted with caution³⁰.

In conclusion, our findings reported no association between Q192R and L55M polymorphisms of PON1 and major depression and bipolar disorder, suggesting that this polymorphism might not play a role in the physiopathology of mood disorders. There were also no significant associations between the polymorphisms of the PON1 gene and the clinical and the demographic characteristics of patients. Prospective studies with larger sample sizes evaluating the different stages of patients investigating the connection between PON1 polymorphisms and treatment outcomes will help us to clarify the relationship between PON1 and mood disorders.

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Conflict of interest

The authors do not have an affiliation with or financial interest in any organization that might pose a conflict of interest that are directly relevant to the contents of the paper.

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