

Paraoxonase1 192 (*PON1* 192) Gene Polymorphism and Serum Paraoxonase Activity in Panic Disorder Patients

HANDE ATASOY¹, SEDA GÜLEÇ-YILMAZ¹, ARZU ERGEN², UZAY GÖRMÜŞ³,
ÖZLEM KÜÇÜKHÜSEYİN², BURAK DALAN⁴, KEYSER KUSAT OL⁵, DICLE BİLGE ÖGÜT⁶,
HÜSEYİN GÜLEÇ⁶, BUĞRA ÇETİN⁶, ÖZLEM TIMIRCI-KAHRAMAN²,
BURCU ÖRMECİ⁷, NAZLI UÇUNOĞLU¹, GULDAL INAL GULTEKİN² and TURGAY İSBİR⁸

Departments of ¹Molecular Medicine and ⁸Medical Biology, Yeditepe University, Istanbul, Turkey;

²Institute of Experimental Medicine, Department of Molecular Medicine, Istanbul University, Istanbul, Turkey;

³Department of Biochemistry, Faculty of Medicine, Istanbul Bilim University, Istanbul, Turkey;

⁴Yeditepe University Hospital, Istanbul, Turkey;

⁵Department of Biochemistry, Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, Turkey;

⁶Erenkoy Mental Research and Training Hospital, Istanbul, Turkey;

⁷Department of Neurology, Yeditepe University Hospital, Istanbul, Turkey

Abstract. *Background/Aim: Reactive oxygen species (ROS) are involved in the development of certain neuropsychiatric disorders. Paraoxonase 1 (PON1) activity has been suggested to be adversely related to oxidative stress in plasma. The purpose of the present study was to demonstrate the relationship between serum PON1 activity and PON1 192 polymorphism in panic disorder (PD). Materials and Methods: Fourty-two patients with PD and 46 healthy controls were included in this study. PON1 192 genotypes were determined by polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) analysis. PON1 activity was measured by spectrophotometric assay of p-nitrophenol production following the addition of paraoxon. Results: PON1 192 AA genotype and A allele in PD were significantly higher than in the control group, whereas the B allele was found to be significantly higher in the control group. Patients with panic disorder have lower PON1 activity than the control group. Conclusion: The PON1 192 AA genotype may increase the risk of PD depending on lipid peroxidation.*

Panic disorder (PD) is an anxiety disorder characterized by recurrent, unexpected panic attacks and a series of long-term

symptoms and attitude between the attacks (1). There are many studies indicating that reactive oxygen species (ROS) are involved in the initiation and development of a wide range of neuropsychiatric disorders. Recently, it has been claimed that free radicals may have a serious role in the pathogenesis of PD. According to a study in which oxidant and antioxidant parameters have been measured in PD, the anti-oxidant levels observed in PD are higher than the control group. A positive relationship between disease severity and the oxidant and antioxidant levels was demonstrated in the same study (2). Another study evaluated the oxidative and antioxidative parameters in PD. Oxidants were found in higher levels in the patient group than in the control group, while there was no statistical significant difference with regard to antioxidant parameters (3).

Human paraoxonase (PON) is a Ca⁺⁺-dependent esterase synthesized in the liver. PON is related to high-density lipoprotein (HDL) (4). PON1 has two main roles: detoxifying organophosphate compounds, such as paraoxon, and protecting low-density lipoprotein (LDL) by hydrolysis of lipid peroxides (5, 6).

The molecular weight of the PON1 enzyme is 43 kDa and this protein consists of 354 amino acids (7). The PON1 enzyme is encoded by the *PON1* gene on the chromosomal 7q21.3-q22.1 region (8). The gene demonstrates two comon polymorphisms, one being a change from glutamine (Gln) to arginine (Arg) at position 192, and the second a change from methionine to leucine at position 55 (9, 10). The paraoxonase activity of the Gln-192 (A allele) isoform has been reported to be lower than that of the Arg-192 (B allele) isoform (11).

PON1 confers antioxidant properties in HDL with which it is associated by reducing the accumulation of lipid

Correspondence to: Professor Dr. Turgay İsbir, Chairman, Department of Medical Biology, Yeditepe University Faculty of Medicine, Yeditepe Üniversitesi Yerleşimi, İnönü Cad. 26 Ağustos Yerleşkesi, 34755 Kayışdağı-Ataşehir, Istanbul, Turkey. Tel/Fax: +90 5332823726/+90 2165780000 (ext. 1263), e-mail: turgay.isbir@yeditepe.edu.tr, tisbir@superonline.com

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peroxidation products. PON1 activity has been suggested to be adversely related to oxidative stress in plasma. Serum PON1 activity is affected by nutrition, several environmental factors and *PON1* polymorphisms (12, 13).

The aim of this study was to determine the *PON1* 192 gene polymorphisms and PON1 activity of PD patients in a Turkish cohort and whether there was any association between this polymorphism and PD.

Materials and Methods

Subjects. The study group consisted of 42 patients with PD and 46 healthy controls. Patients were selected from the Erenköy Mental and Neurological Disease Training and Research Hospital. They were diagnosed with PD, with or without agoraphobia, according to the DSM-IV diagnostic criteria.

DNA extraction. Whole blood samples were taken from all participants using vials containing EDTA (ethylene diamine tetra acetic acid). Genomic DNA was extracted from peripheral whole blood using the iPrep PureLink gDNA Blood Kit with the iPrep Purification Instrument (Invitrogen, Life Technologies, Grand Island, NY, USA). All purified DNA samples were stored at +4°C.

Genotype analysis. For the *PON1* 192 gene polymorphism, the sense primer 5' TAT TGT TGC TGT GGG ACC TGA G 3' and the antisense primer 5' CAC GCT AAA CCC AAA TAC ATC TC 3', which contained the 192 polymorphic region of the human *PON1* gene, were used. The polymerase chain reaction (PCR) mixture contained 100 ng of DNA template, 0.5M of each primer, 1.5 mM of magnesium chloride, 200 µM of dNTPs and 1U of Taq DNA polymerase (Bioron, Ludwigshafen, Germany). After denaturing the DNA for 5 minutes at 94°C, the reaction mixture was subject to 35 cycles of denaturation for 1 minute at 95°C, 1 minute annealing at 60°C and 1 minute extension at 72°C for the 192 genotype. The 99-bp PCR product was digested with 8 U BspPI restriction endonuclease (Thermo Fisher Scientific, Waltham, MA USA) overnight at 55°C and the digested products were separated by electrophoresis on a 3% agarose gel and visualized using ethidium bromide. The B genotype (arginine) contains a unique *BspI* restriction site, which results in 66-bp and 33-bp products, while the A genotype (glutamine) does not contain a restriction site thus allowing the 192 genotype to be determined (9).

Quantification of paraoxonase activity. Paraoxonase activities were measured according to Furlong *et al.* (14). The assay buffer contained 0.132 M Tris-HCl (pH 8.5), 1.32 mM CaCl₂ and 2.63M NaCl. Addition of 200 µl of 6 mM freshly prepared paraoxon (O, O-diethyl-O-p-nitrophenylphosphate; Sigma, Poole, UK) and 40 µl of serum initiated the assay. The rate of generation of p-nitrophenol was determined at 37°C with the use of a continuously recording spectrophotometer at 405 nm. A molar extinction coefficient of 18.05×10^3 was used for the calculation of p-nitrophenol using paraoxon as substrate. Paraoxonase activity is expressed as units/liter.

Statistical analysis. Statistical analyses were performed using the SPSS software package, version 21.0 (SPSS Inc, Chicago, IL, USA). The Student's *t*-test was used for mean age comparison between patients with PD and the healthy control group and analysis

Table I. Distribution of *PON1* 192 genotype and allele frequencies in the study groups.

<i>PON1</i> 192 polymorphism	Patient (n=42)	Control (n=46)
Genotypes		
AA	39 (92.9%)*	23 (50%)
AB	1 (2.4 %)	12 (26.1 %)**
BB	2 (4.8%)	11 (23.9%)*
Alleles		
A	79 (94.04%)*	58 (63.04%)
B	5 (5.96%)	34(36.96%)*

n: number of subjects. **p*=0.001, ***p*=0.015, ****p*=0.002.

Table II. *PON1* activity (U/l) in study groups.

	Patient (n=42)	Control (n=46)
<i>PON1</i> Activity (U/l)	105.79±101.38	162.77±102.14*

n: Number of subjects. **p*=0.01.

of variance (ANOVA) for comparison of PON activities between patients with PD and the healthy control group. Differences in the distribution of *PON1* genotypes between patients with PD were tested using either the Chi-square or the Fisher exact test. If the expected values in any cell of the 2-by-2 contingency table were less than 5, the Fisher's exact test was used. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated to estimate the risk for PD. All results were considered significant when the *p*-value was less than 0.05.

Results

Serum PON1 activity and *PON1* 192 genotypes and allele frequencies were analyzed in 42 PD and 46 control individuals. The demographic characteristics of patients with PD and controls, including gender, age, respiratory attack and agoraphobia status, were analyzed. The number of female patients with PD was 27 (64.3%), while the number of male patients with PD was 15 (35.7%). The number of PD patients with agoraphobia was 21 (67.74%). The number of PD patients with respiratory attack was 22 (70.96%). The mean age of patients with PD was found to be 34.52±10.29.

Genotype and allelic frequencies of the *PON1* 192 polymorphisms for the PD and control groups are shown in Table I. It has been observed that the frequency of the AA genotype (*p*=0.001) and the incidence of the A allele (*p*=0.015) in patients with PD was significantly higher than in the control group. Also, the frequency of the AB genotype (*p*=0.002), BB genotype (*p*=0.015) and B allele (*p*=0.001) in the control group was significantly higher than in the PD group.

Table III. *PON1* activity (U/l) according to *PON* 192 genotypes in the two study groups.

	Patient			Control		
	AA (n:39)	AB (n:1)	BB (n:2)	AA (n:23)	AB (n:12)	BB (n:11)
PON activity (U/l)	84.90±13.66	277.00±85.33	427.45±60.34	133.55±17.79*	199.03±24.63**	184.32±25.72**

n: Number of subjects. * $p=0.034$, Compared to patients with AA genotype. ** $p=0.011$, Compared to controls with AA genotype.

The serum PON1 activity was then compared in the PD patients and the control group (Table II). In terms of PON1 activity, patients with PD showed lower PON1 activity than the control group ($p=0.01$, 95% CI: 13.81-100.15).

The *PON1* 192 polymorphism and correlated serum PON1 activity in PD patients were compared to the control group as shown in Table III. According to *PON1* genotype within the study groups, the PON1 activity in AA genotype individuals in the control group was significantly higher than the AA genotype individuals in the PD group ($p=0.034$, 95% CI=4.00-93, 27). Due to the low frequency of patients having AB and BB genotype, these genotypes were excluded from the statistical analysis within the patient group. The PON1 activity was significantly higher in the AB and BB genotype individuals in the control group compared to the AA genotype individuals in the control group ($p=0.011$, 95% CI=15.98-114, 98).

Discussion and Conclusion

The human body has a number of endogenous free-radical scavenging systems, including paraoxonase (15). The possible explanation is that the central nervous system (CNS) may be more vulnerable to ROS-mediated injury than other tissues mainly because of the high rate of oxidative metabolic activity and a high concentration of readily oxidizable substrate (16). Increased production of ROS and/or decreased detoxification ability of CNS cells might cause increased oxidative stress in neuronal and glial cells leading to abnormal communication patterns of neurons (3). Bilici *et al.* have suggested that patients with major depression, especially melancholics, had elevated antioxidant enzyme levels and lipid peroxidation (17). Herken *et al.* reported that both adenosine deaminase (ADA) and xanthine oxidase (XO) levels were significantly higher in the patients (3). Thus, it may be suggested that patients with PD might be affected by the alterations in the purine or antioxidant enzyme metabolism. Kuloglu *et al.* proposed that free radicals might play an important role in the pathogenesis of PD (2).

This study included 42 patients with PD. The number of female patients was higher than the number of male patients.

Which is of note, as the prevalence of PD is twice as high in women then in male patients (18).

We observed that the frequency of the AA genotype and the incidence of the A allele in patients with PD is significantly higher than in the control group. Also, the frequency of AB genotype, BB genotype and B allele in the control group is significantly higher than in the patients' group. No significant association was reported between Alzheimer and the *PON1* 192 genotype (19).

However, Kucukali *et al.* found that the AA genotype was significantly higher in the control group with schizophrenic patients (20). This is the first study investigating the paraoxonase enzyme genotype related to PON1 enzyme levels in serum samples of PD patients and healthy individuals in terms of antioxidant activity. We found statistically significant higher PON1 levels in the control group compared to PD patients. Kuloğlu *et al.* have compared antioxidant processes using glutathione peroxidase (GPx), catalase (CAD), superoxide dismutase (SOD) and malondialdehyde (MDA), by products of lipid peroxidation, in 20 PD patients and 20 healthy controls. They found GPx, SOD and MDA levels to be significantly higher in PD (2). Herken *et al.* compared oxidant xanthine oxidase (XO) and nitric oxide (NO) levels between 32 PD and 20 control subjects and found XO oxidant levels to be higher in the PD group than in the control group. On the other hand, there was no statistically significant difference regarding antioxidant parameters (SOD and NO levels) (3). According to Işıl *et al.* MDA's oxidant level was found to be high in the PD group and anti-oxidant parameter (total antioxidant capacity, TAC; paraoxonase, PON; arylesterase, ARE) levels lower than the control group (21). To make such an assumption concrete, about the different genotypes and serum PON1 activity in PD, further and larger studies are needed.

According to our study, there are significant differences in distribution of *PON1* 192 genotypes between PD patients and healthy individuals. The AA genotype showed significantly lower serum PON1 activities in the PD group compared to controls. When the control group was tested for PON1 activity, AB and BB genotype individuals showed significantly higher activity than the AA genotype individuals. Our results confirm the evidence that the *PON1* 192 AA variant is associated with the low level of PON1 activity in the serum.

Low serum PON1 activity in PD with AA genotypes allows us to suggest that there may be an important relationship between AA homozygosity and the development of PD, probably through alterations in lipid peroxidation.

This study was the first to assess the relationship of *PON1* polymorphisms and PD in a Turkish cohort. Our results suggests that the AA genotype causes low efficacy both in genotype and PON1 activity in terms of antioxidant capacity. Therefore, The PON1 192 AA genotype may increase the risk of PD depending on lipid peroxidation

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