

Paraoxonase 1 Polymorphisms in Patients With Primary Glomerulonephritis

A Single-center Study in Turkey

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Introduction. Human paraoxonase 1 (PON1) is an enzyme related with high-density lipoprotein cholesterol. The link between genetic polymorphisms of PON1 and hyperlipidemia and increased lipid oxidation may explain these complications in the course of glomerular diseases. In this study, we aimed to investigate *PON1* 192 and *PON1* 55 polymorphisms in patients with primary glomerulonephritis and healthy individuals.

Materials and Methods. Eighty-six patients with biopsy-proven primary glomerulonephritis and 50 healthy controls were included in the study. Clinical characteristics, lipid profile, paraoxonase activity, and PON1 genotypes (*PON1* 192 and *PON1* 55) of all of the participants were studied.

Results. Histopathological diagnoses of the patients were membranoproliferative glomerulonephritis (53.5%), focal segmental glomerulosclerosis (33.7%), and membranous nephropathy (12.8%). The patients had lower PON1 activity levels than the healthy controls. No differences were observed in *PON1* 192 genotypes between the two groups. However, the controls were more likely to carry *PON1* 55 LM genotype (odds ratio, 4.10; 95% confidence interval, 1.96 to 8.61; $P < .001$) and M allele (odds ratio, 3.0; 95% confidence interval, 1.45 to 6.19; $P = .003$) compared to the patients with primary glomerulonephritis. There was a marked elevation in the frequency of *PON1* 55 LL genotype in the patients compared to the controls (odds ratio, 0.33; 95% confidence interval, 0.16 to 0.68; $P = .003$).

Conclusions. This preliminary study shows that the LL genotype might be a risk factor for the development of primary glomerulonephritis and the M allele might be a protective factor against its progression.

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INTRODUCTION

Human paraoxonase 1 (PON1) is a serum enzyme related to high-density lipoprotein cholesterol (HDL).¹ Paraoxonase 1 is important in the detoxification of both organophosphate insecticides and nerve gases and pro-atherogenic

phospholipid peroxides generated during the oxidative modification of low-density lipoprotein cholesterol (LDL).¹ Serum PON1 activity is affected by diet, environmental factors, and PON1 polymorphisms.² Paraoxonase 1 has 2 genetic polymorphisms revealing different amino acid

substitutions, one involving glutamine (A genotype) and arginine (B genotype) at position 192 and the other leucine (L genotype) and methionine (M genotype) at position 55. The A and M genotypes are associated with low serum PON1 activity and the B and L genotypes are associated with high serum PON1 activity.²

Recent studies focus on the pathogenesis and progression of kidney diseases. Focal segmental glomerulosclerosis (FSGS), membranoproliferative glomerulonephritis (MPGN), and membranous glomerulopathy are the most common glomerular diseases that are related to early-onset persistent hyperlipidemia and have a progressive course.³ Hyperlipidemia, increase in lipid oxidation reactions, and defects in antioxidant status may lead to glomerulosclerosis and progression of the glomerular disease in primary glomerulonephritis.^{4,5} No adequate knowledge exists as to whether *PON1* genotype affects the development or the clinical course of primary glomerulonephritis in patients except for 2 reports involving pediatric patients with FSGS and patients with immunoglobulin A nephropathy.⁶⁻⁸ The aim of our study was to compare the frequency of the two polymorphisms of the *PON1* gene between Turkish patients with primary glomerulonephritis and healthy controls.

MATERIAL AND METHODS

Patient Selection and Clinical Investigation

The study was carried out prospectively at Marmara University Medical Faculty between 2002 and 2006. Patients with a biopsy-proven diagnosis of primary glomerulonephritis (n = 86) and healthy controls (n = 50) were included in the study. The two groups were from a similar ethnic origin. Written informed consent was obtained from all patients before participation in the study and the protocol was approved by the ethics committee of Marmara University Medical Faculty. None of the study participants was obese, smoker, or under any medications known to interfere with lipid metabolism.

Eighty-six patients with biopsy-proven primary glomerulonephritis were included. The histopathological diagnoses were MPGN, FSGS, and membranous glomerulonephritis. The control group consisted of 50 volunteers with no history of hypertension, kidney disease, cardiac disease, hepatic disorder, obesity, or hyperlipidemia. Those

with a family history of kidney disease and those who were receiving treatment with antihypertensive or antilipidemic agents were excluded from the control group.

Biochemical Measurements

The clinical characteristics; serum creatinine, albumin, total cholesterol, LDLC, and HDLC; paraoxonase activity; and *PON1* genotypes (*PON1* 192 and *PON1* 55) of all of the participants were studied. Blood samples were obtained in the morning after overnight fasting at the initiation of study.

Genotyping Method

Blood specimens were collected in tubes containing ethylenediaminetetraacetic acid, and DNA was prepared from leucocyte pellets by sodium dodecyl sulfate lysis, ammonium acetate extraction, and ethanol precipitation.⁹ Paraoxonase 1 genotypes were determined following polymerase chain reaction (PCR) according to previously published protocols.^{10,11} For the 192 polymorphism, sense primer 5' TAT TGT TGC TGT GGG ACC TGA G 3' and antisense primer 5' CAC GCT AAA CCC AAA TAC ATC TC 3', which encompass the 192 polymorphic region of the human *PON1* gene, were used. For the 55 polymorphism, sense primer 5' GAA GAG TGA TGT ATA GCC CCA G 3' and antisense primer 5' TTT AAT CCA GAG CTA ATG AAA GCC 3' were used. The PCR reaction mixture contained 100 ng of DNA template, 0.5 M of each primer, 1.5 mM of magnesium chloride, 200 μM of dNTPs, and 1 U of Taq DNA polymerase (MBI Fermentas, Vilnius, Lithuania). 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 *BspI* restriction endonuclease (MBI Fermentas, Vilnius, Lithuania) overnight at 55°C and the digested products were separated by electrophoresis on a 4% metaphore 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 and the A genotype (glutamine) cannot be cut, allowing the 192 genotype to be determined.¹¹ For the *PON1* 55 polymorphism, the PCR reaction and the cycling conditions were

the same as above. The PCR product (170 bp) was digested with Hsp19211 (Promega, Madison, USA) in the presence of bovine serum albumin (0.1 µg/µL final concentration; 37°C; overnight), and the digested products were separated and identified as above. Allele L (leucine) did not contain the Hsp19211 site whereas M (methionine) contained the Hsp19211 site giving rise to 126-bp and 44-bp products.^{10,12}

Statistical Analyses

Statistical analyses were performed using the SPSS software (Statistical Package for the Social Sciences, version 15.0, SPSS Inc, Chicago, Ill, USA). Continuous values were expressed as mean ± standard deviation. The Mann Whitney U test was used for comparisons. Differences in the distribution of *PON1* 192 and *PON1* 55 genotypes or alleles between cases and controls were tested using the chi-square test. The Fisher exact test was used if the expected values in any cell of the 2-by-2 contingency table was less than 5. Relative risks and 95% confidence intervals (CI) were calculated as the odds ratio (OR). The 1-way analysis of variance test was used for comparison of the PON levels according to both *PON* genotypes. A *P* value less than .05 was considered significant.

RESULTS

There were 86 patients in the primary glomerulonephritis group, of whom 48 were men and 38 were women. The mean age of the patients was 38.7 ± 11.6 years and the mean follow-up period was 40 ± 12 months (range, 6 months and 11 years). The distribution of the histopathological diagnosis of the patients were as follows: MPGN in 46 (53.5%), FSGS in 29 (33.7%), and membranous nephropathy in 11 (12.8%) patients. There were 37 men and 13 women in the control group with the mean age of 37.7 ± 9.3 years. The clinical characteristics and the laboratory results of study groups are shown on Table 1. The mean age and gender distribution of the participants in the two groups were similar. The patients had higher serum total cholesterol, LDLC levels, and serum creatinine levels than the controls, while their serum albumin and PON1 activity levels were lower (Table 1).

The *PON1* 192 and *PON1* 55 gene genotypes and alleles of the study groups are shown in Table 2. No differences were observed in *PON1* 192 genotypes

Table 1. Clinical Characteristics and Laboratory Data of the Study Population*

Characteristics	Study Groups		P
	Glomerulonephritis	Control	
Age, y	38.7 ± 11.6	37.7 ± 9.3	.60
Gender			
Male	48 (55.8)	37 (74.0)	
Female	38 (44.2)	13 (26.0)	.05
Follow-up, mo	40 ± 12
Serum total cholesterol, mg/dL	259 ± 88	187 ± 37	< .001
Serum LDLC, mg/dL	156 ± 64	113 ± 38	<.001
Serum HDLC, mg/dL	42 ± 10	45 ± 12	.12
Serum albumin, g/dL	3.5 ± 0.8	4.2 ± 0.5	< .001
Serum creatinine, mg/dL	1.2 ± 0.7	0.7 ± 0.4	< .001
Serum PON1 activity, U/L	116 ± 70	200 ± 156	< .001

*Values in parenthesis are percents. LDLC indicates low-density lipoprotein cholesterol; HDLC, high-density lipoprotein cholesterol; and PON1, paraoxonase 1.

between the two groups. However, the controls were more likely to carry *PON1* 55 LM genotype (OR, 4.10; 95% CI, 1.96 to 8.61; *P* < .001) and M allele (OR, 3.0; 95% CI, 1.45 to 6.19; *P* = .003) compared to the patients with primary glomerulonephritis. Also, there was a marked elevation in the frequency of *PON1* 55 LL genotype in the patients compared to the controls (OR, 0.33; 95% CI, 0.16 to 0.68; *P* = .003; Table 2). There were no association of *PON1* 192 or *PON1* 55 polymorphism with the PON levels. The distribution of *PON1* polymorphisms in patients

Table 2. Distribution of Paraoxonase 1 Genotypes and Alleles

Characteristics	Study Groups	
	Glomerulonephritis (%)	Control (%)
PON1 192		
Genotypes		
AA	42 (48.8)	27 (54.0)
AB	34 (11.6)	18 (10.0)
BB	10 (39.5)	5 (36.0)
Alleles		
A	118 (68.6)	72 (72.0)
B	54 (31.4)	28 (28.0)
PON1 55		
Genotypes		
LL	54 (62.8)*	18 (36.0)*
MM	23 (26.7)	2 (4.0)
LM	9 (10.5)†	30 (60.0)†
Alleles		
L	117 (68.2)	38 (38.0)
M	55 (31.8)‡	62 (62.0)‡

**P* = .003

‡*P* = .003

†*P* < .001

with MPGN, FSGS, and membranous nephropathy were not significantly different.

DISCUSSION

In this study, we demonstrated the distribution of *PON1* 192 and *PON1* 55 polymorphisms in Turkish patients with primary glomerulonephritis and in healthy control individuals, which has not been studied before.

Hyperlipidemia is usually observed during the course of kidney diseases. Primary glomerular diseases presenting with nephrotic-range proteinuria are more likely to reach high serum levels of cholesterol with a persistent course.³ Total cholesterol levels above 200 mg/dL and LDLC levels above 160 mg/dL were reported in nearly 90% of the patients with a nephrotic state.¹³ Hyperlipidemia accelerates the structural changes in microcirculation involving lipid peroxidation and generation of toxic radicals.¹⁴ Mechanisms against oxidative stress affect the clinical course of the kidney disease. Besides these various alterations in the cellular level, an individual's genetic predisposition may be important in disease frequency and clinical progression.^{4,15} Paraoxonase 1 is important to decrease the pro-atherogenic molecules and its activity is affected by *PON1* polymorphisms.¹⁶ Recently, a report by Sanghera and colleagues drew attention to *PON1* 192 polymorphism by its association with an increased risk of coronary heart disease in some populations.¹⁷ The pathogenetic similarity between atherosclerosis and glomerulosclerosis reminds the investigators of the role of *PON1* polymorphisms in kidney diseases. Therefore, studies investigating genetic factors that might have roles in atherosclerotic and glomerulosclerotic process are important.

The number of studies investigating the distribution of *PON1* genotypes in glomerular diseases are very limited. The first study was done by Frisberg and associates on 47 children from two different ethnic populations—Arab and Jewish—with FSGS.⁶ They reported that homozygosity for the L allele of *PON1* confers susceptibility for developing FSGS in Arab children. The data emphasizing the presence of L allele as a predisposing factor for the development of FSGS was also confirmed by Biyikli and colleagues in Turkish children with FSGS.⁷

The unique study in adults with primary glomerulonephritis was performed by Kovacs

and colleagues.⁸ In a study involving 115 patients with IgA nephropathy, they reported that no differences were observed in the genotype frequency at 3 of the polymorphic sites (Q192R, L55M, and -162C/T) between the patients and controls. The polymorphisms were not related to the course of IgA nephropathy, either. We found that the presence of the L allele of *PON1* 55 polymorphism may also be a risk factor for the development of primary glomerulonephritis in Turkish patients. The prevalence of the LL genotype was observed in 63% of the patients with primary glomerulonephritis and 36% of the control subjects with a significant difference between them. No significant difference was found in the distribution of *PON1* 192 polymorphism. The presence of the A and B alleles are not related to the development of glomerulonephritis in Turkish patients.

CONCLUSIONS

Our study identifies that the presence of the LL genotype may be a risk factor for the development of primary glomerulonephritis, and also the M allele is a protective factor against to primary glomerulonephritis; however, larger studies are required to confirm this. We did not find any relationship between the clinical features of primary glomerulonephritis and *PON1* 192 and *PON1* 55 polymorphisms. Nevertheless, due to the relatively small sample size of each genotype group, these results should be viewed with caution.

CONFLICT OF INTEREST

None declared.

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