The relationship between paraoxanase gene Leu-Met (55) and Gln-Arg (192) polymorphisms and coronary artery disease

Paraoksonaz geninde Leu-Met (55) ve Gln-Arg (192) polimorfizmleleri ile koroner arter hastalığı arasındaki ilişki

Pinar Taskiran, M.D., Sirri F. Cam, M.D., Cevat Sekuri, M.D., Nurullah Tuzun, M.D., Emin Alioglu, M.D., Nuray Altintas, M.D., Afig Berdeli, M.D.

1Celal Bayar University, Faculty of Medicine, Department of Medical Biology and Genetics, Manisa, Turkey
2Kent Hospital, Department of Cardiology, Izmir, Turkey
3Central Hospital, Department of Cardiology, Izmir, Turkey
4Ege University, Faculty of Medicine, Department of Pediatrics, Izmir, Turkey

Amaç: Paraoksonaz (PON1), lipit peroksitleri hidroliz eden, yüksek yoğunluklu lipoproteine bağlı bir esterazdır. PON1, düşük yoğunluklu lipoproteinlerin (LDL) oksidatif modificasyonuna karşı ve aterosklerotik süreçleri önlemede önemli bir rol oynamaktadır. PON1 geninde iki polimorfizm yaygın şekilde çalışılmaktır. Bunlar, 55. kodonda lösinin (L aleli) yerine metiyoninin (M aleli) geçmesi ve 192. kodonda glutaminin (Q aleli) yerine arjininin (R aleli) geçmesidir.

Çalışma planı: Çalışmada, erken koroner arter hastalığı (KAH) tanıları konan 120 hastada (92 erkek, 28 kadın; ort. yaş 48.2±4.3) ve KAH öyküsü olmayan ve elektrokardiyografileri normal bulunan 102 sağlıklı bir bireyde (80 erkek, 22 kadın; ort. yaş 46.8±5.2) PON1 geninde 55 ve 192. kodonlardaki aminoasit değişiklikleri polimeraz zincir reaksiyonu ve kısıtlayıcı enzimler kullanılarak incelendi.

Bulgular: Hasta ve kontrol grupları’nda PON 55 bölgesinde genotip dağılımı MM için sırasıyla %6.7 ve %4.9, LM için %46.7 ve %65.7 bulundu. PON 192 bölgesinde ise genotip dağılımı şöyledi: RR %4.2 ve %2, RQ %40 ve %35.3, QQ %55.8 ve %62.8. PON 55 M alel frekansı hasta grubunda kontrollere göre daha fazla bulunurken (0.3 ve 0.2), 192 R alel frekansı kontrolle göre farklıdır (0.2). PON1 M/L55 polimorfizmi ile KAH arasında anlamlı ilişki görüldü (p=0.017); R/Q192 polimorfizmi ile KAH arasında ise anlamlı ilişki bulunmadı (p=0.445).

Sonuç: Paraoksonaz (PON1) geni, HDL-ile ilişkili postprandial ve post-exercise lipoprotein (VLDL/IDL) yüksekliği ile ilişkili olarak tanımlanır. Paraoksonaz genindeki polimorfizm ve konsantenın farmakolojik olarak kardiyovasküler hastalığı ve koroner arter hastalığı ve diğer kardiyovasküler hastalıkların tedavisinde önemlidir.

Anahtar sözcükler: Kolesterol, HDL/metabolizma; koroner arter hastalığı/enzymoloji/genetik; esterase/genetik; genotip; paraokson/metabolizma; polimeraz zincir reaksiyonu; polimorfizm, genetik.

Objectives: Paraoxonase (PON1) is a high-density lipoprotein (HDL)-associated esterase that hydrolyses lipoperoxides. PON1 serves as a protective factor against oxidative modification of LDL, suggesting that it may play an important role in the prevention of atherosclerotic process. Research has focused on two polymorphisms: leucine (L allele) to methionine (M allele) substitution at codon 55, and glutamine (Q allele) to arginine (R allele) substitution at codon 192.

Study design: We examined amino acid changes at codon 55 and 192 in the PON1 gene by polymerase chain reaction and using restriction enzymes in 120 patients (92 men, 28 women; mean age 48.2±4.3 years) with premature coronary artery disease (CAD) and in 102 healthy subjects (80 men, 22 women; mean age 46.8±5.2 years) with no history of CAD and a normal electrocardiogram.

Results: Distribution of genotypes in the patient and control groups at codon 55 was 6.7% and 4.9% for MM, 46.7% and 29.4% for LM, 46.7% and 65.7% for LL, respectively. The frequencies of genotypes at codon 192 were as follows: 4.2% and 2% for RR, 40% and 35.3% for RQ, and 55.8% and 62.8% for QQ, respectively. While the frequency of PON1 55M allele was higher in the CAD group (0.3 vs. 0.2), PON1 192R allele frequency did not differ (0.2). There was a significant relationship between the PON1 M/L55 polymorphism and CAD (p=0.017), whereas the R/Q192 polymorphism was not associated with CAD (p=0.445).

Conclusion: These data suggest that the PON1 M/L55 polymorphism shows a significant relationship with CAD and the Q/R192 polymorphism is not a major risk factor causing susceptibility to CAD in our population.

Key words: Cholesterol, HDL/metabolism; coronary artery disease/enzymology/genetics; esterases/genetics; genotype; paraoxon/metabolism; polymerase chain reaction; polymorphism, genetic.
Paraoxonase (PON1) is a high-density lipoprotein (HDL)-associated esterase that hydrolyses lipoperoxides.[1] It is synthesized in the liver. Paraoxonase hydrolyzes paraoxon, the active metabolite of the organic phosphorus insecticides parathion.[2,3] PON1 is present in a number of tissues apart from the liver, brain, heart, small intestine, and lung.[4]

The molecular weight of PON1 enzyme is 43 kDa and a 354-amino acid glycoprotein.[6] PON1 is encoded by a single gene on chromosome 7q21.3-q22.1 region.[7] PON1 gene is a member of a multigene family including PON2 and PON3 on the same chromosome. Its hydrophobic N-terminal signal sequence distinguishes PON1 from others.[8]

PON has two main functions: (i) detoxifying several organophosphate compounds such as paraoxon which is a pesticide, and (ii) preventing LDL from oxidation hydrolyzing lipid peroxides.[9]

The enzymatic activity of PON1 varies among individuals. Serum PON1 level is relatively low in the presence of very low HDL concentration.[8] Also, serum PON1 activity has been shown to be low in patients with lipid disorders which increase the risk of atherosclerosis, including MI, familial hypercholesterolemia, fish eye disease, Tangier disease, and diabetes mellitus (DM).[10] Polymorphisms encoding PON1 enzyme in the gene region account for the changes of the activity.[8]

Changes in amino acid in two regions of PON1 gene influence serum PON1 activity. Leu (r) Met and Gln (r) Arg amino acid changes at codon 55 and codon 192, respectively occur.[10]

PON1 prevents cholesterol accumulation in peripheral tissues, having an effect on cholesterol transfer and LDL lipid oxidation products.[10] As a result, PON1 gene is suggested to play a role in the pathogenesis of cardiovascular disease particularly in coronary artery disease (CAD).[11,13]

Many studies were carried out to identify the relationship between PON1 polymorphism and CAD.[11,13-15] Even in these studies, differences were found among ethnic populations, suggesting that gene interaction with the environment and/or another gene might have an effect on the relationship between PON1 enzyme and CAD.[8,13,16] These studies suggested that polymorphisms at codon 55 and 192 were associated with CAD risk.[11,13] Zama et al.[11] showed that PON1 192R allele was an independent risk factor for atherosclerosis. Another study including 200 Asians showed higher incidence of PON1 192R allele in patients with CAD.[13] In addition, another study including 408 patients with DM demonstrated that PON1 55 Leu polymorphism was an independent risk factor for CAD.[18] On the other hand, a Turkish study found a negative relationship between Arg192Gln polymorphism and CAD.[19]

In this study we investigated the relationship of CAD with PON1 Leu55Met and Gln 192 Arg.

PATIENTS AND METHODS

In this study 120 patients with early diagnosis of CAD who visited Cardiology Clinic and 102 healthy subjects (controls) with no history of CAD and normal laboratory parameters and electrocardiogram. Early CAD was defined as ≥50% stenosis in at least one of the main coronary vessels or branches, as assessed by coronary angiography in men ≤55 years of age and in women ≤65 years of age. Coronary angiography was performed using Judkin’s technique. Myocardial infarction was defined according to the World Health Organization (WHO) criteria on the basis of symptoms, elevation of cardiac enzymes, and serial ECG changes. Patients were informed about the study and ethical committee approval was obtained.

Diabetes mellitus, hypertension, hypercholesterolemia and cigarette smoking status were evaluated for coronary risk factors. Traditional biochemical assays were performed to measure triglyceride, total cholesterol, HDL-C, and LDL-C levels. Arterial hypertension is defined as a systolic blood pressure (BP) of ≥140 mmHg and/or diastolic BP of ≥90 mmHg, while DM was defined as a history of diabetes and a glucose level of ≥120 mg/dL. Patients were classified as smokers and non-smokers. Overweight was defined as a body mass index of ≥25 kg/m2. Family history of CAD was evaluated in patient visits and follow-up. Patients with congenital heart disease, cardiomyopathy, heart valve disease, renal or hepatic impairment, and using steroids and a substantial amount of alcohol were excluded.

Genetic analysis. Blood samples were drawn from peripheral vein in the forearm and collected into tubes containing K2 EDTA. Genomic DNA was isolated from 200 μL of 1 mL of peripheral blood samples using the NucleoSpin DNA Isolation Kit. DNA samples were
amplified using polymerase chain reaction. The following primers were used in amplification:

(i) 5’-TATTGTTGCTGTGGGACCTGAG-3’
(forward),

5’-CACGCTAAACCCAAATACATCTC-3’
(reverse) for

PON 192 Q/R polymorphism; (ii) 5’-

TTTAATCCAGAGCTAATGAAAGCC-3’
(reverse) for

PON 55 L/M polymorphism.[20,21]

A 25 μL volume of PZR including 2.5 μL of 10xPZR
buffer, 10 μM of dNTP mixture, 10 pmol/μL of primers,
one unit of Taq DNA polymerase enzyme, 1.0 μL of DNA
matrix and ddH2O was prepared for the reaction.
Polymerase chain reaction was performed at 95 ºC for 5
minutes, at 95 ºC for 40 seconds, at 61 ºC for 1 minute,
at 72 ºC for 1 minute (35 cycles), and at 72 ºC for 10 minutes
using thermocycler.

For polymerase chain reaction products Alw was used
in 192 Q/R polymorphism, while Nla III restrictive
enzymes were used in 55 L/M polymorphism. Fragments
were visualized by a %2-agorase gel electrophoresis under
UV light.

Statistical analysis. Statistical analysis was performed
using SPSS 10.0 version. Variables were expressed in
mean±standard deviation (SD). A P value of ≤0.05 was
considered to be significant. Univariate analyses were
performed by chi-square test and Mann-Whitney U test,
while genotype was defined using Hardy-Weinberg
equilibrium and chi-square test.

RESULTS
Majority of the patients (76.7%) and controls (78.4%)
were male. Family history of CAD, hypertension, DM,
cigarette smoking, overweight, and high level of total
cholesterol, LDL-C and triglycerides were common in
patients with CAD (Table 1).

Genotypes and frequencies for PON 55 M/L and 192
R/Q in patient and control groups are shown in Table 2.
Distribution of genotypes in the patient and control groups
at codon 55 was 6.7% for MM, 46.7% for LM, and 46.7%
for LL. The frequencies of genotypes at codon 192 were as
follows: 4.2% for RR, 55.8% for QQ, and 40% for RQ.
While the frequency of PON1 55M allele was higher in
the CAD group (0.3 vs. 0.2), PON1 192R allele frequency
did not differ (0.2). There was a significant relationship
between the PON1 M/L55 polymorphism and CAD
(p=0.017), whereas the R/Q192 polymorphism was not
associated with CAD (p=0.445).

DISCUSSION
Coronary artery disease is a complex disease with
contributions from both genetic and environmental factors
in developed countries. Epidemiological studies have
shown many risk factors for CAD. Low HLD-C level is

<table>
<thead>
<tr>
<th>Table 1. Demographic characteristics and risk factors of the patients and controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient (n=120)</td>
</tr>
<tr>
<td>n</td>
</tr>
<tr>
<td>----</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Body mass index (kg/m2)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Family history (Coronary artery disease)</td>
</tr>
<tr>
<td>Hypertension</td>
</tr>
<tr>
<td>Cigarette smoking</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
</tr>
<tr>
<td>Single-vessel disease</td>
</tr>
<tr>
<td>Multi-vessel disease</td>
</tr>
</tbody>
</table>
considered to be a leading risk factor. Every 1% reduction in HDL-C level increases the CAD risk by 2-3%. As a result, mechanisms of HDL-C having a protective effect have been extensively investigated. Serum paraoxonase enzyme, an HDL-associated enzyme, accounts for antioxidative capacity of HDL. PON1 hydrolyzes several organophosphates. In vitro studies have shown that HDL-mediated PON1 prevents LDL oxidation and breaks bioactive lipids in oxidized LDL. PON1 which is in the normal artery wall increase the concentrations in atherosclerotic process. Aviram et al. found that PON1 reduced oxidized lipids in atherosclerotic lesions which were sampled from coronary artery or carotid.

PON1 which encodes PON1 enzyme has two major polymorphisms: (i) 55 L/M including methionine instead of leucine (Leu-L) at codon 55, and (ii) 192 Q/R including arginine (Arg-R) instead of glutamine (Gln-Q). In 192 Q/R polymorphism, patients with Gln allele have lower PON1 enzyme activity compared to those with Arg allele. On the other hand, compared to paraoxonase, PON1 activity was to be lower in patients with MM homozygous rather than LL homozygous in 55 L/M polymorphism.

In vitro and in vivo prevention of PON1 oxidation has also been considered to be an independent risk factor for CAD, due to its effect on serum activities of polymorphisms in PON1 gene which encodes PON1. It is on debate whether polymorphisms which encode PON1 enzyme are associated with the CAD risk. While several studies show positive results, some of them do not have any suggestion. It may be explained by the differences in the population, dietary habits, environmental factors, and study designs.

There are many case-control studies suggesting that R polymorphism is associated with CAD rather than Q polymorphism in PON1 192. Some of them have shown that PON1 R allele increase the predisposition of risk factors of CAD including DM, cigarette smoking, and age, whereas others suggest no relationship between 192 R polymorphism and CAD. PON1 55 L alloenzyme is more effective in prevention of LDL oxidation, compared to M alloenzyme. None of the case-control studies have suggested a relationship with PON1 55 L allele and atherosclerosis.

In this study, we found a relationship between PON 55 L/M polymorphism and CAD, but not with 192 Q/R polymorphism. Similarly, another study which included Turkish population reported no significant relationship between PON 192 R/Q polymorphism and CAD. The study included 96 patients with CAD in Gaziantep and found R allele frequency to be 38.5%, and 31% in patients and controls, respectively. In another study in Southeast Anatolia Region, Aynacioglu et al. found the distribution of genotypes as follows: QQ 0.49, QR 0.40, RR 0.11, and LL 0.52, LM 0.39, MM 0.09 in 55 and 192 in the PON gene. The reason for different results may be explained by different inclusion criteria and differences among ethnic groups, and even subjects. Considering the ethnic differences in the gene polymorphisms, polymorphisms which may be related to CAD should be investigated in all subjects with high or low risk in each subgroup.

There were some limitations with respect to the study. First, enzyme activity was not measured; therefore enzyme levels (i.e. phenotypic appearance) were different particularly in heterozygotes, and cigarette smoking and DM PON1 affected activation levels. Second, the sample

| Table 2. Distribution of PON1 genotypes according to the patient and control groups |
|-----------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                                  | Patient (n=120)   | Control (n=102)   | p                 |
| PON1 55 L/M genotypes           | n     %   | n     %   |                   |                   |
| LL                                | 56    46.7 | 67    65.7 |                   |                   |
| LM                                | 56    46.7 | 30    29.4 |                   |                   |
| MM                                | 8     6.7  | 5     4.9  |                   |                   |
| PON1 55 L/M frequency            | 0.7/0.3 | 0.8/0.2 | 0.017             |                   |
| PON1 192 Q/R genotypes           | n     %   | n     %   |                   |                   |
| QQ                                | 67    55.8 | 64    62.8 |                   |                   |
| QR                                | 48    40.0 | 36    35.3 |                   |                   |
| RR                                | 5     4.2  | 2     2.0  |                   |                   |
| PON1 192 Q/R frequency           | 0.8/0.2 | 0.8/0.2 | 0.445             |                   |
size was small in the study. A few number of patients with RR and MM alleles was also another limitation. Studies with large number of patients are needed to confirm the relationship between polymorphisms and coronary artery disease. Even though such limitations, it is still obvious that each study investigating polymorphic patterns within and between communities showing extensive variations may contribute to the general knowledge. In addition, this study is important in respect of the variety of gene regions in CAD which is a multigenic and complex disease. All data is critical in diagnosis and treatment of CAD, a major public health problem, and also taking prevents for patients who are at risk at the early stage of the disease.

In conclusion, these data show a significant relationship between CAD and PON1 M/L 55, while no relationship between 192 Q/R polymorphism and CAD, suggesting that PON 55 L/M may be considered as a risk factor for CAD development.

REFERENCES


