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) polimorfizmleri ile koroner arter hastalığı arasındaki ilişki

Pinar Taskiran, M.D.,[1] Sirri F. Cam, M.D.,[1] Cevat Sekuri, M.D.,[2] Nurullah Tuzun, M.D.,[3] Emin Alioglu, M.D.,[3] Nuray Altintas, M.D.,[4] Afig Berdeli, M.D.[4]

[1]Celal Bayar University, Faculty of Medicine, Department of Medical Biology and Genetics, Manisa, Turkey
[2]Kent Hospital, Department of Cardiology, Izmir, Turkey [3]Central Hospital, Department of Cardiology, Izmir, Turkey
[4]Ege 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 modifikasyonuna karşı ve aterosklerotik süreçleri önlemede önemli bir rol oynamaktadır. PON1 geninde iki polimorfizm yaygın şekilde çalışılmıştır. 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) tanısıı konan 120 hastada (92 erkek, 28 kadın; ort. yaş 48.2±4.3) ve KAH öyküsü olmayan ve elektrokardiyografileri normal bulunan 102 sağlıklı 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'DDnda PON 55 bölgesinde genotip dağılımı MM için sırasıyla %6.7 ve %4.9, LM için %46.7 ve %29.4, LL için ise %46.7 ve %65.7 bulundu. PON 192 bölgesinde ise genotip dağılımı şöyleydi: RR %4.2 ve %2, QR %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 kontrollerle farklılık göstermedi (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: Bulgularımız, PON 55 M/L polimorfizmi ile KAH arasıında ilişki olduğunu, 192 R/Q polimorfizminin toplumumuzda KAH'ye yatkınlık sağlamada risk faktörü olmadığını göstermektedir.

Anahtar sözcükler: Kolesterol, HDL/metabolizma; koroner arter hastalığı/enzimoloji/genetik; esteraz/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 (A allele) to arginine (B 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 QR, 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.

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Corresponding address: Dr.Nurullah Tüzün. Central Hospital Kardiyoloji Bölümü, 35580 Bayraklı, İzmir
Tel: +90 232 341 67 67, e-mail:nurullahtuzun@hotmail.com

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.[4,5]

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 paraoxone 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.[6]

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.[8]

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.[6,15,16] These studies suggested that polymorphisms at codon 55 and 192 were associated with CAD risk.[11,12] Zama et al.[13] 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.[17] 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

Table 1. Demographic characteristics and risk factors of the patients and controls

	Patient (n=120)			Controls (n=102)			
	n	%	Mean±SD	n	%	Mean±SD	*p*
Age			48.2±4.3			46.8±5.2	>0.05
Sex							>0.05
Male	92	76.7		80	78.4		
Female	28	23.3		22	21.6		
Body mass index (kg/m2)			27.1±1.8			23.9±2.2	0.01
Diabetes mellitus	45	37.5		3	2.9		0.01
Family history (Coronary artery disease)	50	41.7		12	11.8		0.01
Hypertension	45	37.5		15	14.7		0.01
Cigarette smoking	78	65.0		37	36.3		0.01
Total cholesterol (mg/dL)			205.6±31.3			178.3±22.6	0.01
HDL-C (mg/dL)			40.7±3.1			43.4±3.8	0.024
LDL-C (mg/dL)			131.5±24.2			121.6±25.2	0.039
Triglyceride (mg/dL)			179.8±71.2			156.2±49.4	0.001
Single-vessel disease	44	36.7		-			
Multi-vessel disease	65	54.2		-			

amplified using polymerase chain reaction. The following primers were us 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 95ºC for 5 minutes, at 95 ºC for 40 seconds, at 61ºC for 1 minute, at 72ºC for 1 minute (35 cycles), and at 72ºC for 10 minutes using thermocycler.

For polymerase chain reaction products Alw was used in 192 Q/R polymorphism, while NIa 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 2. Distribution of PON1 genotypes according to the patient and control groups

	Patient (n=120)		Control (n=102)		
	n	%	n	%	p
PON1 55 L/M genotypes					
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					
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**

considered to be a leading risk factor. [22] Every 1 %-reduction in HDL -C level increase the CAD risk by 2-3%.[23] As a result, mechanisms of HD-C having a protective effect have been extensively investigated. Serum paraoxonase enzyme, an HDL-associated enzyme, accounts for antioxidative capacity of HDL. PON1 hydrolizes several organophosphates. In vitro studies have shown that HDL-mediated PON1 prevents LDL oxidation and breaks bioactive lipids in oxidized LDL.[24] PON1 which is in the normal artery wall increase the concentrations in atherosclerotic process. Aviram et al.[25] 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.[20,26] 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.[27]

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.[8] 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 does not have any suggestion.[11,12,28] 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.[4-6] 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.[29,30] 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.[31]

In this study, we found a relationship between PON 55 L/M polymorphism and CAD, but not with192 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.[19] In another study in Southeast Anatolia Region, Aynacioglu et al.[32] 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

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 between 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 preventions 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

1. Antikainen M, Murtomäki S, Syvänne M, Pahlman R, Tahvanainen E, Jauhiainen M, et al. The Gln-Arg191 polymorphism of the human paraoxonase gene (HUMPONA) is not associated with the risk of coronary artery disease in Finns. J Clin Invest 1996;98:883-5.
2. Jureti_ D, Tadijanovi_ M, Reki_ B, Simeon-Rudolf V, Reiner E, Barici_ M. Serum paraoxonase activities in hemodialyzed uremic patients: cohort study. Croat Med J 2001;42:146-50.
3. Li WF, Furlong CE, Costa LG. Paraoxonase protects against chlorpyrifos toxicity in mice. Toxicol Lett 1995; 76:219-26.
4. La Du BN, Adkins S, Kuo CL, Lipsig D. Studies on human serum paraoxonase/arylesterase. Chem Biol Interact 1993;87:25-34.
5. La Du BN. Structural and functional diversity of paraoxonases. Nat Med 1996;2:1186-7.
6. Durrington PN, Mackness B, Mackness MI. Paraoxonase and atherosclerosis. Arterioscler Thromb Vasc Biol 2001; 21:473-80.
7. Motti C, Dessì M, Gnasso A, Irace C, Indigeno P, Angelucci CB, et al. A multiplex PCR-based DNA assay for the detection of paraoxonase gene cluster polymorphisms. Atherosclerosis 2001;158:35-40.
8. Aviram M. Does paraoxonase play a role in susceptibility to cardiovascular disease? Mol Med Today 1999; 5:381-6.
9. Mackness MI, Durrington PN. HDL, its enzymes and its potential to influence lipid peroxidation. Atherosclerosis 1995;115:243-53.
10. Sanghera DK, Saha N, Aston CE, Kamboh MI. Genetic polymorphism of paraoxonase and the risk of coronary heart disease. Arterioscler Thromb Vasc Biol 1997; 17:1067-73.
11. Ruiz J, Blanché H, James RW, Garin MC, Vaisse C, Charpentier G, et al. Gln-Arg192 polymorphism of paraoxonase and coronary heart disease in type 2 diabetes. Lancet 1995;346:869-72.
12. Serrato M, Marian AJ. A variant of human paraoxonase/arylesterase (HUMPONA) gene is a risk factor for coronary artery disease. J Clin Invest 1995;96:3005-8.
13. Zama T, Murata M, Matsubara Y, Kawano K, Aoki N, Yoshino H, et al. A 192Arg variant of the human paraoxonase (HUMPONA) gene polymorphism is associated with an increased risk for coronary artery disease in the Japanese. Arterioscler Thromb Vasc Biol 1997;17:3565-9.
14. Suehiro T, Nakauchi Y, Yamamoto M, Arii K, Itoh H, Hamashige N, et al. Paraoxonase gene polymorphism in Japanese subjects with coronary heart disease. Int J Cardiol 1996;57:69-73.
15. Imai Y, Morita H, Kurihara H, Sugiyama T, Kato N, Ebihara A, et al. Evidence for association between paraoxonase gene polymorphisms and atherosclerotic diseases. Atherosclerosis 2000;149:435-42.
16. Saha N, Roy AC, Teo SH, Tay JS, Ratnam SS. Influence of serum paraoxonase polymorphism on serum lipids and apolipoproteins. Clin Genet 1991;40:277-82.
17. Pati N, Pati U. Paraoxonase gene polymorphism and coronary artery disease in Indian subjects. Int J Cardiol 1998;66:165-8.
18. Garin MC, James RW, Dussoix P, Blanché H, Passa P, Froguel P, et al. Paraoxonase polymorphism Met-Leu54 is associated with modified serum

concentrations of the enzyme. A possible link between the paraoxonase gene and increased risk of cardiovascular disease in diabetes. J Clin Invest 1997;99:62-6.

19. Aynacıoğlu AS, Kepekçi Y. The human paraoxonase Gln-Argl92 (Q/R) polymorphism in Turkish patients with coronary artery disease. Int J Cardiol 2000;74:33-7.
20. Humbert R, Adler DA, Disteche CM, Hassett C, Omiecinski CJ, Furlong CE. The molecular basis of the human serum paraoxonase activity polymorphism. Nat Genet 1993;3:73-6.
21. Mackness B, Durrington PN, Mackness MI. Human serum paraoxonase. Gen Pharmacol 1998;31:329-36.
22. Castelli WP. Lipids, risk factors and ischaemic heart disease. Atherosclerosis 1996;124 Suppl:S1-9.
23. Gordon DJ, Probstfield JL, Garrison RJ, Neaton JD, Castelli WP, Knoke JD, et al. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. Circulation 1989;79:8-15.
24. Watson AD, Navab M, Hama SY, Sevanian A, Prescott SM, Stafforini DM, et al. Effect of platelet activating factor-acetylhydrolase on the formation and action of minimally oxidized low density lipoprotein. J Clin Invest 1995;95:774-82.
25. Aviram M, Hardak E, Vaya J, Mahmood S, Milo S, Hoffman A, et al. Human serum paraoxonases (PON1) Q and R selectively decrease lipid peroxides in human coronary and carotid atherosclerotic lesions: PON1 esterase and peroxidase-like activities. Circulation 2000; 101:2510-7.
26. Adkins S, Gan KN, Mody M, La Du BN. Molecular basis for the polymorphic forms of human serum paraoxonase/arylesterase: glutamine or arginine at position 191, for the respective A or B allozymes. Am J Hum Genet 1993;52:598-608.
27. McElveen J, Mackness MI, Colley CM, Peard T, Warner S, Walker CH. Distribution of paraoxon hydrolytic activity in the serum of patients after myocardial infarction. Clin Chem 1986;32:671-3.
28. Qin Q, Li YL, Zhao FM, Wang H, Li Y, Cui RZ, et al. Association of paraoxonase polymorphisms and serum homocysteine thiolactone complex with coronary heart disease. Zhonghua Xin Xue Guan Bing Za Zhi 2006;34:803-7. [Abstract]
29. Sentí M, Tomás M, Vila J, Marrugat J, Elosua R, Sala J, et al. Relationship of age-related myocardial infarction risk and Gln/Arg 192 variants of the human paraoxonase1 gene: the REGICOR study. Atherosclerosis 2001; 156:443-9.
30. Herrmann SM, Blanc H, Poirier O, Arveiler D, Luc G, Evans A, et al. The Gln/Arg polymorphism of human paraoxonase (PON 192) is not related to myocardial infarction in the ECTIM Study. Atherosclerosis 1996; 126:299-303.
31. Sanghera DK, Aston CE, Saha N, Kamboh MI. DNA polymorphisms in two paraoxonase genes (PON1 and PON2) are associated with the risk of coronary heart disease. Am J Hum Genet 1998;62:36-44.
32. Aynacıoğlu AS, Cascorbi I, Mrozikiewicz PM, Nacak M, Tapanyigit EE, Roots I. Paraoxonase 1 mutations in a Turkish population. Toxicol Appl Pharmacol 1999; 157:174-7.