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Role of Angiotensin Converting Enzyme, Paraoxonase 1 55, 192 Gene Polymorphisms in Syndrome X and Coronary Heart Disease

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Abstract: Aim of this study was to investigate the possible relationship between ACE and/or PON1 M55L, Q192R genetic polymorphisms and subjects with Coronary Heart Disease (CHD) and/or syndrome X (SX) when compared to the control group. ACE I/D, PON1 M55L and Q192R genetic polymorphisms, Body Mass Index (BMI) and biochemical parameters were investigated in subjects with CHD (n = 19), SX (n = 34) and healthy subjects (n = 26). All of the subjects were nonsmokers. According to the unrelated group t-test results; BMI, HDL-C and TG values were found to be slightly different in SX and control subjects but there was no significant difference in LDL-C and TC values. According to the Mann Whitney U-test results, BMI, TC, HDL-C and LDL-C values were found to be significantly different among CHD and control group subjects, but there was no difference in TG values. The results of this study indicates that ACE, PON1 192 and PON1 55 gene polymorphisms are not related to genetic susceptibility to SX and/or CHD in non-smokers. Obviously, the interpretation of these finding is difficult due to the small sample size and larger group studies are needed for more definitive conclusions

Key words: Angiotensin converting enzyme, paraoxonase 1, syndrome X, coronary heart disease, genetic polymorphism

INTRODUCTION

Coronary Heart Disease (CHD) is one of the most important reasons of morbidity and mortality today's society. The syndrome X disease (SX) which is characterized by a constellation of fasting hyperglycemia, hypertriglyceridemia, low HDL-C, hypertension and/or abdominal obesity is thought to be a potential risk factor for CHD (Norman *et al.*, 2005) and a positive association is reported in several case-control studies. Genes that influence the renin-angiotensin system and LDL oxidation have been investigated in recent years as potential etiologic candidates of cardiovascular and syndrome X diseases. Genetic polymorphisms of paraoxonase locus and Angiotensin I-Converting Enzyme (ACE) were associated with risk for coronary heart disease in some studies (Berkovich *et al.*, 2001; Dakik *et al.*, 1997; Uemura *et al.*, 2000; Thomas *et al.*, 2001; Zaman *et al.*, 2001; Schmidt *et al.*, 1998) but other studies contradicted these results (Mansur *et al.*, 2000; Covolo *et al.*, 2003; Deng *et al.*, 2002; Esparragon *et al.*, 2002; Shadrina *et al.*, 2001; Lim *et al.*, 2002; Gardemann *et al.*, 2000;

Antikainen *et al.*, 1996). The reason for these contradictory results may be due to the fact that the study populations were ethnically different.

The Angiotensin I-Converting Enzyme (ACE) is an ectoenzyme of vascular endothelial cells, also secreted in plasma that plays an important role in cardiovascular homeostasis through angiotensin II formation and bradykinin inactivation. In humans, the levels of plasma and cellular ACE are strongly genetically determined (Covolo *et al.*, 2003).

RAS plays a central role in the regulation of blood pressure, sodium metabolism and renal hemodynamics, with its actions mediated primarily by angiotensin II.

Angiotensin II is a potent mitotic and trophic hormone and bradykinin is a cardioprotective hormone. Dynamic regulation of expression ACE in the heart, along with the diverse biological activity of ACE denotes an active role for this enzyme in many cardiovascular diseases such as heart failure, ventricular remodeling, cardiac hypertrophy and atherosclerosis (Hubacek *et al.*, 2000). The ACE gene, which has been mapped to human chromosome 17q23 and is characterized by an

insertion/deletion polymorphism based on the presence (insertion [I]) or absence (deletion [D]) with intron 16 of a 287-base pair alu repeat sequences, resulting in three genotypes (DD and II homozygote and ID heterozygote) (Eichner *et al.*, 2001).

Genes affecting LDL oxidation are excellent candidate genes for CAD. Paraoxonase (PON) is an HDL-bound ester hydrolase that catalyses the hydrolysis of a number of organic esters and protects LDL from oxidation. Two frequent polymorphisms present at the PON1 gene are the methionine (M allele) leucine (L allele) interchange at position 55 and the arginine (R allele) glutamine (Q allele) interchange at position 192. PON1 activity is considerably lower in patients with myocardial infarction (MI), hypercholesterolemia and diabetes. Genetic polymorphisms at the paraoxonase locus are associated with paraoxonase concentration and activity and risk for CHD with some studies showing increased risk (Aviram *et al.*, 2000; Watzinger *et al.*, 2002; Olivera *et al.*, 2004) and others with contradictory results (Agachan *et al.*, 2004; Suehiro *et al.*, 1996).

Family-based association analysis was performed to investigate whether ACE, PON1 55 and 192 polymorphisms are risk factors for CHD in a number of populations, while some studies showed an association, the others did not. In the present study, we tried to investigate the question, Is there any relationship between ACE, PON1 M55L and Q192R genetic polymorphisms (alone or together) and subjects with CHD and SX as compared to the control group? The answer to this question may refine our understanding of the potential role of genetic variation at ACE I/D, PON1 55 L/M and 192 Q/R on CHD and SX in a cohort of Turkish population.

MATERIALS AND METHODS

This research project was conducted from 2003 to 2004. The study group consisted of 34 subjects with SX and 19 with CHD and the control group consisted of 26 healthy individuals, mostly blood donors with no family history of cardiovascular disease. CHD and SX patients were grouped clinically according to the American College of Cardiology/European Society of Cardiology and world health organization definitions, respectively. Patients were selected from Dr. Siyami Ersek Thoracic and Cardiovascular Surgery Training and Research Center, Istanbul. Before the study, all participants read and signed an informed consent approved by the Siyami Ersek Hospital ethics committee.

The patients received a standard questionnaire containing questions age, family history, the method of treatment and other medical issues. All patients and

control groups were nonsmokers. Blood specimens were collected in tubes containing EDTA and DNA was extracted from leucocytes manually by three-step phenol/chloroform extraction and stored at 4°C (Adkins *et al.*, 1993; Humbert *et al.*, 1993). Polymerase Chain Reaction (PCR) based protocols were used to identify the ACE gene I/D and PON1 gene L55M, Q192R polymorphisms according to studies (Rigat *et al.*, 1990; Agachan *et al.*, 2004). BspI restriction, Hsp92II enzyme were used for RFLP restriction of PON1 L55M and Q192R, respectively. PCR products were separated by 2% (ACE) and 4% agarose (PON) gel electrophoresis, stained with ethidium bromide and visualized under ultraviolet light.

The presence (I allele) or absence (D allele) of the 287 bp Alu repeat in intron 16 of the ACE gene was determined by evaluating the size of DNA fragments after PCR amplification, using the appropriate primers. Due to the fact that 4-5% of samples with the ID genotype could be misclassified as DD with older methods, mistyping of I/D heterozygotes was controlled using insertion specific primers during a second PCR as suggested by Lindpaintner *et al.* (1995).

PCR-RFLP (restriction fragment length polymorphism) based protocol as per Agachan *et al.* (2004) was used to identify the PON1 L55M and PON1 Q192R gene polymorphisms.

Serum total cholesterol (Diasis dds-Germany) and triglyceride (Diasis dds-Germany) concentrations were determined enzymatically. HDL-C (Diasis dds-Germany) was determined in serum by a chemically modified enzymatic method. LDL-C was calculated using a formula by Mattu *et al.* (1995).

Statistical analysis: using SPSS Version 10.0 included the χ^2 test for genotype and allele frequencies comparison. Clinical laboratory data were expressed as Means \pm SD. Mean values were compared between patients with unpaired group t-test and Mann Whitney U test. A level of $p < 0.05$ was considered statistically significant.

The study protocol followed the principles outlined in the Helsinki Declaration.

RESULTS AND DISCUSSION

The ACE allele frequencies were D = 55.4, I = 44.6; D = 57.85, I = 42.15 and D = 61.75, I = 38.25 in CHD, control and SX subjects, respectively as shown in Fig. 1. χ^2 analysis showed no significant difference in ACE allele or genotype distribution among these three groups.

The results of our study using the unrelated group t-test showed a significant difference in BMI, triglyceride and HDL-C values among SX and Control subjects as

shown in Table 1. In those groups, the significance of the association was stronger in BMI values than in triglyceride or HDL-C. According to Mann Whitney U test results; CHD and control subjects did not differ with regards to triglyceride values but significantly differed in LDL-C, BMI, total cholesterol and HDL-C values as seen in Table 2. But this time, the strength of the association was more pronounced in the LDL-C values.

The allele frequencies of PON1 Q192R genotype were Q = 78.9 and R = 21.1, Q = 60.65, R = 39.35 and Q = 77.4, R = 22.6 in CHD, SX and control group, respectively

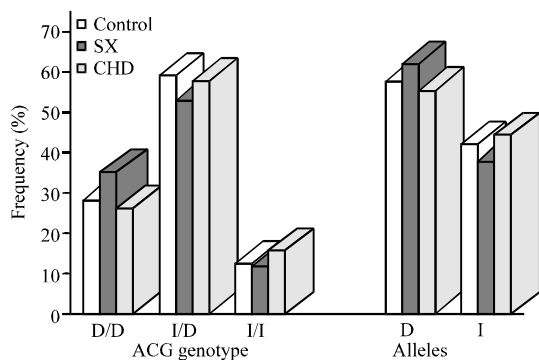


Fig. 1: Frequency distributions ACE genotype (DD, ID, II) and alleles (D, I) in SX, CHD and control groups

Table 1: The relationship between SX and control group

Variables	Groups	Average	SD between groups (\pm)	p-value
BMI	SX	33.85	5.39	<0.01*
	Control	23.71	2.76	
Total cholesterol (mg dL ⁻¹)	SX	207.85	34.84	>0.05
	Control	199.92	37.65	
Triglyceride (mg dL ⁻¹)	SX	167.97	83.35	<0.05*
	Control	83.16	50.02	
HDL-C (mg dL ⁻¹)	SX	48.29	8.54	<0.05*
	Control	56.00	11.59	
LDL-C (mg dL ⁻¹)	SX	129.79	30.80	>0.05
	Control	128.42	34.03	

*According to unrelated group t-test results p<0.05 is statistically significant. BMI: Body Mass Index, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol

Table 2: The relationship between CHD and control group

Variables	Groups	Average	SD between groups (\pm)	p-value
BMI	CHD	28.45	6.3803	<0.05*
	Control	19.02		
Total cholesterol (mg dL ⁻¹)	CHD	17.95	39.8398	<0.05*
	Control	26.69		
Triglyceride (mg dL ⁻¹)	CHD	23.63	13.705	>0.05
	Control	21.64		
HDL-C (mg dL ⁻¹)	CHD	17.97	13.4539	<0.05*
	Control	26.67		
LDL-C (mg dL ⁻¹)	CHD	16.00	33.0194	<0.01*
	Control	28.12		

*According to Mann Whitney U test p<0.05 is statistically significant. BMI: Body Mass Index, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol

(Fig. 2). The allele frequencies of PON1 L55M genotype were L = 65.75 and M = 34.25, L = 69.1 and M = 30.9 and L = 62.5, M = 37.5 in CHD, SX and control group respectively (Fig. 3).

Eichner *et al.* (2001) investigated the relationship between ACE DD genotype and MI in a patient group of 576 males and 124 females. The gene frequencies in this study showed that the most common genotype in the population was the ID genotype. Other studies also reported an association between ACE genetic polymorphism and CHD (Berkovich *et al.*, 2001; Dakik *et al.*, 1997; Uemora *et al.*, 2000). Some studies also found an association between ACE polymorphisms and SX and reported that increased I allele was associated with SX (Thomas *et al.*, 2001). ACE concentration was also found to be associated with plasma triglyceride and total cholesterol levels (Nagi *et al.*, 1998).

In contrast, other studies did not find a statistically significant association between ACE I/D gene

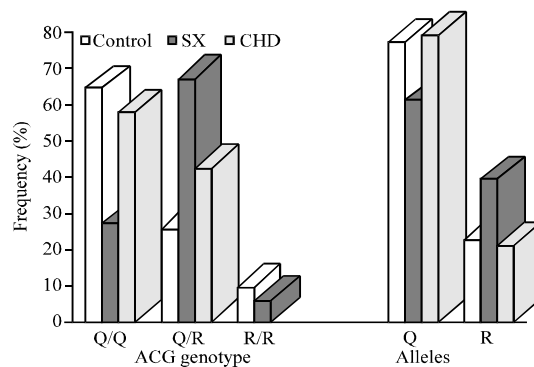


Fig. 2: Frequency distributions PON1 192 genotype (QQ, QR, RR) and alleles (Q, R) in SX, CHD and control groups

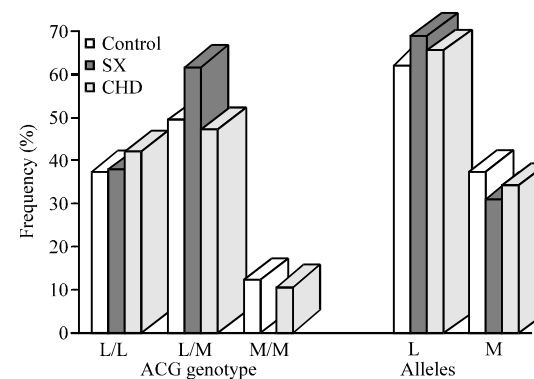


Fig. 3: Frequency distributions PON1 55 genotype (LL, LM, MM) and alleles (L, M) in SX, CHD and control groups

polymorphism and MI and stated that ACE genotype was not related to genetic susceptibility as earlier suggested by Zaman *et al.* (2001) and Fatini *et al.* (2000). There are also further studies that find no association between ACE polymorphism and CHD, as well as ACE polymorphism and SX (Mansur *et al.*, 2000; Covolo *et al.*, 2003; Deng *et al.*, 2002; Esparragon *et al.*, 2002; Shadrina *et al.*, 2001; Lim *et al.*, 2002). This study also agrees with these findings of no significant association between ACE genotype and CHD and SX.

When it comes to studies on PON genetic polymorphism, some studies find that genetic polymorphism at positions 54 and 191 determined by polymerase chain reaction-based restriction enzyme digestion (RFLP) is associated with serum concentration and activity of paraoxonase and with increased risk for coronary heart disease and that especially the LL genotype being significantly associated with the presence and severity of carotid disease (Schmidt *et al.*, 1998). Olivera *et al.* (2004) reported that the PON1 M/L55 mutation (MM genotype) was associated with lower triglyceride levels and the PON2 G/A148 mutation (GG genotype), with higher total and low-density lipoprotein (LDL)-cholesterol levels therefore in contrast to traditional coronary risk factors, the PON1 MM mutation can be considered predictive of protection against CAD. Imai *et al.* (2000) suggested that the Q/R192 is principally associated with both CAD and ischemic stroke in Japanese and one further study showed that the paraoxonase LL genotype at position 55 may present a risk factor for CHD (Watzinger *et al.*, 2002) and antiatherogenic because it hydrolyzes lipid peroxides in human atherosclerotic lesions (Avriam *et al.*, 2000).

Above findings are not universally agreed upon since other reports showed that PON1 gene L55M and Q192R polymorphisms were not associated with CHD. Suehiro *et al.* (1996) investigated the association of paraoxonase (PON) gene polymorphism with both the occurrence of Coronary Heart Disease (CHD) and the severity of coronary artery stenosis in Japanese subjects and did not show that the PON polymorphism is associated with a risk of CHD. Gardemen *et al.* (2000) stated that their findings did not strengthen the hypothesis that the paraoxonase gene polymorphisms were independently associated with coronary heart disease indicating that these gene variations are of little usefulness as genetic markers of cardiovascular disease. The findings of this study also agree with Suehiro *et al.* (1996) and Gardemann *et al.* (2000) findings.

The results of this study support the hypothesis that ACE I/D, PON1 55 L/M and 192 Q/R genotypes are not statistically significantly associated with the genetic susceptibility to SX or CHD in a small cohort of

nonsmoking Turkish population. Obviously, the interpretation of these finding are difficult due to the small sample size and larger group studies are needed for more definitive conclusions.

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