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The *ESR1* gene polymorphisms in patients with coronary artery disease in the southeastern Turkish population

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Aim: The objective of this study was to evaluate the prevalence of the estrogen receptor 1 (*ESR1*) gene variants and their possible association with coronary artery disease (CAD) in Şanlıurfa province.

Materials and methods: We used polymerase chain reaction and restriction enzyme digestion to determine the prevalence of the *ESR1* gene (NM_001122742.1, GI: 2099): c.454-351 A>G (*Xba*I A>G) and c.454-397 T>C (*Pvu*II T>C) polymorphisms in 100 healthy individuals and 80 angiographically confirmed CAD patients.

Results: The body mass index (28.1 ± 5.4), low-density lipoprotein cholesterol (126.1 ± 42 mg/dL), diabetes (n = 13), and hypertension (n = 39) values of the CAD patients were significantly higher than in the control subjects (26.2 ± 3.9, 106.1 ± 26.3, 2, and 18, respectively) ($P < 0.05$). No significant differences were observed in the frequencies of both the c.454-351 A>G and c.454-397 T>C genotype of the *ESR1* gene in CAD patients compared to healthy individuals ($P > 0.05$).

Conclusion: The *ESR1* variants tested in this study were not associated with CAD. Therefore, neither of these 2 variants can be considered as an independent risk factor or a predictor for CAD in the studied Turkish population.

Key words: Coronary artery disease, estrogen receptor 1 gene, polymorphism, polymerase chain reaction, restriction fragment length polymorphism, single nucleotide polymorphism

Introduction

Coronary artery disease (CAD) is a multifactorial disorder. Sex plays an important role in the pathogenesis of CAD by mechanisms that are still mostly unknown to us (1).The sex hormone estrogen has been found as the first potential cardioprotection factor in women, and the binding of estrogen to its receptor triggers estrogenic effects on various tissues (2,3). The estrogen receptor 1 (*ESR1*) gene is located on human chromosome 6 (6p25.1) and consists of 8 exons and 7 introns, spanning 412,779 bp (4). The *ESR1* gene encodes an estrogen receptor α, a ligand-activated transcription factor composed of several domains important for hormone binding, DNA binding, and activation of transcription (5). The effects of estrogens on the vascular system are mediated mainly through *ESR1*, a member of the nuclear hormone receptor super family, acting as a ligand-activated transcription factor (6-8).

A number of studies have indicated that the c.454-351 A>G (*Xba*I, rs9340799) and c.454-397 T>C (*Pvu*II, rs2234693) polymorphic variations in the first intron of the *ESR1* gene are associated with different factors, such as the onset of menopause, bone density, arterial hypertension, and body mass index (BMI) (9-13).

ESR1 is an interesting candidate gene because estrogen has beneficial effects on cardiovascular

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health. Of the polymorphisms identified in the *ESR1* gene, 2 single nucleotide polymorphisms (SNPs), c.454-351 A>G and c.454-397 T>C, are most widely investigated (14,15).

Various studies pointed out that the frequencies of the c.454-351 A>G and c.454-397 T>C genotype and allele were not significantly different between CAD patients and controls in various populations (2,8,14-19). On the other hand, some studies indicated that homozygosity for the alleles of the c.454-351 G and c.454-397 C polymorphisms were significantly associated with increased severity of CAD in males or females in different populations (20-23). These findings suggest that *ESR1* is a novel candidate gene for CAD.

In this study, we aimed to investigate the association of CAD with the frequencies of *ESR1* polymorphisms, and to see whether they are important genetic risk factors for CAD in a Turkish population.

Materials and methods

Study population and DNA extraction

This study was conducted from September 2008 to August 2009 in Turkey. Enrolled into the study were 80 consecutive patients who applied to the Department of Cardiovascular Surgery and Cardiology at Harran University Medical Faculty Hospital with CAD (38 females, mean age 53.07 ± 11.1 years, and 42 males, mean age 56.7 ± 5.9 years) and who had 1 or more coronary lesions causing ≥50% stenosis. The presence and severity of CAD was determined by the Gensini score (24). Exclusion criteria were the presence of any of the following: stable angina pectoris with normal coronary arteries or with <50% stenosis in major coronary arteries; acute coronary syndrome; cerebrovascular disease; known renal, hepatic, or immunologic disorders; obesity secondary to hypothyroidism or Cushing's disease; severe debilitating diseases; and malignancy. From among blood donors, 100 healthy controls (51 females, mean age 54.9 ± 8.6 years, and 49 males, mean age 57.5 ± 9.6 years) who did not have any disease and had no clinical evidence of a family history in first-degree relatives of CAD or other disorders were randomly selected.

Demographic details, including anthropometric measurements, cardiovascular risk factors, and medication use, were recorded for all participants. BMI was calculated as weight in kilograms divided by the square of the height in meters (kg/m^2). The levels of triglycerides (TG), total cholesterol, high-density lipoprotein cholesterol (HDL-c), and low-density lipoprotein cholesterol (LDL-c) were determined by using commercially available assay kits (Abbott®, Abbott Park, Illinois, USA) with an Abbott Aeroset automatic analyzer (Abbott®).

EDTA blood was taken from these individuals, and genomic DNA was extracted from whole blood leukocytes by using the standard salting-out procedure (25).

PCR-RFLP technique

The c.454-351 A>G (g.34720 A>G, rs9340799) and c.454-397 T>C (g.34650 T>C, rs2234693) polymorphic sites of the *ESR1* (NM_001122742.1; GI: 2099) gene were investigated by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. The PCR reactions were performed in 20 µL of reaction volume, including 1X PCR buffer, 2 mM $MgCl_2$, 0.2 mM each of deoxynucleotide triphosphates (dNTPs; Fermentas, St. Leon-Rot, Germany), 0.2 µM of each primer (A: GCCACCCTATCTGTATCTTTTCCTATTCTTC -3, B: TCTCTGCCACCCTGGCGTCGATTATCTGA -3')(26)(BioBasicInc.,Ontario,Canada),40ngofDNA, and 0.5 units of Taq DNA polymerase (Fermentas). The PCR program for the 2 polymorphic sites was performed at 94 °C for 3 min (initial denaturation), then 30 cycles of 94 °C for 50 s, 62 °C for 50 s, 72 °C for 50 s, and a final extension of 72 °C for 5 min.

Ten microliters of PCR product in a 30-µL volume for c.454-351 A>G and c.454-397 T>C was separately digested with 1.5 units of *Xba*I and *Pvu*II restriction enzymes (Fermentas, St. Leon-Rot, Germany) at 37 °C for 2 h.

The digested PCR products were separated on 1.5% agarose gel and analyzed using the AlphaImager Imaging System (AlphaInnotech, San Leandro, California, USA). The digested *ESR1*: c.454-351 A allele yielded 2 fragments of 981 and 393 bp, and the G allele yielded 1 fragment of 1374 bp (Figure 1). The c.454-397 T allele yielded 2 fragments of 936 and 438 bp, and the C allele yielded 1 fragment of 1374 bp (Figure 2).

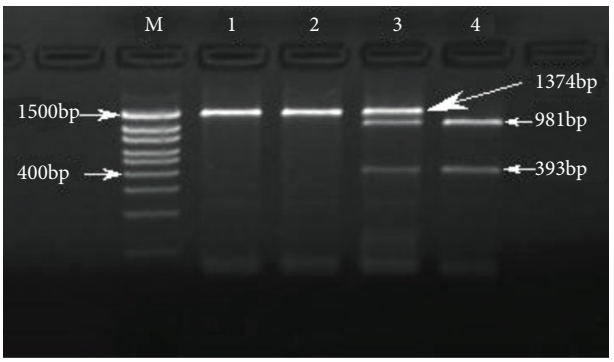

Figure 1. The restriction profile of the *ESR1* gene *Xba*I (c.454-351 A>G). Lane M: DNA marker (100-1500 bp, Bio Basic Inc., Ontario, Canada), lane 1: undigested PCR product, lane 2: GG genotype (homozygous, polymorphic), lane 3: AG genotype (heterozygous), lane 4: AA genotype (homozygous, wild type).

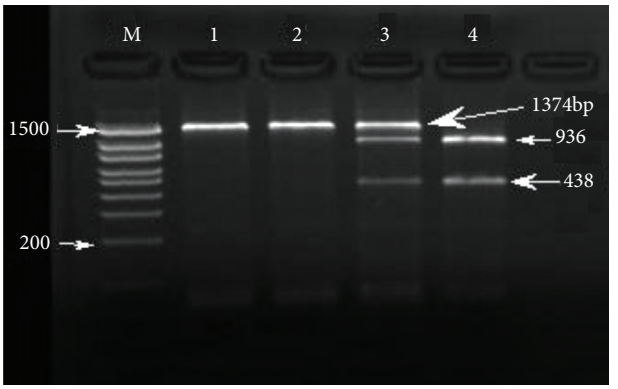

Figure 2. The restriction profile of the *ESR1* gene *Pvu*II (c.454-397 T>C). Lane M: DNA marker (100-1500 bp, Bio Basic Inc.), lane 1: undigested PCR product, lane 2: CC genotype (homozygous, polymorphic), lane 3: TC genotype (heterozygous), lane 4: TT genotype (homozygous, wild type).

Statistical analysis

Student's t-test was used for continuous variables that showed normal distribution according to the one-sample K-S test, and the Mann-Whitney U test was used for variables that did not show normal distribution. Categorical variables were compared by using the chi-square test. Genotype and allele frequencies of *ESR1*: c.454-351 A>G and c.454-397 T>C were tested for Hardy-Weinberg equilibrium by using the chi-square test. Genotype and allele frequencies of these polymorphisms were analyzed with Fisher's exact test. Statistical significance was defined as $P < 0.05$.

Ethics

All patients gave written informed consent before being recruited. The study was approved by the institutional ethics committee.

Results

The baseline characteristics of the patients and controls are presented in Table 1. The frequency of sex and age of patients with CAD and of the healthy controls did not differ between the study groups (P

Table 1. The demographic parameters of the patients with CAD and the control group.

	CAD patients (n = 80)	Controls (n = 100)	P-value
Sex (F/M)	38/42	51/49	[a]0.641
Age (years)	54.9 ± 8.8	56.2 ± 9.2	[b]0.342
BMI (kg/m^2)	28.1 ± 5.4	26.2 ± 3.9	[b]0.006
TG (mg/dL)	164.7 ± 89.0	167.2 ± 47.4	[b]0.810
HDL-c (mg/dL)	40.6 ± 24.5	38.1 ± 9.5	[c]0.985
LDL-c (mg/dL)	126.1 ± 42.5	106.1 ± 26.3	[b]0.001
Diabetes (n)	13	2	[a]0.001
Smoking (n)	15	17	[a]0.760
Hypertension (n)	39	18	[a]0.001
Gensini score (mean ± SD)	48.46 ± 31.46		

Values are mean ± standard deviation (SD).
[a]Chi-square, [b]Student's t-test, [c]Mann-Whitney U test.

Table 2. *ESR1* gene polymorphisms in a Turkish population with CAD and the control group.

SNP genotype/allele	CAD patients (n = 80)	Healthy controls (n = 100)	X^2	OR (95% CI)	*P-value
c.454-351A>G					
Genotypes					
AA	35 (43.8%)	43 (43.0%)		Reference	
AG	31 (38.8%)	46 (46.0%)	0.954	0.743 (0.409-1.350)	0.329
GG	14 (17.5%)	11 (11.0%)	1.570	1.716 (0.732-4.021)	0.210
Alleles					
A	101 (63.1%)	132 (66.0%)		Reference	
G	59 (36.9%)	68 (34.0%)	0.322	1.134 (0.734-1.751)	0.571
c.454-397T>C					
Genotypes					
TT	31 (38.8%)	39 (39.0%)		Reference	
TC	37 (46.2%)	47 (47.0%)	0.010	0.970 (0.538-1.749)	0.920
CC	12 (15.0%)	14 (14.0%)	0.036	1.084 (0.471-2.496)	0.850
Alleles					
T	99 (61.9%)	125 (62.5%)		Reference	
C	61 (38.1%)	75 (37.5%)	0.015	1.027 (0.669-1.577)	0.903

CAD - coronary artery disease, X^2 - chi-squared, OR - odds ratio, CI - confidence interval, SNP - single nucleotide polymorphism. *Chi-square and Fisher's exact tests.

= 0.641 and P = 0.342, respectively). The BMI and LDL-c values and the prevalence of diabetes and hypertension were significantly higher in the CAD group than in the healthy subjects (P = 0.006, P = 0.001, P = 0.001, and P = 0.001, respectively), while there were no statistically significant differences in TG and HDL-c or in the prevalence of current cigarette smoking (P = 0.810, P = 0.985, and P = 0.760, respectively).

The distribution of the genotypes for the c.454-351 A>G and c.454-397 T>C polymorphic sites was consistent with the Hardy-Weinberg equilibrium in the CAD and control groups (P > 0.05). For the

Table 3. The demographic and clinical profiles with and without polymorphic genotype for the *ESR1* gene in patients with CAD.

	c.454-351A>G			**c.454-397T>C**		
Genotype	AA	GG	P-value	TT	CC	P-value
Subjects (n)	35	14		31	12	
Sex (F/M)	13/22	5/9	0.925	12/19	6/6	0.501
Age (years)	54.1 ± 7.9	56.6 ± 9.4	0.350	53.8 ± 8.4	56.1 ± 9.8	0.456
BMI (kg/m^2)	27.7 ± 4.7	29.2 ± 6.6	0.360	27.9 ± 4.8	29.3 ± 5.2	0.406
TG (mg/dL)	183.5 ± 115.1	130.2 ± 64.5	0.111	177.2 ± 106.4	152.0 ± 112.1	0.496
HDL-c (mg/dL)	36.5 ± 7.1	50.6 ± 53.1	0.126	36.6 ± 7.4	52.8 ± 57.1	0.122
LDL-c (mg/dL)	129.3 ± 44.6	118.9 ± 38.2	0.450	123.2 ± 44.0	126.9 ± 42.5	0.805
Diabetes	6	2	0.807	6	2	0.839
Smoking	6	2	0.807	6	3	0.683
Hypertension	16	11	0.037	16	8	0.373

Values are mean ± SD, AA (*Xba*I) - wild-type genotype, TT (*Pvu*II) - wild-type genotype, GG (*Xba*I) - polymorphic genotype, CC (*Pvu*II) - polymorphic genotype.

Table 4. The relation of *ESR1* gene polymorphism genotypes and allele frequencies with CAD and control groups by sex.

SNP c.454-351A>G	CAD patients (n = 80)	Healthy controls (n = 100)	*P-value	SNP c.454-397T>C	CAD patients (n = 80)	Healthy controls (n = 100)	*P-value
Women	38	51		Women	38	51	
Genotypes				**Genotypes**			
AA	13 (34.2%)	22 (43.1%)	Reference	TT	12 (31.6%)	14 (27.5%)	Reference
AG	20 (52.6%)	25 (49.0%)	0.736	TC	20 (52.6%)	26 (51.0%)	0.877
GG	5 (13.2%)	4 (7.8%)	0.411	CC	6 (15.8%)	11 (21.6%)	0.493
Alleles				**Alleles**			
A	46 (60.5%)	69 (68.3%)	Reference	T	44 (57.9%)	54 (52.9%)	Reference
G	30 (39.5%)	33 (31.7%)	0.282	C	32 (42.1%)	48 (47.1%)	0.511
Men	42	49		Men	42	49	
Genotypes				**Genotypes**			
AA	22 (52.4%)	21 (42.9%)	Reference	TT	19 (45.2%)	25 (51.0%)	Reference
AG	11 (26.2%)	21 (42.9%)	0.097	TC	17 (40.5%)	21 (42.9%)	0.818
GG	9 (21.4%)	7 (14.3%)	0.372	CC	6 (14.3%)	3 (6.1%)	0.193
Alleles				**Alleles**			
A	55 (65.5%)	63 (64.3%)	Reference	T	55 (65.5%)	71 (72.4%)	Reference
G	29 (34.5%)	35 (35.7%)	0.867	C	29 (34.5%)	27 (27.6%)	0.310

CAD - coronary artery disease, SNP - single nucleotide polymorphism.
*Chi-square and Fisher's exact tests.

c.454-351 polymorphism, the GG genotype and G allele frequencies in CAD individuals were not significantly higher than those in healthy individuals (17.5% and 36.9% vs. 11.0% and 34.0%, P = 0.210 vs. P = 0.571, respectively) (Table 2). Furthermore, there was no significant difference between CAD patients and healthy controls for the c.454-397 CC genotype (15.0% vs. 14.0%, P = 0.850) or C allele (38.1% vs. 37.5%, P = 0.903) frequencies (Table 2).

No significant difference was found between CAD patients with and without c.454-351 A>G polymorphism (GG genotype and AA genotype wild-type) in terms of sex, age, BMI, TG, HDL-c, LDL-c, diabetes, and current cigarette smoking (P > 0.05 for all), but hypertension was more frequent in the AA genotype (P = 0.037). On the other hand, there was no statistically significant difference between patients with and without the c.454-397 T>G polymorphism (CC genotype and TT genotype wild-type) in terms of all the mentioned risk factors (P > 0.05 for all) (Table 3).

Subgroup analysis by sex did not demonstrate any significant relationship (P > 0.05 for men and P > 0.05 for women). In addition, the subgroup analysis by sex revealed that the relationship between the *ESR1* c.454-397 polymorphism and CAD patients was not significant (P > 0.05 for men and P > 0.05 for women) (Table 4).

Discussion

Coronary heart disease is the leading cause of mortality worldwide (27, 28). Estrogens are known to regulate the cardiovascular system via both systemic effects on circulating factors (e.g. cholesterol, lipoproteins, triglycerides) and via direct effects on the blood vessel wall (e.g. vascular, cell proliferation). However, the genetic factors underlying this form of cardiovascular disease are complex and not clearly established (29).

In investigating the impacts of 2 intronic polymorphisms of the *ESR1* gene on the risk of CAD in a Turkish population, we did not find an association of *ESR1* c.454-351 A>G and c.454-397 T>C genotypes and alleles with CAD. Although the frequencies of the c.454-351 GG genotype (17.5%) and G allele (36.9%) in CAD patients were higher than those in healthy subjects (11.0% and 34.0%, respectively), these differences were not significant

(P > 0.05 for both). Similarly, we did not observe an association between CAD and the frequencies of the *ESR1*: c.454-397 CC genotype and C allele (P > 0.05). On the other hand, no sex-specific differences were detected, either. Thus, our findings for sex are in agreement with the findings of a recent study conducted by Karadag et al (2). Although several studies had been performed on this topic, inconsistent results were obtained from different populations. Xu et al in a Chinese population (16), Boroumand et al in an Iranian population (14, 15), Matsubara et al in a Japanese population (18), and Lluís-Ganella et al in a Spanish population (19) showed that genotype distributions and allele frequencies of c.454-351 A>G or c.454-397 T>C polymorphisms are not significantly different between control subjects and patients (14-16, 18, 19). When the allele frequencies are analyzed separately according to sex, age, cigarette smoking, and hyperlipidemia, there is still no statistically significant difference for both polymorphisms. In the Central Anatolia Region of Turkey, Yilmaz et al indicated that the *ESR1*:c.454-397 T>C polymorphism, but not the c.454-351 A>G polymorphism, was associated with CAD, and the C allele for the c.454-397 T>C polymorphism was found to be an independent determinant of the presence of CAD (26). In another study in a Turkish population (in the Marmara region of Turkey), Karadağ et al. reported that no association was found between the CAD and c.454-397 T>C polymorphism genotypes and alleles and, in addition, subgroup analysis by sex did not demonstrate any significant relation, either (2). This is, to our knowledge, the third study investigating whether *ESR1* polymorphisms are associated with CAD patients in Turkey. Our study is important, though, because it is the first such work in Şanlıurfa Province (southeastern Anatolia region of Turkey). Although there has been controversy about the relationship between *ESR1* polymorphisms and CAD, our findings are similar to those of several previous reports (2,14-16,18,19). It is also worth mentioning that our study is the first study performed in patients with CAD in our population. On the other hand, c.454-351 A>G and c.454-397 T>C genotype distributions were significantly different between the CAD and non-CAD groups in an Israeli population, a Greek population, and in postmenopausal women and in men in a Japanese population (22,23).

Diabetes, hypertension, high LDL-c levels, and the *ESR1*: c.454-397 CC genotype were independent risk factors for CAD, and the c.454-397 CC genotype was associated with the angiographic severity of CAD measured by the number of diseased vessels as well as c.454-351A>G, but no association was found with CAD susceptibility before or after sex stratification. In addition, no association was found between the polymorphisms and the angiographic severity of CAD (16).

Total cholesterol, TG, and HDL-c levels were previously found not to be different among *ESR* genotypes (18). Our results were consistent with data reported previously (16,18). In our study, comparison of CAD patients without the c.454-351 AA wild-type genotype and those with the polymorphic c.454-351 GG genotype showed no association in terms of age, BMI, TG, HDL-c, LDL-c, diabetes, and smoking (P > 0.05), but did show an association in terms of hypertension (P < 0.05). However, we did not find a statistically significant difference between patients without the wild-type TT genotype and those with the polymorphic CC genotype in terms of clinical and demographic variants for the c.454-397 A>G polymorphism (P > 0.05).

The limited number of patients and healthy individuals is the most important limitation of the study. Furthermore, our data indicated that there were no statistically significant differences in TG, HDL-C, and the prevalence of current cigarette smoking between the CAD group and healthy subjects, while the levels of BMI and LDL-C and the frequencies of diabetes and hypertension were significantly higher in the CAD group. This unexpected result is also related to the limited number of subjects.

In conclusion, we found that the *ESR1* gene c.454-351 A>G and c.454-397 T>C polymorphisms are not important risk factors for CAD in a Turkish population in the southeastern Anatolia region of Turkey. However, studies of larger numbers of patients and controls in various populations will be required in order to determine the influence of these genes and other candidate genes and SNPs on the risk of CAD.

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References

1. Ross R. Atherosclerosis: an inflammatory disease. N Engl J Med 1999; 340: 115-26.
2. Karadağ B, Güven M, Hacıoğlu Y, Öz E, Batar B, Domaniç N et al. Relationship between two estrogen receptor-α gene polymorphisms and angiographic coronary artery disease. Anadolu Kardiyol Derg 2009; 9: 267-72.
3. Sasaki M, Kotcherguina L, Dharia A, Fujimoto S, Dahiya R. Cytosine phosphoguanine methylation of estrogen receptors in endometrial cancer. Cancer Res 2001; 61: 3262-6.
4. Menasce LP, White GR, Harrison CJ, Boyle JM. Localization of the estrogen receptor locus (ESR) to chromosome 6q25.1 by FISH and a simple post-FISH banding technique. Genomics 1993; 17: 263-5.
5. Parker MG, Arbuckle N, Dauvois S, Danielian P, White R. Structure and function of the estrogen receptor. Ann N Y Acad Sci 1993; 684: 119-26.
6. Bhalla RC, Toth KF, Bhatty RA, Thompson LP, Sharma RV. Estrogen reduces proliferation and agonist-induced calcium increase in coronary artery smooth muscle cells. Am J Physiol 1997; 272: 1996-2003.
7. Sasaki M, Tanaka Y, Sakuragi N, Dahiya R. Six polymorphisms on estrogen receptor 1 gene in Japanese, American and German populations. Eur J Clin Pharmacol 2003; 59: 389-93.
8. Mansur ADP, Nogueira CCM, Strunz CMC, Aldrighi JM, Ramires JAF. Genetic polymorphisms of estrogen receptors in patients with premature coronary artery disease. Arch Med Res 2005; 36: 511-7.
9. Lehrer S, Rabin J, Kalir T, Schachter BS. Estrogen receptor variant and hypertension in women. Hypertension 1993; 21: 439-41.
10. Losordo DW, Kearney M, Kim EA, Jekanowski J, Isner JM. Variable expression of the estrogen receptor in normal and atherosclerotic coronary arteries of premenopausal women. Circulation 1994; 89: 1501-10.
11. Weel A, Uitterlinden AG, Westendorp ICD, Burger H, Schuit SCE, Hofman A et al. Estrogen receptor polymorphism predicts the onset of natural and surgical menopause. J Clin Endocrinol Metab 1999; 84: 3146-50.
12. Kobayashi S, Inoue S, Hosoi T, Ouchi Y, Shiraki M, Orimo H. Association of bone mineral density with polymorphism of the estrogen receptor gene. J Bone Miner Res 1996; 11: 306-11.
13. Jian WX, Yang YJ, Long JR, Li YN, Deng FY, Jiang DK et al. Estrogen receptor α gene relationship with peak bone mass and body mass index in Chinese nuclear families. J Hum Genet 2005; 50: 477-82.
14. Boroumand M, Ghaedi M, Mohammadtaghvaei N, Pourgholi L, Anvari MS, Davoodi G et al. Lipid profile and inflammatory markers associated with estrogen receptor α PvuII and *XbaI* gene polymorphisms. Transl Res 2009; 153: 288-95.
15. Boroumand M, Ghaedi M, Mohammadtaghvaei N, Pourgholi L, Anvari MS, Davoodi G et al. Association of estrogen receptor α gene polymorphism with the presence of coronary artery disease documented by coronary angiography. Clin Biochem 2009; 42: 835-9.
16. Xu HY, Hou XW, Wang NF, Hui B, Jin JF, Yun S et al. Gender-specific effect of estrogen receptor-1 gene polymorphisms in coronary artery disease and its angiographic severity in Chinese population. Clin Chim Acta 2008; 395: 130-3.
17. Schuit SCE, Oei HHS, Witteman JCM, van Kessel CHG, van Meurs JBJ, Nijhuis RL et al. Estrogen receptor alpha gene polymorphisms and risk of myocardial infarction. JAMA 2004; 291: 2969-77.
18. Matsubara Y, Murata M, Kawano K, Zama T, Aoki N, Yoshino H et al. Genotype distribution of estrogen receptor polymorphisms in men and postmenopausal women from healthy and coronary populations and its relation to serum lipid levels. Arterioscler Thromb Vasc Biol 1997; 17: 3006-12.
19. Lluis-Ganella C, Lucas G, Subirana I, Escurriol V, Tomas M, Senti M et al. Qualitative assessment of previous evidence and an updated meta-analysis confirms lack of association between the *ESR1* rs2234693 (*Pvu*II) variant and coronary heart disease in men and women. Atherosclerosis 2009; 207: 480-6.
20. Almeida S, Hutz MH. Estrogen receptor 1 gene polymorphisms and coronary artery disease in the Brazilian population. Braz J Med Biol Res 2009; 39: 447-54.
21. Rokach A, Pollak A, Rosen L, Friedlander Y, Blumenfeld A, Reznik L et al. Estrogen receptor α gene polymorphisms are associated with the angiographic extent of coronary artery disease. J Clin Endocrinol Metab 2005; 90: 6556-60.
22. Alevizaki M, Saltiki K, Cimponeriu A, Kanakakis I, Xita N, Alevizaki CC et al. Severity of cardiovascular disease in postmenopausal women: associations with common estrogen receptor α polymorphic variants. Eur J Endocrinol 2007; 156: 489-96.
23. Lu H, Higashikata T, Inazu A, Nohara A, Yu WX, Shimizu M et al. Association of estrogen receptor-α gene polymorphisms with coronary artery disease in patients with familial hypercholesterolemia. Arterioscler Thromb Vasc Biol 2002; 22: 817-23.
24. Gensini GG. A more meaningful scoring system for determining the severity of the coronary heart disease. Am J Cardiol 1983; 51: 606.
25. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1998; 16: 1215.
26. Yilmaz A, Menevse S, Erkan AF, Ergun MA, Ilhan MN, Cengel A et al. The relationship of the ESR1 gene polymorphisms with the presence of coronary artery disease determined by coronary angiography. Genet Test 2007; 11: 367-71.

27. Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJL. Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. Lancet 2006; 367: 1747-57.

28. Tünel HA, Manduz Ş, Katrancıoğlu N, Karahan O, Berkan Ö. Determining the risk and frequecy of coronary artery disease in patients who have peripheric vascular occlusive disease and who will have vascular surgery. Türk Göğüs Kalp Damar Cer Derg 2009; 17: 261-6 (in Turkish with English abstract).

29. Girelli D, Martinelli N, Peyvandi F, Olivieri O. Genetic architecture of coronary artery disease in the genome wide era: implications for the emerging "golden dozen" loci. Semin Thromb Hemost 2009; 35: 671-82.