Relationship between two estrogen receptor-α gene polymorphisms and angiographic coronary artery disease

İki östrojen reseptör-α gen polimorfizminin anjiyografik koroner arter hastalığı ile olan ilişkisi

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ABSTRACT

Objective: To investigate the association of estrogen receptor-α *Pvu*II and *Btg*I polymorphisms with angiographic presence and severity of coronary artery disease (CAD).

Methods: Our cross-sectional study included 140 patients with ≥50% coronary stenoses (CAD group) and 47 patients with normal angiograms (CAD-free group) (total n=187, age 59.6±13.2 years; 66 women). *Pvu*II and *Btg*I genotype and allele distributions were determined by standard method of polymerase chain reaction and restriction fragment length polymorphism. The CAD subgroups by the number of diseased vessels were also defined. Variable associations and group differences were analyzed by independent t test, one-way ANOVA, Pearson's Chi-square (χ^2), Spearman's correlation tests and logistic regression analyses.

Results: While there was no association between *Pvu*II polymorphism and angiographic CAD (p=0.384), *Btg*I polymorphism was more prevalent in CAD-free group (23.4% vs. 10% (CAD group), OR=2.75, 95% CI=1.150 to 6.579, p=0.019). This difference was more pronounced in women (28.6% vs. 4.4%; OR=8.6; 95% CI=1.564 to 47.303; p=0.005) compared to men (p=0.391). Logistic regression analysis confirmed *Btg*I polymorphism as the most important predictor for a normal coronary angiogram among parameters such as body mass index, diabetes and age (OR 8.13, 95% CI 1.257 to 52.627, p=0.028). However, no significant association between *Btg*I polymorphism and the number of stenotic arteries was detected.

Conclusion: ESR1 *Pvu*II polymorphism is not associated with angiographically significant CAD. ESR1 *Btg*I polymorphism is strongly associated with the presence of normal coronary angiograms in women, which suggests protective effect. Further confirmation of these findings is required. *(Anadolu Kardiyol Derg 2009; 9: 267-72)*

Key words: Genetics, estrogen receptor alpha, women, coronary artery disease, logistic regression analysis, predictive models

ÖZET

Amaç: Östrojen reseptör-α *Pvu*II and *Btg*I gen polimorfizmlerinin koroner arter hastalığının (KAH) anjiyografik varlığı ve şiddeti ile olan ilişkisinin incelenmesi.

Yöntemler: Enine-kesitli olan çalışmamızda, anjiyografisinde %50 ve üzeri koroner arter stenozu bulunan 140 hasta (KAH grubu) ile koroner anjiyogramı normal olan 47 hasta (KAH olmayan grup) olmak üzere toplam 187 hasta incelendi (yaş ortalaması 59.6±13.2 yıl; 66'sı kadın). *Pvu*II ve *Btg*I genotipleri polimeraz zincir reaksiyonu ve "restriction fragment length polymorphism" yöntemleri yardımı ile saptandı. Koroner arter hastalığı şiddeti hastalıklı damar sayısına göre derecelendirildi. Değişkenler arası bağıntılar ve gruplar arası farklar bağımsız t testi, tek-yönlü ANOVA, Pearson Ki-kare (χ^2), Spearman korelasyon testleri ve lojistik regresyon analizleri ile incelendi.

Bulgular: Grupların karşılaştırılmasında *Pvu*II polimorpfizmi ile anjiyografik KAH arasında herhangi bir anlamlı ilişki bulunmazken (p=0.219), *Btg*I polimorfizminin KAH olmayan grupta daha sık görüldüğü tespit edildi (%23.4'e karşı %10 (KAH), OR=2.75, %95 GA=1.15 - 6.58, p=0.019). Alt grup analizinde bu farkın erkeklerden ziyade (p=0.391) asıl olarak kadınlarda görüldüğü saptandı (%28.6'ya karşı %4.4 (KAH); OR=8.6; %95 GA=1.564 - 47.303; p=0.005). Lojistik regresyon analizinde *Btg*I polimorfizminin vücut kitle indeksi, yaş ve diyabet gibi yan değişkenlere göre normal koroner anjiyogram sonucunu kestirme bakımından daha güçlü bir değişken olduğu teyit edildi (OR 8.13, %95 GA 1.257-52.627, p=0.028). Ancak, *Btg*I polimorfizmi ile KAH şiddeti arasında anlamlı bir ilişki saptanmadı.

Sonuç: Östrojen reseptör-α *Pvu*II polimorfizmi ile anjiyografik KAH arasında bir bağıntı saptanmazken özellikle kadınlarda *Btg*I polimorfizmi ile KAH arasında güçlü bir ters ilişki bulunmaktadır. *Btg*I polimorfizminin kadınlarda KAH'na karşı koruyucu etkisinin olabileceğini gösteren bu bulguların başka çalışmalarda da teyit edilmesi gereklidir. *(Anadolu Kardiyol Derg 2009; 9: 267-72)*

Anahtar kelimeler: Genetik, östrojen reseptör alfa, kadın cinsiyeti, koroner arter hastalığı, lojistik regresyon analizi, prediktif modeller

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Introduction

Coronary artery disease (CAD) is a multifactorial disease with major impact on public health throughout the world. Gender plays an important role in clinical presentation and prognosis of CAD by mechanisms, which are still mostly unknown to us (1). In the search for explanation, the sex hormone estrogen was the first factor suggested as the potential source of cardioprotection in women (2). However, after several large-scale hormone replacement therapy (HRT) trials failed to prove the efficacy of this hormone in primary and secondary prevention of CAD in post-menopausal women, it became obvious that there were other unknown factors involved (3, 4).

Then in 1997, after Sudhir et al. reported a case of 31-year-old man, who presented with extensive premature atherosclerosis and endothelial dysfunction and had destructive homozygous mutation in estrogen receptor 1 (ESR1) gene, the attention of scientific community focused on the role of estrogen receptors in the development of atherosclerosis (5, 6).

Two types of human estrogen receptors are currently defined-α (ESR1) and β (ESR2). Most of the current scientific evidence points out ESR1 as the main receptor linked with the predisposition to atherosclerosis (2).

Estrogen receptor 1 is encoded by a gene located on chromosome 6, locus 6q25.1, comprising 8 exons and 7 introns (7). Out of the reported numerous ESR1 gene variations (single nucleotide polymorphisms (SNP's)) only a few of them have been widely studied in conjunction with their possible relation to CAD. The first ESR1 polymorphism that showed some association with CAD in some early large-scale studies was intron 1 T/C *Pvu*II polymorphism, also known as c.454-397T>C or rs2234693 (8, 9). However, the findings of the most recent large-scale studies didn't support this initial association (10, 11). Since there is ongoing controversy about the role of this SNP in CAD, we wanted to test its relation to angiographic CAD in our patient population, as well.

In our search for other potential SNP candidates, we came across ESR1 exon 8 *Btg*I polymorphism, also known as G594A (substitution of guanine (G) by adenine (A) in location 594) or rs2228480, which was recently shown to carry significant association with susceptibility to migraines (12). In the light of recently presented evidence suggesting inverse relationship between migraines and angiographic CAD we wondered if there were studies investigating the role of *Btg*I polymorphism in CAD, as well (13). However, our literature search did not produce any positive results. To our knowledge, there is not a single reported study that had assessed the relationship of this SNP to the risk of CAD.

Thus, the current study aimed to investigate the association of estrogen receptor-α *Pvu*II and *Btg*I polymorphisms with the angiographic presence and severity of coronary artery disease (CAD) in our patient population.

Methods

Selection of participants

Our study population was selected from patients who presented to our emergency department (ED) or our outpatient cardiology clinic with symptoms and signs suggestive of CAD during the period between March 28, 2005 and June 30, 2006. Among them, we screened all patients who underwent coronary angiography performed sequentially by one particular operator. Patients with no angiographic evidence of CAD (n=47) were enrolled as CAD free group and those who had one or more coronary lesions with ≥ 50% stenosis (n=140) were enrolled as CAD group. Patients with coronary lesions <50% stenosis, previous coronary revascularization (either by percutaneous transluminal coronary angioplasty ± stenting or coronary by-pass grafting), previous HRT and age less than 18 were excluded from the study.

The study was designed as cross-sectional study and conducted in accordance with the Helsinki Declaration of 1975. The study protocol was reviewed and approved by the Ethics Committee at Istanbul University Cerrahpaşa School of Medicine. Written informed consent was obtained from all patients.

Baseline characteristics regarding the demographic and clinical parameters of the enrolled patients were obtained from the medical records of each patient.

Determination of CAD status

The presence of CAD was determined by coronary angiography using the standard catheterization technique. All of the procedures were performed at the Catheterization Laboratory of Department of Cardiology, İstanbul University Cerrahpaşa Faculty of Medicine, Turkey. The degree of luminal narrowing (stenosis) for each lesion was calculated quantitatively using Shimadzu DICOM viewer software. The severity of CAD was additionally graded according to the number of diseased coronary vessels with ≥50% stenosis as one, two or three vessel disease.

Assessment of ESR1 SNP genotypes

Leukocytic DNA samples were extracted from peripheral blood samples of each patient (5ml, EDTA) using the standard method. ESR1 SNP genotypes were detected via standard method of polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) with the help of specific restriction endonucleases. (14, 15) The PCR amplification of the area of interest in ESR1 intron 1 was achieved by using sense primer 5'-CTG CCA CCC TAT CTG TAT CTT TTC CTA TTC TCC-3' and antisense primer 5'-TCT TTC TCT GCC ACC CTG GCG TCG ATT ATC TGA-3'. The amplified PCR product of 1300 bp was further cleaved by 10 Units of *Pvu*II enzyme and analyzed by agarose gel electrophoresis. Genotypes were determined as follows: samples containing only two types of fragments (850 bp and 450 bp) were labeled as genotype TT; samples with only one type of fragments (1300 bp) as genotype CC; and samples with all three types of fragments (1300 bp, 850 bp and 450 bp) were defined as genotype TC (Fig. 1).

Polymerase chain reaction amplification of the area of interest in ESR1 exon 8 was achieved by using sense primer 5'-GAG GAG ACG GAC CAA AGC CAC -3' and antisense primer 5'-GCC ATT GGT GTT GGA TGC ATG -3'. The amplified PCR product of 227 bp was further cleaved by 10 Units of *Btg*I enzyme and analyzed by agarose gel electrophoresis. The genotypes of

the samples was determined as follows: samples containing two types of fragments (129bp and 98bp) were of genotype GG; samples with only one type of fragment (227 bp) represented genotype AA and samples containing all three types of fragments (227bp, 129bp and 98bp) corresponded to genotype AG (Fig. 2).

Statistical analysis

All of the statistical analyses were performed using SPSS 17.0 software for Windows (Chicago, IL, USA). Categorical variables are expressed as counts (percentages) and continuous variables were expressed as mean ± SD. The relations among demographic and clinical parameters, genotypes and allele frequencies of the groups were analyzed by independent t test, one-way ANOVA, Pearson's Chi-square (χ^2), Spearman's correlation tests and multiple logistic regression analyses. The parameters of logistic regression model used for the prediction of normal angiographic result included age as independent variable and BMI and diabetes as dependant variables. Results were considered statistically significant for $p<0.05$.

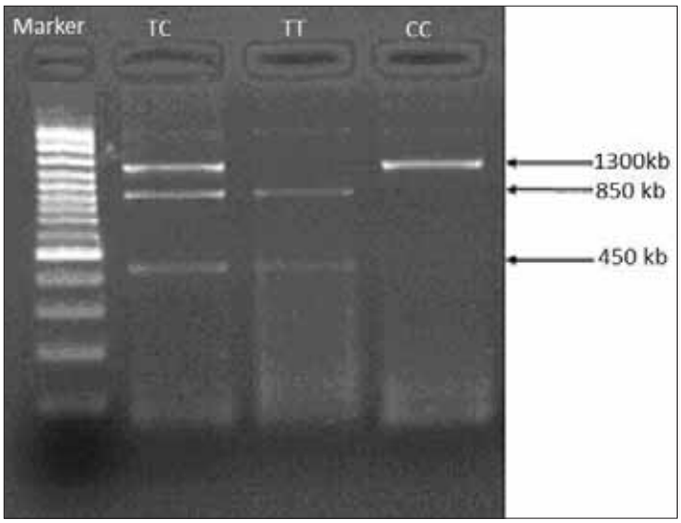

Figure 1. Detection of ESR1 *Pvu*II genotypes by agarose gel electrophoresis Samples containing two types of fragments (850pb and 450 bp) have genotype TT; samples with only one type of fragment (1300 bp) represent genotype CC; and samples with all three types of fragments (1300 bp, 850 bp and 450 bp) are of genotype TC

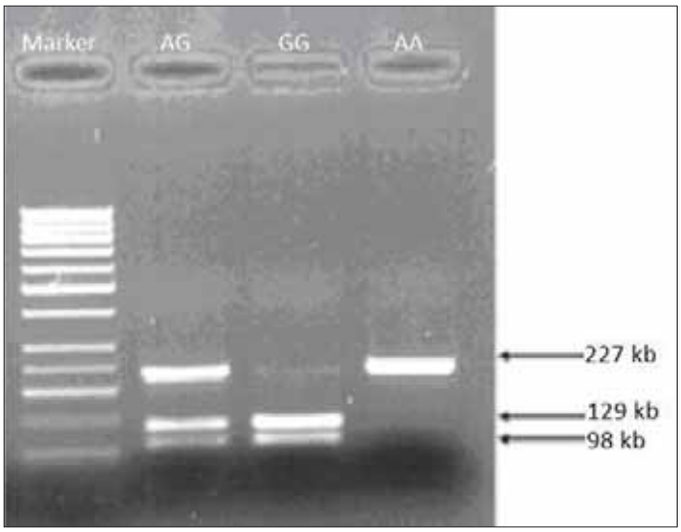

Figure 2. Detection of ESR1 *Btg*I genotypes by agarose gel electrophoresis Samples containing two types of fragments (129 bp and 98 bp) have genotype GG; samples with only one type of fragment (227 bp) represent genotype AA; and samples with all three types of fragments (227 bp, 129 bp and 98 bp) are of genotype AG

Results

Demographic and clinical characteristics

Our study population included 187 participants, 66 of whom were women (35.3%). The mean age was 59.5±13.1. The rest of the main characteristics of patients are summarized in Table 1. The CAD-free and CAD groups differed in respect to age (p=0.042), body mass index (BMI) (p<0.001) and presence of diabetes mellitus (DM) (p=0.009). Comparison of CAD-free group and subgroups according to the extent of CAD is provided in Table 2.

The distribution of patients by their initial clinical diagnosis based on their presenting symptoms and signs was as follows: asymptomatic (silent ischemia) (n=4; 2%); atypical angina pectoris (n=40, 22%); stable angina pectoris (n=36, 19%); unstable angina pectoris (USAP) (n=56, 30%); non-Q wave myocardial infarction (MI) (n=23; 12%); and Q-wave MI (n=28; 15%).

ESR1 polymorphism genotypes and allele frequencies

Genotype distributions were in Hardy Weinberg equilibrium in all of the groups (CAD-free group, CAD group and CAD subgroups).

No difference was found between the CAD free and CAD groups in respect of *Pvu*II polymorphism genotypes and alleles (p=0.219) (Table 3). Subgroup analysis by gender did not demonstrate any significant relation, neither (p=0.42 for men and p=0.52 for women).

When we reviewed ESR1 exon 8 *Btg*I polymorphism genotype distributions we have noticed that the number of patients with genotype AA was too low (n=1 (2%) in the control group and n=1 (0.7%) in the CAD group) to warrant reliable comparisons between the groups. In order to minimize the statistical error in our calculations we decided to merge geno type AA and genotype AG cases into a combined AA+AG group and use it as a reference in all of our analyses.

*Btg*I polymorphism (AA+AG) genotypes as well as allele A frequencies were more prevalent in the CAD-free group than the CAD group (23.4% vs. 10%, OR 2.75, 95% CI 1.15 to 6.58, p=0.019 and 12.8% vs. 5.4%, p=0.064, respectively) (Table 3).

The subgroup analysis by gender revealed that the relationship between ESR1 *Btg*I polymorphism and the angiographic presence of CAD was mainly confined to women (28.6% vs. 4.4%; OR= 8.6; 95% CI= 1.564 to 47.303; p=0.005), rather than men (p=0.391) (Table 4).

We also conducted multivariate logistic regression analysis in which ESR1 *Btg*I polymorphism proved to be a strong predictor of having normal coronary anatomy on angiograms in comparison to covariates such as BMI, age and diabetes mellitus (β=2.096; OR 8.13, 95% CI 1.257 to 52.627, p=0.028) (Table 5).

Table 1. Demographic and clinical characteristics of participants

Parameters	All patients (n=187)	CAD-free group (n=47)	CAD group (n=140)	*p
Age, years	59.6±13.2	56.2±15.1	60.7±12.3	0.042[a]
Women, n(%)	66 (35.3)	21 (44.7)	45 (32.1)	0.12[b]
BMI, kg/m^2	28.8±3.1	27.2± 3.3	29.3±2.8	<0.001[a]
Hyperlipidemia, n(%)	37 (19.8)	7 (14.9)	30 (21.4)	0.331[b]
Hipertension, n(%)	94 (50.3)	24 (51.1)	70 (50)	0.9[b]
Cigarette smoking, n(%)	50 (26.7)	12 (25.5)	38 (27.1)	0.829[b]
Diabetes mellitus, n(%)	56 (29.9)	7 (14.9)	49 (35)	0.009[b]
Family history of CAD, n(%)	24 (12.8)	4 (8.5)	20 (14.3)	0.306[b]

Data are presented as Mean±SD and proportion/percentage
[a]Comparison of CAD group and CAD-free group by independent samples t test
[b]Comparison of CAD group to CAD-free group by Pearson's Chi-square test
*p is statistically significant for values <0.05
BMI - body mass index, CAD - coronary artery disease

Table 2. Comparison of demographic and clinical characteristics of participants by CAD subgroups

Parameters	CAD-free group (n=47)	CAD subgroups			*p	F[a]
		One-vessel (n=58)	Two-vessel (n=39)	Three-vessel (n=43)		
Age, years	56.2±15.1	56.9±12.6	62.5±11.9	64.2±11	0.005[a]	4.476
Women, n(%)	21 (44.7)	20 (34.5)	12 (30.8)	13 (30.2)	0.447[b]	-
BMI kg/m^2	27.2±3.3	28.7±2.8	29.1±2.7	30.3±2.9	<0.001[a]	8.532
Hyperlipidemia, n(%)	7 (14.9)	17 (29.3)	6 (15.4)	7 (16.3)	0.184[b]	-
Hipertension, n(%)	24 (51.1)	26 (44.8)	18 (46.2)	26 (60.5)	0.432[b]	-
Cigarette smoking, n(%)	12 (25.5)	17 (29.3)	13 (33.3)	8 (18.6)	0.467[b]	-
Diabetes mellitus, n(%)	7 (14.9)	16 (27.6)	14 (35.9)	19 (44.2)	0.018[b]	-
Family history of CAD, n(%)	4 (8.5)	6 (10.3)	7 (17.9)	7 (16.3)	0.48[b]	-

Data are presented as Mean±SD and proportion/percentage
[a]Comparison of CAD subgroups and CAD-free group by One-way ANOVA analysis
[b]Comparison of CAD subgroups and CAD-free group by Pearson's Chi-square test
*p is statistically significant for values <0.05
BMI - body mass index, CAD - coronary artery disease

The relation of SNP's to CAD severity

The correlation between ESR1 SNP genotypes and the severity of CAD defined by the number of diseased coronary vessels was also assessed.

ESR1 *Pvu*II polymorphism did not exert any significant correlation with the number of diseased arteries (Spearman's rho= -0.059; p= 0.423).

No correlation between the severity of CAD and *Btg*I genotypes was found, as well (Spearman's rho= 0.100; p=0.172) (Table 3).

Subgroup analyses by gender also did not reveal any significant association of genotypes with CAD severity (p=0.577 and p=0.569 for *Pvu*II; and p=0.094 and p=0.529 for *Btg*I in women and men, respectively) (Table 4).

Discussion

While our study did not show any association of *Pvu*II polymorphism with the prevalence and severity of angiographic CAD, it revealed significant inverse relationship between ESR1 exon 8 A/G *Btg*I polymorphism, also known as G594A or rs 2228480, and angiographic presence of CAD in women. In the multivariate logistic regression model, which also included covariates such as age, DM and BMI, allele A appeared to be the strongest predictor of normal coronary anatomy in women who underwent coronary angiography for symptoms suggesting significant CAD. To our knowledge, this is the first study to report such an association. However, these findings are not sufficient for us to label this association as a direct or indirect causation. Large-scale case control matched studies are needed to assess the nature of this relationship by examining potential direct and indirect interactions between various variables.

The gender selective character of this cardioprotective effect suggests that *Btg*I polymorphism could be an important factor in gender-specific pathophysiology of atherosclerosis. Thus, further research is warranted.

Table 3. The relation of ESR1 *Pvu*II and *Btg*I polymorphism genotype and allele frequencies with the presence and severity of CAD

	CAD-free group (n=47)	CAD group (n=140)	One-vessel disease (n=58)	Two-vessel disease (n=39)	Three-vessel disease (n=43)	Correlation coefficient[b]	*p
ESR1 *Pvu*II							
TT, n(%)	8 (17.1)	40 (28.6)	23 (39.6)	7 (18)	10 (23.2)		
TC, n(%)	29 (61.7)	68 (48.6)	25 (43.1)	24 (61.5)	19 (44.1)	-	0.219[a]
CC, n(%)	10 (21.2)	32 (22.8)	10(17.3)	8 (20.5)	14 (32.7)	-0.059[b]	0.423[b]
C allele frequency, %	52.1	47.1	38.8	51.3	54.6		0.219[a]
T allele frequency, %	47.9	52.9	61.2	48.7	45.4	-0.059[b]	0.423[b]
ESR1 *Btg*I							
AA +AG, n(%)	11(23.4)	14 (10)	5 (8.6)	3 (7.7)	6 (13.9)	-	0.019[a]
GG, n(%)	36(76.6)	126 (90)	53 (91.4)	36 (92.3)	37 (86.1)	0.100[b]	0.172[b]
A allele frequency, n(%)	12.8	5.4	4.3	3.8	8.1	-	0.064[a]
G allele frequency, n(%)	87.2	94.6	95.7	96.2	91.9	0.099[b]	0.178[b]

[a]Comparison of CAD-free group to CAD group by Pearson's Chi-square test
[b]Spearman's correlation between polymorphism genotypes / allele frequencies and the severity of CAD by the number of diseased vessels
*p is statistically significant for values <0.05
CAD - coronary artery disease

Table 4. The relation of ESR1 *Btg*I polymorphism genotypes and allele frequencies with the presence and severity of CAD by gender

Women	**CAD-free group (n=21)**	**CAD group (n=45)**	**One-vessel (n=20)**	**Two-vessel (n=12)**	**Three-vessel (n=13)**	**Correlation coefficient[b]**	**p***
AA +AG, n(%)	6 (28.6)	2 (4.4)	0(0)	0(0)	2 (15.4)	-	0.005[a]
GG, n(%)	15 (71.4)	43 (95.6)	20(100)	12(100)	11 (84.6)	0.208[b]	0.094[b]
A allele frequency, %	14.2	2.2	0	0	7.6	-	0.005[a]
G allele frequency, %	85.8	97.8	100	100	92.4	0.208[b]	0.094[b]
Men	**CAD-free group (n=26)**	**CAD group (n=95)**	**One-vessel (n=38)**	**Two-vessel (n=27)**	**Three-vessel (n=30)**	**Correlation coefficient[b]**	**p***
AA +AG, n(%)	5 (19.2)	12 (12.6)	5 (13.2)	3 (11.1)	4 (13.3)	-	0.391[a]
GG, n(%)	21(80.8)	83 (87.4)	33 (86.8)	24 (88.9)	26 (86.7)	0.058[b]	0.529[b]
A allele frequency, %	11.5	7.8	6.5	5.6	8.3	-	0.521[a]
G allele frequency, %	88.5	92.2	93.5	94.4	91.7	0.057[b]	0.535[b]

[a]Comparison of CAD-free group to CAD group by Pearson's Chi-square test
[b]Spearman's correlation between polymorphism genotypes / allele frequencies and the severity of CAD by the number of diseased vessels
*p is statistically significant for values <0.05
CAD - coronary artery disease

Table 5. Multivariate logistic regression model predicting the presence of normal coronary angiogram in women

Variable	p*	Odds Ratio	95% CI
Intercept	0.09	-	-
Age	0.025	0.945	0.9 to 0.993
BMI	0.436	0.915	0.733 to 1.143
Diabetes mellitus	0.119	0.338	0.086 to 1.323
A allele	0.028	8.133	1.257 to 52.627

* p is statistically significant for values <0.05
BMI - body mass index, CI- confidence interval

The lack of linear correlation between *Btg*I polymorphism and severity of CAD found in our study could be explained with the possibility of ESR1 *Btg*I polymorphism being more influential on the prevention of atherosclerosis rather than on its rate of progression. However, we should bear in mind that the presence of relatively smaller number of patients in subgroups (by number of diseased vessels) may weaken the reliability of statistical results and only large-scale studies may overcome this problem.

There is also previous evidence of ESR1 *Btg*I polymorphism having straight association with the susceptibility to migraines (12). In the light of the findings of Ahmed et al. (13), who reported a significant inverse association between migraines and the

severity of angiographic coronary disease, the findings of our study become more important by suggesting possible presence of common genetic factor (ESR1 *Btg*I polymorphism) affecting both of these conditions but in opposite directions (13). This possibility requires us to conduct further research on a larger scale.

Our study didn't show any association of *Puv*II polymorphism with the prevalence and severity of angiographic CAD. No gender specific differences were detected, either. Thus, our findings are in agreement with the findings of a recent large-scale study (4868 participants) conducted by Koch et al., in which no significant association between ESR1 intron 1 polymorphisms (-397T/C and -351A/G) and the susceptibility to MI was demonstrated (10).

Another large-scale study reported similar findings regarding the influence of ESR1 -397T/C polymorphism on the risk of cardiovascular disease (CVD) as well as on reproductive organ cancers and hip fracture (11). The results of this impressively large scale study (total of 23,122 participants) and the findings of the meta-analysis of 8 other large-scale studies deepened the controversy about the nature and magnitude of the association of *Puv*II polymorphism with CAD initially suggested by several large scale studies (8, 9).

Study limitations

One of the limitations of our study was the relatively small sample size of the groups. However, it is important to realize that this study is the first one to investigate the relation of *Btg*I polymorphisms to CAD. Funding for larger scale studies can only be justified after the findings of studies like ours are reported.

There were also discrepancies between the groups in respect of demographic and clinical characteristics such as age, BMI and DM, because this was not a case controlled study in which those parameters could be matched. However, the weight of these parameters on the outcomes of our study was found to be clinically insignificant on the multiple logistic regression analysis.

We were also limited by the lack of information regarding the *Btg*I polymorphism genotype distributions in the general Turkish population. That would have provided a chance to compare the genotype frequencies of our groups to those of the general population. The allele frequencies in our control (12.8%) and CAD group (5.4%) are comparable to those reported in other general European and Asian populations ranging from 4.2% to 29.2% (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=2228480). However, population based epidemiological studies are needed to clarify the prevalence of ESR1 SNPs in the general Turkish population.

Conclusions

The findings of our study demonstrate that ESR1 intron1 *Puv*II polymorphism is not associated with the presence and extent of angiographic CAD. However, ESR1 exon 8 *Btg*I polymorphism, also known as G594A or rs 2228480, is inversely associated with the presence of angiographically significant CAD especially in women suggesting cardioprotective effect. No such relationship was detected in men, which suggests that ESR1 *Btg*I polymorphism has gender specific effect. Larger scale studies are warranted to confirm these findings. It is also worthwhile to investigate the role of ESR1 *Btg*I polymorphism in the pathophysiology of the inverse relationship between the susceptibility to migraines and angiographic CAD in larger scale studies.

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