ARCHIVES OF THE TURKISH SOCIETY OF CARDIOLOGY

TURKISH SOCIETY OF CARDIOLOGY

Association of polymorphisms in the sex hormone genes with the presence and severity of coronary artery disease

Cinsiyet hormonu genlerindeki polimorfizmlerin koroner arter hastalığı ve ciddiyeti ile ilişkisi

ORIGINAL ARTICLE
KLİNİK ÇALIŞMA

ABSTRACT

Objective: Coronary artery disease (CAD) is an important public health problem worldwide. Therefore, it is important to identify the molecular mechanisms and the candidate gene polymorphisms involved in the development of CAD. In this study, we focused on 2 polymorphisms of the atherosclerosis-related genes, *ESR1* and *CYP19A1*.

Methods: Unselected 339 individuals who underwent coronary angiography were divided into 2 groups: those with normal coronary arteries (≤30% stenosis) and those with critical disease (≥50% stenosis). Individuals were genotyped for *CYP19A1* rs10046 C/T and *ESR1* rs2175898 A/G polymorphisms using hybridization probes in real-time PCR. In addition, Gensini and SYNTAX scores were assessed.

Results: *ESR1* polymorphism was significantly associated with CAD in men (p=0.036) via G allele carriage. Multiple logistic regression analyses showed that *ESR1* rare allele carriage was associated with CAD presence (Odds ratio=2.12, 95% confidence interval 1.01–4.1, p=0.025), adjusted for age, HDL-C, LDL-C and smoking status in the male group. *CYP19A1* rs10046 T allele carriers had a 2.84-fold increased risk for complex CAD in multiple logistic regression analysis (p=0.016). Furthermore, the univariate analysis of variance indicated that T allele carriage of rs10046 polymorphism was associated with increased SYNTAX and Gensini scores (p<0.05). Female patients who were *ESR1* G allele carriers with CAD had higher adiponectin levels (p=0.005), whereas HbA1c levels were associated with T allele of *CYP19A1* in the CAD group (p=0.004) and male CAD group (p=0.018).

Conclusion: The *CYP19A1* and *ESR1* polymorphisms were associated with the presence and severity of CAD. These gene polymorphisms warrant further studies for the elucidation of their contribution to CAD development.

Keywords: Coronary artery disease, genetics, estrogen receptor alpha, single nucleotide polymorphism, aromatase

ÖZET

Amaç: Koroner arter hastalığı (KAH) dünyada önemli bir halk sağlığı sorunudur. Hastalığın etiyolojisinin altında yatan klasik risk faktörleri tanımlanmış olsa da bu faktörleri etkileyen moleküler mekanizmalar tamamen aydınlatılamamıştır. Bu nedenle, hastalığın gelişimi ve tedavisinde rol oynayabilecek aday gen polimorfizmlerinin tanımlanması önem taşımaktadır. Bu bilgiler ışığında, bu çalışmada ateroskleroz ile ilişkili iki gen olan *ESR1* ve *CYP19A1* gen polimorfizmleri araştırılmıştır.

Yöntemler: 339 KAH ve KAH olmayan bireye ait periferik kan örneğinden DNA izolasyonu yapılmış ve çalışmaya katılan bireyler *CYP19A1* rs10046 (C/T) ve *ESR1* rs2175898 (A/G) polimorfizmleri için hibridizasyon probları kullanılarak genotiplenmiştir. Kan örnekleri koroner anjiyografiden önce alınarak biyokimyasal analizler yapılmıştır. Bunun yanında, çalışmaya katılan bireylerin koroner anjiyografi sonuçlarına göre Gensini ve SYNTAX skorları belirlenmiştir.

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Received: January 30, 2021
Accepted: April 25, 2021

Cite this article as: Çoban N, Özuynuk AS, Fahri Erkan A, Kaya A, Ekici B, Güçlü-Geyik F, et al. Association of polymorphisms in the sex hormone genes with the presence and severity of coronary artery disease. Turk Kardiyol Dern Ars 2022;50:22-33.

DOI: 10.5543/tkda.2022.17203

Bulgular: *ESR1* polimorfizmi G allel taşıyıcılığı erkeklerde KAH ile ilişkili bulunmuştur (p=0.036). Yaş, HDL-K, LDL-K düzeyleri ve sigara içme durumuna göre ayarlama yapılan lojistik regresyon analizinde, *ESR1* G allel taşıyıcılığının erkeklerde KAH ile ilişkili olduğu gösterilmiştir (OR=2.12, [%95 GA 1.01–4.1] p=0.025). Yapılan lojistik regresyon analizlerinde, *CYP19A1* rs10046 T allel taşıyıcısı bireylerde kompleks KAH riskinin 2.84 kat arttığı görülmüştür (p=0.016). Buna ek olarak, *CYP19A1* rs10046 T alel taşıyıcılığı, SYNTAX ve Gensini skorları ile ilişkili bulunmuştur (p<0.05). *ESR1* G allel taşıyıcılığı kadın hastalarda yüksek adiponektin düzeyi (p=0.005) ile ilişkili bulunurken *CYP19A1* T alleli, HbA1c düzeyleri ile KAH hastalarında (p=0.004) ve erkek KAH hastalarında (p=0.018) ilişkili bulunmuştur.

Sonuç: *CYP19A1* ve *ESR1* polimorfizmleri KAH şiddeti ve KAH varlığı ile ilişkili bulunmuştur. Çalışılan gen polimorfizmlerin KAH gelişimindeki etkilerinin belirlenebilmesi için daha çok çalışmaya ihtiyaç bulunmaktadır.

Anahtar Kelimeler: Koroner arter hastalığı, genetik, östrojen reseptör alfa, tek nükleotid polimorfizmi, aromataz

Coronary artery disease (CAD), which is a complex multifactorial disease, is the most common cause of morbidity and mortality worldwide. Although classical risk factors such as smoking, diabetes, and hypertension are well established, the contribution of genetic factors and their mechanisms are still not completely understood.[1] Therefore, much attention has been paid on understanding the cardiovascular risk factors associated with inherited genetic factors and underlying pathogenesis. Studies on the disease have shown that the development of CAD is associated with numerous factors, including sex hormones.[2,3]

The physiological effects of estrogen depend on 2 distinct receptors; estrogen receptor α (ER-α) and estrogen receptor β, which is encoded by estrogen receptor 1 (*ESR1*) and estrogen receptor 2 (ESR2) genes, respectively.[4] *ESR1*, located on chromosome 6q25.1-q25.2, includes 8 exons and 7 introns and is expressed in endothelial, smooth muscle, and myocardial cells.[5] The product of *ESR1*, ER-α is involved in vasodilation and plays a role in the depletion of cardiac cell apoptosis.[6,7] Moreover, in an animal study, it has been shown that *ESR1* is the main mediator of the atheroprotective effect of estrogen.[8] In a previous study, *ESR1* polymorphism located in the intronic region of the gene, rs2175898 (reference SNP ID number 2175898), was significantly associated with body mass index (BMI) and waist circumference, which are important risk factors for CAD.[9] RegulomeDB data indicates that rs2175898 polymorphism has regulatory effects on gene expression.[10] Hence, these features made *ESR1* and the polymorphisms of this gene a focus of interest in CAD.

Cytochrome P-450 19A1, (*CYP19A1*), located on chromosome 15q21.2 encodes aromatase, which is responsible for the biosynthesis of C18 estrogens from C19 androgens.[11] Whether genetic variations in this gene, such as single nucleotide polymorphisms (SNPs), can alter aromatase activity has been studied.[12,13] The results of different studies on rs10046 polymorphism, located in the 3' untranslated region (3'-UTR) of the *CYP19A1*, indicated that the ratio of estradiol to testosterone and/or estrone to androstenedione were the highest for the TT genotype.[14-16] Moreover, *CYP19A1* polymorphisms, rs700518, and rs10046 were associated with hypertension in a sex-specific manner.[12,17]

In this study, we aimed to determine whether the *ESR1* rs2175898 (NM_000125.3:c. 644-4838C>T) and *CYP19A1* rs10046 (NC_000015.10: g.51210789G>A) polymorphisms are associated with the prevalence and severity of CAD in our angiographically determined study group, stratifying by sex and severity.

ABBREVIATIONS

3'-UTR	3' untranslated region
Apo	Apolipoprotein
BMI	Body mass index
CAD	Coronary artery disease
CI	Confidence interval
CRP	C-reactive protein
CYP19A1	Cytochrome P-450 19A1
ER-α	Estrogen receptor α
ESR1	Estrogen receptor 1
ESR2	Estrogen receptor 2
HbA1c	Glycated hemoglobin
HDL-C	High-density lipoprotein cholesterol
LDL-C	Low-density lipoprotein cholesterol
OR	Odds ratio
SNPs	Single nucleotide polymorphisms
TC	Total cholesterol
TG	Triglycerides

METHODS

Study population

The study samples were recruited from Ufuk University School of Medicine Cardiology Department between 2014 and 2017; all individuals were 18 years old or older who underwent invasive coronary angiography because of stable angina pectoris, ischemia, and acute

coronary syndrome. A total of 339 Turkish individuals were selected (185 patients with ≥50% luminal stenosis narrowing and 154 non-CAD controls [≤30% stenosis]) were genotyped for *CYP19A1* rs10046 C/T and *ESR1* rs2175898 A/G genotype analysis. Two independent operators evaluated coronary luminal narrowing following the guidelines of the American College of Cardiology/American Heart Association (ACC/AHA) for the classification of coronary lesions.[18] The synergy between percutaneous coronary intervention with Taxus and Cardiac Surgery scores (SYNTAX score) and Gensini scores were calculated for each sample following angiography to evaluate the complexity and severity of CAD.[19,20] Patients with a SYNTAX score ≥23 and <33 are considered to have moderately complex CAD, whereas those with a SYNTAX score ≥33 were considered to have highly complex CAD.[20] For further analysis using this definition, patients with CAD were grouped, and those with a SYNTAX score ≥23 were classified as patients with complex CAD.

Whole sample collection and analysis processes were conducted in compliance with the ethical guidelines of the Declaration of Helsinki and approved by the institutional review board at İstanbul University (Approval Date: February 28, 2014; Approval Number: 444). The written informed consents were obtained from all the participants, and all experiments were performed in accordance with the approved guidelines and regulations.

Inclusion and exclusion criteria

Patients with a history of previous coronary bypass graft surgery, ongoing decompensated (New York Heart Association Functional Class IV) heart failure, advanced hepatic or renal failure, a life expectancy of less than a year, and known malignancy (except basal cell carcinoma and squamous cell carcinoma of the skin in full remission), and those with an active infection or inflammatory disease or a rheumatologic disease were excluded from the study. Patients with a previous history of coronary brachytherapy and those diagnosed with familial hypercholesterolemia were also excluded from the study.

Measurement of risk factors

The presence of CAD risk factors was determined using the criteria of the European Society of Cardiology: a hypertensive condition was attributed when the systolic blood pressure values were ≥140 mm Hg and/or diastolic blood pressure values ≥90 mm Hg in at least 2 separate measurements; patients were considered smokers if they smoked cigarettes, regardless how many per day and non-smokers if they never smoked or had stopped smoking at least one year before sample collection.[21] The weight of individuals was measured in underwear without shoes using a scale. BMI was calculated by dividing the body weight in kilograms by the height in meter squared (kg/m^2). In the classification of obesity, individuals with a BMI ≥30 kg/m^2 were considered obese.

Blood serum samples were collected before coronary angiography and stored at -80oC until analysis. Any lipemic, icterus, and hemolysis specimens were excluded. Analyses of biochemical parameters were determined in 2 central laboratories. Concentrations of total cholesterol (TC), fasting triglycerides (TG), glucose, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured by UniCel DxC 800 (Beckman Coulter, USA). Concentrations of apolipoprotein (Apo) A-I, ApoE, and C-reactive protein (CRP) were determined immunonephelometrically using BN ProSpec analyzer (Siemens Healthcare GmbH, Germany). Adiponectin was measured immuneturbidimetrically using Randox kits (Randox Laboratories, United Kingdom) by Cobas c501 chemistry analyzer (Roche Diagnostics, Germany).

Genetics analysis

Determination of the ESR1 rs2175898 and CYP19A1 rs10046 genotypes

DNA was extracted from peripheral blood leucocytes using inorganic method.[22] The study patients were genotyped for the *CYP19A1* rs10046 C/T (minor allele T) and *ESR1* rs2175898 A/G (minor allele G) genotypes. Genotyping was performed using hybridization probes in real-time PCR LightCycler 480 device (Roche Life Science, USA). DNA amplification was set up in 96 well plates (ABGENE, UK) Typical 10 µL PCR reaction consisted of 2 µL LightCycler 480 Genotyping Master (Roche Life Science, USA) ready mix, 0.2 µL probes, and 0.2 µL primers, 6.6 µL distilled water. Genomic DNA 1 µL (15 ng) was added to the PCR mixture. PCR was carried out on LightCycler 480 using the following conditions: 95°C for 10 min, 95°C for 5 s, 61°C for 5 s, 72°C for 7 s (45 cycles). Melting curve analysis was assessed using the LightCycler480 genotyping software.

Statistical analysis

The genotypic distributions were compared using the chi-squared test. Hardy-Weinberg equilibrium was computed to the expected genotype distribution. All variables were tested for the normality of their distribution and found to be normally distributed except TG.

Table 1. Characteristics of groups stratified according to CAD status and sex

Characteristics	Non-CAD (n=154) mean±SD; %	CAD (n=185) mean±SD; %	p	Male (n=187) mean±SD; %	Female (n=152) mean±SD; %	p
Age (years)	58.34±11.74	63.19±10.57	<0.001**	60.74±11.91	61.30±10.67	0.657
BMI (kg/m^2)	28.68±3.80	28.70±3.88	0.979	28.51±3.94	28.91±3.70	0.352
Total cholesterol (mg/dL)	202.1±47.15	193.91±44.91	0.105	190.08±42.81	206.90±48.30	0.001**
HDL cholesterol (mg/dL)	42.06±9.22	39.23±10.00	0.008**	37.88±9.46	43.76±9.10	<0.001**
LDL cholesterol (mg/dL)	119.42±36.56	113.63±36.01	0.146	111.96±34.37	121.55±38.04	0.016**
Fasting triglyceride (mg/dL)*	142.10±1.63	149.59±1.71	0.362	150.38±1.70	141.06±1.63	0.256
Fasting glucose (mg/dL)	114.29±43.69	125.67±47.69	0.023**	115.62±36.70	126.43±55.25	0.032**
HbA1c (%)*	5.99±1.81	6.51±1.41	<0.001**	6.09±1.19	6.21±1.24	0.390
Apolipoprotein A-I (mg/dL)	0.45±0.52	0.47±0.52	0.726	0.43±0.51	0.50±0.53	0.258
C-reactive protein (mg/L)	1.95±1.66	2.54±2.94	0.065	2.45±2.74	2.03±1.99	0.187
Adiponectin (µg/mL)	7.51±5.74	7.91±6.41	0.591	5.85±4.33	10.07±7.13	<0.001**
Apolipoprotein E (mg/dL)	3.85±3.74	4.02±3.80	0.714	3.44±3.53	4.55±3.96	0.018**
Stenosis (%)	10.52 ±10.15	78.18 ±17.09	<0.001**	57.56±34.26	34.99±35.77	<0.001**
SYNTAX score	0.02±0.18	17.91±12.51	<0.001**	12.42±13.42	6.53±11.31	<0.001**
Gensini score	2.49±3.54	67.82±53.19	<0.001**	48.14±54.00	25.84±44.36	<0.001**
Hypertension†	46.8	46.2	0.918	48.9	43.4	0.313
Obesity†	39.0	36.2	0.603	35.3	40.1	0.360
Type 2 diabetes mellitus†	31.8	50.3	0.001**	38.5	46.1	0.161
Current smokers†	44.8	42.2	0.625	41.7	45.4	0.496
Lipid lowering drug usage†	30.5	49.2	<0.001**	42.8	38.2	0.389
Usage of antidiabetic drugs†	26.0	40.5	0.005**	33.2	34.9	0.740
Sex, male†	39.0	68.6	<0.001**	–	–	–

*log-transformed variables expressed in geometric values.
†Categorical variables are presented in percentages.
** Statistically significant p values (p≤0.05).
BMI: body mass index; CAD: coronary artery disease; HbA1c: glycated hemoglobin; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

Owing to skewed distribution, TG was logarithmically transformed for analyses and expressed as geometric means. A two-tailed t-test was used to compare continuous variables and expressed as means and standard deviation, and categorical variables were compared using the chi-squared test. Multiple logistic regression analyses were used to derive maximum odds ratio (OR) estimates and associated 95% confidence intervals (CIs), adjusted for several risk factors as confounders. Univariate analysis of variance was used for genotypes and expressed as estimated marginal means and 95% CIs, adjusted for different CAD risk factors. The confounders in the analyses were selected from the associated CAD risk factors. A p value <0.05 was considered significant. All statistical analyses were performed using SPSS version 23.0 (IBM Corp., Armonk, NY, USA) software.

RESULTS

Study characteristics and frequencies of SNPs

The demographic and clinical characteristics of patients with and without CAD are summarized in Table 1. The sample consisted of 185 patients with CAD (mean age 63.2±10.6 years, 68.6% male) and 154 individuals without CAD (mean age 58.3±11.7 years, 39.0% male). In addition, demographic and clinical characteristics of the male and female groups are shown in Table 1. The levels of TC, HDL-C, and ad-

Table 2. Genotype and allele frequencies of *CYP19A1* rs10046 C/T and *ESR1* rs2175898 A/G polymorphisms in the study population, CAD, and non-CAD groups

Variables	Study population	CAD	Non-CAD	p
CYP19A1 Genotype frequency				
CC, % (n)	33.1 (85)	32.6 (45)	33.6 (40)	0.866
CT, % (n)	42.0 (108)	43.5 (60)	40.3 (48)	
TT, % (n)	24.9 (64)	23.9 (33)	26.1 (31)	
CT+TT, % (n)	66.9 (172)	67.4 (93)	66.4 (79)	0.864
Allele frequency				
C, %	53.4	54.5	53.8	0.898
T, %	46.6	45.5	46.2	
ESR1 Genotype frequency				
AA, % (n)	51.6 (175)	48.1 (89)	55.8 (86)	0.202
AG, % (n)	40.1 (136)	41.6 (77)	38.4 (59)	
GG, % (n)	8.3 (28)	10.3 (19)	5.8 (9)	
AG+GG, % (n)	48.4 (164)	51.9 (96)	44.2 (68)	0.156
Allele frequency				
A, %	71.7	68.9	75	0.081
G, %	28.3	31.1	25	

Frequency of alleles was computed using gene-counting method.
CAD: coronary artery disease.

Table 3. Genotype and allele frequencies of *ESR1* rs2175898 A/G and *CYP19A1* rs10046 C/T polymorphisms stratified according to sex

	Male			Female		
Variables	**Non-CAD**	**CAD**	**p**	**Non-CAD**	**CAD**	**p**
ESR1 Genotype frequency						
AA, % (n)	63.3 (38)	48.0 (61)	0.142	51.1 (48)	48.3 (28)	0.722
AG, % (n)	30.0 (18)	41.0 (52)		43.6 (41)	43.1 (25)	
GG, % (n)	6.7 (4)	11.0 (14)		5.3 (5)	8.6 (5)	
AG+GG, % (n)	36.7 (22)	52.0 (66)	0.050**	48.9 (46)	51.7(30)	0.738
Allele frequency*						
A, %	78.3	68.5	0.050**	72.9	69.8	0.601
G, %	21.7	31.5		27.1	3.2	
CYP19A1 Genotype frequency						
CC, % (n)	28.3 (13)	33.0 (31)	0.820	37.0 (27)	31.8 (14)	0.745
CT, % (n)	43.4 (20)	42.5 (40)		38.3 (28)	45.5 (20)	
TT, % (n)	28.3 (13)	24.5 (23)		24.7 (18)	22.7 (10)	
CT+TT, % (n)	71.7 (33)	67.0 (63)	0.572	63.0 (46)	68.2 (30)	0.570
Allele frequency						
C, %	50.0	55.3	0.503	56.2	54.5	0.809
T, %	50.0	44.7		43.8	45.5	

*The frequency of alleles was computed using the gene-counting method.
** Statistically significant p values (p≤0.05).
CAD: coronary artery disease.

Table 4. Associations of *ESR1* rs2175898 genotype in multiple logistic regression for CAD, stratified according to sex

	Total		Male		Female	
Model	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**
Sex, male	3.608 (2.202–5.911)	<0.001**				
Age	1.048 (1.026–1.071)	<0.001**	1.041 (1.012–1.071)	0.006**	1.062 (1.026–1.099)	0.001**
HDL-C	0.983 (0.958–1.009)	0.200	0.983 (0.949–1.019)	0.362	.985 (0.948–1.024)	0.455
LDL-C	0.999 (0.992–1.006)	0.797	0.998 (0.988–1.007)	0.628	1.001 (0.991–1.010)	0.901
Smoking status	0.942 (0.589–1.507)	0.802	0.850 (0.445–1.622)	0.622	1.091 (0.545–2.183)	0.806
ESR1 AG+GG*	1.521 (0.950–2.434)	0.081	2.123 (1.009–4.101)	0.025**	1.012 (0.506–2.024)	0.974

*Referent for AG+GG genotypes was AA genotype.
** Statistically significant p values (p≤0.05).
The model comprised 339 individuals, 185 with CAD.
CI: confidence interval; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; OR: odds ratio.

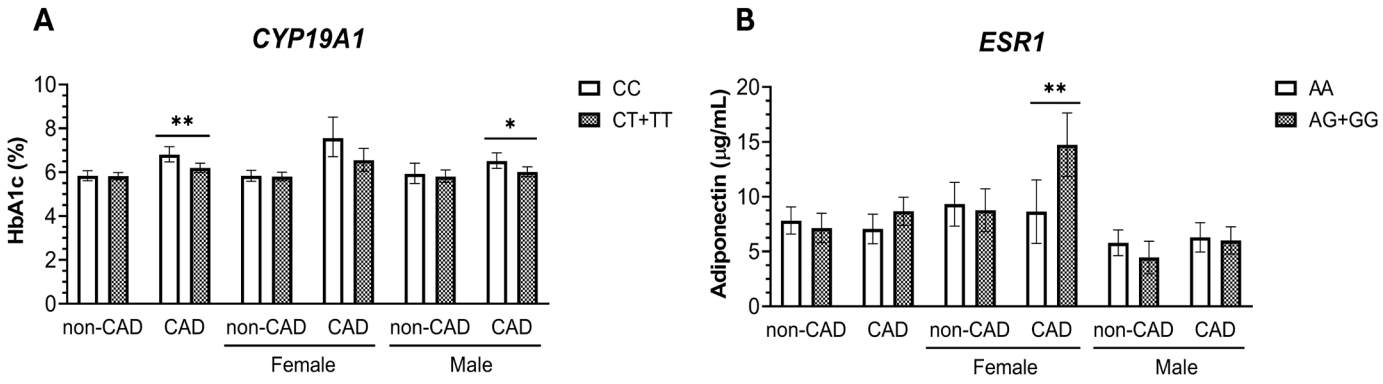

Figure 1. A, B. Association between biochemical parameters and polymorphisms. Estimated marginal means and 95% confidence intervals for (A) Glycated hemoglobin (HbA1c) and (B) adiponectin serum levels are shown across the genotypes of (A) *CYP19A1* rs10046 and (B) *ESR1* rs2175898 polymorphisms. (A) The univariate analysis of variance (ANOVA) conducted to identify the association between rs10046 polymorphism and HbA1c levels, age, sex, body mass index, and antidiabetic drug usage status parameters were used as confounders. (B) Age, sex, obesity, and type 2 diabetes mellitus status parameters were used as confounders in univariate ANOVA for the determination of the association between serum adiponectin levels and *ESR1* polymorphism. *p<0.05, **p<0.01

iponectin along with stenosis percentage and Gensini and SYNTAX scores were found to be significantly different among the male and female groups (Table 1). The genotype distributions of *CYP19A1* rs10046 C/T and *ESR1* rs2175898 A/G are presented in Table 2. The genotype distributions of all the SNPs were in the Hardy-Weinberg equilibrium. No statistically significant difference was found in genotype distributions and allele frequencies between CAD and non-CAD groups (p>0.05) for rs2175898 and rs10046 polymorphisms (Table 2).

Biochemical parameters and their associations with *ESR1* rs2175898 and *CYP19A1* rs10046 polymorphisms

In this study, *CYP19A1* rs10046 polymorphism T allele carriers showed lower HbA1c levels than those with CC genotype in the CAD group according to two-tailed t-test (data not shown, p=0.007). The association between HbA1c levels and *CYP19A1* rs10046 polymorphism has remained statistically significant in the univariate analysis of variance in which age, sex, BMI, and antidiabetic drug usage were used as confounders (Figure 1A, p=0.004). In addition, when the

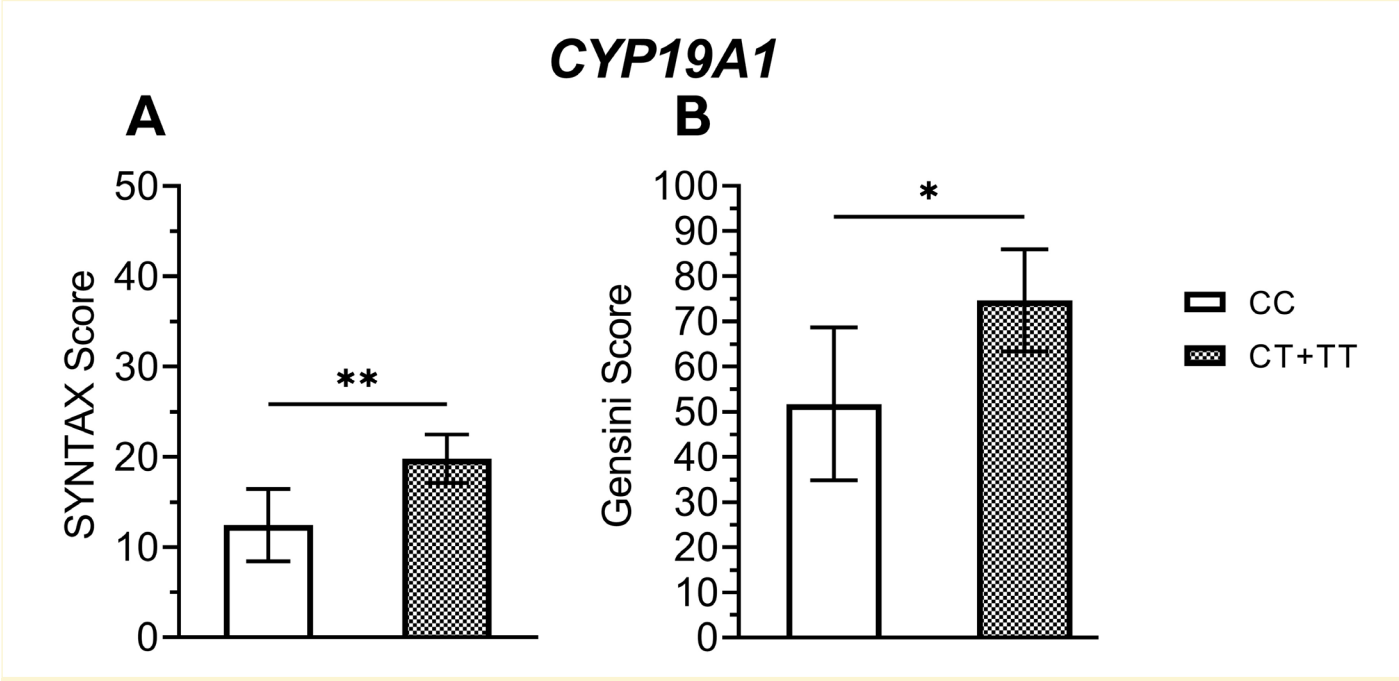

Figure 2. A, B. Estimated marginal means and 95% confidence intervals for (A) SYNTAX and (B) Gensini scores are shown across genotypes of *CYP19A1* rs10046 in patients with coronary artery disease after adjustment for age, sex, smoking status, body mass index, hypertension, fasting glucose, triglyceride, and high-density lipoprotein levels (*p<0.05, **p<0.01).

Table 5. Frequencies of *CYP19A1* and *ESR1* polymorphisms in CAD using SYNTAX scores

Polymorphisms	SYNTAX <23	SYNTAX ≥23	p
CYP19A1 genotypes			
CAD (n=257)			
CC % (n)	36.2 (76)	19.1 (9)	0.025*
CT+TT % (n)	63.8 (134)	80.9 (38)	
ESR1 genotypes			
CAD (n=339)			
AA % (n)	52.6 (144)	47.7 (31)	0.481
AG+GG % (n)	47.4 (130)	52.3 (34)	

*Statistically significant p values (p≤0.05).
CAD: coronary artery disease.

CAD and non-CAD groups were divided according to sex, HbA1c levels were associated with CC genotype in the male CAD group when compared with T allele carriers in the univariate analysis of variance which age, BMI, and antidiabetic drug usage used as confounders (Figure 1A, p=0.018). Moreover, the G allele carriers of *ESR1* rs2175898 polymorphism had higher adiponectin levels than non-carriers in the female CAD group according to the two-tailed t-test (data not shown, p=0.004). For further testing, a univariate analysis of variance in which age, obesity, and type 2 diabetes mellitus (T2DM) status parameters used as confounders was performed, and adiponectin levels remained significantly associated with G allele carriage of *ESR1* polymorphism (Figure 1B, p=0.005). The T allele carriage of *CYP19A1* rs10046 and *ESR1* rs2175898 polymorphisms were not associated with lipid profiles, BMI, glucose, ApoA-I, ApoE, and CRP levels in the CAD and non-CAD groups (data not shown, p>0.05).

Associations of the *ESR1* rs2175898 A/G polymorphism with CAD

ESR1 rs2175898 polymorphism was significantly associated with CAD in the male group via G allele carriage (p=0.050, Table 3). After adjustment for sex, HDL-C, LDL-C, age, and smoking status, multiple logistic regression analysis showed that the *ESR1* rs2175898 G allele carriage in the male group significantly elevated OR for CAD when it is compared with A homozygotes (OR=2.12, 95% CI 1.01-4.1, p=0.025) (Table 4). No significant association was found between the *ESR1* rs2175898 polymorphism and hypertension, diabetes, and other CAD risk factors in the male and female groups using a chi-squared test (data not shown, p>0.05). In addition, no significant association was found between *CYP19A1* rs10046 polymorphism and CAD presence in the chi-squared test (Tables 2 and 3).

Associations of the *CYP19A1* rs10046 C/T polymorphism with CAD severity

The genotype distributions of *CYP19A1* rs10046 C/T and *ESR1* rs2175898 A/G in CAD subgroups classified according to the SYNTAX score are shown in Table 5. *CYP19A1* rs10046 T allele carriage was significantly associated with a higher SYNTAX score than the CC genotypes (p=0.025) (Table 5). No significant association was found between the *ESR1* polymorphism and SYNTAX score using the chi-squared test (Table 5). After adjustment for sex, age, smoking status, BMI, HDL-C, and LDL-C levels, logistic regression analysis showed that *CYP19A1* rs10046 T allele carriers exhibit increased risk for complex CAD compared with CC genotypes (OR=2.84, 95% CI 1.21-6.63, p=0.016). In addition, univariate analysis of variance showed that T allele carriage of rs10046 polymorphism is significantly associated with increased SYNTAX (p=0.004) and Gensini (p=0.032) scores compared with the CC genotype, adjusted for the same confounders in multiple logistic regression analysis (Figure 2).

DISCUSSION

CAD is one of the most common cardiovascular diseases and is one of the leading causes of death at present.[23] Lipid accumulation and inflammatory response in macrophages are two key factors in the pathogenesis of CAD. Sex differences in CAD are well established; however, the mechanisms remain unclear. Therefore, we examined the association between CAD and the variations in sex hormone related genes in men and women. In this study, a significant association was found between *ESR1* rs2175898 polymorphism and CAD presence in the male group, whereas *CYP19A1* rs10046 polymorphism was associated with complexity of CAD. *ESR1* polymorphism was associated with serum adiponectin levels in female patients independent from obesity and T2DM status, whereas *CYP19A1* polymorphism was associated with HbA1c levels.

The cytochrome P450 enzyme aromatase, *CYP19A1*, is required for estrogen biosynthesis in both premenopausal and postmenopausal women.[24] Molecular epidemiology studies were done with a relatively small number of common *CYP19A1* polymorphisms. Paradisi et al.[25] have shown that different sex-dependent relationships exist between androgens and cardiovascular disease or insulin resistance. Polycystic ovary syndrome has been associated with insulin resistance and an increased risk of cardiovascular death or myocardial infarction (MI) in women.[26] The biological relevance of one of the *CYP19A1* SNPs was supported by an association with plasma estradiol levels.[27] Only a few studies have investigated whether *CYP19A1* polymorphisms are associated with blood pressure and hypertension, which are risk factors for CAD.[17,28,29] The results of these studies were found to be inconsistent.[17,28,29] In addition, in a recent study, conducted in the Chinese Han population, several *CYP19A1* polymorphisms were associated with T2DM, which is a risk factor for cardiovascular diseases.[30] Our previous findings in the Turkish Adult Risk Factor (TARF) study confirmed the fact that T allele was associated with hypertension in men, independent of waist girth in the Turkish population.[31] The role of polymorphisms of the *CYP19A1* gene in cardiovascular disease was investigated previously in some ethnic groups such as Greek, Han, and Uygur populations.[32,33]] Meng et al.[32] have suggested that the *CYP19A1* rs2236722 and rs4646 polymorphisms are associated with CAD and circulating sex hormone levels in the Chinese population. A study in the Greek population showed that the rs10046 (C/T) polymorphism of the *CYP19A1* gene exhibits correlation with CAD, and individuals with the C allele have an increased probability of manifesting the disease.[33] In another study, the identification of a *CYP19A1* polymorphism was associated with reduced risk of coronary heart disease.[34] Although there are a number of studies that tested the effects of genetic variations in the *CYP19A1* gene on CAD, whether the *CYP19A1* gene is associated with CAD remains controversial. The results of a study conducted by Bampali et al.[33] differed from the findings presented in our study as the T allele was associated with CAD severity. This rather contradictory result may be because of the different sample sizes and the inclusion criteria of the study groups. However, in this study, although no differences were observed in genotype distributions between the CAD and non-CAD groups, which is similar to the TARF study, we observed that the *CYP19A1* rs10046 T allele was associated with angiographic severity of CAD according to both the SYNTAX and Gensini scores. This result may be explained by the fact that the two studies have different diagnostic methods for CAD. Although the results of clinical and non-invasive tests were used in the TARF study for the CAD and non-CAD classification; in this study, coronary angiography which determines the severity of the disease was used along with non-invasive tests. Therefore, although the association between polymorphism and CAD severity could be defined in this study, this evaluation could not be made in the TARF study as there was no data about the severity of the disease. As a result, our findings revealed that the *CYP19A1* T allele is a risk factor for CAD severity, independent of sex, age, smoking status, BMI, hypertension, HDL-C, TG, and glucose.

Further investigations in the large discovery and replication samples are necessary to confirm this increased CAD severity risk.

The other important and widely studied candidate gene for the cardiovascular system is *ESR1*. Estrogen deficiency is a major player in the pathogenesis of CAD. The role of polymorphisms of the *ESR1* gene in cardiovascular disease development was investigated in several populations.[35-38] In a study conducted in the Turkish population, a significant association was found between CC genotype of *ESR1* c.454-397T>C polymorphism and increased risk for CAD, independent of known CAD risk factors.[39] A meta-analysis study suggested that the *ESR1* PvuII polymorphism may be associated with CAD susceptibility, especially among Asian populations.[40] On the contrary, previous studies have reported generally inconsistent results regarding the role of *ESR1* genetic variation in CAD risk.[35,41] There is a limited number of studies on *ESR1* rs2175898 polymorphism, and this variation was not evaluated in patients with CAD. Our results revealed a sex-specific correlation between CAD and *ESR1* rs2175898 polymorphism. In this study, it was observed that the *ESR1* rs2175898 G allele carriage was associated with CAD presence in men. The sex-specific role of *ESR1* polymorphisms in cardiovascular diseases has been documented previously, and the results were quite varying. In the recent TARF study, we observed that G allele carriage of *ESR1* rs2175898 polymorphism was associated with high TC levels in the male group.[42] Furthermore, in previous studies, the relation between other risk factors of CAD, such as hypertension and *ESR1* polymorphisms were investigated, and sex-specific associations were found. Kelly et al.[43] have identified strong and consistent associations between *ESR1* gene variants and blood pressure salt sensitivity in men. Lehtimäki et al.[36] have shown that the *ESR1* gene is an interesting candidate in the pathogenesis of acute coronary events among elderly men. Shearman et al.[44] have shown that male carriers of the PvuII CC genotype had a substantially increased risk and morbidity rate of MI and severe atherosclerosis. However, Hayashi et al.[45] have found that the TT genotype of PvuII was associated with higher arterial stiffness in female patients. These results indicate that *ESR1* polymorphisms could have sex-dependent inverse effects.

In this study, serum adiponectin levels of female patients who were *ESR1* G allele carriers and had CAD were significantly higher than those with AA genotype of rs2175898 polymorphism. Adiponectin, as previously shown, has protective effects against cardiovascular diseases and is found in lower concentrations in the serum of individuals with T2DM and obesity.[46,47] A study that investigated the association between serum adiponectin levels and *ESR1* PvuII polymorphism found that women with the CC genotype had the highest serum adiponectin levels compared with other genotypes.[48] *ESR1* polymorphisms can affect the expression levels of the gene, such as in the case of TT genotype of PvuII polymorphism, which results in lower expression levels of *ESR1* mRNA.[49] Altered gene expression of *ESR1* could be the reason behind the changed serum adiponectin levels because of its regulatory effects on the adipogenesis-related genes in visceral adipose tissue. RegulomeDB data for rs2175898 polymorphism indicate that this polymorphism could alter the gene expression as it is located on a site that includes DNA motif for ASCL2, which is a transcription factor.[10] In our study, similar to the results of PvuII, men who were carriers of the G allele of rs2175898 polymorphism had increased risk for CAD, whereas G allele carriage in women resulted in higher serum adiponectin levels, which could be interpreted as protective against CAD.

Limitations

Our study had several limitations. Further studies with larger sample size are necessary to better state the role of *ESR1* and *CYP19A1* gene polymorphisms in CAD. Larger sample size may be required to observe the statistical significance for small effect genes that might have been missed in this study. In addition, although no significant coronary stenosis was observed in individuals who were determined as the control group via coronary angiography, they did not fully represent healthy individuals, and it can be assumed that individuals without CAD have additional risk factors than the general population. However, as invasive coronary angiography cannot be performed in healthy individuals, those who did not undergo angiography cannot be assumed to be healthy. The non-CAD and CAD groups were not equal in terms of the number of men and women because CAD is more prevalent among men than women. In addition, in this study, both stable angina and acute coronary syndromes were included in the CAD group, which could have resulted in heterogeneity. Moreover, the study population represented a relatively older population, and the effects observed in this study did not project all individuals with these polymorphisms. As these polymorphisms are within protein coding genes, another limitation of this study was that the circulating gene products were not evaluated. This is a factor that should be considered in future studies. Furthermore, analyses of other SNPs of *ESR1* and *CYP19A1* along with the functional studies are needed for the elucidation of these genes in CAD.

CONCLUSION

Our results revealed a sex-specific association between CAD and *ESR1* rs2175898 polymorphism and that *CYP19A1* rs10046 was associated with angiographic severity of CAD using both the Gensini and SYNTAX scoring systems. In addition, it appears that *ESR1* polymorphism influences adiponectin levels in female patients, whereas *CYP19A1* polymorphism alters the HbA1c levels in patients with CAD. Although the molecular mechanism leading to these associations remains to be addressed, our findings may contribute to the risk assessment of severe CAD.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of İstanbul University (Approval Date: February 28, 2014; Approval Number: 444).

Informed Consent: Written informed consent was obtained from all participants of this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – N.Ç.; Design – N.Ç.; Supervision – N.Ç.; Resources N.Ç., F.G.G., E.A.; Materials – A.F.E, B.E., F.G.G.; Data Collection and/or Processing – N.Ç., A.F.E., B.E.; Analysis and/or Interpretation – N.Ç., A.S.O., G.C.; Literature Search – A.S.O., A.K., E.A., G.C., N.E.Ü.; Writing– N.Ç., A.S.Ö.; Critical Review – N.Ç., A.S.Ö., A.F.E., A.K., B.E., F.G.G., E.A., G.C., N.E.Ü.

Funding: This study was supported by The Research Support Unit of İstanbul University (Grant no. 47372).

Conflict of Interest: None.

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