Gender specific association of *ABCA1* gene R219K variant in coronary disease risk through interactions with serum triglyceride elevation in Turkish adults

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Abstract

Objective: ATP binding cassette transporter A1 *(ABCA1)* controls the reverse cholesterol transport. Some *ABCA1* variants are correlated with serum high-density lipoprotein cholesterol (HDL-C) and other lipid concentrations. We aimed to explore the relationship of *ABCA1* gene with both the lipid profile and coronary heart disease (CHD) risk.

Methods: Selected 627 individuals of the Turkish Adult Risk Factor Study were genotyped for *ABCA1* R219K polymorphism using PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) method. Student's t-test, one-way ANOVA, Chi-square test, linear and logistic regression was used for statistical analysis.

Results: We demonstrated a gender-specific effect of the R219K polymorphism on plasma lipids and CHD. In men, while homozygosity of the K allele was associated with increased plasma low-density lipoprotein cholesterol (LDL-C) ($p<0.05$) and total cholesterol concentrations ($p<0.05$), carriage of this allele was associated with higher HDL-C concentrations ($p<0.05$) after adjustment for associated risk factors, but not with CHD. In women, however, without being related to HDL-C levels, each 219K allele was associated with 10% higher triglycerides (TG) concentrations ($p<0.05$). R219K heterozygosity in women independently doubled (95% CI 1.00; 3.80) the odds ratio for CHD risk in regression models, after adjustment for several variables. Interaction of TG elevation (>140 mg/dL) with CHD was demonstrated in female 219RK genotype carriers.

Conclusion: R219 allele of the *ABCA1* gene independently confers CHD risk in heterozygote Turkish women, not via reduced HDL-C, but interacting with elevated TG expressed by the 219K allele, but not in men. *(Anadolu Kardiyol Derg 2014; 14: 18-25)*

Key words: *ABCA1* gene variants, coronary heart disease risk, gender difference, phospholipids, serum triglycerides, Turkish population, regression analysis

Introduction

Serum high-density lipoprotein (HDL) cholesterol is inversely correlated with risk of coronary heart disease (CHD) (1). The antiatherogenic function of HDL-C is generally attributed to its pivotal role in reverse cholesterol transport, a process that delivers excess cholesterol from macrophages within the arterial wall to the liver for disposal (2, 3). The first step in this process is mediated by ATP binding cassette transporter A1 (*ABCA1*), a member of the ATP-binding cassette family that stimulates efflux of cholesterol and phospholipids, particularly to lipid-poor apolipoprotein A-I (Apo A-I), the initial step in the formation of nascent HDL-C (4). Although there are multiple mechanisms by which HDL-C can be atheroprotective, the relative activity of *ABCA1* plays a major role in this process (5). Therefore, it is suggested that *ABCA1* is a protein that plays a key role in regulating plasma lipid metabolism and a major factor in CHD risk protection (6, 7).

A number of common polymorphisms have been reported both in the coding and promoter regions of the *ABCA1* gene (7, 8). These polymorphisms in the *ABCA1* gene have been associated with HDL-C concentrations (8-10) and CHD risk in some of epidemiological and case-control studies (11, 12). The K allele of the R219K polymorphism (Arg219Lys, rs2230806) has been associated with decreased triglyceride (TG) concentrations (11, 13) and increased HDL-C concentrations (9, 11, 12), consistent with a net increase in transporter function; the rare allele of the R219K, on the other hand, was found to be associated with

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increased risk of CHD (14). Because findings on the relationship between *ABCA1* R219K polymorphism and CHD have been inconclusive, a meta-analysis was recently conducted comprising over 9400 cases and over 16.000 controls (12, 15). Ethnicity explained much of the heterogeneity, and the K219 allele was found a protective factor against CHD in Asians [OR 0.69 (95%CI 0.55; 0.86)] but not a significant one in Caucasians (OR 0.87) (15).

Among other *ABCA1* polymorphisms, R219K variant's association with HDL-C was examined by Hodoğlugil et al. (10) on a large sample of Turkish adults, and an association was lacking in men. The KK genotype of R219K polymorphism alone showed no association with elevated HDL-C but an association was observed in combination with TT genotype of C-14T polymorphism. However, investigators did not examine the variant's association with TG concentrations and CHD. The allele frequency in their study group was found to be similar to our (0.38 for the rare allele).

Turkish adults are prone to metabolic syndrome (MetS) (16) and women display a high burden of CHD (17) and diabetes for which elevated TG and proinflammatory state appear pivotal (18, 19).

We, therefore, explored the role of *ABCA1* gene in predisposing Turkish adults to CHD in a nested case-control study comprising 627 genotyped individuals for the R219K polymorphism. This polymorphism was selected due to its location on a functional region of *ABCA1*. The specific aim of this study was to examine the relation of the R219K polymorphism with both the lipid concentrations and CHD in a randomly selected sample of the Turkish Adult Risk Factor Study (TARF) cohort, representative of Turkish adults (20).

Methods

Study population

Participants of this study are derived from the cohort of the *Turkish Adult Risk Factor Study*, a prospective survey on the prevalence of cardiac disease and risk factors in a representative sample of adults in Turkey conducted periodically in 59 communities throughout all geographical regions of the country. Partial logistic support was provided by the Turkish Ministry of Health. The methodology of this paper, based on the survey 2003/04, has been previously described in detail (20). Data were obtained for history of the past years via a questionnaire, physical examination of the cardiovascular system and recording of a resting electrocardiogram. Economic restraints limited the sample of this nested case-control study to 627 participants who attended the survey period 2008-2010 over a third of whom was randomly selected among those identified as CHD and the remainder from participants without CHD. Participants gave written informed. The study protocol was approved by the Ethic Committee.

Definitions

Obesity was defined as a BMI (body mass index) ≥30 kg/m^2. Individuals with *diabetes* were diagnosed with criteria of the American Diabetes Association (21), namely when plasma fasting glucose was ≥126 mg/dL (or 2-h postprandial glucose >200 mg/dL) and/or the current use of diabetes medication. Oral glucose tolerance test was not performed. Individuals with MetS were identified when 3 out of the 5 criteria of the National Cholesterol Education Program (ATP III) (22) were met, modified for prediabetes (fasting glucose rather than 110-125 mg/dL (23) and further for abdominal obesity using as cutpoint ≥95 cm (instead of 102 cm) in men, as assessed in the Turkish Adult Risk Factor study (24).

Diagnosis of CHD was based on the presence of typical exertional angina pectoris, of a history of myocardial infarction with or without accompanying Minnesota codes of the ECG (25), or on a history of myocardial revascularization. Among women, typical angina and age >45 years were prerequisite for a definitive diagnosis when angina was isolated. ECG changes of "ischemic type" of greater than minor degree (Codes 1.1-2, 4.1-2, 5.1-2, 7.1) were considered as myocardial infarct sequelae or myocardial ischemia, respectively, as previously published (17, 24). CHD diagnosis was based on history of interventional procedures in nearly one-quarter of patients.

Measurement of risk factors

Self-reported cigarette smoking was categorized into non-smokers who included former smokers (discontinuance of 3 months or more) and current smokers (regularly 1 or more cigarettes daily). An average daily alcohol intake of 2 mL of ethanol was considered as using alcohol. Weight was measured without shoes in light indoor clothes using a scale. Body mass index was calculated as weight divided by height squared (kg/m^2). Waist circumference was measured with a tape (Roche LI95 63B 00), the subject standing and wearing only underwear, at the level midway between the lower rib margin and the iliac crest.

Blood samples were collected after an 11-hour or longer fasting. Samples were shipped on cooled gel packs to Istanbul to be stored at-75°C, until analyzed at a central laboratory. Serum concentrations of total cholesterol, fasting TG, glucose, and HDL-C (directly without precipitation) were determined using enzymatic kits from Roche Diagnostics with a Hitachi 902 autoanalyzer (Roche Diagnostics, Mannheim, Germany). Concentrations of Apo A-I and B were measured by Behring kits and nephelometry (BN Prospec, Behring Diagnostics, Westwood, MA). Within-run variation of serum glucose, lipid, lipoprotein and Apo A-I and B variables ranged from 1% to 2.4%, and day-to-day variations from 1.5% to 4%.

Genetics analysis

The detection of R219K polymorphism using RFLP (restriction fragment length polymorphism)

DNA was extracted from peripheral blood leukocytes using a QIAmp^R DNA Maxi KIT (Qiagen, Hilden, Germany). A 243-bp fragment of exon 7 of the *ABCA1* gene involving nucleotide 1051 of the DNA sequence was amplified by polymerase chain reac-

tion (PCR), using the following primers: R219K Forward: 5′-tca taa tcc tct tct gct ag-3′ and R219K Reverse: 5′-cag tta gca agt cta cgc a-3′. PCR was initiated with predenaturation by first heating the samples for 3 min at 94 °C. Genomic DNA was subjected to 35 cycles of denaturation at 94°C for one minute, annealing at 59°C for one minute, and extension at 72°C for one minute, followed by a final extension at 72°C for 10 minutes. After amplification, an aliquot of 10 µl of PCR product was digested with 10 U of the restriction enzyme *Xag*I (MBI Fermentas, Lithuania) at 37°C for more than three hours. The R219 allele was resistant to digestion, whereas the 219K allele was digested into 155 bp and 88 bp fragments (11). The fragments obtained after digestion were analyzed by electrophoresis on 2% agarose gels (Fig. 1). The bands were visualized by staining with ethidium bromide.

Statistical analysis

All statistical analyses were performed using Windows SPSS version 10.0 software (SPSS Inc, Chicago, IL, USA). The genotypic distributions were compared using the x^2-test. Hardy-Weinberg equilibrium was computed to the expected genotype distribution. A p-value of <0.05 was considered statistically significant. Due to skewed distributions, fasting TG were logarithmically transformed for analyses and expressed as geometric means and standard deviation (S.D.). Multinominal regression analyses were performed for interactions between SNP and gender. Interactions between single nucleotide polymorphism (SNP) and studied traits, however, were examined by using univariate analysis. Two-tailed *t* test and analysis of variance (ANOVA) test were used to compare continuous variables and expressed as means and standard deviation (S.D.), while categorical variables were compared using the chi-square test. In one-way ANOVA and two-tailed *t* test, drug usage for lipids was excluded. One-way analysis of covariance (ANCOVA) was performed with TG, HDL-C, LDL-C (low-density lipoprotein cholesterol) and total cholesterol as dependent variables and the following factors as independent variables: *ABCA1* genotype, adjusted for age, waist circumference, lipid lowering medication and CHD in men and women. Linear regression analyses were performed with TG, HDL-C, LDL-C and total cholesterol each as dependent variable adjusted for pooled 219K allele carriage, age, waist circumference, and lipid lowering medication, separately in men and women. Logistic regression models were used to estimate odds ratios (ORs) and associated 95% confidence intervals (CIs) for CHD, adjusted for risk factors and/or interaction with a triglyceride cutoff and expressed per 1 SD increment of continuous variables.

Results

Study characteristics

The biometric parameters and characteristics of the participants of the study population are shown in Table 1. The sample comprised of selected 627 (mean age; 58.9±12.8, 45% male) Turkish adults. The prevalence of CHD and type 2 diabetes were 35.1% and 18.7%, respectively. Of all the subjects, 10.4% were being treated with lipid-lowering drugs. Control subjects were composed of 407 individuals in whom CHD (n=220 cases) had not been identified among the TARF study participants. The main characteristics of the study population stratified by gender are presented in Table 1. Significant differences were observed between genders for various parameters.

Allele and genotype distributions of ABCA1 R219K polymorphism

The genotype frequencies of the *ABCA1* R219K polymorphism, determined for 627 participants, disclosed the following distribution: 36.4% (n=228), 50.4% (n=316) and 13.2% (n=83) for the RR, RK and KK genotypes, respectively. The 219K allele frequency was found to be 0.38 and the carrier (RK+KK) of rare allele was found to be 0.63. Genotype distribution of the *ABCA1* R219K polymorphism was in Hardy-Weinberg equilibrium for our study population.

Association of the ABCA1 R219K variant with biochemical variables

We examined the relationship between concentrations of biochemical variables and the R219K polymorphism of the ABCA1 gene in a total of 627 individuals (men=282, women=345). Table 2 shows serum concentrations of the biochemical lipid variables after adjustment for age, waist circumference, lipid lowering medication and CHD. Men carrying 219K alleles had higher HDL-C (p=0.035), after adjusting for confounders, than RR

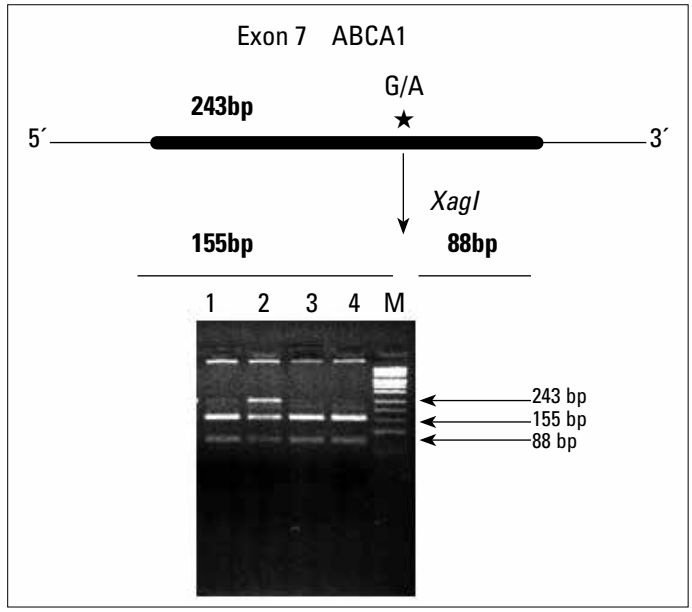

Figure 1. Determination of the R219K genotype by PCR amplification and restriction analysis. In the upper part, the G/A polymorphism position is indicated by an asterisk. When the nucleotide A is present, a XagI restriction site is created. In the lower part, 4% agarose gel electrophoresis of XagI digested PCR products is shown. After cleavage with XagI, either a 155 and 88bp fragment (homozygous AA, lanes 1,3 and 4), 243, 155, and 88 bp fragments (heterozygous GA, line 2) are produced. M- Puc8 marker

PCR - polymerase chain reaction

Table 1. Characteristics of the participants

Variables	Males (n)	Females (n)	**P
Age, years	58.9±12.8 (282)	56.7±12.3 (345)	0.03
Body mass index, kg/m^2	27.7±4.4 (213)	30.6±5.9 (361)	<0.001
Waist circumference, cm	96.3±11.9 (267)	94±13.7 (330)	0.037
Total cholesterol, mg/dL	184±43.8 (272)	194.5±42.5 (335)	0.003
HDL-C, mg/dL	43.3±12.7 (272)	49.7±12 (337)	<0.001
LDL-C, mg/dL	107.2±34.5 (259)	111.6±34.2 (307)	0.132
Fasting triglycerides, mg/dL*	136.1±1.75 (270)	135.8±1.71 (332)	0.966
Fasting glucose, mg/dL	109.6±44.4 (259)	105.2±40.8 (330)	0.218
Apolipoprotein A-I, mg/dL	133.8±24.1 (156)	145.4±27.4 (200)	<0.001
Apolipoprotein B, mg/dL	97.1±27.5 (157)	101.7±26 (204)	0.105
Systolic blood pressure, mmHg	121.0±21.9 (267)	128.2±23.6 (332)	0.01
Diastolic blood pressure, mmHg	76.6±11.7 (267)	78.8±11.9 (332)	0.026
Diabetes mellitus, %(n)	19.9 (56)	17.7 (61)	0.486
Obesity, %(n)	27.2 (58)	52.1 (136)	<0.001
Coronary heart disease, %(n)	40.4 (114)	30.7 (106)	0.011
Cigarette smoking, %(n)	28.9 (77)	14.2 (47)	<0.001
Alcohol usage, %(n)	10.2 (27)	1.2 (4)	<0.001
Lipid-lowering medication, %(n)	9.9 (28)	10.7 (37)	0.745
Antihypertensive medication, %(n)	30.1 (85)	38.3 (132)	0.034
Medication for diabetes, %(n)	13.1 (37)	12.8 (44)	0.892

Continuous variables are presented as mean±SD and dichotomous variables as percentages.
*Geometric mean values.
**A two-tailed t-test and Chi-square-test
HDL-C - high-density lipoprotein cholesterol; LDL-C - low-density lipoprotein cholesterol

homozygotes; they also tended to lower TG concentration. However, in male, LDL-C (p=0.01) and total cholesterol (p=0.05) concentrations were substantially higher in 219KK homozygotes. In ANCOVA, the K allele carriage of this polymorphism was associated significantly with higher serum TG concentration (p=0.04) in women. In relation to TG concentration, but not to LDL-C and total cholesterol, the ABCA1 R219K polymorphism had significant gender-by-genotype interaction (p=0.049).

Further analyses were performed to explore the relationship between this polymorphism and TG, total cholesterol, HDL-C, LDL-C concentrations using ANCOVA test (data not shown). In model, gender-specific covariance analysis by controlling for age, waist circumference, BMI, physical activity, lipid lowering medication, menopausal status and CHD showed that the associations remained the same in women and men (p<0.05).

Separate analyses in non-obese (BMI <30 kg/m^2) men were associated with higher HDL-C concentrations in 219K allele carriers when compared to RR genotype (45.3±13.1 and 40.3±11, respectively, p=0.016).

A summary overview on net percent changes of main lipid concentrations of the pooled 219K allele carriage is provided in Table 3 which indicates a 14%-increase in fasting TG in women and an 8%-increase in HDL-C in men. This corresponds to approximately 10% increase in TG in women per each 219K allele carried. The increase in HDL-C in men was not concomitant with lower but rather with a tendency to higher LDL-C and to lower triglyceride levels when compared with R219 homozygotes.

Associations of ABCA1 R219K polymorphism with CHD risk

We investigated the distribution of the clinical and biochemical characteristics of genotypes of R219K polymorphism in the CHD and there were no significant associations (data not shown).

The allele and genotype frequencies of R219K polymorphism were examined in 220 cases of CHD and 407 without CHD. The 219K allele frequencies of the cases with and without CHD were 0.39 and 0.37, respectively. The 219K allele carrier prevailed in 59.6% in men and 74.5% for CHD in women. Frequencies of *ABCA1* R219K genotypes (RR, RK and KK) were 25.5% (n=27), 64.1% (n=68) and 10.4% (n=11) in women with CHD (p=0.012) and 40.4% (n=46), 44.7% (n=51) and 14.9% (n=17) in men with CHD (p=0.53), respectively. In relation to CHD, the *ABCA1* R219K polymorphism had significant gender-by-genotype interaction (p=0.009). The R219K polymorphism was found to be associated with CHD only in women using the chi-square test. In women, the frequency of the 219RK genotype was higher in the group with CHD (64.1%, n=68) than without (46.8%, n=112) (p=0.01). Further analyses adjusted by genetic models of R219K polymor-

Table 2. Adjusted mean (±SE) serum fasting TG, HDL-C, LDL-C and total cholesterol values stratified to the *ABCA1* genotype and gender

Variables	RR	RK	KK	*p	RK+KK	**P
Females n	118	180	47		227	
Total cholesterol, mg/dL	193.1±3.9 (111)	193.9±3.1 (172)	198.1±6 (47)	0.773	194.8±2.8 (219)	0.712
HDL-C, mg/dL	49.7±1.1 (111)	49.3±0.9 (172)	50.4±1.7 (47)	0.847	49.4±0.8 (219)	0.925
LDL-C, mg/dL	114.1±3.2 (103)	110.3±2.6 (159)	110.3±5.2 (40)	0.650	110.3±2.3 (199)	0.353
Fasting triglycerides, mg/dL¶	124.2±1.05 (107)	139±1.04 (171)	146.6±1.08 (47)	0.09	140.6±1.04 (218)	0.04
Apo A-I, mg/dL	141.5±24.6 (55)	149.2±25.3 (93)	141.5±40.6 (29)	0.193	147.4±29.6 (122)	0.197
Apo A-I/HDL-C ratio	2.93±0.08 (55)	3.0±0.06 (93)	3.06±0.11 (29)	0.523	3.0±0.05 (122)	0.26
Current smokers, % (n)	12.7 (15)	12.2 (22)	10.6 (5)	0.951	11.9 (27)	0.806
Males n	110	136	36		172	
Total cholesterol, mg/dL	179.7±4.3 (105)	183.2±3.9 (128)	200.8±7.5 (34)	0.05	187±3.5 (161)	0.191
HDL-C, mg/dL	41.1±1.2 (105)	43.8±1.1 (128)	46.4±2.1 (34)	0.063	44.4±0.97 (161)	0.035
LDL-C, mg/dL	102.2±3.5 (98)	107.3±3.1 (122)	123.1±5.9 (34)	0.010	110.7±2.8 (155)	0.056
Fasting triglycerides, mg/dL¶	144.2±1.05 (103)	131.5±1.05 (128)	131.5±1.09 (34)	0.376	131.5±1.04 (162)	0.162
Apo A-I, mg/dL	131.6±24.5 (56)	136.9±24.6 (63)	135.8±22.4 (21)	0.476	136.7±23.9 (84)	0.228
Apo A-I/HDL-C ratio	3.16±0.08 (56)	3.08±0.08 (63)	3.04±0.12 (21)	0.655	3.07±0.06 (84)	0.384
Current smokers, % (n)	35.5 (39)	21 (29)	25 (9)	0.225	22 (38)	0.068

Values were adjusted for age, waist circumference, lipid lowering medication and CHD, data on Apo A-I and current smoking only for age but after exclusion of participants using lipid lowering drugs. ¶Geometric mean values (SE).
p values obtained by ANCOVA for comparisons p* among R219K genotypes, p** RR genotype versus K allele carriers (RK+KK genotypes).
In relation to TG (not to LDL-C and total cholesterol) concentrations; the *ABCA1* R219K polymorphism had gender-by-genotype interaction (p=0.049).
Apo - apolipoprotein; HDL-C - high-density lipoprotein cholesterol; LDL-C - low-density lipoprotein cholesterol; TG - triglyceride

Table 3. Adjusted* effect (in %) of pooled 219K allele carriage in men and women**

Variables	Women		Men	
	Net change	*P*	Net change	*P*
HDL-C	-1	0.76	+8	0.03
Fasting triglycerides¶	+14	<0.03	-9.5	0.13
LDL-C	-3	0.42	+8.2	0.06
Total cholesterol	+1	0.66	+4	0.20

*Values were adjusted for age, waist circumference and lipid lowering medication.
¶log-transformed.
**K allele carriers (RK+KK genotypes) compared with RR genotype.
HDL-C - high-density lipoprotein cholesterol; LDL-C - low-density lipoprotein cholesterol

phism were performed for CHD in women (Table 4). In logistic regression analysis, after adjusting for age, current smoking, alcohol usage, waist circumference, TG, systolic blood pressure and diabetes, the overdominant genetic model (RK genotype vs the RR+KK genotypes) of R219K polymorphism showed significantly higher odd ratios for CHD in women [OR 1.95 (95%CI 1.00-3.80, p=0.059)] (Table 4, model 1). Then we divided the group as having blood triglyceride levels over and under 140 mg/dL (140 mg/dL was chosen as the critical level in Turkish population (26) and repeated the same logistic regression analysis including these two groups as covariants. Interaction of TG elevation (>140 mg/dL) with CHD was demonstrated in female 219RK genotype carriers [OR 2.42 (95%CI 1.06-5.5, p=0.035, Table 4, model 2)].

The analysis after adjustment for the same covariates by a recessive genetic model for R219K polymorphism showed no association with CHD in women (OR=0.57, 95%CI; 0.21-1.55, p= 0.27).

Discussion

The *ABCA1* gene R219K polymorphism, shown to be related to serum lipids, displayed a sex interaction in a nested case-control sample of Turkish adults with respect to both lipid levels and the CHD risk. Male 219K allele carriage, compared with RR homozygotes, was associated with significantly increased HDL-C, a tendency to reduced TG and no association regarding multivariably-adjusted CHD risk. In contrast, 219K allele carriage in women was associated by a mean 10% elevation in fasting TG concentrations per each 219K allele, without being related to HDL-C levels. Female R219K heterozygosity constituted a significant independent factor doubling the CHD risk which could be demonstrated to an interaction of the R219 allele with triglyceride elevation induced by the 219K allele. These findings were consistent with the hitherto reported enhanced proinflammatory state, prominent role of serum TG (18) and high metabolic and coronary disease risk (17, 19).

Among other *ABCA1* polymorphisms, R219K variant's association with HDL-C was examined by Hodoglugil at el. (10) on a large sample of Turkish adults, and an association was lacking in men (as well as in women in whom this was confirmed herein). However, investigators did not examine the variant's

Table 4. Multiple logistic regression analysis for the adjusted association of *ABCA1* R219K variant with CHD in men and women

	Male, n*= 108/260		Female, n*= 100/318	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Model 1				
Age, 11 years	1.94 (1.44-2.61)	<0.001	2.40 (1.75-3.34)	<0.001
Diabetes mellitus, yes/no	2.50 (1.22-5.14)	0.012	3.57 (1.75-7.26)	<0.001
Waist circumference, 12 cm	1.25 (1.07-1.51)	0.164	1.36 (1.01-1.80)	0.039
Fasting TG¶, 65%	1.20 (0.97-1.49)	0.101	1.23 (0.98-1.53)	0.076
Systolic BP, 24 mmHg	1.61 (1.15-2.28)	0.007	1.27 (0.93-1.77)	0.128
Current vs never smokers	1.79 (0.79-4.03)	0.162	0.45 (0.14-1.43)	0.174
Alcohol usage, yes/no	1.71 (0.63-4.69)	0.296	9.75 (0.63-151)	0.103
219RK**	0.87 (0.46-1.61)	0.647	1.95 (1.00-3.80)	0.05
219KK**	1.10 (0.45-2.71)	0.834	0.57 (0.21-1.55)	0.271
Model 2				
219RK***+Triglycer<140 mg/dL	0.78 (0.35-1.75)	0.558	1.57 (0.67-3.72)	0.302
219RR***+TG>140 mg/dL	1.25 (0.53-2.92)	0.607	0.74 (0.3-1.83)	0.513
219RK***+TG>140 mg/dL	1.06 (0.45-2.75)	0.892	2.42 (1.06-5.5)	0.035

*Lower sample (n=578) number in this analysis was due to the exclusion of participants with missing values. ¶log-transformed values;
**Referent model 1: RR homozygotes.
***Referent in Model 2: RR+KK homozygotes + Triglyceride <140 mg/dL or *ABCA1* RK+ Triglyceride <140 mg/dL, respectively. In relation to CHD, the *ABCA1* R219K polymorphism had significant gender-by-genotype interaction (p=0.009). p values were obtained, after adjusting for age, current smoking, alcohol usage, waist circumference, TG, systolic blood pressure and diabetes. n-number of CHD /subjects at risk.
BP - blood pressure; CHD - coronary heart disease; TG - triglycerides

association with triglycerides and CHD. The 219K allele frequency of 0.38 in the Turks sample lies between frequencies reported as 0.25 in white subjects and 0.46 in Asians subjects (12, 27-29).

Interaction between ABCA1 R219K, elevated TG and sex

The finding of 219K allele carriage being independently associated with higher TG concentration in women can be accounted for by serum TG being strong independent covariate of total phospholipids (30) and the knowledge that dietary long-chain polyunsaturated fatty acids arachidonic acid and docosahexaenoic acid contribute to plasma phospholipids more in women than men, and mainly in populations with low dietary n-3 fatty acid intake (30, 31) to which Turks belong. 219RK genotype, gender and elevated TG interacted in this study sample to impact CHD. The reason why males with 219RK genotype and elevated TG were not associated with excess CHD risk is likely that phospholipids on HDL-C have been observed in (as yet unpublished) prospective analyses to be protective against CHD in men, as distinct from women.

Variances were found in decreased TG concentrations associated with the 219K allele carriers in European populations and Chinese patients with CHD (11, 32) and in overwhelmingly male Japanese patients with and without CHD (33) who tended to higher TG levels in carriers of the 219K allele may well be ascribed to the stated interacting factors, along with the CHD status of the sample studied.

Our results were in favor of association between higher HDL-C and the 219K allele carriers in the non-obese men. In the normal-weight group, the 219K allele was observed to be significantly associated with higher HDL-C concentrations in general population or men, similar to the other published work (9, 34). The absence of obesity is required for the appearance of the association between KK genotype and HDL-C in our study as well as in others.

On the other hand, Villard et al. (35) reported that R219K polymorphism significantly modulates the capacity of whole-plasma to mediate cholesterol efflux from human macrophages in a sex-dependent manner. Furthermore, Villard et al. (35) demonstrated impact of the R219K on plasma total cholesterol, LDL-C and Apo A-I concentrations but not HDL-C and TG concentrations in men or women subjects. Such contradistinction between studies may reflect differences in population size, study design, gender, genetic heterogeneity or indeed the dietary environment of the population study.

Role of involvement of cellular phospholipids' efflux

As in our study, Brousseau et al. (14) reported also that the 219K allele might promote the development of CHD. A recent meta-analysis demonstrated ethnic differences in the protective and risk conferring alleles of the *ABCA1* R219K polymorphism for CHD (12). While in some studies, 219K allele carriers were found to be protective for CHD both in Asians and Caucasians (12), others indicated that the *ABCA1* 219K allele is a protective factor of CHD in Asians, but not in Caucasians (15, 36).

According to a proposed a novel hypothesis on systemic inflammation, oxidized phospholipids and fatty acids metabolized from arachidonic acid affect endothelial and small intestinal cells to induce cytokines that influence macrophages or hepatocytes (37). The ensuing acute phase reaction is opposed by normally functioning HDL, which, however, may lose its anti-inflammatory properties during a chronic acute phase reaction. Gut flora was found to metabolize phospholipids, a process recently implicated to promote cardiovascular disease (38). Thus, inflammation and oxidized phospholipids are closely linked to *ABCA1* R219K polymorphism, gender and CHD risk.

Study limitations

The cross-sectional design of the study may be no more than a minor limitation. Focusing on the effect of *ABCA1* R219K polymorphism, without assessing the combined effect of other interacting genetic factors on analyses might limit the interpretation of our study. Though based on a representative cohort, our study sample differs from the general population in selectively having a higher proportion of CHD patients. Residual confounding, though unlikely to be of relevance to the main elicited findings, cannot be ruled out. The relatively large sample of both genders harboring CHD in adequate numbers, a study sample having a high prevalence of MetS, and, foremost, the evaluation of the CHD risk after adjustment for conventional risk factors forms the strengths of the present study.

Clinical implications

The herein described interaction of the common allele of this *ABCA1* variant with elevated TG inducing a doubling of the CHD risk entails huge clinical implications. Worldwide millions of obese women with elevated triglycerides, typically illustrated by hypertriglyceridemic waist phenotype (39), are at high cardiovascular risk, which might be reduced if recognized. Clinicians need to identify such a constellation and to urge women to avoid weight gain and hypertriglyceridemia. Genotyping for the *ABCA1* R219K polymorphism might yield practical benefit in prevention.

Conclusion

In conclusion, R219K polymorphism as a common *ABCA1* variant influences lipid concentrations and the CHD risk gender-specifically among Turkish adults. Women disclosed a mean nearly 10% elevation in fasting TG concentrations for carriage of each 219K allele, without concomitantly being related to HDL-C levels, thus contributing to the proinflammatory state commonly found in them. Heterozygosity of this variant in women was associated independently with a 2-fold CHD risk which was attributable to an interaction of the R219 allele with elevated serum TG induced by the 219K allele. The gene-gender-triglyceride interaction is likely linked primarily to the phospholipid efflux function of *ABCA1*. Further studies are needed in this direction.

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