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

\textbf{Neslihan Çoban, Altan Onat, Evrim Kömürçü-Bayrak, Çağrı Güleç, Günay Can, Nihan Erginel-Ünaltuna}

Department of Genetics, Institute for Experimental Medical Research, Istanbul University; Istanbul-Turkey
Departments of \textsuperscript{1}Cardiology and \textsuperscript{2}Public Health, Cerrahpaşa Medical Faculty, Istanbul University; Istanbul-Turkey

\textbf{ABSTRACT}

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

\textbf{Methods:} Selected 627 individuals of the Turkish Adult Risk Factor Study were genotyped for \textit{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.

\textbf{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.

\textbf{Conclusion:} R219 allele of the \textit{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. (\textit{Anadolu Kardiyoj Derg} 2014; 14: 18-25)

\textbf{Key words:} \textit{ABCA1} gene variants, coronary heart disease risk, gender difference, phospholipids, serum triglycerides, Turkish population, regression analysis

\textbf{Introduction}

Serum high-density lipoprotein (HDL) cholesterol is inversely correlated with risk of coronary heart disease (CHD) (1). The anti-atherogenic 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 (\textit{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 \textit{ABCA1} plays a major role in this process (5). Therefore, it is suggested that \textit{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 \textit{ABCA1} gene (7, 8). These polymorphisms in the \textit{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...
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 obesity was defined as a BMI (body mass index) ≥30 kg/m² (18, 19). Obesity was defined as a BMI (body mass index) ≥30 kg/m². 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/m²). 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 cool 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® 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 XagI (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 x²-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
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/m²) 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).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Males (n)</th>
<th>Females (n)</th>
<th><strong>P</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>58.9±12.8 (282)</td>
<td>56.7±12.3 (345)</td>
<td>0.03</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.7±4.4 (213)</td>
<td>30.6±5.9 (361)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>96.3±11.9 (267)</td>
<td>94±13.7 (330)</td>
<td>0.037</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>184±43.8 (272)</td>
<td>194.5±42.5 (335)</td>
<td>0.003</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>43.3±12.7 (272)</td>
<td>49.7±12 (337)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>107.2±34.5 (259)</td>
<td>111.6±34.2 (307)</td>
<td>0.132</td>
</tr>
<tr>
<td>Fasting triglycerides, mg/dL</td>
<td>136.1±1.75 (270)</td>
<td>135.8±1.71 (332)</td>
<td>0.966</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>109.6±44.4 (259)</td>
<td>105.2±40.8 (330)</td>
<td>0.218</td>
</tr>
<tr>
<td>Apolipoprotein A-I, mg/dL</td>
<td>133.8±24.1 (156)</td>
<td>145.4±27.4 (200)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Apolipoprotein B, mg/dL</td>
<td>97.1±27.5 (157)</td>
<td>101.7±26 (204)</td>
<td>0.105</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>121.0±21.9 (267)</td>
<td>128.2±23.6 (332)</td>
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</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>76.6±11.7 (267)</td>
<td>78.8±11.9 (332)</td>
<td>0.026</td>
</tr>
<tr>
<td>Diabetes mellitus, % (n)</td>
<td>19.9 (56)</td>
<td>17.7 (61)</td>
<td>0.486</td>
</tr>
<tr>
<td>Obesity, % (n)</td>
<td>27.2 (58)</td>
<td>52.1 (136)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Coronary heart disease, % (n)</td>
<td>40.4 (114)</td>
<td>30.7 (106)</td>
<td>0.011</td>
</tr>
<tr>
<td>Cigarette smoking, % (n)</td>
<td>28.9 (77)</td>
<td>14.2 (47)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alcohol usage, % (n)</td>
<td>10.2 (27)</td>
<td>1.2 (4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lipid-lowering medication, % (n)</td>
<td>9.9 (28)</td>
<td>10.7 (37)</td>
<td>0.745</td>
</tr>
<tr>
<td>Antihypertensive medication, % (n)</td>
<td>30.1 (85)</td>
<td>38.3 (132)</td>
<td>0.034</td>
</tr>
<tr>
<td>Medication for diabetes, % (n)</td>
<td>13.1 (37)</td>
<td>12.8 (44)</td>
<td>0.892</td>
</tr>
</tbody>
</table>

Continuous variables are presented as means±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

Table 1. Characteristics of the participants

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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)].

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 et al. (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
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).

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

<table>
<thead>
<tr>
<th>Model 1</th>
<th>Male, n= 108/260</th>
<th>Female, n= 100/318</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Age, 11 years</td>
<td>1.94 (1.44-2.61)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes mellitus, yes/no</td>
<td>2.50 (1.22-5.14)</td>
<td>0.012</td>
</tr>
<tr>
<td>Waist circumference, 12 cm</td>
<td>1.25 (1.07-1.51)</td>
<td>0.164</td>
</tr>
<tr>
<td>Fasting TG, 65%</td>
<td>1.20 (0.97-1.49)</td>
<td>0.101</td>
</tr>
<tr>
<td>Systolic BP 24 mmHg</td>
<td>1.61 (1.15-2.28)</td>
<td>0.007</td>
</tr>
<tr>
<td>Current vs never smokers</td>
<td>1.79 (0.79-4.03)</td>
<td>0.162</td>
</tr>
<tr>
<td>Alcohol usage, yes/no</td>
<td>1.71 (0.63-4.69)</td>
<td>0.296</td>
</tr>
<tr>
<td>219RK**</td>
<td>0.87 (0.46-1.61)</td>
<td>0.647</td>
</tr>
<tr>
<td>219KK**</td>
<td>1.10 (0.45-2.71)</td>
<td>0.834</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Model 2</th>
<th></th>
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<td></td>
<td>Male, n= 108/260</td>
<td>Female, n= 100/318</td>
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<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>219RK***+Triglycer&lt;140 mg/dL</td>
<td>0.78 (0.35-1.75)</td>
<td>0.558</td>
<td>1.57 (0.67-3.72)</td>
</tr>
<tr>
<td>219RR***+TG&gt;140 mg/dL</td>
<td>1.25 (0.53-2.92)</td>
<td>0.607</td>
<td>0.74 (0.3-1.83)</td>
</tr>
<tr>
<td>219RK***+TG&gt;140 mg/dL</td>
<td>1.06 (0.45-2.75)</td>
<td>0.892</td>
<td>2.42 (1.06-5.5)</td>
</tr>
</tbody>
</table>

*Lower sample (n=578) number in this analysis was due to the exclusion of participants with missing values. **log-transformed values; ***Referent model 2: RR homogyzotes.

bp - blood pressure; CHD - coronary heart disease; TG - triglycerides
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 gender-triglyceride interaction is likely linked primarily to the phospholipid efflux function of ABCA1. Further studies are needed in this direction.

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.

Authorship contributions: Concept - N.E.Ü., N.Ç.; Design - N.Ç.; Supervision - N.E.Ü.; Resource - A.O., N.E.Ü.; Material - A.O.; Data collection&/or processing - N.Ç., A.O.; Analysis &/or interpretation - N.Ç.; Literature search - N.Ç., A.O.; Writing - N.Ç.; Critical review - N.E.Ü., A.O.

Acknowledgments
This study was supported by The Research Support Unit of Istanbul University as the project no T-608/17032005. We thank the Turkish Society of Cardiology and the pharmaceutical companies AstraZeneca, Pfizer and Sanofi-Aventis (Istanbul) that have supported financially the Turkish Adult Risk Factor survey. The partial logistic support of the Turkish Ministry of Health is acknowledged.
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