The role of ATP-binding-cassette-transporter-A1 (ABCA1) gene polymorphism on coronary artery disease risk

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ATP-binding cassette transporter A1 (ABCA1) plays a pivotal role in intracellular cholesterol removal and exerts a protective effect against atherosclerosis. The role of genetic factors in susceptibility to coronary artery disease (CAD) is not clear. The aim of this study was to evaluate for the first time the possible association between R219K gene polymorphism and coronary artery disease in an Iranian adult population. A total of 207 consecutive patients with CAD (group A) and 94 patients without CAD (group B) were studied. We determined the presence of the R219K variant in the ABCA1 gene by polymerase chain reaction (PCR) and restriction analysis in 301 patients with and without CAD. The distribution of genotypes among the 2 groups was significantly different (P = 0.009). In univariate analysis (with genotype AA as reference), the GG genotype was associated with a significantly increased risk of CAD (P = 0.002; odds ratio (OR) = 2.761; 95% confidence interval (CI) = 1.418–5.374), but the GA genotype did not show a significant association (P = 0.234) (data not shown). A multivariate logistic regression analysis (using sex as clinically significant variable, and using age, diabetes mellitus, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein (HDL), smoking, body mass index (BMI), and genotype as statistically significant variables) was used to determine independent associations and adjusted ORs. The GG genotype (compared with the AA genotype) was an independent predictor of CAD (OR = 2.856, 95% CI = 1.307–6.241; P = 0.009), followed by BMI (P = 0.034; OR = 1.100; 95% CI = 1.007–1.200). The GG genotype in the ABCA1 gene is independently associated with CAD in Iranian patients. (Translational Research 2010;155:185–190)

Abbreviations: ABC = ATP-binding cassette; ABCA1 = ABC transporter A1; BMI = body mass index; CAD = coronary artery disease; HDL = high-density lipoprotein; HDL-C = HDL-cholesterol; LDL = low-density lipoprotein; LDL-C = LDL-cholesterol; PCR = polymerase chain reaction; SNP = single nucleotide polymorphism

The ATP-binding cassette (ABC) transporter is a member of the ABC family of proteins that are involved in the transmembrane transport of a variety of different molecules, which include bile acids, steroid hormones, ions, amino acids, sugars, vitamins, metabolic products, lipids, sterols, and drugs.1,2 This process plays an important role in maintaining cellular cholesterol homeostasis and exerts a protective

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Effect against atherosclerosis. Several studies have consistently reported an association between the R219 K polymorphism and coronary artery disease (CAD). Yokoyama suggested that ABC1 expression is induced by cholesterol loading and cAMP treatment and is reduced upon subsequent cholesterol removal by apolipoproteins. These proteins bind ATP and use the energy to drive the transport of various molecules across all cell membranes. ABCA1 mutation carriers have markedly higher incidence of coronary artery disease (CAD) compared with noncarriers. Polymorphisms in ABCA1 are associated with either increased or decreased CAD.

Translational Significance

The aim of this study was to evaluate the possible association between R219 K gene polymorphism and CAD in an Iranian adult population. Our study supported a major role for the ABCA1 gene as a risk factor for coronary artery disease.

Materials and Methods

Study population. A total of 207 consecutive patients with CAD (group A) and 94 patients without CAD (group B) who were referred to our cardiac clinic from March 2006 through April 2008 were enrolled into the study. They were selected after a physical examination and appropriate laboratory tests, which included chest X-ray and resting electrocardiogram. The patients involved in the study had been admitted to the cardiothoracic surgery unit of the Tehran Heart Center for coronary bypass graft surgery, myocardial infarction, and coronary angioplasty. All patients gave their written informed consent for plasma sampling, storage, and genetic analysis; the local ethics committee at Tehran University of Medical Sciences approved the study protocol.

The following variables were determined for each patient: age, sex, smoking habits, body mass index (BMI; according to Quetelet equation by using the following formula: BMI = weight in kilograms/height in meters squared), total cholesterol, low-density lipoprotein (LDL), HDL, triglycerides, and single-nucleotide polymorphism (SNP) in ABCA1 gene. The diagnosis of diabetes was based on criteria from the American Diabetes Association.

Lipid analysis. Blood samples were collected in ethylenediaminetetraacetic acid-containing tubes from all participants after an overnight fast. The plasma total cholesterol was measured with an enzymatic colorimetric procedure. HDL-C was determined after precipitation of apoB-containing lipoproteins with phosphotungstic acid and magnesium. LDL-cholesterol (LDL-C) was calculated using the Friedewald formula. Triglycerides were measured using an enzymatic colorimetric method using lipase, glycerol kinase, and glycerol-3-phosphate oxidase.

R219K polymorphism analysis. Genomic DNA was isolated from peripheral blood leukocytes according to a standard salting-out method, with minor modifications. A 166-bp fragment of exon 7 of the ABCA1 gene was amplified by polymerase chain reaction (PCR), using the following primers: The sense primer was 5′-GCAAGGCTACCAGTTACATTTGACAAG-3′ and the antisense primer was 5′-GATTGGCTTGATGGTGG-3′. Then, the DNA was

**AT A GLANCE COMMENTARY**

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Background


ABCA1 mutation carriers have markedly higher incidence of coronary artery disease (CAD) compared with noncarriers. Polymorphisms in ABCA1 are associated with either increased or decreased CAD.

Translational Significance

The aim of this study was to evaluate the possible association between R219 K polymorphism and CAD in an Iranian adult population. Our study supported a major role for the ABCA1 gene as a risk factor for coronary artery disease.
amplified for 35 cycles with denaturation at 95 °C for 1 min, annealing at 62 °C for 1 min, and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 10 min. After amplification, an aliquot of 4 μL of PCR product was digested with 5 U of the restriction enzyme XbaI (Fermentas, Inc., Glen Burnie, Md) at 37 °C for more than 3 h. The fragments obtained after digestion were analyzed by electrophoresis on 10% polyacrylamide gel electrophoresis. The bands were visualized by staining with ethidium bromide.22 The R219 K polymorphism is the result of a nucleotide change G to A at position 1051 of the complementary DNA sequence, and it results in the substitution of lysine for arginine at amino acid 219 of the ABCA1 protein. After digestion of the 166-bp fragment obtained by PCR, the following 3 possible genotypes were distinguished: homozygous GG (RR; 166 bp), heterozygous GA (RK; 166, 101, and 65 bp), and homozygous AA (KK; 101 and 65 bp).

Statistical analysis. Data were analyzed using SPSS statistical software (SPSS Inc., Chicago, Ill). All tests were 2 sided and \( P < 0.05 \) was considered statistically significant. The significance of the differences between group means was tested by the Student \( t \)-test and differences in proportions were assessed by the chi-square test.

Checks for deviation of single-locus genotypes from Hardy-Weinberg equilibrium were based on the Fisher exact test. A multivariate binary logistic regression was then performed to determine the independent association between the genotype and CAD status. Sex and smoking as clinically significant variables, as well as the statistically significant variables extracted from the univariate analysis, were included in a regression. The genotype was coded by considering the AA genotype as the reference category, and the relevant ORs were defined accordingly.

RESULTS

Table I shows the demographic, clinical, and laboratory data of patients. The groups were similar in some variables (gender, cholesterol, and statins) except for diabetes (\( P = 0.001 \)), BMI (\( P < 0.001 \)), HDL-C (\( P = 0.024 \)), LDL-C (\( P = 0.038 \)), smoking (\( P < 0.001 \)), and age (\( P < 0.001 \); the results could be a result of the selection criteria). The distribution of genotypes between the 2 groups was significantly different (\( P = 0.009 \)) (Table I).

The frequency of AA, GA, and GG genotypes among the studied group was 28.5%, 37.2%, and 34.3% in patients with CAD; and 41.5%, 40.4%, and 18.1% in patients without CAD, respectively (Table II).

An analysis of our results showed that the frequency of GG genotype was significantly higher in CAD group in comparison with the non-CAD group (34.3% vs 18.1%, respectively).

In a univariate analysis (with genotype AA as reference), the GG genotype was associated with a significantly increased risk of CAD (\( P = 0.002; \) OR = 2.761; 95% CI = 1.418–5.374), but the GA genotype did not show a significant association (\( P = 0.234; \) data not shown).

The R219 K polymorphism frequency distribution in the patients with and without CAD was consistent with the Hardy-Weinberg equilibrium. The frequency of G allele in CAD patients was 52.9% as compared with the patients without CAD (38.3%). An analysis of our results showed that the frequency of G allele in comparison with the A allele was significantly different in 2 groups (OR = 1.809; 95% CI = 1.273–2.572; \( P = 0.001 \); data not shown).

In a binary logistic regression using the study group (ie, CAD vs non-CAD) as the dependent variable and using age, BMI, HDL, LDL, smoking, diabetes mellitus, and R219 K genotype as covariates, the GG genotype (compared with the AA genotype) significantly and independently increased the odds of having CAD (OR = 2.856, 95% CI = 1.307–6.241; \( P = 0.009 \)). The only other significant variables were age (\( P < 0.001; \) OR = 0.863; 95% CI = 0.816–0.914), and BMI (\( P = 0.034; \) OR = 1.100; 95% CI = 1.007–1.200) with a significant independent effect (Table III).

DISCUSSION

The relationship between the ABCA1 gene polymorphism and CAD has been the subject of many recent studies. The current study is the first to examine ABCA1 gene polymorphism in Iranian patients with CAD. Although findings on the association between R219 K polymorphism and many disorders are documented,23 inconsistent results have been also observed for other ABCA1 gene polymorphisms. In the current study, we had 2 groups of patients with and without CAD.

The results of our study showed that the GG genotype is strongly associated with increased risk of CAD. The GG genotype (vs the AA genotype) independently increased the risk of CAD in patients 2.856-fold, whereas the GA genotype did not alter the risk significantly. This pattern suggests a recessive mode of inheritance in allele G of the R219 K variant, as also has been suggested in other studies.24,25

HDL-C is a major independent factor involved in the development of premature CAD. (26,27). The antiatherogenic function of HDL has been attributed to its role in reverse cholesterol transport, and the protein ABCA1 plays a crucial role in its initial step.28 Several studies have showed that the G allele in the ABCA1 gene causes low HDL-C levels, increased triglycerides, depressed levels of cholesterol efflux, and an increased
risk of CAD. Specifically, the R219 K variant was indicated to play a crucial role in lipid metabolism and atherosclerosis.

Harada et al provided evidence that the I/M 823 variant, not the R/K 219 variant, in the ABCA1 gene is a determinant of the HDL level. In addition, these authors showed the importance of this gene on lipid metabolism in Japanese patients with CAD. Tregouet et al found that the ABCA1 R219 K variant is associated with myocardial infarction risk. Liu et al suggested that the -191 G/C SNP in the promoter region of the ABCA1 gene is associated with increased CAD. Hong et al found that the ABCA1 G2265 T variant may lead to decreased HDL-C. Albrecht et al showed that ABCA1 gene expression was significantly elevated in atherosclerotic plaques. Porchay et al suggested that ABCA1 gene polymorphisms modulate HDL-C concentrations in an interaction with BMI, and thus, they might influence cardiovascular risk in the general population.

Xiao et al showed that their results exhibited an interaction of PON1 A/B192 and ABCA1 R219 K on serum lipid levels in 680 patients with stroke. Brousseau et al observed that the K allele of the R219 K variant is an antiatherogenic allele with increased cholesterol efflux activity. Hodoglugil et al evaluated polymorphisms in the ABCA1 gene in Turks, which is a population characterized by low HDL levels. They observed that the rare alleles of the C14 T and V771 M polymorphisms were associated with higher HDL levels in men. We observed that HDL-C in the CAD group was higher than the non-CAD group. Thus, the R219 K variant could affect HDL-C levels and could influence the risk of atherosclerosis. This result may help explain the relationship between ABCA1 gene polymorphism and HDL-C.

### Table I. Demographic, clinical, and laboratory data of the patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>A group (n = 207)</th>
<th>B group (n = 94)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M:F)</td>
<td>172:35</td>
<td>56:38</td>
<td>0.230</td>
</tr>
<tr>
<td>Age (years)</td>
<td>49.1 ± 4.68</td>
<td>55.1 ± 8.65</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.66 ± 3.75</td>
<td>25.76 ± 3.53</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>DM (%)</td>
<td>74 (35.7)</td>
<td>16 (17)</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>106 (51.2)</td>
<td>27 (28.7)</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>162.8 ± 44.35</td>
<td>174.42 ± 56.26</td>
<td>0.056</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>180.65 ± 95.27</td>
<td>169.2 ± 150.73</td>
<td>0.795</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>89.7 ± 38.25</td>
<td>99.70 ± 39.48</td>
<td>0.038*</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>41.36 ± 10.53</td>
<td>44.75 ± 14.79</td>
<td>0.024*</td>
</tr>
<tr>
<td>Statins (%)</td>
<td>71 (37.5)</td>
<td>146 (70.5)</td>
<td>0.407</td>
</tr>
<tr>
<td>R219 K genotypes (AA:GA:GG)</td>
<td>59 (28.5):77 (37.2):71 (34.3)</td>
<td>39 (41.5): 38 (40.4): 17 (18.1)</td>
<td>0.009†</td>
</tr>
</tbody>
</table>

Abbreviations: DM, diabetes mellitus; TG, triglycerides.
Notes: Data are shown as mean ± SD or frequency (%) when appropriate.
*P < 0.05.
†P < 0.01.

### Table II. Allele and genotype frequencies of R219 K gene polymorphism in patients with and without CAD

<table>
<thead>
<tr>
<th>Genotype</th>
<th>A group (n = 207) n (%)</th>
<th>B group (n = 94) n (%)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>59 (28.5)</td>
<td>39 (41.5)</td>
<td>NS</td>
</tr>
<tr>
<td>GG</td>
<td>71 (34.3)</td>
<td>17 (18.1)</td>
<td>0.032*</td>
</tr>
<tr>
<td>GA</td>
<td>77 (37.2)</td>
<td>38 (40.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G allele</td>
<td>219 (52.9)</td>
<td>72 (38.3)</td>
<td>NS</td>
</tr>
<tr>
<td>A allele</td>
<td>195 (47.1)</td>
<td>116 (61.7)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Abbreviation: NS, nonsignificant.
Notes: The distribution and comparison of alleles and genotypes, as well as the frequency of the R219 K gene polymorphism in each, was made using a chi-square test, the Fisher exact test, and the maximum likelihood ratio.
*P < 0.05.

### Table III. Results of multivariate binary logistic regression

<table>
<thead>
<tr>
<th>P value</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>&lt;0.001†</td>
<td>0.863</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>0.240</td>
<td>0.592</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.034*</td>
<td>1.100</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>0.068</td>
<td>0.563</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>0.065</td>
<td>0.977</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>0.235</td>
<td>0.995</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.057</td>
<td>0.502</td>
</tr>
<tr>
<td>ABCA1(R219 K) genotypes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG vs AA</td>
<td>0.009†</td>
<td>2.856</td>
</tr>
<tr>
<td>GA vs AA</td>
<td>0.964</td>
<td>1.015</td>
</tr>
</tbody>
</table>

Abbreviation: NS, nonsignificant.
Notes: The distribution and comparison of alleles and genotypes, as well as the frequency of the R219 K gene polymorphism in each, was made using a chi-square test, the Fisher exact test, and the maximum likelihood ratio.
*P < 0.05.
†P < 0.01.
However, in several previous studies, no association has been found. The reason for these conflicting results is unclear. The racial difference of the subject populations is 1 possible reason. The authors would like to stress 2 important issues in this discrepancy. First, caution should be exercised in extrapolating an association found in 1 population to others. The presence or absence of an observed association in any ethnic, racial, or geographic population may be related to many other factors, which include gene–gene and gene–environmental interactions. The potential effect of ABCA1 gene polymorphism on various outcomes is no exception. Second, the importance of matching the groups (either clinically or during analysis) in as many potential confounders as possible should not be underestimated. Such a close match between the groups increases the odds for the outcome of interest to be genuinely linked to the potential variable being investigated.

In conclusion, we showed, for the first time in an Iranian population, that the GG genotype of the R219 K gene is independently associated with CAD. The clinical effect of this study could help to identify subjects susceptible to the development of CAD. The patients with the GG genotype seem to be more prone to CAD based on these results. Hence, the potential value of identifying a patient with the GG genotype could lead to early therapeutic intervention and the reduction of subsequent progression of CAD.

However, the cross-sectional nature of the current study prevents us from drawing cause-and-effect conclusions. Longitudinal studies are needed to determine whether our results came from mere associations or point-to-causal relationships.

REFERENCES

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