Associations between common polymorphisms of adenosine triphosphate-binding cassette transporter A1 and coronary artery disease in a Tunisian population


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Summary

Background. — The adenosine triphosphate-binding cassette transporter A1 (ABCA1) protein plays an important role in the first step of the reverse cholesterol transport system.

Aims. — We studied the association of four polymorphisms in the ABCA1 gene (G1051A, G2706A, G2868A and −565C/T) with lipid profile and coronary artery disease.

Methods. — Overall, 316 Tunisian patients underwent coronary angiography. Genotyping was performed using polymerase chain reaction-restriction fragment length polymorphism analysis. Lipid and apolipoprotein concentrations were measured.

KEYWORDS
ABCA1 polymorphisms; Coronary artery disease; Haplotype; Tunisian

Abbreviations: ABCA1, adenosine triphosphate-binding cassette transporter A1; ApoAl, apolipoprotein AI; ApoB, apolipoprotein B; CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ORs, odds ratios; SCS, significant coronary stenosis.

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ABCA1 polymorphisms and coronary artery disease

Background

Low concentrations of plasma high-density lipoprotein cholesterol (HDL-C) are associated with an increased risk of atherosclerotic complications [1]. An important mechanism underlying the antiatherogenic properties of high-density lipoprotein is its role in reverse cholesterol transport, the pathway that facilitates the transfer of cholesterol from peripheral tissues back to the liver [2]. The adenosine triphosphate-binding cassette transporter A1 (ABCA1) has been identified as the mediator of the initial step of reverse cholesterol transport because it facilitates the efflux of phospholipids and cholesterol from peripheral cells to lipid-free apolipoprotein A1 (ApoAI), creating nascent high-density lipoprotein particles [3]. The human gene for ABCA1 is composed of 50 exons spanning 149 kb of genomic sequence. It has been mapped to the region q31 of chromosome 9 [4] and encodes a 2261 amino acid protein with a predicted molecular weight of 220 kDa. Human ABCA1 is primarily expressed in placenta, liver, lung, adrenal glands and foetal tissues [5].

Although HDL-C concentration is strongly influenced by environmental factors [6], research has been focused increasingly on genetic causes leading to reduced HDL-C. Deficiency of ABCA1 has been identified as the molecular cause of Tangier disease, a rare condition with very low concentrations of HDL-C, excessive accumulation of cholesteryl esters in tissue macrophages and the reticuloendothelial system, with an increased risk of premature coronary disease [7]. Many common genetic variations of ABCA1 have been reported to be associated with variations in serum lipid concentrations (particularly HDL-C) and may also be associated with coronary artery disease risk, but this association is still controversial among several populations [8–11].

To our knowledge, there are no published data on the screening of the ABCA1 gene or on its association with significant coronary stenosis (SCS) among the Tunisian population.
In this study, four variants identified in ABCA1 gene (G1051A [rs2230806], G2706A [rs2066718], G2868A [rs4149312] and —565C/T [rs2422493]) were screened in a Tunisian population.

Materials and methods

Study population

Sampling procedures for this study have been described previously in detail [12]. Briefly, the study comprised 316 patients who underwent coronary angiography because of myocardial infarction (n=113), angina (n=169), thoracic pain (n=18) or heart failure (n=16) in the Cardiology Department at Sahliou University Hospital, Sousse, Tunisia. Patients were subdivided into two groups: those with SCS and those without SCS. SCS patients were those who had significant coronary artery stenosis, which was defined as a luminal narrowing of ≥ 50% in at least one major coronary artery. Patients without SCS were those without any significant coronary artery stenosis (< 50%).

Data on lifestyle factors were collected using an interviewer-administered questionnaire, which included questions about personal history, presence of disease, drug intake (if any), cigarette smoking and alcohol consumption. Patients taking lipid-lowering drugs were excluded. Diabetes mellitus was defined as fasting glucose > 7 mmol/L or currently receiving antidiabetic medication. The smoking status of an individual was assigned ‘yes’ if they were smoking currently or had given up < 3 months previously. Hypertension was defined as blood pressure > 140/90 mmHg or currently on antihypertensive medication. Dyslipidaemia was defined as low-density lipoprotein cholesterol (LDL-C) concentration ≥ 4.1 mmol/L and/or HDL-C concentration ≤ 1 mmol/L and/or triglyceride concentration ≥ 1.71 mmol/L. Informed consent was taken from all the participants. The study was approved by the local medical ethics committee.

Measurement of serum lipids and apolipoproteins

After overnight fasting and before coronary angiography, blood was collected from each subject. Serum total cholesterol, triglyceride and HDL-C concentrations were determined by a standard method using the Synchrom CX7 Clinical System (Beckman, Fullerton, CA, USA). LDL-C concentration was calculated using the Friedewald formula [13] when the triglyceride concentration was < 4 mmol/L; otherwise, LDL-C concentration was measured directly using the Synchrom CX7 Clinical System (Beckman, Fullerton, CA, USA). Serum ApoA1 and apolipoprotein B (ApoB) concentrations were determined using the IMMAGE Immunochemistry System (Beckman, Fullerton, CA, USA), based on immunonephelometric quantitation. ApoB/ApoA1 and total cholesterol/HDL-C ratios were calculated. We considered elevation of these atherogenicity ratios as > 0.86 and > 4.5, respectively, and hypoApoA1 as < 0.9 g/L.

DNA extraction and ABCA1 genotyping

Genomic DNA was extracted from ethylenediamine-tetraacetic acid-treated whole blood samples using a salting-out method [14]. The genotypes for each ABCA1 polymorphism (G1051A [R219K], G2706A [V771M], G2868A [V825I] and —565C/T [—477C/T]) were determined by polymerase chain reaction-restriction fragment length polymorphism analysis. The G1051A and —565C/T polymorphisms were genotyped following Assmann and Nofer [2]; the G2706A and G2868A polymorphisms were genotyped as described by Miller and Miller [1]. The polymerase chain reaction was carried out using a deoxyribonucleic acid thermal cycler (LP×2 Thermal Cycler, Thermo Electron Corporation, Milford, NE, USA). After initial denaturation at 95 °C for 5 min, the polymerase chain reaction was carried out for 35 cycles, each cycle comprising a denaturation at 95 °C for 45 s, an annealing temperature as described previously [1,2] for 45 s and 72 °C for 45 s, with a final extension time of 7 min at 72 °C. Shortly, each polymerase chain reaction product of G1051A, G2706A, G2868A and —565C/T polymorphisms was digested using StyI (2U), BsaAI (2U), BsaI (3U) and AciI (2U), respectively. Polymerase chain reaction products and the digested products were resolved by 2% agarose gel electrophoresis and visualized by ethidium bromide staining.

Statistical analysis

Statistical analysis was performed by SPSS 16.0 for Windows. The biological variables were compared by one-way analysis of variance then by Student’s t-test or Fisher’s exact test and their values were reported as means ± standard deviations. A chi-square analysis was performed to determine the Hardy–Weinberg equilibrium of the polymorphism studied in both groups with one degree of freedom. Genotype and allele frequencies were compared by a chi-square test. Pairwise linkage disequilibrium coefficients were expressed as D’, which is the ratio of unstandardized coefficient to its minimal/maximal value, and estimated using a single nucleotide polymorphism analyser programme [15]. Odds ratios (ORs) were calculated as a measure of the association of the each ABCA1 genotype with the phenotype. For each OR, two-tailed p values and 95% confidence intervals (CIs) were calculated; p was considered to be significant when it was < 0.05. Adjusted ORs for potential confounders were determined using logistic regression analysis and corresponding p values were reported.

Results

Population characteristics

Clinical and biochemical characteristics of the study population are given in Table 1. Patients with SCS had significantly lower HDL-C (p = 0.040) and ApoA1 (p = 0.009) concentrations, significantly higher triglyceride concentrations (p = 0.022) and a higher ApoB/ApoA1 ratio (p = 0.036) than patients without SCS. All variables with a p value < 0.25 between the two studied groups were considered as confounding factors for further OR adjustment.
**Table 1**  Clinical and biochemical characteristics of the study population.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>With SCS (n = 212)</th>
<th>Without SCS (n = 104)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex ratio (men/women)</td>
<td>1.97</td>
<td>1.26</td>
<td>0.010</td>
</tr>
<tr>
<td>Age (years)</td>
<td>60.6 ± 10.6</td>
<td>59.4 ± 11.9</td>
<td>0.38</td>
</tr>
<tr>
<td>Smoker</td>
<td>120 (56.6)</td>
<td>47 (45.2)</td>
<td>0.013</td>
</tr>
<tr>
<td>Diabetic</td>
<td>73 (34.4)</td>
<td>23 (22.1)</td>
<td>0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>97 (45.7)</td>
<td>46 (44.2)</td>
<td>0.35</td>
</tr>
<tr>
<td>History of myocardial infarction</td>
<td>91 (42.9)</td>
<td>13 (12.5)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Dyslipidaemia</td>
<td>33 (15.6)</td>
<td>9 (8.6)</td>
<td>0.027</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.029 ± 1.192</td>
<td>5.025 ± 1.087</td>
<td>0.970</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>1.614 ± 1.117</td>
<td>1.340 ± 0.647</td>
<td>0.022</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>0.961 ± 0.283</td>
<td>1.030 ± 0.276</td>
<td>0.040</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>3.440 ± 1.167</td>
<td>3.330 ± 1.039</td>
<td>0.37</td>
</tr>
<tr>
<td>ApoAI (g/L)</td>
<td>1.160 ± 0.379</td>
<td>1.290 ± 0.437</td>
<td>0.009</td>
</tr>
<tr>
<td>ApoB (g/L)</td>
<td>1.153 ± 0.376</td>
<td>1.110 ± 0.413</td>
<td>0.46</td>
</tr>
<tr>
<td>ApoAI/ApoB</td>
<td>1.057 ± 0.493</td>
<td>0.920 ± 0.290</td>
<td>0.036</td>
</tr>
<tr>
<td>Total cholesterol/HDL-C</td>
<td>5.630 ± 2.040</td>
<td>5.410 ± 1.890</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Data are mean ± standard deviation or number (%). ApoAI: apolipoprotein AI; ApoB: apolipoprotein B; SCS: significant coronary stenosis; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.

**ABCA1 genotypes**

The prevalence of the four ABCA1 polymorphisms (G1051A, G2706A, G2868A and −565C/T) was evaluated in 316 subjects. Each of these polymorphisms was found to be in the Hardy–Weinberg equilibrium. Genotype and allele frequencies are shown in Table 2. The prevalence of homozygous individuals for the G2706 allele was significantly higher (p = 0.009) in the SCS group. No mutated genotype for this polymorphism was found in the group without SCS. No significant difference was observed in genotype frequencies between the two groups for the other three polymorphisms. No allele frequency difference was observed between patients with versus without SCS for any polymorphism.

**Associations between ABCA1 polymorphisms and serum lipid concentrations**

The lipid profile of the studied polymorphisms was investigated according to the genotype distributions. Only individuals with the 1051AA genotype and A allele carriers (genotypes GA+AA) had a significantly higher mean HDL-C concentration than individuals with the 1051GG genotype (1.08 ± 0.35, p = 0.042 and 1.11 ± 0.34, p = 0.032, respectively, vs 0.93 ± 0.37 in the study population). There were no significant differences in mean serum HDL-C concentrations in either genotype of the −565C/T, G2706A and G2868A polymorphisms. No statistically significant differences in other lipids variables were seen in any genotype.

In addition, A allele carriers (genotypes GA+AA) of the G2706A polymorphism seemed to have a low risk of hypo-HDLaemia (OR 0.39, 95% CI 0.19—0.81, p = 0.012). The risk of atherogenicity ratio elevation, hypoHDLaemia and hypoApoAI associated with the four polymorphisms was not significant.

**Associations of ABCA1 polymorphisms with the presence of stenosis**

Table 3 shows the ORs for significant stenosis for the four polymorphisms of ABCA1. After adjustment for confounding variables, only the carriers of the G2706A allele were
Table 3  Odds ratios for significant stenosis according to ABCA1 polymorphisms.

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>Crude ORs</th>
<th>Adjusted ORs&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>G1051A</td>
<td>GG</td>
<td>1</td>
</tr>
<tr>
<td>GA+AA</td>
<td>0.81</td>
<td>—0.50—1.30</td>
</tr>
<tr>
<td>G2706A</td>
<td>GG</td>
<td>1</td>
</tr>
<tr>
<td>GA+AA</td>
<td>0.59</td>
<td>—0.19—0.75</td>
</tr>
<tr>
<td>G2868A</td>
<td>GG</td>
<td>1</td>
</tr>
<tr>
<td>GA+AA</td>
<td>1.17</td>
<td>—0.66—2.07</td>
</tr>
<tr>
<td>–565C/T</td>
<td>CC</td>
<td>1</td>
</tr>
<tr>
<td>CT+TT</td>
<td>1.16</td>
<td>—0.68—1.98</td>
</tr>
</tbody>
</table>

ABCA1: adenosine triphosphate-binding cassette transporter A1; CI: confidence interval; OR: odds ratio.
<sup>a</sup> Adjusted for age, sex, diabetes, smoking status, hypertension, dyslipidaemia.
<sup>b</sup> All p values are calculated by comparison with wild type genotypes.

Table 4  Odds ratios for significant stenosis according to smoking status.

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>Smokers</th>
<th>Non-smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR&lt;sup&gt;a&lt;/sup&gt;</td>
<td>95% CI</td>
</tr>
<tr>
<td>G1051A</td>
<td>GG</td>
<td>1</td>
</tr>
<tr>
<td>GA+AA</td>
<td>0.97</td>
<td>—0.44—2.13</td>
</tr>
<tr>
<td>G2706A</td>
<td>GG</td>
<td>1</td>
</tr>
<tr>
<td>GA+AA</td>
<td>0.48</td>
<td>—0.09—0.83</td>
</tr>
<tr>
<td>G2868A</td>
<td>GG</td>
<td>1</td>
</tr>
<tr>
<td>GA+AA</td>
<td>1.72</td>
<td>—0.62—4.72</td>
</tr>
<tr>
<td>–565C/T</td>
<td>CC</td>
<td>1</td>
</tr>
<tr>
<td>CT+TT</td>
<td>1.21</td>
<td>—0.50—2.91</td>
</tr>
</tbody>
</table>

CI: confidence interval; OR: odds ratio.
<sup>a</sup> Adjusted for age, sex, diabetes, hypertension, dyslipidaemia.
<sup>b</sup> All p values are calculated by comparison with wild type genotypes.

associated with a decreased risk of significant stenosis (OR 0.66, 95% CI 0.22—0.92, p = 0.029), although the frequency of the G2706A allele was low. The interaction of polymorphisms with other risk factors was studied, and a significant interaction of the G2706A polymorphism with smoking and diabetes was noted. In fact, in the binary logistic regression model, we found Exp(B) 1.22, CI 1.02—1.73 (p = 0.044) for smoking by G2706A and Exp(B) 1.62, CI 1.05—2.485 (p = 0.026) for diabetes by G2706A, hence the study population was stratified according to smoking and diabetes status.

Table 5  Odds ratios for significant stenosis according to diabetes.

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>Diabetes</th>
<th>No diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR&lt;sup&gt;a&lt;/sup&gt;</td>
<td>95% CI</td>
</tr>
<tr>
<td>G1051A</td>
<td>GG</td>
<td>1</td>
</tr>
<tr>
<td>GA+AA</td>
<td>0.83</td>
<td>—0.34—1.98</td>
</tr>
<tr>
<td>G2706A</td>
<td>GG</td>
<td>1</td>
</tr>
<tr>
<td>GA+AA</td>
<td>0.53</td>
<td>—0.03—0.86</td>
</tr>
<tr>
<td>G2868A</td>
<td>GG</td>
<td>1</td>
</tr>
<tr>
<td>GA+AA</td>
<td>1.15</td>
<td>—0.44—3.00</td>
</tr>
<tr>
<td>–565C/T</td>
<td>CC</td>
<td>1</td>
</tr>
<tr>
<td>CT+TT</td>
<td>0.78</td>
<td>—0.28—2.13</td>
</tr>
</tbody>
</table>

CI: confidence interval; OR: odds ratio.
<sup>a</sup> Adjusted for age, sex, smoking status, hypertension, dyslipidaemia.
<sup>b</sup> All p values are calculated by comparison with wild type genotypes.
The protective effect of G2706A seemed to be more significant in smokers than non-smokers (OR 0.48, 95% CI 0.10–0.83, p = 0.021) and in those with versus without diabetes (OR 0.53, 95% CI 0.03–0.86, p = 0.007) (Tables 4 and 5).

No significant association was observed with the risk of significant stenosis for the −565C/T polymorphism or the two polymorphisms in the coding region (G1051A, G2868A), neither in study population nor according to smoking or diabetic status.

Linkage disequilibrium and haplotype analysis

The G1051A polymorphism was in significant linkage disequilibrium with G2706A (D' 0.27, p = 0.0074) and G2868A (D' = 0.28, p = 0.039). When the four ABCA1 polymorphisms were combined, the haplotype model (AAGC), possessing G1051A, G2706A, G2868A and −565C/T polymorphisms, seemed to be the most protective. In fact, it occurred more frequently in patients without SCS than in the SCS group (0.079 vs 0.030). The haplotype model (GGAT) occurred more frequently in SCS patients than in those without SCS (0.044 vs 0.016).

On haplotype analysis, after adjustment for confounding factors, the (AAGC) haplotype consistently showed the lowest risk of significant stenosis compared with the wild haplotype (possessing all common alleles: GGGC) (OR 0.5, 95% CI 0.29–0.96, p = 0.048). The (GGAT) haplotype increased the risk of significant stenosis (OR 1.26, 95% CI 1.03–1.56, p = 0.025) compared with the wild haplotype.

The risks of hypoHDLaemia and hypoApoA1 associated with the (AAGC) haplotype were, respectively, OR 0.69, 95% CI 0.48–0.95 (p = 0.025) and OR 0.65, 95% CI 0.46–0.92 (p = 0.037). By comparison, the risks of hypoHDLaemia and hypoApoA1 associated with the (GGAT) haplotype were, respectively, OR 1.26, 95% CI 0.76–2.10 (p = 0.36) and OR 0.7, 95% CI 0.48–1.02 (p = 0.06). None of the other haplotype combinations was associated with a significant change in HDL-C or ApoA1 concentrations, atherogenicity ratio elevation or the risk of significant stenosis.

Discussion

In the present study, we did not find associations between the ABCA1 variants studied and lipid profile. Only the AA genotype of the G1051A polymorphism was significantly associated with increased concentrations of HDL-C (p = 0.042). The effect of the genotype on HDL-C remains controversial, with some studies showing an association between the genotype of these polymorphisms and higher concentrations of HDL-C [16–20], and others showing no effect [9,16,21–27].

The role of variation in ABCA1 in vascular risk remains controversial [11,18,22,23,25,26,28]. Neither the −565C/T single nucleotide polymorphism nor the two coding polymorphisms (G1051A, G2868A) were associated with a risk of significant stenosis in our study. We found that only the G2706A variant was associated with a decreased risk of significant stenosis (OR 0.658, p = 0.029), without pronounced effects on plasma lipids. Many studies are in agreement with this result [17,20,29], although Frikke-Schmidt et al. reported an increased risk of ischaemic heart disease [18]. Emerging evidence suggests that ABCA1 gene variants can exert phenotypic effects on atherosclerosis, independent of changes in plasma lipid concentrations [9,11,17,23]. The association of G2706A polymorphisms with significant stenosis without a major change in the overall HDL-C concentration suggests that this polymorphism probably exerts its effects principally by HDL particle modification, with decreases in the relative fraction of large HDL particles that are believed to be cardioprotective, or by modifying reverse cholesterol transport in macrophages. In fact, it was reported that cholesterol efflux from cells in the arterial wall that have the potential to transform into foam cells — primarily macrophages — is directly relevant to atherosclerosis [30]. The absence of ABCA1 leads to significant changes in the morphology, properties and functional activities of macrophages, resulting in increases in cholesterol deposition and the response to chemotactic factors [31]. The selective inactivation of ABCA1 in the macrophages of hyperlipidaemic mice leads to a marked increase in atherosclerosis and foam cell accumulation, demonstrating the antiatherogenic properties of ABCA1, independent of plasma lipid and HDL-C concentrations [32]. However, it has been shown that the contribution of monocyte/macrophage ABCA1 to overall plasma HDL-C concentration is minimal [33].

Another result of our study is that only the A allele of the G2706A variant appeared to be more protective for smokers than non-smokers (OR 0.48, p = 0.021 vs OR 0.74, p = 0.055). Smoking increases the rate of oxidation of lipoprotein particles, and it might be possible that this oxidative stress can be alleviated in part through the ABCA1 pathway, as ABCA1 has been reported to mediate the cellular secretion of α-tocopherol (the active form of vitamin E with antioxidant properties) [29]. Thus, it might be possible that subjects carrying the 2706A allele are more protected against lipoprotein oxidation and subsequent risk of atherosclerosis. Further studies will be necessary to confirm this hypothesis. This protective effect also seemed to be more significant in diabetic patients than in those without diabetes (OR 0.53, p = 0.007 vs OR 0.75, p = 0.35). This result may be explained by the fact that impaired ABCA1 may play a role in the cardiovascular disease that is the major cause of morbidity and mortality in both type 1 and type 2 diabetes [33–35]. In fact, two features of diabetes are elevated glucose and fatty acids [36,37]. It was reported that the reactive carbonyls glyoxal and glycoaldehyde, induced by prolonged hyperglycaemia [38,39], acutely and severely impair the ABCA1 pathway in cultured macrophages, presumably by directly damaging the ABCA1 protein. In addition, fatty acids destabilize the ABCA1 protein by activating a signalling pathway that phosphorylates ABCA1 and enhances its degradation [40–43].

In our study, in haplotype analysis, after adjustment of the confounding variables, the most protective (AAGC) haplotype was associated with a decreased risk of significant stenosis (OR 0.5, p = 0.048), with a 33% decreased risk of hypoHDLaemia and a 36% decreased risk of hypoApoA1. We also showed that the (GGAT) haplotype increased risk of significant stenosis (OR 1.26, p = 0.025), hypoHDLaemia (OR 1.26, p = 0.36) and hypoApoA1 (OR 0.7, p = 0.060).

Some limitations should be noted in our study. Only four polymorphisms in the ABCA1 gene were studied. It is possible that other single nucleotide polymorphisms in the
gene might have more potent associations with HDL-C or subclinical atherosclerosis, or that haplotype analysis with additional genotyping might increase the power to find these potential associations. Additionally, the size of the study population may have limited our power to perform haplotype and interaction analyses.

In conclusion, we showed that only the A allele of the G2706A polymorphism seemed to be associated with a reduced risk of significant stenosis without important modification of serum HDL-C concentration, and that it appears to be more protective for smokers and diabetic patients. We found that (AAGC) is a protective haplotype whereas (GGAT) is associated with an atherogenic effect in a Tunisian population.

Conflict of interest statement
None.

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