In-Depth Haplotype Analysis of ABCA1 Gene Polymorphisms in Relation to Plasma ApoA1 Levels and Myocardial Infarction

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Objective—By regulating the cellular cholesterol efflux from peripheral cells to high-density lipoprotein, the ABCA1 protein is suspected to play a key role in lipid homeostasis and atherosclerosis. Twenty-six polymorphisms of the ABCA1 gene were genotyped and tested for association with plasma levels of ApoA1 and myocardial infarction (MI) in the ECTIM study.

Methods and Results—In addition to single-locus analysis, a systematic exploration of all possible haplotype effects was performed, with this exploration being performed on a minimal set of “tag” polymorphisms that define the haplotype structure of the gene. Two polymorphisms were associated with plasma levels of ApoA1, 1 in the promoter (C-564T) and 1 in the coding (R1587K) regions, whereas only 1 polymorphism (R219K) was associated with the risk of MI. However, no haplotype effect was detected on ApoA1 variability or on the risk of MI.

Conclusion—ABCA1 gene polymorphisms but not haplotypes are involved in the variability of plasma ApoA1 and the susceptibility to coronary artery disease. (Arterioscler Thromb Vasc Biol. 2004;24:1-7.)

Key Words: XXXXX ■ XXXXX ■ XXXXX ■ XXXXX ■ XXXXX ■ XXXXX

Low levels of plasma high-density lipoprotein cholesterol (HDL-C) are associated with an increased risk of atherosclerotic complications.1–3 A few environmental factors are known to affect HDL-C metabolism and plasma levels, such as physical activity,4,5 alcohol consumption,6 and cigarette smoking.7,8 The known influence of these environmental factors on the risk of coronary heart disease may be partly mediated by their effect on HDL-C metabolism. However, polymorphisms in the cholesterol ester transfer protein,9,10 lipoprotein lipase,11,12 apolipoprotein (Apo) A-I,13 and scavenger receptor class B type I14 genes have been shown, to some extent at least, to affect plasma HDL-C levels. However, their contribution to clinical endpoints is, at most, very weak.

Because of the recognition of its implication in Tangier disease,15 a rare form of genetic HDL deficiency, the ATP-binding cassette transporter 1 (ABCA1) has been investigated extensively and is now recognized as a key player in the reverse transport of cholesterol from peripheral cells to the liver and other organs.16–19 ABCA1 is a member of the ABC membrane transporters family, which comprises proteins translocating a wide variety of substrates across cellular membranes.20 It has been suggested that ABCA1 participates in the efflux of free cholesterol from peripheral cells, including macrophage-derived foam cells, and contributes to the formation of mature HDL by facilitating the lipidation of circulating nascent ApoA-I particles with free cholesterol at the plasma membrane.16–21,22 Functional deficiency of ABCA1 could therefore induce an atherogenic decrease in HDL-C levels, incriminating the ABCA1 gene as a candidate for atherosclerotic complications. This hypothesis has been supported by recent studies showing that in addition to rare mutations,23–25 common polymorphisms in the ABCA1 gene also affect HDL-C concentrations and could be of clinical importance.23,25–29 These results were not, however, entirely consistent; in particular, a dissociation was shown between the effect of these polymorphisms on HDL-C and other circulating lipid variables.23,26 Although ABCA1 polymorphisms may have only a weak effect on commonly measured lipid parameters such as HDL-C, they may have a stronger impact on more specific phenotypes such as apolipoproteins.

The aim of this study was to investigate whether ABCA1 gene polymorphisms could be associated with the risk of myocardial infarction (MI) and the variability of ApoA1
levels. For this purpose, a molecular screening of the ABCA1 gene was first performed to identify all common polymorphisms in the coding and promoter regions of the gene in individuals of European origin. Identified polymorphisms were further genotyped in the ECTIM Study,\(^3\) a case-control study of MI, and tested for association with plasma ApoA-I levels and with MI. Association analysis was performed by use of our recently proposed haplotype-based analysis method\(^1\) in order to better characterize the contribution of ABCA1 gene haplotypes to ApoA-I variability and the risk of MI.

**Methods**

**Screening of the ABCA1 Gene for Detection of Polymorphisms**

The molecular screening of the ABCA1 gene was performed on genomic DNA obtained from 32 individuals of European origin. The entire coding sequence and the promoter region of the ABCA1 gene were analyzed as well as six intronic sequences flanking exons. Primary polymerase chain reaction (PCR) amplification primers were designed using Primer Software (ggc package) and specific amplification was achieved using the selected PCR primers. Sequencing reactions were performed in 10 μL, using 2 μL of purified PCR product, 10 μmol/L amplification primer forward or reverse from PCR reaction (0.5 μL), and 1.5 μL of BigDye terminator kit (Applied Biosystems). All reactions were cleaned using G5 Sephadex as recommended by the supplier (Applied Biosystems). The traces were analyzed with the package Phred Phrap Polyped and Consed (Washington University). The polymorphisms were then blasted against the protein to identify the amino acid changes.

**Genotyping of the ABCA1 Gene Polymorphisms**

All genotyped SNPs were analyzed in different sets of 2, 3, or 4 SNPs. For each set of SNPs, primary PCR amplification primers were designed using Primer Software (ggc package). The single base extension (SBE) oligonucleotides were chosen 20 bp upstream or downstream of the polymorphic site so that the 5' end terminates 1 base before the polymorphic site. A thymine (T) tail was 5' added to the second, third, and fourth SBE primers to allow separation of each marker according to its length. The T-tails were, respectively, 0T, 6T, 12T, and 18T. Specific amplification of the genomic regions containing the SNPs was achieved using the PCR primers. After SBE reaction, a size-standard labeled on a fifth dye (Applied Biosystems) was added to 3 μL of the SBE reaction in a final volume of 15 μL. The samples were processed using an ABI 3700 as recommended by the supplier (Applied Biosystems). The samples were processed with Genscan for size-standard definition and allele pic recognition. The pictures information was exported to a matrix file (Genotype software, Applied Biosystems) in which the markers data were stored. Alleles were determined from the presence of 1 or 2 pics in two different colors.

**Study Population**

All SNPs identified in the promoter and translated coding regions were then genotyped in the ECTIM study. The ECTIM study design has been described elsewhere.\(^3\) The initial study population has been extended by the recruitment of further population samples from the United Kingdom in Belfast and Glasgow.\(^4\) The results reported here are based on samples of subjects from populations covered by WHO MONICA Project registers in the United Kingdom: Belfast (Northern Ireland) and Glasgow (Scotland). Patients (33% females) aged 25 to 64 (for males) and 25 to 69 years (for females) (mean age±SD: 56±8.1 years) were recruited from 3 and 9 months (2 years for females in Belfast) after the index MI. Controls of the same sex and comparable age were recruited from the lists of general practitioners in the same areas in the United Kingdom (mean age±SD: 57±8.0 years). In the present analysis, DNA was available for 800 cases and 776 controls. However, haplotype analyses were performed on smaller numbers of individuals because of missing genotypic data at polymorphisms. Each participant provided written informed consent. The methodology used to assess plasma ApoAI levels has been described previously.\(^3\)

**Statistical Analysis**

Allele frequencies were estimated by gene counting. Departure from Hardy-Weinberg equilibrium was tested in each center using a χ² with 1 degree of freedom (df), and pair-wise linkage disequilibrium (LD) was estimated using a log-linear model analysis.\(^5\) The extent of LD was expressed as D², which is the ratio of the unstandardized coefficient to its minimal/maximal value.\(^6\) For case-control comparisons, controls with coronary heart disease were excluded. For each polymorphism, allelic odds ratios (ORs) were calculated by a logistic regression adjusted for age, sex, and center. Association between plasma ApoAI levels with each ABCA1 gene polymorphisms was performed by classical linear regression analysis assuming additivity of the allele effects after adjusting for age, sex, center, and case/control status. Heterogeneity of effects between cases and controls was tested by introducing the corresponding interaction term.

To reduce the haplotype dimension and to select the most informative and parsimonious haplotype configuration in terms of prediction of the phenotypic variability, we applied our maximum likelihood method to all possible 1 to k-loci combinations of polymorphisms that could be derived from the set of k “tag” polymorphisms. For each model, including the model with no polymorphism, an information criteria, AIC,\(^7\) was calculated. All AIC values were rescaled by subtracting the minimum AIC value obtained over all models explored. Following a rule derived by extensive Monte Carlo simulation,\(^8\) all models with a rescaled AIC ≤2 could be considered as “equivalent” to the model leading to the minAIC. Among those, the most parsimonious one corresponding to the minimal haplotype configuration was retained. Note that this “best” model could be the one without any polymorphism.

Haplotype analyses were adjusted for sex, age, center, and ApoAI level (for case-control comparison) or case-control status (for ApoAI levels).

**Results**

**Identification of ABCA1 Gene Polymorphisms**

The present report focused on the analysis of 16 polymorphisms located in the promoter region and 10 polymorphisms in the translated coding region of the gene.

Characteristics of the identified ABCA1 gene polymorphisms are reported in Table 1. In particular, the 777rpt polymorphism was characterized by 4 different alleles de-
pending on the size of the repeat region: 19, 23, 28, or 32 nucleotides. These will be referred to as allele 1, 2, 3, and 4, respectively. Allele 1 was carried by only 1 subject (case) who was then eliminated from analyses. Among the 10 polymorphisms in the coding region, 6 induced an amino acid change and 4 were synonymous. No deviation from Hardy-Weinberg equilibrium was observed for any polymorphism. No allele frequency difference was observed between Belfast and Glasgow for any polymorphism.

Detailed description of the LD pattern can be found at our web site (http://genecanvas.ecgene.net). Polymorphisms in the promoter region were strongly associated, with LD being complete for most of the pairs. Because the A-1652G, the G-1506C, and the G-407 polymorphisms were nearly completely concordant (<1% of recombinants) with the A-1814G, the 777rpt, and the G-564T polymorphisms, respectively, they were not considered for analysis. Strong LD was also present in the coding region for 1 cluster of polymorphisms (R219K, G316G, I680I, V771 mol/L, V825I, I883 mol/L) located in exons 8 to 17. Outside the region encompassing this set of polymorphisms, LD was weak or absent. This is likely the consequence of the large size of the hABCA1 gene sequence, which is distributed over a region of almost 150 kb. LD between the promoter and the coding polymorphisms was very weak. Therefore, haplotype analysis was performed in the promoter and the coding regions separately.

### Table 1. Description and Frequency of ABCA1 Gene Polymorphisms in the 2 Centers From the United Kingdom

<table>
<thead>
<tr>
<th>Name</th>
<th>5' Flanking</th>
<th>Nucleotide Change</th>
<th>3' Flanking</th>
<th>Belfast (N=888)</th>
<th>Glasgow (N=725)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-1814G</td>
<td>CAGCTCTCGT</td>
<td>A/g</td>
<td>GATAACAGGC</td>
<td>0.339</td>
<td>0.366</td>
</tr>
<tr>
<td>C-1801T</td>
<td>TAAAGGAGCC</td>
<td>C/t</td>
<td>CGCCACACCA</td>
<td>0.443</td>
<td>0.433</td>
</tr>
<tr>
<td>A-1652G</td>
<td>CACTGGGCC</td>
<td>A/g</td>
<td>GCTCAGATCC</td>
<td>0.350</td>
<td>0.364</td>
</tr>
<tr>
<td>G-1506C</td>
<td>CTCTTCTATG</td>
<td>G/c</td>
<td>GTCTGTCTG</td>
<td>0.203</td>
<td>0.205</td>
</tr>
<tr>
<td>C-1395T</td>
<td>TGAATGTCTG</td>
<td>C/t</td>
<td>ATGCAGTGTTG</td>
<td>0.428</td>
<td>0.425</td>
</tr>
<tr>
<td>G-1252A</td>
<td>TGCCCTTCAA</td>
<td>G/a</td>
<td>GTGGCTACAA</td>
<td>0.095</td>
<td>0.088</td>
</tr>
<tr>
<td>C-1217T</td>
<td>AGGTAGGAGA</td>
<td>C/t</td>
<td>CTGGTG6G6CT</td>
<td>0.120</td>
<td>0.121</td>
</tr>
<tr>
<td>−1034ins/del</td>
<td>ATATTAGAC</td>
<td>±AT</td>
<td>ATGGTGTGTA</td>
<td>0.210</td>
<td>0.220</td>
</tr>
<tr>
<td>T-940G</td>
<td>GCAAACAGAG</td>
<td>T/g</td>
<td>AAGTTGAGGG</td>
<td>0.486</td>
<td>0.483</td>
</tr>
<tr>
<td>G-803A</td>
<td>AAATTAAAGA</td>
<td>G/a</td>
<td>GGCTGTGCCT</td>
<td>0.108</td>
<td>0.093</td>
</tr>
<tr>
<td>−777rpt/23nt*</td>
<td>CTGTGTTTTTCTGGTTGTTTCTTC</td>
<td></td>
<td></td>
<td>0.527</td>
<td>0.518</td>
</tr>
<tr>
<td>−777rpt/28nt</td>
<td>CTGTGTTTTTCTGGTTGTTTCTTC</td>
<td></td>
<td></td>
<td>0.267</td>
<td>0.272</td>
</tr>
<tr>
<td>−777rpt/32nt</td>
<td>CTGTGTTTTTCTGGTTGTTTCTTC</td>
<td></td>
<td></td>
<td>0.206</td>
<td>0.210</td>
</tr>
<tr>
<td>C-564T</td>
<td>GAGACTGTC</td>
<td>G/t</td>
<td>GCTTCCCCCT</td>
<td>0.456</td>
<td>0.472</td>
</tr>
<tr>
<td>C-407C</td>
<td>GCGAAAGCA</td>
<td>G/c</td>
<td>GATTAGAGG</td>
<td>0.455</td>
<td>0.459</td>
</tr>
<tr>
<td>C-302T</td>
<td>CTCTTACAGC</td>
<td>C/t</td>
<td>GCCTGACACG</td>
<td>0.196</td>
<td>0.189</td>
</tr>
<tr>
<td>G-278C</td>
<td>GGGAAGGAGG</td>
<td>G/c</td>
<td>AGCAGACCG</td>
<td>0.435</td>
<td>0.424</td>
</tr>
<tr>
<td>C-14T</td>
<td>GGAACTGTC</td>
<td>G/t</td>
<td>CGGCAAAAC</td>
<td>0.335</td>
<td>0.346</td>
</tr>
<tr>
<td>R219K</td>
<td>GGGCTACCA</td>
<td>G/a</td>
<td>GGAAGACTG</td>
<td>0.283</td>
<td>0.278</td>
</tr>
<tr>
<td>G316G</td>
<td>AGGGAGGGG</td>
<td>G/a</td>
<td>CTGAGATCA</td>
<td>0.114</td>
<td>0.095</td>
</tr>
<tr>
<td>I680I</td>
<td>ACAACAGCT</td>
<td>G/a</td>
<td>CTGTGTTTTA</td>
<td>0.129</td>
<td>0.118</td>
</tr>
<tr>
<td>V771 mol/L</td>
<td>GGGAGACTG</td>
<td>G/a</td>
<td>TGCGTTTCAC</td>
<td>0.029</td>
<td>0.034</td>
</tr>
<tr>
<td>V825I</td>
<td>CACCACTTCG</td>
<td>G/a</td>
<td>TCTCAAGAT</td>
<td>0.056</td>
<td>0.060</td>
</tr>
<tr>
<td>I883 mol/L</td>
<td>AGAAGAAGAT</td>
<td>A/g</td>
<td>TGAGAAAGTA</td>
<td>0.137</td>
<td>0.126</td>
</tr>
<tr>
<td>L1122L</td>
<td>GAAGAACCAG</td>
<td>G/t</td>
<td>TGGAAGAAGG</td>
<td>0.023</td>
<td>0.017</td>
</tr>
<tr>
<td>E1172D</td>
<td>GGAGACCTGA</td>
<td>G/c</td>
<td>AGTGAGACGC</td>
<td>0.026</td>
<td>0.027</td>
</tr>
<tr>
<td>T1427T</td>
<td>GGAGACACAC</td>
<td>G/a</td>
<td>CTCGACACCT</td>
<td>0.069</td>
<td>0.083</td>
</tr>
<tr>
<td>R1587K</td>
<td>CTGAGACCA</td>
<td>G/a</td>
<td>AAAATGAGTC</td>
<td>0.216</td>
<td>0.226</td>
</tr>
</tbody>
</table>

*1 subject carried an additional GTTT deletion, leading to an allele of 19 nucleotides.
†In the pooled sample (cases + controls).

Numbering of polymorphisms in the 5' region is from the start of transcription and is based on the −777rpt/32nt sequence; the nucleotide before the position designates the common allele. For insertion/deletion, the common allele is also indicated first. Polymorphisms in translated exons are designated by the codon number and the single letter amino acid codes (the letter before the codon number indicates the common allele).

Association Analysis of ABCA1 Gene Polymorphisms With MI

To simplify the presentation, the genotype frequency distributions of all the polymorphisms investigated are not re-
portrayed here but can be found at our web site. No significant heterogeneity in the association of MI with any polymorphism was observed between males and females.

**Promoter Region**

None of the polymorphisms was associated with MI by univariate analysis. As a consequence of the strong LD between polymorphisms, a limited number of haplotypes was inferred from the genotypic data (Table 2). Ten haplotypes with a frequency ranging from 0.01 to 0.50 accounted for >95% of all chromosomes. The haplotypic structure of the promoter could be fully characterized by a subset of 5 "tag" polymorphisms, A-1814G, A-1034I/D, T-940G, G-803A, and C-14T (Table 2). Globally, haplotype frequency distribution did not differ between patients and controls ($\chi^2 = 13.12$ with 9 df; $P=0.15$). Results of the full exploration of all models are available online. The most parsimonious and informative model was the one including no polymorphism, which is the model specifying no haplotype effect.

**Coding Region**

By single-locus analysis, the R219K polymorphism was associated with MI, with K219 allele being associated with a decreased risk consistently in the 2 centers (population adjusted OR = 0.80 [0.68 to 0.94], $P=0.007$).

Sixteen haplotypes with a frequency >0.01 were inferred from the genotypic data of all individuals and accounted for >96% of all chromosomes (Table 3). The most frequent haplotype (≈50%) was the one combining the most frequent allele at each site. The frequency of the other haplotypes ranged from 0.012 to 0.084. Because of the weak LD between polymorphisms of the coding region, a subset of 8 "tag" polymorphisms was required to characterize the inferred haplotypic structure (Table 3). After adjusting for sex, age, center, and ApoA1 levels, the whole haplotype frequency distribution did not differ between cases and controls ($\chi^2 = 17.28$ with 15 df; $P=0.302$). The "best" model encountered in the systematic exploration was the model with the R219K polymorphism alone, suggesting that apart from the raw effect of the R219K polymorphism, no other polymorphism was associated with the risk of MI.

Finally, when investigating whether this association could be modulated by potential moderators including alcohol, body mass index, and smoking, we observed that it was mainly present in nonsmokers (OR = 0.67 [0.53 to 0.84], $P<10^{-3}$) but not in smokers, OR = 0.95 [0.73 to 1.22], $P=0.66$; test for interaction $P=0.05$).

**Association Analysis of ABCA1 Gene Polymorphisms With ApoA1 Levels**

Plasma levels of ApoA1 were lower in cases than in controls (mean ± SEM: 1.41 ± 0.01 versus 1.57 ± 0.01 mg/mL, $P<10^{-4}$) and were higher in females than in males (1.56 ± 0.01 versus 1.42 ± 0.01 mg/mL, $P<10^{-4}$), irrespective of clinical status. Full description of the association between each ABCA1 gene polymorphism and ApoA1 levels can be found at our web site.

**Promoter Region**

By single-locus analysis, the C-564T polymorphism was the only polymorphism of the promoter region significantly associated with plasma ApoA1 levels. The 564T allele was associated with increased plasma ApoA1 levels (1.47 versus 1.49 versus 1.51; $R=0.5%$; $P=0.015$, in CC, CT, and TT, respectively). The association was homogeneous in patients and controls (data not shown). The test for a global haplotypic effect was not significant ($\chi^2 = 12.38$ with 8 df, $P=0.135$). The "best haplotypic model" with respect to ApoA1 levels was the model including the T-940G polymorphism alone, whose association with ApoA1 levels was borderline in univariate analysis ($P=0.089$, data available at our web site). Note that the C-564T was not included in the subset of 5 "tag" polymorphisms, which explains why it was not selected by haplotype analysis. However, the T-940G polymorphism was in strong LD with the C-564T polymorphism. These results suggested that variation in the promoter influenced plasma

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### TABLE 2. Haplotype Structure Defined by the ABCA1 Promoter Gene Polymorphisms (N=917)

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>Controls N=465</th>
<th>Cases N=452</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-1814G</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>C-1801T</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>C-1395T</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>G-1252A</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>C-1217T</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>−1034I/D</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>T-940G</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>G-803A</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>−777rpt</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>C-564T</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>C-302T</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>G-278C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>C-14T</td>
<td>T</td>
<td>T</td>
</tr>
</tbody>
</table>

The haplotype structure of the promoter region of the ABCA1 gene can be completely defined by a subset of 5 “tag” polymorphisms indicated in boxes. An alternative subset of 5 “tag” polymorphisms could have been selected by replacing the T-940G polymorphism by the −777rpt.
Apolipoprotein A1 (ApoA1) levels, but it was not possible from haplotype analysis to clearly identify the potentially functional polymorphisms.

**Coding Region**

By single-locus analysis, the R1587K polymorphism was the only one in the coding region that was significantly associated with plasma levels of ApoA1. The association was homogeneous in patients and controls, with the K1587 allele being significantly associated with decreased ApoA1 levels. The association was not significant (\(\chi^2 = 20.57\) with 15 df, \(P = 0.151\)). Because the most informative and parsimonious model with regard to plasma ApoA1 levels was the model including the R1587K polymorphism alone, it could be deduced from the principle of parsimony that the association between plasma ApoA1 and the R1587K polymorphism alone, it could be deduced from the principle of parsimony that the association between plasma ApoA1 levels and haplotype variability in the coding region was caused by only the R1587K polymorphism.

Note that similar results were observed when haplotype analyses were repeated after excluding individuals who were using lipid-lowering drugs (data available at our web site).

**Discussion**

Much attention has been focused on the association of ABCA1 gene polymorphisms with different phenotypes including lipid variables and clinical endpoints.23,25–29,39 Several studies have consistently reported an association between the R219K polymorphism and coronary artery disease.23,26,27 This polymorphism has been shown to be associated with triglycerides23 but not with HDL-C.26,27 Inconsistent results were also observed for other ABCA1 gene polymorphisms including V825I, I883 mol/L, and E1172D. One of the objectives of this work was to investigate the association of ABCA1 gene polymorphisms with plasma ApoA1 levels. To this end, a molecular screening of the promoter and coding regions of the ABCA1 gene was performed, which led to the identification of already known polymorphisms and new ones mainly in the promoter region. However, our molecular screening failed to identify 2 previously identified polymorphisms located in the promoter region (G-191C and C-17G).29 It cannot be excluded that our sample size of 32 healthy individuals was too small to identify these polymorphisms that have been identified in patients with coronary artery disease and have also shown to be associated with an increase of coronary events. The polymorphisms identified were then genotyped in the ECTIM study30 and tested for association with MI and ApoA1 levels. The single-locus analysis may provide misleading results if particular combinations of nucleotides on the same chromosome, ie, haplotypes, constitute the relevant genetic units with regard to the phenotypes. These haplotypes may serve as markers for unknown functional variants or may define functional units whose effects cannot be predicted from what is known of the effect of each variant. Our strategy of haplotype analysis was first to define the haplotypic variability of the ABCA1 gene by identifying “tag” polymorphisms and then to perform haplotype–phenotype association analysis by use of these “tag” polymorphisms. Because LD was very weak between the promoter and coding regions, haplotype analysis was performed separately within these 2 blocks, as we have already performed in previous work.31 However, it cannot be excluded that some functional haplotypes might be defined by some polymorphisms that are not in LD.31

Because of the strong LD within the promoter region of the ABCA1 gene, the haplotypic diversity of this region was rather low, with 5 “tag” polymorphisms (A-1814G, A-1034I/D, T-940G, G-803A, and C-14T), among the 14 polymorphisms identified, defining 11 frequent haplotypes.
This set of 5 “tag” polymorphisms was not unique because the T-940G position could have been replaced by a repeat polymorphism at position 777 without changing the results of the analysis (data not shown). No single-locus or haplotype effect was observed on the risk of MI. The C-564T polymorphism was marginally associated with ApoA1 levels, with the T allele being associated with increased levels. Note that the C-564T polymorphism was in strong LD with the C-14T polymorphism that was shown to be associated with HDL-C in a Chinese population.²⁹

Unlike what was observed in the promoter region, the LD was rather weak between polymorphisms located in the coding region; most of these polymorphisms are relatively rare. Of the 10 identified polymorphisms in this region, 8 were required to characterize the 16 main haplotypes inferred. The R1587K polymorphism that was not in LD with any other polymorphism was the only one associated with plasma ApoA1 variability. The K1587 allele was associated with decreased plasma ApoA1 levels. No significant association of this polymorphism was observed with MI, although carriers of the K1587 allele (RK and KK) were at slightly decreased risk of MI (OR: 0.76; 95% CI: 0.61 to 0.94; P = 0.013). These results are quite consistent with those of a previous study that also showed that the R1587K polymorphism was associated with HDL-C but not with coronary artery disease.²³ No other polymorphism within the coding region seemed to modulate the variability of plasma ApoA1. Conversely, the R219K polymorphism was associated with MI but not with ApoA1 levels. The K219 allele was associated with a decreased risk of MI (OR: 0.80; 95% CI: 0.68 to 0.94; P = 0.007) as previously reported.²³,²⁶,²⁷ Interestingly, the protective effect of the K219 allele was more pronounced in nonsmokers than in smokers, as previously observed.²⁷

ABCA1 shows limited interaction with the major HDL species isolated from plasma.¹³ This may explain why in our study the association between the ABCA1 polymorphisms and HDL-C was consistent but weaker than that observed between the polymorphisms and ApoA1 (data not shown).

Overall, these results in addition to those of other studies suggest an implication of ABCA1 polymorphisms in the variability of plasma ApoA1 and the susceptibility to coronary artery disease. In the presence of ApoA1, ABCA1 promotes cellular efflux of cholesterol and ABCA1 cross-links to lipid-poor ApoA1. One explanation for the observed association would be that the ABCA1 polymorphisms affect the interaction between ABCA1 and ApoA1; however, we have no experimental proof that this is the case. There are also a number of inconsistencies in the results that we are unable to explain for the moment, especially the fact that R1587K affects plasma ApoA1 but appears unrelated to MI and conversely that R219K is associated with MI but does not affect plasma ApoA1 levels. It is important to keep in mind that because the ECTIM Study was designed to recruit MI survivors within defined geographic areas, a survival bias could obviously result from the high early lethality of MI. An unbiased picture could be provided only by a prospective study in which the outcome also includes MI deaths.

Although haplotype analyses have the advantages of reducing the number of statistical tests to be performed when investigating the interaction between several polymorphisms,³¹ a simulation study⁴⁰ has recently shown that the power for detecting moderate haplotypic effects (OR = 2) was quite moderate even in large samples (N = 1000). Therefore, it cannot be excluded that our analysis failed to identify haplotype effects because of a lack of power, especially if haplotype effects deviated from the assumed hypothesis of additivity.

It is remarkable that the R1587K polymorphism was not in LD with any other polymorphisms in the gene, implying that its direct identification was required to detect its effect on plasma ApoA1 levels. A general conclusion that we can draw from this observation is that in a number of instances, genetic markers may be useless and that direct identification of a “functional” polymorphism may be required. This suggests an exhaustive analysis of candidate gene polymorphisms as opposed to an analysis based on some selected markers. In addition, we must note that for a polymorphism like R1587K, haplotype analysis was useless and even blurred an association that was evident with the polymorphism alone. It is also interesting to mention that despite the large number of polymorphisms within the ABCA1 gene, no haploypic effect was found in the present analyses unlike what has been observed for other genes.¹⁰,³¹

Acknowledgments

We are grateful to all investigators of the ECTIM study.

References


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David-Alexandre Tregouet, Sylvain Ricard, Viviane Nicaud, Isabelle Arnould, Stéphane Soubigou, Marie Rosier, Nicolas Duverger, Odette Poirier, Sandrine Macé, Frank Kee, Caroline Morrison, Patrice Denèfle, Laurence Tiret, Alun Evans, Jean-Francois Deleuze and Francois Cambien

*Arterioscler Thromb Vasc Biol.* published online February 12, 2004;

*Arteriosclerosis, Thrombosis, and Vascular Biology* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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