Clinical Investigation and Reports

Common Genetic Variation in ABCA1 Is Associated With Altered Lipoprotein Levels and a Modified Risk for Coronary Artery Disease

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Background—Low plasma HDL cholesterol (HDL-C) is associated with an increased risk of coronary artery disease (CAD). We recently identified the ATP-binding cassette transporter 1 (ABCA1) as the major gene underlying the HDL deficiency associated with reduced cholesterol efflux. Mutations within the ABCA1 gene are associated with decreased HDL-C, increased triglycerides, and an increased risk of CAD. However, the extent to which common variation within this gene influences plasma lipid levels and CAD in the general population is unknown.

Methods and Results—We examined the phenotypic effects of single nucleotide polymorphisms in the coding region of ABCA1. The R219K variant has a carrier frequency of 46% in Europeans. Carriers have a reduced severity of CAD, decreased focal (minimum obstruction diameter 1.81 ± 0.35 versus 1.73 ± 0.35 mm in noncarriers, \( P = 0.001 \)) and diffuse atherosclerosis (mean segment diameter 2.77 ± 0.37 versus 2.70 ± 0.37 mm, \( P = 0.005 \)), and fewer coronary events (50% versus 59%, \( P = 0.02 \)). Atherosclerosis progresses more slowly in carriers of R219K than in noncarriers. Carriers have decreased triglyceride levels (1.42 ± 0.49 versus 1.84 ± 0.77 mmol/L, \( P = 0.001 \)) and a trend toward increased HDL-C (0.91 ± 0.22 versus 0.88 ± 0.20 mmol/L, \( P = 0.12 \)). Other single nucleotide polymorphisms in the coding region had milder effects on plasma lipids and atherosclerosis.

Conclusions—These data suggest that common variation in ABCA1 significantly influences plasma lipid levels and the severity of CAD. (Circulation. 2001;103:1198-1205.)

Key Words: ABC transporters ▪ coronary disease ▪ lipids ▪ genetics

HDL cholesterol (HDL-C) was first suggested to protect against the development of coronary artery disease (CAD) 25 years ago. Since then, a strong inverse relationship between plasma HDL-C levels and CAD has been confirmed in a large number of epidemiological studies. Low plasma HDL-C is now generally accepted as a strong independent risk factor for the development of premature atherosclerosis.

A rare form of genetic HDL deficiency is Tangier disease, which has been diagnosed in 60 patients worldwide and is associated with an almost complete absence of HDL-C. We and others have recently identified mutations in the ABCA1 gene as the molecular defect in Tangier disease and familial HDL deficiency associated with reduced cholesterol efflux (FH-A).

Individuals heterozygous for mutations in the ABCA1 gene have decreased HDL-C, increased triglycerides (TG), and an increased risk of CAD. Specific variants associated with complete or near-complete loss of ABCA1 function are not found at a high frequency in patients presenting with low HDL-C. However, the extent to which common variation in the ABCA1 gene influences these phenotypes in the general population is uncertain. Thus, we sought to address whether variants having milder effects on ABCA1 function influence plasma lipid levels and risk of CAD.

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Dr Hayden serves as Chair of the Scientific Advisory Board and Dr Kastelein serves as a Medical Consultant for Xenon Genetics, Inc. Figure I can be found Online Only at www.circulationaha.org.

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We identified numerous single-nucleotide polymorphisms throughout the coding region (cSNPs) of the ABCA1 gene and examined the phenotypic effects of 9 nonsynonymous (ie, those that change an amino acid) cSNPs in a large, ethnically uniform cohort. We report here that a common genetic variant in ABCA1 may influence these clinical outcomes in the general population.

Methods

Subjects
During the course of sequencing 16 Tangier disease and FHA probands, we identified 16 cSNPs. Sequencing 16 individuals yields a 97% chance of identifying a variant present at a frequency ≥10%. Thus, it is likely that all the common ABCA1 cSNPs have been identified. We studied the effects of these cSNPs on the baseline lipid parameters of the cohort of 804 Dutch men with proven CAD who participated in the Regression Growth Evaluation Statin Study (REGRESS); these subjects were described previously.11 The mean segment diameter (MSD) measures the average luminal diameter along the vessel and reflects diffuse atherosclerotic differences. The minimum obstruction diameter (MOD) represents the smallest vessel diameter at an obstructed site and assesses focal atherosclerotic changes. Larger MSD and MOD measurements reflect less vessel occlusion. Events during the study (death, myocardial infarction, unscheduled coronary angioplasty or bypass surgery [PTCA, CABG], and stroke/transient ischemic attack) were also examined.

cSNP Screening
We identified a restriction enzyme whose cleavage pattern was altered by each variant or employed a mismatch technique allowing restriction fragment length polymorphism analysis. The conditions of all assays are described in Table 1.

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<tr>
<th>Variant</th>
<th>pmol of Each Oligo</th>
<th>Forward Oligo (5′−3′)*</th>
<th>Reverse Oligo (5′−3′)*</th>
<th>Annotation Temperature, °C</th>
<th>Enzyme</th>
<th>Wild-type Allele</th>
<th>Variant Allele</th>
<th>Product, bp</th>
<th>% Agarose Gel for Resolution</th>
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<td>GAATTCCTCACGGTTGATTTGCTGAC</td>
<td>GAATTCCTCACGGTTGATTTGCTGAC</td>
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<td>EcoN I</td>
<td>177</td>
<td>1.5</td>
<td>107, 70</td>
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<td>(R219K)</td>
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<td>GATTGGCTTCAGGATGTTGCTGAC</td>
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<td>107, 70</td>
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<td>EcoR V</td>
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<td>EcoR V</td>
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<tr>
<td>(E1172D)</td>
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<td>55</td>
<td>EcoR V</td>
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<td>94, 35</td>
<td>2.5</td>
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</table>

*Bold indicates mismatch in oligo to create restriction site.

We avoided stratification by ethnicity or other demographic factors. All individuals gave informed consent.

CAD Measurements
Computer-assisted quantitative coronary angiography was performed as previously described. The mean segment diameter (MSD) measures the average luminal diameter along the vessel and reflects diffuse atherosclerotic differences. The minimum obstruction diameter (MOD) represents the smallest vessel diameter at an obstructed site and assesses focal atherosclerotic changes. Larger MSD and MOD measurements reflect less vessel occlusion. Events during the study (death, myocardial infarction, unscheduled coronary angioplasty or bypass surgery [PTCA, CABG], and stroke/transient ischemic attack) were also examined.

Cellular Cholesterol Efflux
Cholesterol efflux was measured in a series of Dutch individuals with HDL-C less than the fifth percentile, essentially as described...
previously. Measurements are reported as the percentage efflux relative to the average of 2 healthy controls included within the same experiment. All individuals had an efflux in the normal range (>60% of controls). None had mutations in ABCA1 associated with Tangier disease or FHA.

Statistics
The baseline characteristics of the patients with each genotype for each cSNP were compared using 1-way ANOVA and the χ² test. Subsequent comparisons between carriers and noncarriers were made using a t test. Probability values unadjusted for multiple comparisons are presented to allow readers to reach their own conclusions regarding significance. The cumulative event incidence was compared using the log-rank test. The relationships between age and HDL-C or efflux were investigated using a linear regression model, and the slopes of the regression lines were compared using a covariance analysis (interaction between age and genotype). Randomization to placebo and pravastatin in the REGRESS cohort was assessed by χ² analysis and was equivalent for all genotypes except R1587K, in which a lower proportion of carriers was randomized to pravastatin. Events during the trial were also analyzed for the placebo and pravastatin subgroups separately, with similar effects in each subgroup. Thus, the combined results are presented. All lipid levels are expressed in mmol/L, and all values are reported as the mean ± SD. The population-attributable risk for R219K is calculated from the sum of each genotype frequency multiplied by its risk (relative to KK). The population-attributable risk is calculated as

\[ \text{Population-attributable risk} = \sum \text{genotype frequency} \times \text{risk} \]

For replication studies, KK and RR genotypes were compared by 1-tailed t test to test for the specific differences seen in the REGRESS cohort. Although each cohort was small, statistical power was increased by combining the results in a meta-analysis (Meta 5.3).

Results
Identification and Distribution of ABCA1 cSNPs
A total of 16 cSNPs were identified in the 6.8-kb coding region (~1 cSNP every 425 bp). Because nonsynonymous cSNPs are most likely to be associated with functional effects, we focused on those 10 (Table 2). The nonsynonymous cSNPs are nonrandomly distributed throughout the protein (Figure 1).

The R219K Polymorphism Is Associated With a Decreased Severity of CAD
The G1051A polymorphism results in the substitution of lysine for arginine at amino acid 219 of the ABCA1 protein (R219K; Table 2). There were no significant differences in blood pressure (systolic and diastolic), plasma glucose levels, or smoking behavior between the genotypes. BMI was slightly higher in heterozygotes compared with either homozygous genotype.

The K allele of the R219K polymorphism was associated with a decreased severity of CAD (Table 3), as indicated by an increased MSD and MOD. The angiographic data were paralleled by differences in clinical events. A smaller percentage of individuals homozygous for the K allele had a myocardial infarction before the trial, although this did not reach significance (Table 3). Carriers had 29% fewer events (death, myocardial infarction, unscheduled PTCA or CABG, stroke, or transient ischemic attack) during the study compared with noncarriers (Figure 2, P = 0.07). Furthermore, total events (prior myocardial infarction or event during the trial) were significantly reduced in KK compared with RR individuals (odds ratio for KK, 0.45; 95% CI, 0.22 to 0.91). Conversely, this translates to a 2-fold increased risk (odds ratio, 2.2; 95% CI, 1.1 to 4.4) for RR individuals relative to KK.

From the increased relative risk associated with the RR genotype compared with the KK genotype, the population-attributable risk was calculated. For the R219K variant, the population-attributable risk is 5.3%, suggesting that the frequency of CAD events would be 5.3% lower if all individuals carried the KK genotype.

If the K allele of the R219K variant is protective against CAD, we might expect its frequency to be reduced in this cohort, which was selected for CAD. Indeed, the genotype frequencies observed for this variant are not consistent with Hardy-Weinberg equilibrium (P = 0.004), with fewer KK individuals than would be expected (observed, 36; expected, 51, P = 0.04).

Association of the R219K Polymorphism With Plasma Lipid Levels
TG were significantly lower in the carriers of the K allele (Table 4). We previously showed that decreased ABCA1 function is associated with increased TG levels. There were no differences in mean HDL-C levels in the group as a whole (Table 4).

The phenotype of individuals heterozygous for ABCA1 mutations becomes more pronounced in older individuals. Therefore, we further examined HDL-C levels in age-defined subgroups. In individuals younger than the median age of the cohort (56.7 years), carriers (RK + KK) had a trend toward increased HDL-C compared with noncarriers (0.91 ± 0.22

### Table 2. Frequencies of ABCA1 cSNPs

<table>
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<tr>
<th>Nucleotide Change</th>
<th>Amino Acid Change</th>
<th>Exon</th>
<th>Carrier Frequency</th>
<th>Allele Frequency</th>
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<td>0.003</td>
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*Number of alleles screened.
†Only observed in French-Canadian individuals.
mmol/L in RK+KK versus 0.88±0.20 mmol/L in RR, \( P = 0.12 \) that was no longer evident in those above the median age (0.94±0.23 mmol/L for RK+KK versus 0.96±0.24 mmol/L in RR, \( P = 0.37 \)). Linear regression analysis of HDL-C and age showed that in RR individuals, HDL-C was positively correlated with age. In contrast, this relationship was not apparent in carriers (Figure 3A), such that the HDL-C difference between the genotypes was lost in the older individuals (\( P \) comparing slopes = 0.04).

The changes in HDL-C with age are matched by trends in cholesterol efflux with age (Figure 3B). In RR individuals (n=16), cholesterol efflux increased with age, whereas in RK+KK individuals (n=22), efflux decreased with age (\( P \) comparing slopes = 0.07). In younger individuals, cholesterol efflux and HDL-C were increased in KK compared with RR individuals, which suggests that the R219K variant may be especially protective against premature CAD.

Age Subgroup Analysis Indicates CAD Progresses More Slowly in R219K Carriers
In the noncarriers, MOD and MSD decreased significantly with age, reflecting increased atherosclerosis in the older individuals (1.77±0.34 versus 1.69±0.35 mm, \( P < 0.0001 \), and 2.75±0.36 versus 2.65±0.38 mm, \( P = 0.006 \) for MOD and MSD, respectively, in younger versus older noncarriers). In contrast, in carriers of the R219K variant, these measurements do not significantly change with age (1.83±0.36 versus 1.78±0.34 mm, \( P = 0.30 \), and 2.79±0.37 versus 2.75±0.37 mm, \( P = 0.18 \), for MOD and MSD, respectively, in younger versus older carriers). Thus, vascular disease progresses more slowly with age in carriers of R219K compared with noncarriers (Figure 1; can be found Online at www.circulationaha.org).

Replication Cohorts Show the R219K Variant Is Associated With Decreased TG and Increased HDL-C
To confirm and replicate the relationship observed between the R219K variant and plasma lipid levels, we genotyped this variant in 3 small cohorts of European subjects. For every KK individual identified, an RR individual matched for age, sex, and BMI was selected from the same cohort.

In each of the cohorts, HDL-C was increased 10% to 15% in KK compared with RR individuals, regardless of the

### TABLE 3. CAD in R219K Carriers Compared With Controls

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<th></th>
<th>RR</th>
<th>RK</th>
<th>KK</th>
<th>Carriers (RK+KK)</th>
<th>RK vs RR</th>
<th>KK vs RR</th>
<th>RK+KK vs RR</th>
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<td>424</td>
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<td>366</td>
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<td>0.22</td>
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<td>MSD, mm</td>
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<td>2.77±0.37</td>
<td>2.78±0.40</td>
<td>2.77±0.37</td>
<td>0.01</td>
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<td>MOD, mm</td>
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<td>1.81±0.35</td>
<td>1.85±0.35</td>
<td>1.81±0.35</td>
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<td>MI before trial, % (n)</td>
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<td>47.1 (155)</td>
<td>33.3 (12)</td>
<td>45.8 (167)</td>
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<tr>
<td>Events during trial, % (n)</td>
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<td>13 (41)</td>
<td>11 (4)</td>
<td>12 (45)</td>
<td>0.10</td>
<td>0.49</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>Total events, % (n)</td>
<td>59 (248)</td>
<td>52 (170)</td>
<td>39 (14)</td>
<td>50 (184)</td>
<td>0.06*</td>
<td>0.03†</td>
<td>0.02‡</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SD or % (n).

*RK vs RR: odds ratio, 0.75; 95% CI, 0.56–1.01.
†KK vs RR: odds ratio, 0.45; 95% CI, 0.22–0.91.
‡RK+KK vs RR: odds ratio, 0.72; 95% CI, 0.54–0.95.
presence or absence of CAD in the cohort (Table 5). Furthermore, TG were reduced in KK individuals in each of the cohorts compared with the matched RR pairs. Because trends were evident in each of the cohorts, we combined the results in a meta-analysis to increase statistical power. HDL-C was significantly increased in homozygous carriers compared with noncarriers (P = 0.02). Furthermore, there was a strong trend toward decreased TG in KK individuals compared with RR individuals.

Other ABCA1 cSNPs Influence Plasma Lipid Levels and Risk of CAD

Carriers of the V825I cSNP (n = 103 VI + 4 II) had no obvious differences in lipid levels or baseline MSD or MOD (Table 6), but they did have a significantly increased number of events during the trial (44% versus 33% in noncarriers, P = 0.0008; odds ratio, 2.31; 95% CI, 1.41 to 3.83).

Although there were no differences in mean lipid levels between the genotypes in carriers of the 1883M cSNP (IM+MM, Table 6), MM individuals (n = 14) had an increased progression in MOD (mean change, 0.53±0.79 versus 0.11±0.25 mm in noncarriers, P < 0.001) and a cardiac event rate double that of the II individuals (n = 320; 21.4% versus 10.6%, P = 0.19). The genotype frequencies of this variant in the REGRESS population were not consistent with Hardy-Weinberg equilibrium (P < 0.01), with too few heterozygotes observed. These findings contrast with those of a recent report that suggests that homozygous carriers of this cSNP have increased HDL-C.14

Carriers of R1587K (RK+KK) had decreased HDL-C compared with noncarriers in an allele dose-dependent trend (0.86±0.16, 0.91±0.23, and 0.94±0.23 mmol/L, respectively, for 58 KKs, 288 RKs, and 433 RRs; Table 6). On multiple regression analysis including age, BMI, smoking, and TG as covariates, the R1587K genotype remained a significant predictor of HDL-C (P = 0.03). However, no significant differences in CAD or events during the trial were evident in carriers compared with noncarriers.

No homozygous carriers were detected for any of the rare cSNPs (<10%). Heterozygous carriers of V399A had a trend toward higher HDL-C compared with noncarriers. Interestingly, no coronary events were observed in the VA group

### TABLE 4. Baseline Demographics and Lipid Levels in the REGRESS Cohort by R219K Genotype

<table>
<thead>
<tr>
<th></th>
<th>RR</th>
<th>RK</th>
<th>KK</th>
<th>RK+KK</th>
<th>RR vs RK</th>
<th>KK vs RR</th>
<th>RK+KK vs RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>424</td>
<td>330</td>
<td>36</td>
<td>366</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>57±8</td>
<td>55±8</td>
<td>57±7</td>
<td>55±8</td>
<td>0.0007</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.8±2.6</td>
<td>26.3±2.7</td>
<td>25.5±2.3</td>
<td>26.2±2.7</td>
<td>0.01</td>
<td>0.50</td>
<td>0.09</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.02±0.86</td>
<td>6.07±0.89</td>
<td>5.89±0.85</td>
<td>6.06±0.89</td>
<td>0.44</td>
<td>0.38</td>
<td>0.60</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>0.92±0.22</td>
<td>0.93±0.23</td>
<td>0.92±0.20</td>
<td>0.93±0.23</td>
<td>0.54</td>
<td>1</td>
<td>0.81</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>4.27±0.75</td>
<td>4.35±0.83</td>
<td>4.33±0.82</td>
<td>4.35±0.83</td>
<td>0.17</td>
<td>0.65</td>
<td>0.19</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.84±0.77</td>
<td>1.78±0.78</td>
<td>1.42±0.49</td>
<td>1.74±0.76</td>
<td>0.29</td>
<td>0.001</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Values are mean±SD.
(versus 14% in VVs). Carriers of V399A had half the frequency of a positive family history of CAD (22.2% versus 49.4%, P=0.18) and trends toward an increased baseline MOD (Table 6) and less progression in MSD (0.05±0.10 mm, 0.08±0.19 mm in noncarriers, P=0.16) during the trial. However, because the number of carriers was small, conclusions regarding the relationship of this variant to increased HDL-C and decreased CAD cannot be drawn.

Carriers of the V771M (n=37 VM) had decreased focal atherosclerosis (MOD) compared with noncarriers (Table 6) and a trend toward less diffuse atherosclerosis (increased MSD). Carriers of V771M had no difference in lipid levels compared with noncarriers. However, all but 2 carriers of V771M were also carriers of R219K.

Carriers of the other 3 rare variants (T774P, K776N, and E117SD) showed no significant differences in lipid levels or CAD compared with their respective noncarriers (Table 6).

No carriers of S1731C were detected in the REGRESS population. This variant was initially found in 1 of our French Canadian FHA families (FHA28). The presence of this variant in individuals heterozygous for the R2144X ABCA1 mutation was associated with further significantly decreased HDL-C compared with R2144X carriers without this polymorphism (0.16±0.04 mmol/L, n=2, versus 0.64±0.14 mmol/L, n=10; P=0.0009). In unaffected family members, although carriers of S1731C (n=6) had slightly lower HDL-C compared with noncarriers (n=14, 1.03±0.22 versus 1.09±0.23 mmol/L), the difference was not statistically significant. This variant has been identified in other French Canadians.

### TABLE 6. ABCA cSNPs in REGRESS Case-Control Cohorts

<table>
<thead>
<tr>
<th>Carrier</th>
<th>MOD, mm</th>
<th>Carrier</th>
<th>MSD, mm</th>
<th>Carrier</th>
<th>HDL-C, mmol/L</th>
<th>Carrier</th>
<th>TG, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>V825I</td>
<td>1.74±0.37 (107)</td>
<td>1.77±0.35 (573)</td>
<td>0.39</td>
<td>2.70±0.38</td>
<td>2.75±0.38</td>
<td>0.21</td>
<td>0.91±0.23</td>
</tr>
<tr>
<td>I883M</td>
<td>1.74±0.38 (100)</td>
<td>1.75±0.36 (320)</td>
<td>0.71</td>
<td>2.69±0.38</td>
<td>2.73±0.36</td>
<td>0.41</td>
<td>0.91±0.22</td>
</tr>
<tr>
<td>R1587K</td>
<td>1.77±0.34 (346)</td>
<td>1.76±0.37 (433)</td>
<td>0.75</td>
<td>2.73±0.39</td>
<td>2.74±0.36</td>
<td>0.64</td>
<td>0.90±0.22</td>
</tr>
<tr>
<td>V399A</td>
<td>1.92±0.32 (9)</td>
<td>1.73±0.35 (540)</td>
<td>0.13</td>
<td>2.73±0.40</td>
<td>2.71±0.37</td>
<td>0.89</td>
<td>1.03±0.28</td>
</tr>
<tr>
<td>V771M</td>
<td>1.89±0.38 (37)</td>
<td>1.76±0.37 (598)</td>
<td>0.045</td>
<td>2.83±0.49</td>
<td>2.73±0.37</td>
<td>0.13</td>
<td>0.91±0.20</td>
</tr>
<tr>
<td>T774P</td>
<td>1.63±0.31 (4)</td>
<td>1.76±0.36 (621)</td>
<td>0.47</td>
<td>2.85±0.34</td>
<td>2.73±0.37</td>
<td>0.52</td>
<td>0.85±0.07</td>
</tr>
<tr>
<td>K776N</td>
<td>1.92±0.33 (5)</td>
<td>1.78±0.34 (546)</td>
<td>0.48</td>
<td>2.95±0.48</td>
<td>2.76±0.37</td>
<td>0.36</td>
<td>0.94±0.28</td>
</tr>
<tr>
<td>E117SD</td>
<td>1.80±0.39 (34)</td>
<td>1.77±0.36 (610)</td>
<td>0.67</td>
<td>2.78±0.35</td>
<td>2.74±0.37</td>
<td>0.42</td>
<td>0.93±0.23</td>
</tr>
</tbody>
</table>

Values are mean±SD (n).

The Phenotypic Effects of R219K Are Independent of Other cSNPs

There is linkage disequilibrium between cSNPs in the ABCA1 gene. Two rare cSNPs (V771M and K776N) are most commonly found in individuals carrying the R219K K allele. If all V771M and K776N carriers are excluded, the results are unaltered, with increased MOD (1.80±0.35 versus 1.73±0.35 mm, P=0.006) and MSD (2.76±0.36 versus 2.70±0.37 mm, P=0.02) and lower mean TG levels (1.71±0.75 versus 1.84±0.77 mmol/L, P=0.02) in carriers of R219K (n=329) compared with noncarriers (n=422).

The I883M and R1587K cSNPs are also often seen in carriers of R219K. We identified R219K carriers who do not also carry either the I883M or R1587K genotype (n=62) and compared them with the group of individuals who do not carry any of the 3 variants (n=116). MSD was still significantly increased in R219K carriers compared with noncarriers (2.81±0.37 versus 2.69±0.36 mm, P=0.04); MOD was increased in carriers (1.78±0.39 versus 1.73±0.38 mm); and TG remained significantly decreased in carriers (1.67±0.76 versus 1.97±0.74 mmol/L, P=0.02). Thus, the effects of the R219K variant described herein are not due to other cSNPs that are found in linkage disequilibrium with it.

The V825I cSNP was found to be in linkage disequilibrium with I883M. The relative risk of the V825I carriers adjusted for I883M genotype was 2.31 (95% CI, 0.78 to 6.85). Because the effects of the I883M variant were only evident in homozygous carriers, the number of individuals was too few to correct for V825I genotype.

The E1172D cSNP was found exclusively in carriers of the R1587K variant. Excluding carriers of E1172D (n=34), a trend toward decreasing HDL-C with the R1587K K allele was still evident (0.87±0.18 mmol/L in KK, 0.92±0.23 mmol/L in RK, and 0.94±0.23 mmol/L in RR, P=0.19). It is likely this no longer remained significant because the number of KK individuals was decreased by 50%. No significant differences in lipid levels or CAD were observed for E1172D carriers compared with R1587K heterozygotes without E1172D. Thus, the effects of the R1587K cSNP are

### TABLE 6. ABCA cSNPs in REGRESS
not due to the nonfunctional E1172D variant, with which it is in linkage disequilibrium.

Discussion

Here we present a complete cSNP analysis of the ABCA1 gene, providing evidence that common genetic variations within ABCA1 are associated with altered plasma lipid levels and risk of CAD. The R219K variant, with a carrier frequency of 46% in European populations, is associated with a decreased severity of CAD, which manifests as decreased focal and diffuse atherosclerosis, with less progression and decreased coronary events. The increased risk associated with the wild-type allele may account for up to 5% of the population risk of coronary events. The phenotypic effects of the remaining cSNPs are less striking than those of R219K. Further analysis from additional large cohorts will be required to validate these findings.

Both the finding of decreased TG and of increased HDL-C in younger carriers of the R219K K allele is consistent with the decreased CAD observed in carriers of the variant.15,16 TG levels showed similar trends in our replication groups, and increased HDL-C levels in R219K carriers were observed in our independent populations. No obvious difference in cholesterol efflux level between carriers (n=2) and noncarriers (n=4) was detected; this was probably influenced by the small numbers and the ~15% interassay coefficient of variation in the efflux assay, which makes it impossible to detect small differences in efflux. The phenotypic effects of this variant are opposite to those in individuals heterozygous for ABCA1 mutations, suggesting this variant is associated with a gain of normal ABCA1 function and increased RCT.

The lack of obvious differences in HDL-C in carriers of different cSNPs (R219K, V771M, and I883M), together with clear differences in CAD, suggests that stimulating the RCT pathway can increase the net flux of cholesterol toward the liver without altering steady-state plasma HDL-C levels. This increase in reverse cholesterol transport (RCT) activity may directly reduce the development of atherosclerosis without necessarily altering plasma lipid levels.

The mechanism underlying the decreased TG in carriers of the R219K variant is unknown. Cholesterol ester transfer protein activity results in the equilibration of the core components of lipoprotein particles.17 Cholesterol esters are transferred from HDL to TG-rich lipoproteins, while TG are transferred in reverse. Increased ABCA1 activity, resulting in increased HDL-C, might trigger increased cholesterol ester/TG exchange. Hepatic lipase efficiently hydrolyzes the TG component of HDL.18 Thus, increased transfer of TG to HDL may ultimately increase TG catabolism. Alternatively, alterations in ABCA1 activity have been suggested to alter intracellular lipid transport.19,20 Several genes involved in lipid metabolism are differentially regulated in ABCA1-deficient mice.19 Changes in intracellular cholesterol and phospholipid metabolism triggered by increased ABCA1 activity21 might lead to the diversion of fatty acids from TG synthesis to phospholipid synthesis, resulting in decreased TG secretion by the liver and reduced plasma TG levels.

The phenotype in individuals heterozygous for ABCA1 mutations is modified by age. In heterozygotes, the phenotype is more pronounced in older individuals.9 This suggests that ABCA1 activity may normally increase with age but that this is blunted in R219K heterozygotes. Age-related increases in the expression and activity of P-glycoprotein, another ATP-binding cassette transporter, have been described.22,23 In the present study, we show that the R219K polymorphism was also associated with an altered relationship between age and HDL-C. In noncarriers, there was a general increase in cholesterol efflux and HDL-C with age, which is suggestive of increased ABCA1 function. However, in carriers of the K allele, this age-dependent increase in both HDL-C and efflux was not evident, suggesting this variant is already associated with maximal efflux levels and is not responsive to regulation by age.

This high frequency of cSNPs emphasizes the importance of verifying that putative mutations observed within the gene are not, in fact, cSNPs. Of note, the V399A and I883M variants were shown to cosegregate on a mutation-bearing chromosome in one of the initial Tangier families described.6 The authors suggested that 1 of these 2 variants was likely the functional mutation. Yet, here we show that the V399A variant was associated with a trend toward increased HDL-C. Furthermore, we show that I883M is a common variant that is possibly associated with an increased risk of CAD in the homozygous state, although no differences in HDL-C were evident. Neither variant was associated with the marked decrease in HDL-C seen in individuals heterozygous for ABCA1 mutations. Thus, without proper analysis of missense changes in a large, ethnically matched cohort, cSNPs can be inappropriately confused with disease-causing mutations.

The distribution of cSNPs was not random (Figure 1); they were found away from known functional domains, such as the ATP-binding cassettes and regions where mutations cluster.9 The one exception to this pattern was the I883M variant, which was located just N-terminal of the first ATP-binding cassette region, where several mutations have been shown to occur (amino acids 909 to 937).24 Because this variant was associated with little functional effect, it might demarcate the border of the region in which structural alterations significantly impair ATP-binding cassette function. Similarly, the region containing the V771M, T774P, and K776N variants is unlikely to be critical to ABCA1 function, because a high degree of polymorphism is tolerated without functional effects.

We showed that common ABCA1 cSNPs are associated with altered plasma lipid levels and severity of atherosclerosis. Specifically, the frequent R219K variant is associated with a decreased severity of atherosclerosis, a decreased risk of coronary events, decreased TG, and increased HDL-C, which is consistent with a gain of function in ABCA1. These effects were independent of any other cSNPs found in association with R219K and were seen both in different measures of CAD and in multiple cohorts. These findings emphasize the importance of common genetic variation in ABCA1 in the general population in determining plasma lipid levels and severity of CAD.

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References
Common Genetic Variation in ABCA1 Is Associated With Altered Lipoprotein Levels and a Modified Risk for Coronary Artery Disease


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