Common ABCA1 variants, HDL levels, and cellular cholesterol efflux in subjects with familial low HDL

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Abstract HDL promotes cholesterol efflux from peripheral cells via ABCA1 in the first step of reverse cholesterol transport (RCT). We investigated whether the early steps of RCT were disturbed in subjects with familial low HDL and an increased risk for early atherosclerosis. Cholesterol efflux from monocyte-derived macrophages to lipid-free apolipoprotein A-I (apoA-I; %) was measured in 22 patients with familial low HDL without Tangier disease mutations and in 21 healthy controls. In addition, we defined the different alleles of ABCA1 using single-nucleotide polymorphism haplotypes and measured ABCA1 and ABCG1 mRNA transcript levels in cholesterol-loaded macrophages. Similar ABCA1-mediated cholesterol efflux levels were observed for macrophages derived from control subjects and from low-HDL subjects. However, when efflux of cholesterol was estimated as cholesterol efflux to apoA-I (%)/relative ABCA1 mRNA expression level, cholesterol removal was significantly ($P = 0.001$) lower in the low-HDL group. Cholesterol-loaded macrophages from low-HDL subjects showed significantly increased levels of ABCA1 mRNA but not of ABCG1 mRNA and were more often carriers of the rare ABCA1 alleles L158 and R219K. These results suggest that defective ABCA1 function in cholesterol-loaded macrophages is one potential contributor to the impaired RCT process and the increased coronary heart disease risk in subjects with familial low HDL.—Soro-Paavonen, A., J. Naukkarinen, M. Lee-Rueckert, H. Watanabe, E. Rantalà, S. Soderlund, A. Hiukka, P. T. Kovanen, M. Jauhiainen, L. Peltonen, and M-R. Taskinen. Common ABCA1 variants, HDL levels, and cellular cholesterol efflux in subjects with familial low HDL. J. Lipid Res. 2007. 48: 1409–1416.

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The principal antiatherogenic function of HDL is its ability to promote the efflux of cholesterol from peripheral cells and transport it to the liver for excretion, process termed reverse cholesterol transport (RCT) (1). This concept was further confirmed once a homozygous defect in ABCA1 [Mendelian Inheritance in Man (MIM) 205400 and 600046] was discovered to cause Tangier disease, a condition characterized by a virtual absence of HDL in plasma and a resultant accumulation of cholesteryl esters within cells (2–4). The ABCA1 protein mediates the efflux of phospholipids and unesterified cholesterol from peripheral cells to HDL. A lipid-poor apolipoprotein A-I (apoA-I)-phospholipid complex, the preβ-HDL particle, is the most effective cholesterol acceptor in ABCA1-mediated efflux (5). The activity of ABCA1 in the liver is the major contributor to the generation of HDL, accounting for ~80% of the circulating HDL reserve (6, 7). Based on these estimations of the contribution of liver and intestine to the HDL plasma pool, it can be stated that the monocyte/macrophage ABCA1 only minimally affects plasma HDL levels (8). Therefore, hepatic ABCA1 is critical in maintaining the circulation of mature HDL particles by direct lipidation of lipid-poor apoA-I, as it slows the HDL catabolism in the kidney and prolongs its residence time in plasma.

In subjects heterozygous for a defective allele of the ABCA1 gene, cholesterol efflux is decreased to approximately half of that in normal individuals (9, 10). The heterozygotes also have increased carotid artery intima-
media thickness (IMT) and low high density lipoprotein-cholesterol (HDL-C) and thus are at increased risk for the development of early atherosclerosis (11). The association between ABCA1 variants and HDL-C levels was initially reported in French-Canadian families with familial hypopalphalipoproteinemia (3) and subsequently in Dutch, North American, and Danish study cohorts (9, 12–14). Recently, it was shown that up to 10% of people in the lowest HDL-C percentile are heterozygous for rare mutations in ABCA1 (14). However, reports also exist of the lack of association between ABCA1 variants and the level of HDL-C (15–17).

In addition to ABCA1, macrophages also express other ABC transporters such as ABCG1 (18, 19). In contrast to ABCA1, which interacts with the lipid-poor preβ-HDL, ABCG1 transports cellular cholesterol predominantly to the large spherical HDL2 and HDL3 particles (19–21). It is likely that a synergistic action of ABCA1 and ABCG1 is needed for an effective RCT process and the resultant protection against atherosclerosis (22).

The deleterious clinical phenotype of patients with low levels of HDL-C could be the result of decreased cholesterol efflux from peripheral cells to HDL, a defect of nascent HDL as a cholesterol acceptor, or a combination of both. We analyzed the efficiency of cholesterol efflux from peripheral cells to HDL, a defect of HDL-C (15–17).

The study comprised two cohorts. First, functional efflux studies were performed using cultured macrophage foam cells derived from 43 male subjects: 22 low-HDL subjects and 21 control subjects. The clinical and biochemical characteristics of the study populations participating in the efflux study are shown in Table 1. Second, genotyping of single-nucleotide polymorphisms (SNPs) using the array technology was carried out in 72 individuals (including those participating in the efflux study): 28 affected family members with low HDL (of whom 6 were women), 19 unaffected family members (of whom 12 were women), and 25 control subjects (of whom 4 were women). The clinical and biochemical parameters of the 72 individuals are provided in supplementary Table I.

Analytical methods

Fasting levels of lipids, apolipoproteins, glucose, and insulin were measured by standardized techniques as described previ-
ously (17, 23). Preβ-HDL was quantified by crossed immunoelectrophoresis (24, 25) and expressed as a percentage of the sum of α-HDL and preβ-HDL areas. The preβ-HDL mass is expressed in absolute amount (mg/dl apoA-I present in preβ-HDL in serum). HDL particle size was determined by native gradient gel electrophoresis (26). Cholesterol ester transfer protein (CETP) and phospholipid transfer protein (PLTP) activities were measured as described previously (23), and PLTP mass was measured using the PLTP ELISA method (27).

DNA was extracted from blood samples and used for genotyping of SNPs using the array technology (28). SNPs were chosen by interrogating the HapMap data and other public databases and on the basis of the polymorphisms identified in our earlier sequencing effort (17). All genotyped SNPs were in Hardy-Weinberg equilibrium.

**Measurement of apoA-I-mediated cholesterol efflux from cultured macrophages**

Human monocyte-derived macrophages were obtained from human whole blood by cell culturing. Fasting blood (60 ml) was drawn into tubes containing citrate as an anticoagulant. Buffy coat was promptly separated by low-speed centrifugation (1,500 g) at room temperature. Diluted buffy coat was then layered over Ficoll-Paque and centrifuged again (2,000 g, 40 min), and the mononuclear cells were recovered as a cell layer. Subsequently, three washes with PBS were performed to eliminate platelets from the mononuclear pellet. Finally, the cell pellet was suspended in DMEM. Mononuclear cells were plated onto 24-well plates (2 million cells per well) and allowed to stay in the wells for 1 h for attachment, after which macrophage medium with Macrophage Colony-stimulating Factor (M-CSF) was added. The medium was then changed every 2–3 days. After 7 days, when the monocytes had been phenotypically converted to macrophages, they were labeled with [3H]cholesteryl linoleate by incubating them for 48 h with 25 μg/ml [3H]cholesterol linoleate-acetyld-LDL. This loading procedure induced the formation of a cellular pool of radioactively labeled cholesterol esters. To induce ABCA1-dependent cholesterol efflux from the radiolabeled macrophages, purified human lipid-free apoA-I (10 μg/ml; kindly provided by Dr. Peter Lerch of the Swiss Red Cross) was added to the medium as cellular cholesterol acceptor (29). After incubation for 16 h, an interval within which efflux is linear (30), incubation medium was collected. Radioactivity in the medium and in washed cells was determined by scintillation counting, and the fractional cholesterol efflux (as percentage) was calculated as dpm_{medium}/(dpm_{cell} + dpm_{medium}) × 100. Efflux values to incubation medium in the absence of apoA-I were subtracted from those in the presence of apoA-I.

**Expression analyses**

Monocyte-derived macrophages were collected for mRNA extraction 1) after cholesterol loading, 2) after efflux in the absence of apoA-I, and 3) after efflux in the presence of apoA-I. Macrophage mRNA was extracted and quantified as described previously (31). Quantitative PCR, using the SYBR-Green assay (Applied Biosystems), was done to measure the relative abundance of transcripts. Two-step RT-PCR was done using the TaqMan Gold RT-PCR kit. Primer sequences are available on request. To compare the relative ABCA1 and ABCG1 expression levels at different stages, the relative mRNA expression in each experiment was normalized against the expression level of the housekeeping gene GAPDH. The reliability of GAPDH as a housekeeping gene was evaluated by studying the expression of GAPDH in a sample of 47 fat biopsies assayed on Affymetrix U133 Plus 2.0 chips according to standard protocols. Samples were obtained from individuals from the same dyslipidemic families as the monocyte-derived macrophages used in this study. Correlation of housekeeping genes to any possible confounders, such as age, sex, body mass index (BMI), HDL, total cholesterol (TC), and triglyceride (TG), was evaluated by the Pearson correlation using SPSS 11.0 statistical software (SPSS, Inc., Chicago, IL). No correlation to any of these variables could be identified for GAPDH expression, and the distribution of expression values of GAPDH in the macrophage samples was stable and comparable between the low-HDL and control groups.

**Carotid artery ultrasonography**

B-mode ultrasound imaging was used to measure the intima-media complex of the carotid artery wall with a Hewlett-Packard (Andover, MA) Image Point M2410A ultrasound system as described previously (32). The average of all mean IMT measurements (mean IMT) over 28 sites (the far wall and the near wall of six arterial segments: right and left common carotid artery, carotid bulb, and proximal internal carotid artery) was chosen as the primary outcome variable. A single physician (A.H.) carried out all ultrasound examinations. To estimate the intraobserver variability in the scannings, 10 subjects were scanned twice on two different occasions. The intraobserver repeatability for mean IMT was 0.971 with a SEM of 0.029.

**Statistical methods**

Statistical comparisons of clinical and biomedical parameters were performed with SPSS 11.0 for Windows. Differences in continuous variables between groups were analyzed by ANOVA. P < 0.050 (two-tailed t-test) was considered significant. Variables with skewed distribution were log_{10}-transformed before the analyses. A Chi-square test with Fisher’s exact test was used to determine significant differences in the allele frequencies and categorical variables between groups. Bivariate correlations between the continuous variables were calculated using the parametric Pearson correlation analysis. Adjustment for BMI and waist circumference was performed by treating these factors as covariates in the General Linear Model Univariate analysis and saving the nonstandardized residual value as the adjusted variable. Adjustment for statin use was performed by treating this as a fixed factor in the General Linear Model Univariate analysis. Multivariate stepwise linear regression analysis was conducted by removing the independent variables from the model until the best-fitting model with the maximum multiple R² was achieved.

**RESULTS**

**Clinical characteristics of the study subjects**

Clinical and biochemical characteristics of the study subjects are presented in Table 1 and supplementary Table I. Low-HDL subjects and controls had similar age distribution. By definition, HDL-C and apoA-I were decreased in the low-HDL group. They also had significantly lower total preβ-HDL mass and smaller mean HDL particle size than the controls. The low-HDL subjects had increased levels of TG and TC, and they had higher waist circumference and BMI than the controls. Hypertension was recorded in nine low-HDL subjects and five controls, and six low-HDL subjects were currently smokers compared with one in the control group. Mean IMT was increased significantly in the low-HDL group (1.02 ± 0.21 vs. 0.86 ± 0.17 mm; P = 0.007). This difference remained statistically significant. 

**Defective cholesterol efflux in familial low HDL**
after adjustment for BMI ($P = 0.022$) and marginally significant after adjustment for waist circumference ($P = 0.053$). From the two lipid transfer proteins analyzed, significantly increased CETP activity was observed among the low-HDL subjects, whereas PLTP activity did not differ between the groups. PLTP mass was lower in low-HDL subjects. The prevalence of CHD was high within the low-HDL group (17 individuals), and 13 of them were on statin medication. The lipid values originally used for the phenotype determination in the recruitment phase were taken before lipid-lowering medication was started, but the values presented in the text and tables were acquired during medication. Because statins affect lipid status and can cause bias in the interpretation of the metabolic data, we divided the individuals within the low-HDL group into two subgroups based on their statin use. All 15 affected low-HDL subjects treated with statins had CHD, whereas among nonusers, 3 were CHD patients. Those who were treated with statins had similar cholesterol efflux (%) from macrophages compared with that among nonusers. As expected, TC was decreased significantly in the statin users. The two subgroups did not differ in age, HDL-C, apolipoprotein, or preβ-HDL levels. The $P$ values for the between-group comparisons in Table 1 are presented before and after adjustment for statin use. None of the study subjects was diabetic.

Macrophage cholesterol efflux to apoA-I and its relation to ABCA1 mRNA expression in cholesterol-loaded macrophages

We first investigated whether monocyte-derived macrophages of low-HDL subjects would display signs of dysfunction in the initial step of RCT. As shown in Fig. 1A, the low-HDL subjects ($n = 22$) displayed similar ABCA1-mediated cholesterol efflux to lipid-free apoA-I (calculated as percentage efflux) as the control group ($n = 21$) ($5.1 \pm 1.3$ vs. $5.7 \pm 1.3$; $P = 0.133$). This result did not change after adjustment for statin therapy. There were no differences in cholesterol efflux levels in the low-HDL subjects with CHD ($n = 16$) compared with those without CHD ($n = 6$) (see supplementary Table II). In contrast, when cholesterol efflux was normalized to macrophage protein content (dpm in medium/μg cell protein/18 h), the rate of efflux was slightly slower in the low-HDL group compared with that in the control group (Fig. 1B). Importantly, the ability to become loaded with cholesteryl esters after incubation with acetyl-LDL did not differ between the macrophages derived from the low-HDL subjects and those derived from the control subjects (data not shown).

We next analyzed steady-state transcripts of ABCA1 and ABCG1 in the cultured monocyte-macrophages. Sufficient amounts of mRNA were available from only 10 low-HDL and 11 control subjects. Notably, the relative ABCA1 expression in cholesterol-loaded macrophages was significantly higher in the low-HDL group than in the control group (Fig. 2A). The absolute ABCA1 expression levels decayed after cholesterol efflux to apoA-I, and the differences between the groups disappeared. Unlike the transcript levels of ABCA1, the relative ABCG1 transcript levels did not differ significantly between the groups either in macrophages after cholesterol loading or in macrophages after the efflux period (data not shown). In light of this clear difference in ABCA1 transcript levels but no clear difference in percentage efflux to apoA-I, we next expressed efflux by cholesterol efflux to apoA-I (%)/relative ABCA1 mRNA expression. This ratio was significantly reduced in the low-HDL group compared with the controls ($3.93 \pm 2.30$ vs. $7.85 \pm 2.14$; $P < 0.001$) (Fig. 2B). This difference remained statistically significant after adjustment for BMI ($P = 0.004$) and waist circumference ($P = 0.016$) but not after adjustment for lipid medication. ABCG1 expression in macrophages of low-HDL subjects followed the same trend as that of ABCA1, being highest in the cholesterol-loaded cells and declining during the efflux process (data not shown).

Next, we analyzed potential correlations between efflux (using cholesterol efflux percentage to apoA-I) and various parameters listed in Table 1. In the low-HDL group, cholesterol efflux showed a positive association with circulating preβ-HDL levels ($r = 0.504$, $P = 0.017$), whereas no correlation could be observed in the control group. Cholesterol efflux did not correlate significantly with mean IMT or HDL-C in the low-HDL group or in the control group (data not shown). The mean IMT was strongly positively correlated with age in both low-HDL subjects ($r = 0.709$, $P < 0.001$) and control subjects ($r = 0.746$, $P < 0.001$). In the multivariate linear regression model for mean IMT, the variables that entered the final model were
age, cholesterol efflux, and smoking, with an adjusted multiple $R^2$ of 0.613 (in low-HDL and control subjects pooled together; $n = 43$) (individual correlation coefficients are presented in Table 2).

### Allelic diversity of the ABCA1 gene and HDL levels

Motivated by the observed differences in ABCA1 expression, we first addressed the allelic diversity of the genes in the efflux experiment ($n = 21$) by genotyping 15 intragenic SNPs of the ABCA1 gene. Analysis of the genotypes revealed an association of two SNPs with the ABCA1-mediated cholesterol efflux (rs2740492, $P = 0.030$; rs3858075, $P = 0.018$). The genotyping was subsequently extended to include the nonaffected family members ($n = 72$). Three SNPs differed significantly between the low-HDL family members and control subjects (L158 (rs2230805), $P = 0.004$; R219K (rs2230806), $P = 0.005$; and rs2297409, $P = 0.006$), so that the rare alleles of each of these SNPs were overrepresented among low-HDL subjects (Table 3). The variants L158 and R219K showed significant association with low HDL-C levels ($P = 0.009$ and $P = 0.007$, respectively), and T1427 showed association with high HDL-C ($P = 0.028$). One SNP (L158) showed association with lower cholesterol efflux ($P = 0.009$), and one SNP (rs4149341) showed association with increased mean IMT ($P = 0.025$). We constructed allelic haplotypes of the three SNPs that showed association with either high or low HDL-C levels (L158, R219K, and T1427) using PHASE version 2.0 software. Because of the linkage disequilibrium between these variants, only five different haplotypes could be identified in our study sample. The carrier status for the most common haplotype correlated with the HDL-C levels in a dose-dependent manner: the mean HDL-C level of individuals with two copies was 1.30 mmol/l, that for individuals with one copy was 1.06 mmol/l, and that for individuals with no copies was 0.85 mmol/l ($P = 0.025$, Kruskal-Wallis test). The likelihood of an individual belonging to the low-HDL group when carrying zero, one, or two copies of this allelic

### Table 2. Multivariate regression analysis for mean IMT and predictor variables when low HDL subjects ($n = 22$) and control subjects ($n = 21$) were pooled together ($n = 43$)

| Independent Variables       | Standard Coefficient | SEM   | $P$  |
|----------------------------|----------------------|-------|-----|
| Age                        | 0.547                | 0.001 | <0.001 |
| Cigarette years            | 0.356                | 0.001 | 0.003 |
| Cholesterol efflux (%)     | -0.227               | 0.007 | 0.042 |
| Adjusted multiple $R^2$    |                      |       | 0.613 |

Variables excluded from the model were preβ-HDL (%), apoAI, apoA-II, HDL-C, BMI, TC, apoB, CETP activity, PLTP activity, systolic blood pressure, TG, homeostasis model assessment of insulin resistance (HOMA IR), HDL particle size, waist circumference, and statin use.

### Table 3. Comparisons of the ABCA1 allele frequencies between 47 low-HDL family members (19 unaffected and 28 affected subjects) and 25 control subjects, and ABCA1 genotype effects on cholesterol efflux, HDL-C, and mean IMT in the pooled study group ($n = 72$)

| Single-Nucleotide Polymorphism | Allele Frequencies and Mean IMT $P^c$ | Allele Frequencies in Low-HDL Family Members Versus Control Subjects $P^b$ | Allele Frequencies in High HDL Family Members Versus Control Subjects $P^a$ |
|-------------------------------|---------------------------------------|-------------------------------------------------|----------------------------------|
| rs2472459                     | [rs, rs, rs]                           | NS                                             | NS                               |
| rs2246293                     | [rs, rs, ns]                           | NS                                             | NS                               |
| rs2516516                     | [ns, ns, ns]                           | NS                                             | NS                               |
| rs1809978                     | [ns, ns, ns]                           | NS                                             | NS                               |
| rs2740492                     | [ns, ns, ns]                           | NS                                             | NS                               |
| rs3858075                     | [ns, ns, ns]                           | NS                                             | NS                               |
| rs1929842                     | [ns, ns, ns]                           | NS                                             | NS                               |
| rs2230805                     | [L158 (0.45 vs. 0.32) 0.004] 0.009     | NS                                             | NS                               |
| rs2298086                     | [R219K (0.40 vs. 0.08) 0.005] 0.007     | NS                                             | NS                               |
| rs2487037                     | [ns, ns, ns]                           | NS                                             | NS                               |
| rs2297409                     | [ns, ns, ns]                           | NS                                             | NS                               |
| rs2066716                     | [T1427 (0.11 vs. 0.32) 0.053] 0.003     | NS                                             | NS                               |
| rs2230808                     | [R158 (0.30 vs. 0.04) 0.006] 0.007     | NS                                             | NS                               |
| rs2066881                     | [ns, ns, ns]                           | NS                                             | NS                               |
| rs4149341                     | [ns, ns, ns]                           | NS                                             | NS                               |

For statistically significant differences, the corresponding allele frequencies are given in parentheses (in low-HDL family members vs. control subjects), followed by Chi-square $P$ value, with Fisher’s exact test for the differences in allele frequencies between low-HDL family members and control subjects.

ANOVA $P$ value for the ABCA1 genotype effect on HDL-C.

ANOVA $P$ value for the ABCA1 genotype effect on mean IMT.
haplotype was 80, 48, or 30%, respectively. The observed differences in the allele frequencies also remained statistically significant when tested in the study group used in functional analyses for whom cholesterol efflux to apoA-I was measured (22 low-HDL and 21 control subjects). Interestingly, the carrier status of the haplotype containing the R219K variant was not associated with the cholesterol efflux activity, implying that this particular variant does not result in the disturbed function of ABCA1 in macrophages but rather tags the allelic variant related to serum HDL-C level regulation, possibly at the hepatic level.

Interestingly, the carriers of the rare alleles in L158 had decreased efflux (%), like noncarriers in the whole study sample (4.6 ± 1.0 vs. 5.7 ± 1.3; \( P = 0.009 \)). They also had lower HDL-C (1.00 ± 0.35 vs. 1.29 ± 0.46; \( P = 0.011 \)). The relative ABCA1 expression was not significantly different between carriers and noncarriers. The efflux (%) was not changed significantly between carriers and noncarriers of the rare alleles in R219K. However, the carriers had lower HDL-C (0.99 ± 0.34 vs. 1.30 ± 0.46; \( P = 0.008 \)) and increased relative ABCA1 expression (1.61 ± 0.31 vs. 1.02 ± 0.46; \( P = 0.013 \)).

**DISCUSSION**

This study is the first comprehensive analysis of the macrophage cholesterol efflux efficiency in patients with familial low HDL-C, not representing Tangier patients. The ABCA1-dependent cholesterol efflux was measured in a foam cell model measuring 1) cholesterol loading of macrophages isolated from the study subjects and 2) unloading of macrophage-derived foam cells by incubation with lipid-free apoA-I as a primary cholesterol acceptor. We found that macrophages derived from low-HDL subjects expressed similar fractional cholesterol efflux (%) as those isolated from the control subjects. However, their cholesterol efflux estimated as percentage efflux (to apoA-I) relative to the levels of ABCA1 transcripts was reduced significantly. Although low-HDL subjects had markedly increased mean IMT, the arterial thickening was not associated with decreased cholesterol efflux. This finding might be related to the relatively small number of subjects participating in the cholesterol efflux study.

The efflux methodology that we applied to measure ABCA1-facilitated cholesterol efflux to apoA-I has been used widely previously (33, 34). Currently, different methods can be used to calculate the amount of cholesterol efflux from macrophage foam cells to acceptors. In this study, two of the most common approaches were used: 1) cholesterol efflux to apoA-I expressed as fractional efflux (%), and 2) cholesterol efflux to apoA-I expressed as the release of radioactivity to medium relative to cell protein (dpm/µg protein/18 h). Interestingly, although cholesterol efflux calculated as a percentage did not differ between the low-HDL subjects and the control group, there was a trend for reduced efflux (\( P = 0.044 \)) among the low-HDL subjects when the efflux data were normalized to the cell protein. This discrepancy could be attributable to statistical limitations (i.e., comparison of two parameters with scale differences, and the relatively limited number of individuals per group attributable to the analytical complexity of the study). Next, we determined the abundance of ABCA1 transcript after loading of the macrophages and demonstrated significantly higher expression levels in the foam cells derived from low-HDL subjects. To compare the efflux between the two groups, we expressed efflux (as a percentage or normalized for the cellular protein) relative to ABCA1 transcript and found that macrophage foam cells from the low-HDL subjects had reduced efflux of cholesterol compared with the control subjects. The following possibilities must be considered to explain this result. First, it is possible that the transcript levels do not correlate with the ABCA1 protein abundance, because posttranscriptional regulation is an important determinant of ABCA1 activity (35). Second, the ABCA1 protein in the low-HDL subjects may be abnormally unstable; therefore, the observed increase in mRNA expression could reflect a compensatory mechanism caused by a faster degradation rate of the polypeptide. Finally, the observed higher transcript level of ABCA1 may reflect a defective efflux in specific ABCA1 variants in the macrophages of low-HDL patients. The relative mRNA expression levels of ABCG1 in macrophages after either cholesterol loading or apoA-I-mediated efflux were not significantly different between low-HDL subjects and control subjects. However, as observed with ABCA1, the expression of ABCG1 was highest in the macrophage foam cells and decreased significantly after cholesterol efflux, also reflecting the known regulatory effects of cellular cholesterol loading and unloading on both genes (36).

It has been shown that the most effective acceptor of ABCG1-derived cholesterol is the mature, spherical HDL, and not the lipid-poor apoA-I or preβ-HDL (20, 22). This finding may explain the early data indicating that individuals with high levels of large HDL2 particles are protected from atherosclerosis (37, 38). In mice with a targeted disruption of ABCG1, the absence of functional protein causes an accumulation of neutral lipids and phospholipids in macrophages and hepatocytes (39). The human ABCG1 gene is located on chromosome 21q22.3 (40), and to date, eight transcriptional variants have been characterized. However, no human disorders attributable to either heterozygous or homozygous defects in the ABCG1 gene have been described. In turn, some data exist that ABCG1 is overexpressed in macrophages from Tangier patients compared with control macrophages (41).

When addressing the allelic diversity of the ABCA1 gene, we observed that low-HDL subjects carried rare ABCA1 alleles significantly more often than control subjects. Four SNPs differed in allele frequency between the low-HDL and control groups. A previous study in a separate sample of Finnish families with between the low-HDL and control groups. A previous study in a separate sample of Finnish families with between the low-HDL and control groups. A previous study in a separate sample of Finnish families with low-HDL-C reported no effect of the ABCA1 locus on HDL-C levels (16). Negative findings for the common ABCA1 variants were also reported in the Veterans Affairs HDL Intervention Trial and the Framingham Offspring Study (15). In turn, in healthy subjects and CHD patients of European
and French-Canadian origin, the common variant R219K was associated with increased levels of HDL, decreased TGs, and reduced severity of atherosclerosis (42, 43). In a recent elegant approach, a systematic screening of ABCA1 in a large Danish population sample revealed that of the subjects in the lowest percentile of HDL-C levels, 10% are heterozygous for ABCA1 mutations and that common ABCA1 SNPs in fact do contribute to HDL-C concentrations at the population level (14). In this study, we genotyped five of the SNPs reported in the Danish population, of which three were associated with HDL-C levels (ANOVA \( P < 0.050 \)). Of these, T1427 gave corresponding results in both data sets, showing association with increased HDL-C.

The variant R219K showed association with low serum HDL levels but not with the cholesterol efflux in our study sample. Interestingly, the L158 polymorphism (rs2230805) did correlate with percentage efflux; however, being a synonymous SNP, it is in itself unlikely to be functional. Although the common ABCA1 haplotype did not associate with cholesterol efflux but did relate to serum HDL levels, it could be important for the liver-specific processing of ABCA1, thus explaining why it did not correlate with the level of macrophage cholesterol efflux.

A number of study limitations should be considered. First, the limited number of subjects available for the cholesterol efflux experiments may attenuate the statistical power. The variable yield of cells (monocytes) isolated for the efflux experiments and ABCA1 mRNA evaluation is another possible flaw. Complete sequencing of the ABCA1 gene would possibly identify carriers of rare variants in a larger study sample, but as previous sequencing in these Finnish low-HDL families failed to find any novel, rare variants, we are confident that our haplotype analysis based on 15 tagging SNPs exposes the detectable allelic diversity in our samples. Finally, lack of simultaneous data on the actual ABCA1/G1 protein level is a weakness. Specifically, in addition to defining transcript levels of ABCA1 transporters, additional studies are necessary to determine the correlation between cholesterol efflux and the levels of ABCA1/G1 proteins in low-HDL subjects. Because accurate quantification of ABC transporter protein levels cannot be achieved with semiquantitative Western blot analyses, the development of an ELISA-type immunoassay would be necessary for such purposes.

In summary, based on our data, we conclude that 1) macrophages derived from Finnish subjects with familial low HDL displayed similar cholesterol-loading capacity but decreased cholesterol efflux to apoA-I via the ABCA1 pathway after loading; 2) familial low HDL in these families is not caused by rare mutations in ABCA1; rather, the common allelic variants are associated with HDL-C levels, as indicated by the dose-dependent contribution of the haplotype carrying the common R219K change in these subjects; and 3) relative ABCA1 mRNA expression in cholesterol-loaded macrophages was increased significantly in the low-HDL group. We propose that defective ABCA1 function in cholesterol-loaded macrophages is one potential contributor to the impaired RCT process in low-HDL subjects.

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