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

Susanne M. Clee, Aeilko H. Zwinderman, James C. Engert, Karin Y. Zwarts, Henri O. F. Molhuizen, Kirsten Roomp, J. Wouter Jukema, Michel van Wijland, Marjel van Dam, Thomas J. Hudson, Angela Brooks-Wilson, Jacques Genest, Jr, John J. P. Kastelein and Michael R. Hayden

Circulation. 2001;103:1198-1205
doi: 10.1161/01.CIR.103.9.1198
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7322. Online ISSN: 1524-4539

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

Susanne M. Clee, BSc; Aeilko H. Zwinderman, PhD; James C. Engert, PhD; Karin Y. Zwarts; Henri O.F. Molhuizen, PhD; Kirsten Roomp, MSc; J. Wouter Jukema, MD, PhD; Michel van Wijland, MSc; Marjel van Dam, MD; Thomas J. Hudson, MD; Angela Brooks-Wilson, PhD; Jacques Genest, Jr, MD; John J.P. Kastelein, MD, PhD; Michael R. Hayden, MB, ChB, PhD

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.[1] Since then, a strong inverse relationship between plasma HDL-C levels and CAD has been confirmed in a large number of epidemiological studies.[2,3] Low plasma HDL-C is now generally accepted as a strong and independent risk factor for the development of premature atherosclerosis.

A rare form of genetic HDL deficiency is Tangier disease,[4] 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 ATP-binding cassette transporter 1 gene (*ABC1*, *ABCA1*) as the molecular defect in Tangier disease[5–7] and familial HDL deficiency associated with reduced cholesterol efflux (FHA).[5,8]

Individuals heterozygous for mutations in the *ABCA1* gene have decreased HDL-C, increased triglycerides (TG), and an increased risk of CAD.[9] 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.[9] 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.

Received December 28, 2000; revision received January 23, 2001; accepted January 23, 2001.

From the Centre for Molecular Medicine and Therapeutics, University of British Columbia, Vancouver, Canada (S.M.C., K.Y.Z., M.R.H.); the Department of Medical Statistics, Leiden University Medical Center, Leiden, the Netherlands (A.H.Z.); Montreal Genome Centre, McGill University Health Centre, Montreal, Canada (J.C.E., T.J.H.); the Department of Vascular Medicine, Academic Medical Centre, Amsterdam, the Netherlands (H.O.F.M., M.v.W., M.v.D., J.J.P.K.); Xenon Genetics, Inc, Vancouver, Canada (K.R., A.B.-W.); the Department of Cardiology, Leiden University Medical Center and Interuniversity Cardiology Institute, Leiden, the Netherlands (J.W.J.); and the Cardiovascular Genetics Laboratory, McGill University Health Centre, Montreal, Canada (J.G.).

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

This article originally appeared Online on February 12, 2001 (*Circulation*. 2001;103:r13-r20).

Correspondence to Michael R. Hayden, Centre for Molecular Medicine and Therapeutics, 980 W. 28th Avenue, Vancouver, BC, Canada V5Z 4H4. E-mail mrh@cmmt.ubc.ca

TABLE 1. Methods for Restriction Fragment Length Polymorphism Screening of *ABCA1* cSNPs

Variant	pmol of Each Oligo	Forward Oligo (5′→3′)* Reverse Oligo (5′→3′)*	Annealing Temperature, °C	Enzyme	Product, bp Wild-type Allele Variant Allele	% Agarose Gel for Resolution
G1051A	20	GTATTTTTGCAAGGCTACCAGTTACATTTGACAA	60	EcoN I	177	1.5
(R219K)		GATTGGCTTCAGGATGTCCATGTTGGAA			107, 70	
T1591C	27.5	GCTGCTGTGATGGGGTATCT	57	Hph I	117, 103, 48, 33	1.5
(V399A)		ACCTCACTCACACCTGGGAA			220, 48, 33	
G2706A	27.5	CAAGTGAGTGCTTGGGATTG	57	BsaA I	98, 252	2
(V771M)		TGCTTTTATTCAGGGACTCCA			350	
A2715C	27.5	GTGATCCCAGCGTGGTGTTTGTCTT	55	Hph I	56, 69, 95	2
(T774P)		GAAAGGCCAGAGGTACTCACAGCGAAGATCTTGAG**G**G			56, 161	
G2723C	12	TCGTTTTATTCAGGGACTCCA	55	Bgl II	269, 80	2
(K776N)		CAAGTGAGTGCTTGGGATTG			349	
G2868A	27.5	CCCATGCACTGCAGAGATTC	57	Bsa I	149, 237	2
(V825I)		GCAAATTCAAATTTCTCCAGG			386	
A3044G	27.5	GAGAAGAGCCACCCTGGTTCCAACCAGAAGAG**G**AT	55	EcoR V	94, 35	2.5
(I883M)		AAGGCAGGAGACATCGCTT			129	
G3911C	27.5	GAGCAGTTCTGATGCTGGCCTGGGCAGCGACCA**C**GA	55	BssS I	104, 37	2
(E1172D)		TCTGCACCTCTCCTCCTCTG			141	
G5155A	27.5	CAGCTTGGGAAGATTTATGACAGGACTGGACAC**G**A	55	BssS I	114, 31	2
(R1587K)		ATGCCCCTGCCAACTTAC			145	
C5587G	20	GTGCAATTACGTTGTCCCTGCCACACT	60	MnI I	82, 35	3
(S1731C)		CCATACAGCAAAAGTAGAAGGGCTAGCACA			117	

*Bold indicates mismatch in oligo to create restriction site.

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 *ABCA1* cSNP, R219K, is associated with decreased TG, increased HDL-C and, importantly, a decreased progression of atherosclerosis and a reduced risk of coronary events, suggesting that common genetic variants 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%.[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]

For replication studies with the R219K variant, we genotyped 3 smaller cohorts. Because reliable, standardized information on CAD was not available on all cohorts, we did not include CAD in the replication analysis. The cohorts comprised individuals of European descent with familial hypercholesterolemia seen at the lipid clinic in Vancouver, a group of French Canadians with CAD and low HDL-C (<0.86 mmol/L), and a random sample of French Canadians without clinical manifestations of CAD who were unselected for plasma lipid levels. All individuals known to be diabetic or have the apoE2 allele, a body mass index (BMI) >30 kg/m^2, or TG>5 mmol/L were excluded. Comparisons were performed on a case-control basis to avoid stratification by ethnicity or other demographic factors. All individuals gave informed consent.

CAD Measurements

Computer-assisted quantitative coronary angiography was performed as previously described.[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.

To screen the V399A, V771M, T774P, I883M, and E1172D cSNPs, TaqMan-based assays[12] were developed. Fluorogenic hybridization probes for each allele were labeled with different fluorescent reporter dyes (6-carboxy-fluorescein or 6-carboxy-4,7,2′,7′-tetrochloro-fluorescein) at the 5′ terminus and with a common quencher dye (6-carboxy-N,N,N′,N′,tetramethyl rhodamine) at the 3′ terminus. The fluorescence from each reaction was normalized to the signal from the no-template controls.[13] The difference in the measured fluorescence intensity between the 2 probes allows for accurate allele calling compared with genotype standards included on each plate.

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.[8] 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 χ^2 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 covariance analysis (interaction between age and genotype). Randomization to placebo and pravastatin in the REGRESS cohort was assessed by χ^2 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 [sum−1]/sum.

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 a 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.

TABLE 2. Frequencies of *ABCA1* cSNPs

Nucleotide Change	Amino Acid Change	Exon	REGRESS Carrier Frequency	REGRESS Allele Frequency	n*
Nonsynonymous					
G1051A	R219K	7	46.3	0.254	1588
T1591C	V399A	11	1.6	0.008	1098
G2706A	V771M	16	5.8	0.029	1270
A2715C	T774P	16	0.6	0.003	1250
G2723C	K776N	16	0.5	0.003	1106
G2868A	V825I	17	15.7	0.081	1364
A3044G	I883M	18	23.8	0.136	840
G3911C	E1172D	24	5.3	0.026	1288
G5155A	R1587K	35	44.3	0.259	1566
C5587G†	S1731C	38	0	0	558
Synonymous			From sequencing		
G869A	None	6	62.5	0.38	32
C1331T	None	9	31.3	0.19	32
G1343A	None	9	25	0.133	32
T3554G	None	22	12.5	0.059	32
G4676A	None	30	6.3	0.06	32
C6842T	None	49	6.3	0.033	32

*Number of alleles screened.
†Only observed in French-Canadian individuals.

From the increased relative risk associated with the RK and RR genotypes 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.[9] 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.[9] 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

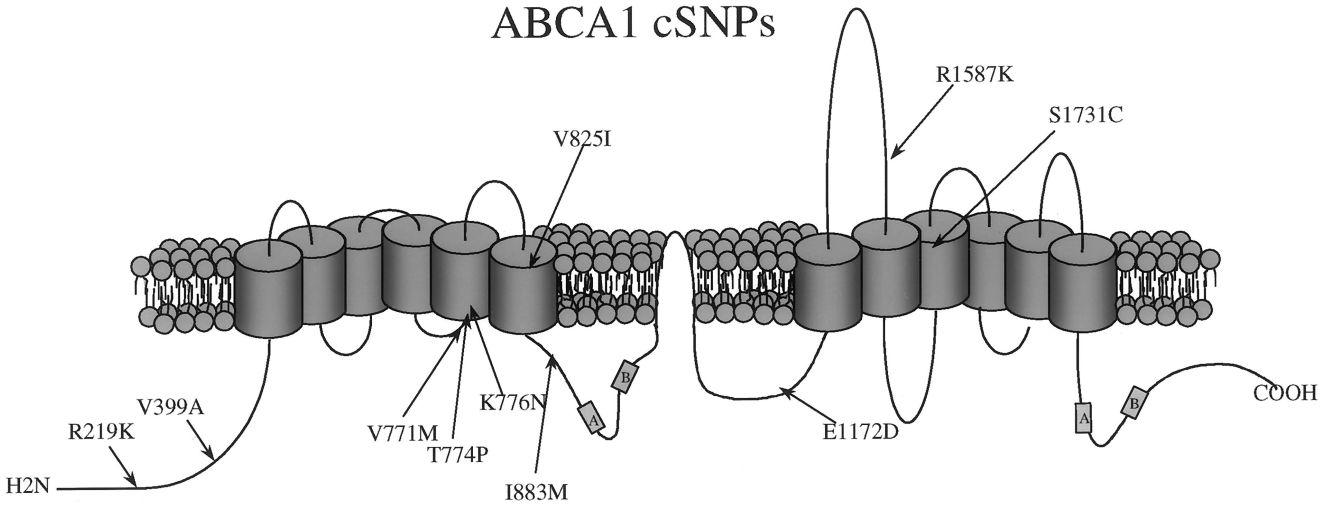

Figure 1. Schematic diagram of the ABCA1 protein, showing locations of nonsynonymous cSNPs. No variants are located in the ATP-binding cassette motifs (walker A and B domains [A, B]) or segments just N-terminal to the first transmembrane or the far C-terminal domain, where mutations tend to cluster. In contrast, 3 variants cluster near the start of the fifth transmembrane domain.

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 I; 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

					P		
	RR	RK	KK	Carriers (RK+KK)	RK vs RR	KK vs RR	RK+KK vs RR
n	424	330	36	366			
MSD, mm	2.70±0.37	2.77±0.37	2.78±0.40	2.77±0.37	0.01	0.22	0.005
MOD, mm	1.73±0.35	1.81±0.35	1.85±0.35	1.81±0.35	0.002	0.05	0.001
MI before trial, % (n)	48.3 (205)	47.1 (155)	33.3 (12)	45.8 (167)	0.71	0.12	0.48
Events during trial, % (n)	17 (71)	13 (41)	11 (4)	12 (45)	0.10	0.49	0.09
Total events, % (n)	59 (248)	52 (170)	39 (14)	50 (184)	0.06*	0.03†	0.02‡

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.

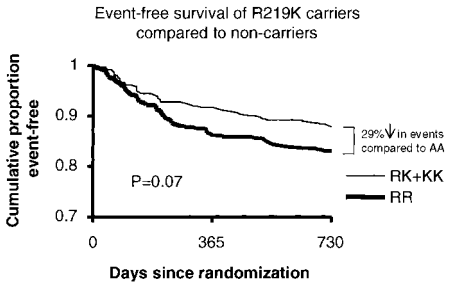

Figure 2. Event-free survival curves for R219K carriers (RK+KK; thin line) and noncarriers (RR; thick line). Curves represent the cumulative proportion of cohort that remained event-free during the trial. Carriers of R219K had a 29% increased event-free survival compared with noncarriers.

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 I883M 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]

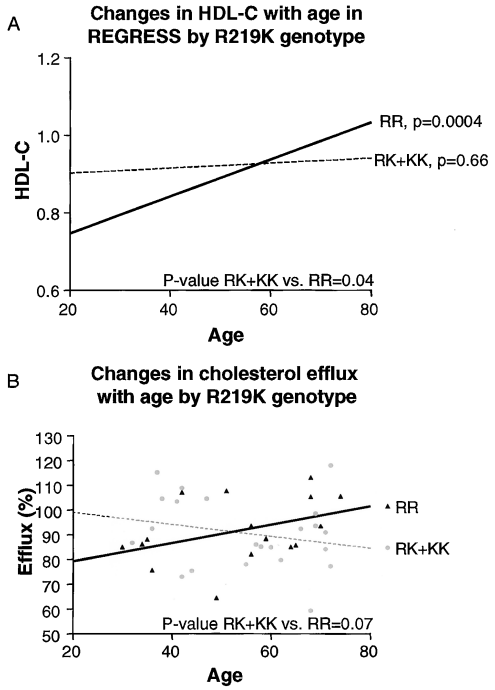

Figure 3. Changes in HDL-C and efflux with age by R219K genotype. A depicts the relationship between HDL-C and age in R219K carriers (RK+KK; dashed line) compared with noncarriers (RR; solid line). HDL-C increased significantly with age in noncarriers but not in carriers. B depicts the relationship between cholesterol efflux and age in carriers and noncarriers. Consistent with the findings for HDL-C, cholesterol efflux increased with age in noncarriers (gray circles and dashed line) but not in carriers (triangles and solid line).

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

					P		
	RR	RK	KK	RK+KK	RK vs RR	KK vs RR	RK+KK vs RR
n	424	330	36	366			
Age, y	57±8	55±8	57±7	55±8	0.0007	1	0.03
BMI, kg/m^2	25.8±2.6	26.3±2.7	25.5±2.3	26.2±2.7	0.01	0.50	0.09
Total cholesterol, mmol/L	6.02±0.86	6.07±0.89	5.89±0.85	6.06±0.89	0.44	0.38	0.60
HDL-C, mmol/L	0.92±0.22	0.93±0.23	0.92±0.20	0.93±0.23	0.54	1	0.81
LDL-C, mmol/L	4.27±0.75	4.35±0.83	4.33±0.82	4.35±0.83	0.17	0.65	0.19
TG, mmol/L	1.84±0.77	1.78±0.78	1.42±0.49	1.74±0.76	0.29	0.001	0.08

Values are mean±SD.

TABLE 5. Mean HDL-C and TG in Replication Case-Control Cohorts

	KK	RR	Number of KK-RR Pairs	*P*
HDL-C, mmol/L				
European FH	1.27±0.29	1.15±0.28	24	0.08
French Canadian CAD	0.78±0.05	0.70±0.06	6	0.02
French Canadian no CAD	1.59±0.28	1.38±0.38	4	0.20
All cohorts combined				0.02
TG, mmol/L				
European FH	1.55±0.83	1.77±0.80	24	0.18
French Canadian CAD	2.38±0.97	3.94±2.16	6	0.07
French Canadian no CAD	1.26±0.09	1.75±0.93	4	0.17
All cohorts combined				0.08

Values are mean±SD. FH indicates familial hypercholesterolemia.

(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 versus 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 E1172D) 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 (FHA2[8]). 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.

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 (n=29) 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

	MOD, mm			MSD, mm			HDL-C, mmol/L			TG, mmol/L		
	Carrier	Noncarrier	*P*	Carrier	Noncarrier	*P*	Carrier	Noncarrier	*P*	Carrier	Noncarrier	*P*
V825I	1.74±0.37 (107)	1.77±0.35 (575)	0.39	2.70±0.38	2.75±0.38	0.21	0.91±0.23	0.93±0.22	0.42	1.86±0.84	1.80±0.76	0.49
I883M	1.74±0.38 (100)	1.75±0.36 (320)	0.71	2.69±0.38	2.73±0.36	0.41	0.91±0.22	0.91±0.21	0.97	1.75±0.77	1.82±0.75	0.42
R1587K	1.77±0.34 (346)	1.76±0.37 (433)	0.75	2.73±0.39	2.74±0.36	0.64	0.90±0.22	0.94±0.23	0.03	1.79±0.76	1.81±0.78	0.77
V399A	1.92±0.32 (9)	1.73±0.35 (540)	0.13	2.73±0.40	2.71±0.37	0.89	1.03±0.28	0.92±0.23	0.15	1.71±0.63	1.82±0.78	0.68
V771M	1.89±0.38 (37)	1.76±0.35 (598)	0.045	2.83±0.49	2.73±0.37	0.13	0.91±0.20	0.92±0.22	0.58	1.98±0.79	1.78±0.76	0.11
T774P	1.63±0.31 (4)	1.76±0.36 (621)	0.47	2.85±0.34	2.73±0.37	0.52	0.85±0.07	0.93±0.22	0.50	1.90±1.04	1.82±0.77	0.84
K776N	1.92±0.33 (3)	1.78±0.34 (546)	0.48	2.95±0.48	2.76±0.37	0.36	0.94±0.28	0.93±0.22	0.93	2.25±0.94	1.76±0.76	0.26
E117SD	1.80±0.39 (34)	1.77±0.36 (610)	0.67	2.78±0.35	2.74±0.37	0.42	0.93±0.23	0.94±0.23	0.89	1.80±0.90	1.77±0.76	0.80

Values are mean±SD (n).

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,[9] 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] Cholesteryl 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 cholesteryl 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 activity[21] 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.

Acknowledgments

This work was supported by the Canadian Institutes of Health Research (S.M.C., M.R.H.), the Heart and Stroke foundation of

Canada (M.R.H.), the Canadian Networks of Centers of Excellence (NCE Genetics; M.R.H., T.J.H.), and Xenon Genetics, Inc. Dr Molhuizen and K. Zwarts were supported by the Netherlands Heart Foundation, and K. Zwarts was also supported by Dr Saal van Zwanenbergstichting. Dr Hayden is an established investigator of the BC Children's Hospital and is an MRC-PMAC Senior Scientist. We would like to thank the REGRESS Investigators for their contribution. The REGRESS study was sponsored by Bristol Myers Squibb. Carole Dore and Yannick Renaud are thanked for their assistance.

References

1. Miller GJ, Miller NE. Plasma high-density-lipoprotein concentration and development of ischaemic heart disease. *Lancet*. 1975;1:16–19.
2. Wilson PWF, Abbott RD, Castelli WP. High density lipoprotein cholesterol and mortality: the Framingham Heart Study. *Arteriosclerosis*. 1988;8:737–741.
3. Goldbourt U, Yaari S, Medalie JH. Isolated low HDL cholesterol as a risk factor for coronary heart disease mortality: a 21 year follow-up of 8000 men. *Arterioscler Thromb Vasc Biol*. 1997;17:107–113.
4. Fredrickson DS, Altrocchi PH, Avioli LV, et al. Tangier disease: combined clinical staff conference at the National Institutes of Health. *Ann Intern Med*. 1961;55:1016–1031.
5. Brooks-Wilson A, Marcil M, Clee SM, et al. Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. *Nat Genet*. 1999;22:336–345.
6. Bodzioch M, Orsó E, Klucken J, et al. The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. *Nat Genet*. 1999;22:347–351.
7. Rust S, Rosier M, Funke H, et al. Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. *Nat Genet*. 1999;22:352–355.
8. Marcil M, Brooks-Wilson A, Clee SM, et al. Mutations in the ABC1 gene in familial HDL deficiency with defective cholesterol efflux. *Lancet*. 1999;354:1341–1346.
9. Clee SM, Kastelein JJP, van Dam M, et al. HDL cholesterol levels and coronary artery disease in heterozygotes for ABCA1 mutations are predicted by cholesterol efflux levels and influenced by age. *J Clin Invest*. 2000;106:1263–1270.
10. Summers KM. DNA polymorphisms in human population studies: a review. *Ann Hum Biol*. 1987;14:203–217.
11. Jukema JW, Bruschke AVG, van Boven AJ, et al. Effects of lipid lowering by pravastatin on the progression and regression of coronary artery disease in symptomatic men with normal to moderately elevated serum cholesterol levels: the Regression Growth Evaluation Statin Study (REGRESS). *Circulation*. 1995;91:2528–2540.
12. Holland PM, Abramson RD, Watson R, et al. Detection of specific polymerase chain reaction product by utilizing the 5′–>3′ exonuclease activity of Thermus aquaticus DNA polymerase. *Proc Natl Acad Sci U S A*. 1991;88:7276–7280.
13. Livak KJ, Marmaro J, Todd JA. Toward fully automated genome-wide polymorphism screening. *Nat Genet*. 1995;9:341–342.
14. Wang J, Burnett JR, Near S, et al. Common and rare ABCA1 variants affecting plasma HDL cholesterol. *Arterioscler Thromb Vasc Biol*. 2000;20:1983–1989.
15. Zilversmit DB. Atherogenic nature of triglycerides, postprandial lipemia, and triglyceride-rich remnant lipoproteins. *Clin Chem*. 1995;41:153–158.
16. Manninen V, Tenkanen L, Koskinen P, et al. Joint effects of serum triglyceride and LDL cholesterol and HDL cholesterol concentrations on coronary heart disease risk in the Helsinki Heart Study: implications for treatment. *Circulation*. 1992;85:37–45.
17. Tall AR. Metabolic and genetic control of HDL cholesterol levels. *J Intern Med*. 1992;231:661–668.
18. Eisenberg S. High density lipoprotein metabolism. *J Lipid Res*. 1984;25:1017–1058.
19. McNeish J, Aiello RJ, Guyot D, et al. High density lipoprotein deficiency and foam cell accumulation in mice with targeted disruption of ATP-binding cassette transporter-1. *Proc Natl Acad Sci U S A*. 2000;97:4245–4250.
20. Orsó E, Broccardo C, Kaminski WE, et al. Transport of lipids from Golgi to plasma membrane is defective in Tangier disease patients and ABC1-deficient mice. *Nat Genet*. 2000;24:192–196.
21. Lawn RM, Wade DP, Garvin MR, et al. The Tangier disease gene product ABC1 controls the cellular apolipoprotein-mediated lipid removal pathway. *J Clin Invest*. 1999;104:R25–R31.
22. Gupta S. P-glycoprotein expression and regulation: age related changes and potential effects on drug therapy. *Drugs Aging*. 1995;7:19–29.
23. Witkowski JM, Miller RA. Increased function of p-glycoprotein in T lymphocyte subsets of aging mice. *J Immunol*. 1993;150:1296–1306.
24. Hayden MR, Clee SM, Brooks-Wilson A et al. Cholesterol efflux regulatory protein, Tangier disease and familial high-density lipoprotein deficiency. *Curr Opin Lipidol*. 2000;11:117–122.