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Article

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Age and residual cholesterol efflux affect HDL cholesterol levels and coronary artery disease in *ABCA1* heterozygotes

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Introduction

Low HDL cholesterol (HDL-C) levels are an important risk factor for coronary artery disease (CAD). Epidemiological studies have shown strong inverse relationships between HDL-C and CAD (1), and although often seen in association with other lipid abnormalities, isolated low HDL-C is an independent risk factor for CAD (2, 3).

Glomset first proposed that the primary antiatherogenic function of HDL might be related to its key role in the transport of cholesterol from peripheral cells to the liver (4). However, until recently, little has been understood about the initial step of reverse cholesterol transport, namely, the removal of cholesterol from peripheral cells. In addition, there has been no direct investigation of the relationship between efflux of cholesterol to plasma HDL-C levels and risk of CAD (5, 6).

Tangier disease (TD), originally described by Fredrickson et al. in 1961, is associated with a near absence of HDL cholesterol and apoAI, hepatosplenomegaly, neuropathy, and marked cholesterol ester deposition within tissues (7). Biochemically, TD is associated with decreased cellular cholesterol and phospholipid efflux (8, 9). We and several others have recently reported mutations in the *ABCA1* (*ABC1*) gene as the underlying cause of TD (10–15). ABCA1 is a member of the large family of ATP binding cassette transporters, known to be involved in the energy-dependent transport of a variety of substrates (16). We have also shown that (17) familial hypoalphalipoproteinemia (FHA) with decreased cholesterol efflux (18) is allelic to TD (10) and due to heterozygosity for mutations in the *ABCA1* gene.

The phenotype of heterozygosity for mutations in the *ABCA1* gene has not been clearly defined. As many factors, both genetic and environmental, influence plasma HDL-C levels and contribute to low HDL-C values, unambiguous identification of heterozygotes for *ABCA1* mutations has until now been impossible. Individuals from TD kindreds presumed to be heterozygous have shown a range of phenotypes, and much overlap with unaffected individuals has been seen (19–21), possibly reflecting the fact that some individuals had been misclassified. Indeed, the inability to uniquely identify heterozygous individuals created difficulty in mapping the gene for TD (15). Studies in obligate heterozygotes have also been limited to small numbers (22), often within a single family (19), and thus restricted in the ability to analyze the phenotypic expression with different mutations and over a range of ages.

We have now identified a cohort of 77 individuals in whom heterozygosity has been defined by mutation identification in the *ABCA1* gene. For the first time, it is now possible to characterize the phenotype in mutation-defined heterozygotes and to compare this with a large number of unaffected family members, thus controlling for other genetic and environmental factors. Furthermore, variation in ABCA1 activity as defined by levels of cholesterol efflux can be correlated with plasma HDL-C and risk of CAD.

Methods

Identification of subjects. Subjects heterozygous for mutations in the *ABCA1* gene were individuals identified from seven TD and FHA families described previously (10, 23). In addition, heterozygous individuals from three new TD families (TD3–5) and one new FHA kindred (FHA6) were included. The second mutation has not been identified in one of the TD kindreds (TD4); however, a marker immediately adjacent to *ABCA1* cosegregates with the low HDL phenotype (data not shown). Individuals bearing the affected haplotype were considered heterozygotes. The presence or absence of mutations identified by genomic sequencing of probands from each family was subsequently confirmed in family members by RFLPs, to define heterozygous and unaffected individuals, respectively.

Families with TD have been ascertained on the basis of the clinical features of TD, and all heterozygotes available from each family were included. There has been no selection on the basis of HDL-C levels or CAD status of these individuals. Two of the six FHA probands (FHA2-301 and FHA3-301) were referred to the clinic on the basis of CAD. The remaining probands were identified solely on the basis of low HDL-C. Again, all heterozygotes from the FHA families were included, with no selection for HDL-C levels or CAD.

Our control cohort comprises unaffected members of the 11 families. These individuals share a genetic background with the heterozygotes, and environmental factors are expected to be similar among family members. Thus, many additional factors that may influence HDL-C are controlled for, and the phenotypic differences between heterozygotes and unaffected individuals can be largely attributed to variation in *ABCA1* gene activity.

All subjects gave informed consent to their participation in this study, and the genetic analysis protocol was approved by the Ethics Committees of the University of British Columbia, the Academic Medical Centre in Amsterdam, and the Clinical Research Institute of Montreal, as described elsewhere (10).

Lipid and cholesterol efflux measurements. Lipid levels in ABCA1 heterozygotes were measured as described previously (10, 23), at standardized lipid clinics in Vancouver, Montreal, and Amsterdam. LDL cholesterol was calculated by the method of Friedewald et al. (24), modified to account for lipid measurements in mmol/l.

Cellular cholesterol efflux from fibroblast cultures was measured as described previously (10, 23). Briefly, fibroblast cultures were established from skin biopsies of subjects and healthy controls. Efflux was measured over 24 hours in the presence of purified apoAI and was calculated as the percent of free cholesterol in the medium after incubation. Each experiment was performed in triplicate wells and averaged. Measurements are reported as the percentage efflux in each subject relative to the average values of two healthy controls included as standards within the same experiment. Individual experiments were repeated at least twice, and the average relative efflux over all experiments was used. Note that the number of heterozygotes with efflux measurements is less than the number of mutations, as not all TD families have efflux measured in heterozygous carriers of each mutation.

Statistics. In analysis of the heterozygotes, differences in mean baseline demographics and lipid levels between groups were compared by Student's t test. Comparisons of frequency either between the male/female ratio or of distributions across various percentile ranges were made using the χ^2 test. Analysis of potential interactions between affected status and either sex or body mass index (BMI) were performed using a general linear model. Statistical analysis was performed using Prism (version 3.00; Graphpad Software for Science Inc., San Diego, California, USA) or Systat (version 8.0; SPSS Inc., Chicago, Illinois, USA). All values are reported as mean ± SD.

Results

ABCA1 heterozygotes have decreased HDL cholesterol and an increased risk for CAD. Our cohort comprised 77 individuals from 11 families identified as heterozygous for mutations in the *ABCA1* gene. A comparison of mean lipid levels in heterozygotes with mean levels in all available unaffected family members (n = 156) is presented in Table 1. As predicted, heterozygotes have an approximately 40–45% decrease in HDL-C and apoAI and a mild (~10%) decrease in apoAII compared with unaffected family members. Mean triglycerides (TG) were increased by approximately 40% in heterozygotes compared with

Table 1
Characterization of heterozygotes

	TD Patients	Heterozygotes	Unaffected family members	*P* value heterozygotes vs. unaffected	*P* value patients with TD vs. unaffected
Number	5	77[A]	156[A]		
Age (years)	43.4 ± 9.0	42.5 ± 19.6	39.9 ± 21.0	0.35	0.71
Range	31–56	5–81	4–86		
Male/female	3/2	33/44	82/74	0.16	0.74
TC (mmol/l)	2.34 ± 1.03	4.52 ± 1.12	4.71 ± 1.07	0.23	<0.0001
TG (mmol/l)	1.95 ± 0.97	1.66 ± 1.59	1.20 ± 1.03	0.03	0.11
HDL (mmol/l)	0.08 ± 0.05	0.74 ± 0.24	1.31 ± 0.35	<0.0001	<0.0001
LDL (mmol/l)	1.37 ± 1.02	3.03 ± 0.99	2.84 ± 0.87	0.171	0.0003
ApoAI (g/l)	0.03 ± 0.04 (3)	0.92 ± 0.32 (61)	1.43 ± 0.26 (55)	<0.0001	<0.0001
ApoAII (g/l)	0.10 ± 0.08 (2)	0.35 ± 0.08 (46)	0.39 ± 0.08 (43)	0.01	<0.0001
ApoB (g/l)	0.89 ± 0.53 (2)	0.93 ± 0.25 (52)	0.94 ± 0.33 (42)	0.88	0.84
CHD ≥ 20 years	20% (1/5)	12.9% (8/62)	4.1% (5/122)	0.03	0.10
Odds ratio (95% CI)				3.47 (1.08–11.09)	5.85 (0.55–62.4)
Age of onset	38	48.9 ± 8.6	60.4 ± 12.8	0.08	

[A]For TC, TG, and LDL, *n* = 76 for heterozygotes, 153 for unaffected family members. For apoAI, apoAII, and apoB, *n* is given in parentheses after the mean.

unaffected family members and were further increased in patients with TD. Unlike patients with TD, there is no significant decrease in either total cholesterol (TC) or LDL cholesterol in heterozygotes, and apoB levels were not different in heterozygotes from controls. Mean HDL-C levels in carriers of each of the mutations were similarly reduced by approximately 40–50% compared with unaffected family members (Table 2).

We further examined the heterozygote phenotype by calculating the percentage of individuals falling within a given range of age- and sex-specific percentiles (25, 26). Much variability in the heterozygote phenotype was evident. As shown in Figure 1, although a significantly higher percentage of heterozygotes had HDL-C less than the fifth percentile for age and sex compared with unaffected controls (65% vs. 5%; $P < 0.0001$), 5% of heterozygotes had HDL greater than the 20th percentile, with HDL-C ranging up to the 31st percentile for age and sex. Thus in some individuals, clearly the phenotype is less severe. A broad distribution of TG levels was also evident (Figure 1). A significantly lower percentage of heterozygous individuals had TG below the 20th percentile for age and sex ($P = 0.03$), and a significantly larger percentage had TG greater than 80th percentile ($P = 0.005$) compared with unaffected family members, but substantial overlap between the two distributions was seen.

Another important question is whether individuals heterozygous for *ABCA1* mutations are at an increased risk of developing CAD. Studies on obligate TD heterozygotes have reported conflicting findings (19, 22). In our large cohort, symptomatic vascular disease was over three times as frequent in the adult heterozygotes as in unaffected family members (Table 1). Interestingly, the presentation of vascular disease was generally more severe in the heterozygotes than in their unaffected family members (Table 3). Heterozygotes had myocardial infarctions (five, one fatal) and severe vascular disease requiring multiple interventions, whereas in unaffected individuals, CAD was manifest as angina in two cases and as a transient ischemic attack at the age of 80 in another. Furthermore, the mean age of onset was on average a decade earlier in heterozygotes compared with unaffected controls (Table 1).

Cholesterol efflux, HDL cholesterol levels and CAD. We next sought to directly assess the relationship between cholesterol efflux levels, HDL-C, and CAD. We have

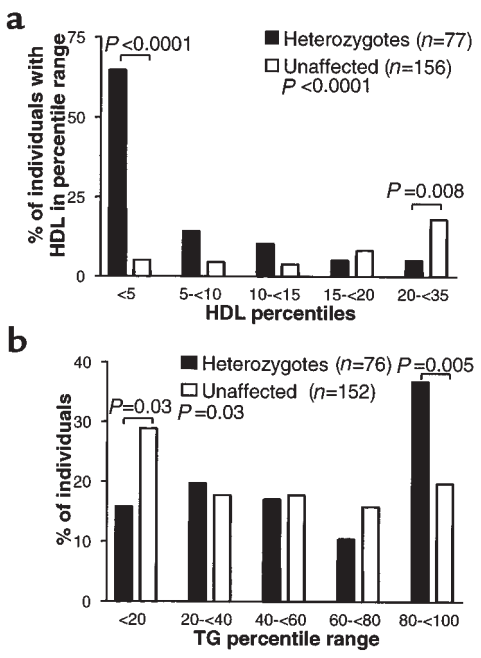


Figure 1
The percent of heterozygotes or unaffected family members with HDL-C and TG within a given range of percentiles for age and sex, based on the Lipid Research Clinics criteria (25), are shown. A broad distribution of HDL-C levels was seen in the heterozygotes, extending up to the 31st percentile for age and sex. There is much overlap in the distribution of TG between heterozygotes and unaffected family members, although a larger portion of heterozygotes have TG greater than or equal to the 80th percentile for age and sex.

Table 2
HDL-C by mutation

Family	Mutation	HDL-C in heterozygotes: mean ± SD (*n*)	HDL-C in unaffected family members: mean ± SD (*n*)	HDL-C in heterozygotes: % of unaffected	Age- and sex-matched population median:[A] mean ± SD	CAD in heterozygotes
FHA1	Del L 693	0.79 ± 0.20 (8)	1.22 ± 0.35 (11)	64.8	1.39 ± 0.08	-
FHA2	R2144X	0.56 ± 0.23 (12)	1.07 ± 0.22 (20)	52.3	1.34 ± 0.19	+
FHA3	Del E,D 1893,94	0.77 ± 0.24 (8)	1.44 ± 0.38 (9)	53.5	1.30 ± 0.17	+
FHA4	R909X	0.59 ± 0.26 (5)	1.04 ± 0.37 (9)	56.5	1.39 ± 0.24	-
FHA5	M1091T	0.48 ± 0.48 (4)	1.37 ± 0.43 (6)	35.0	1.56 ± 0.05	+
FHA6	P2150L	0.61 ± 0.07 (7)	1.05 (1)	58.1	1.30 ± 0.22	+
TD1	ivs25+1G→C	0.78 ± 0.06 (4)	1.35 ± 0.29 (70)	57.8	1.22 ± 0.22	-
TD4	Del C 6825→2145X	0.91 ± 0.10 (2)	1.00 ± 0.05 (3)	91.0	1.31 ± 0.16	-
TD5	CTC6952-4TT→2203X	0.80 ± 0.20 (3)	1.65 (1)	48.5	1.39 ± 0.19	-
TD1	C1477R	0.82 ± 0.18 (9)	1.35 ± 0.29 (70)	60.7	1.37 ± 0.14	+
TD2	Q597R	0.82 ± 0.07 (5)	None available	-	1.39 ± 0.17	-
TD3	T929I	1.01 ± 0.18 (8)	1.48 ± 0.42 (26)	68.2	1.33 ± 0.19	-
TD4	unidentified	0.74 ± 0.05 (2)	1.00 ± 0.05 (3)	73.5	1.49 ± 0.09	+

[A]Calculated based on the mean age- and sex- specific 50th percentile levels in the LRC population.

previously shown that individuals heterozygous for *ABCA1* mutations have decreased cholesterol efflux (23); however, the extent to which variations in cholesterol efflux are directly related to HDL-C levels is unknown. Relative cholesterol efflux in individuals heterozygous for an *ABCA1* mutation was plotted against the mean HDL-C levels observed in the carriers of that mutation, expressed as a percentage of the unaffected members within that family (Figure 2). Efflux measures were not available in heterozygotes of some mutations from TD families in which efflux has only been measured in the TD probands. Cholesterol efflux levels associated with each mutation strongly predict the corresponding HDL-C levels in our families, accounting for 82% of the variation in HDL-C (r^2=0.82; P = 0.005). Furthermore, in one large family (FHA2), in which efflux has been measured in three independent heterozygotes, an r^2 value of 0.81 was obtained when individual plasma HDL-C levels were plotted against individual efflux measurements. Using the regression equation of mean HDL-C levels in the heterozygotes on the efflux level of the heterozygous carrier (P = 0.02), we can estimate the relationship between expected changes in ABCA1 efflux activity and HDL-C levels. From this, we would predict that each 8% change in efflux levels would be associated with a 0.1 mmol/l change in HDL-C.

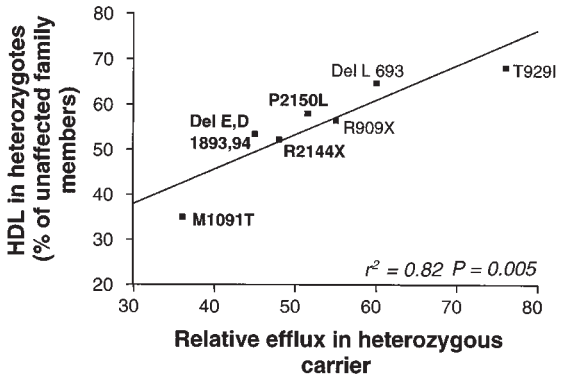


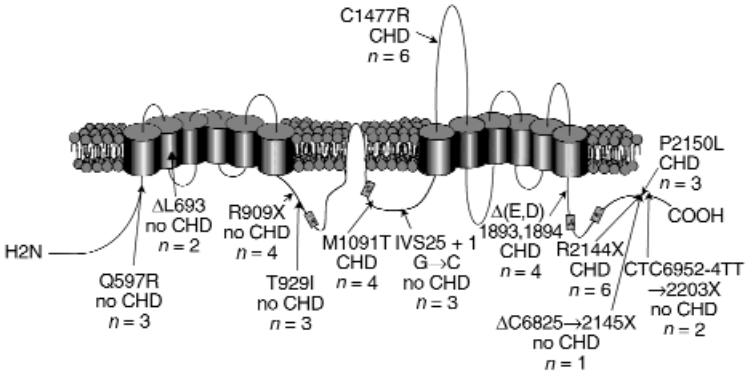
Figure 2
Average HDL in heterozygotes for each mutation (expressed as a percentage of mean HDL in the unaffected members of that family) are plotted against the efflux levels measured in a heterozygous carrier of each mutation. Efflux levels are highly correlated with levels of HDL cholesterol and are associated with 82% of the variation in HDL-C.

Relative cholesterol efflux levels are also related to CAD within the family. Families with clearest evidence for premature CAD had individuals with the lowest cholesterol efflux (Table 2; Figure 2, boldface). These data suggest that the level of residual ABCA1 function is a critical determinant of both HDL-C levels and risk of CAD.

ABCA1 mutation type and location do not influence the severity of phenotype in heterozygous individuals. We have previously noted that the phenotypic presentation of our FHA heterozygotes was more severe than that of our TD heterozygotes (27), and we initially noted more deletions and premature truncations of the protein in our FHA families than in our TD families (10, 23). Thus, with our identification of several different *ABCA1* mutations, and as residual ABCA1 activity is an important predictor of severity of the phenotype, we sought to examine whether the nature of the mutation influenced the phenotypic expression of mutations in the *ABCA1* gene. Severe mutations were defined as deletions, those that caused premature truncation of the protein or disrupted natural splicing of the protein, and would be expected to result in a nonfunctional allele. Missense mutations, on the other hand, result in the change of only a single amino acid and may result in a protein product that still retains partial activity.

Lipid levels were compared in heterozygous carriers of severe and missense mutations. Although there was a trend toward decreased HDL-C levels in carriers of severe compared with missense mutations, this did not reach significance (0.78 ± 0.26 vs. 0.70 ± 0.23; P = 0.18). A range of HDL-C levels in individual missense and severe mutations was observed (Table 2). No significant

Figure 3
A schematic diagram of the ABCA1 protein, illustrating the location of mutations in the heterozygotes and the presence of CAD in carriers of that mutation. The number of heterozygotes aged 40 years or older that may be expected to have developed CAD is included. The number of unaffected family members greater than 40 years old is 69.



differences in TG were evident between carriers of missense and severe mutations (1.77 ± 2.15 vs. 1.55 ± 1.01; $P = 0.58$). Interestingly, the M1091T missense mutation is the most severe mutation both by effects on efflux and HDL-C levels, with a more severe phenotype than even early truncations of the protein (e.g., R909X).

The site of mutation (e.g., NH_2-terminal or COOH-terminal) within the ABCA1 protein did not influence the phenotype (Figure 3). The presence of CAD is seen in carriers of mutations in different domains of the protein. Patients with mutations on both alleles manifest with splenomegaly alone or in association with CAD (TD1; data not shown). Thus, the phenotype appears to be mutation specific and most likely dependent on remaining ABCA1 function of the wild-type allele and residual function of the mutant allele, similar to what has been shown for mutations in *ABCR*, a close homologue of *ABCA1* (28).

The phenotype of mutations in the ABCA1 gene is modified by age. One factor influencing phenotypic expression that became apparent in our families was age. This was first brought to our attention in two of the families initially investigated (23). In family FHA3, although heterozygous individuals in older generations all had HDL-C levels less than the fifth percentile for age and sex, those in the youngest generation had a much more variable phenotype, with HDL-C ranging up to the 20th percentile. In family FHA1, the same pattern was observed.

We compared the distribution of individuals across HDL-C percentile ranges in those less than 30 versus those from 30 to less than 70 years of age (Figure 4). A significantly larger percentage of individuals 30–70 years of age had HDL-C less than the fifth percentile than did those less than 30 years. Mean HDL-C decreases in heterozygotes greater than 30 years of age compared with those less than 30 years of age, whereas there is no significant change in unaffected controls (Table 4). Similar results are seen in males and females separately and are seen at both pre- and postmenopausal ages in women (Figure 5). TG increase with age in both heterozygotes and unaffected family members.

Assessment of the influences of sex and BMI on the phenotypic expression of ABCA1 mutations. Females are known to have elevated HDL-C and decreased TG compared with males (26). Thus, we sought to address whether the phenotype of *ABCA1* heterozygotes was influenced by sex. HDL-C is significantly lower than unaffected controls in both heterozygous males and

Table 3
Coronary artery disease

Individual	Mutation	Exon	Disease (age of onset)	Other risk factors
TD proband				
TD1	C1477R, ivs25+1G→C	30, intron 25	CHD (38)	-
ABCA1 heterozygotes				
TD4-201	unidentified	-	MI (<58)	-
FHA5-215	M1091T	22	MI (61)	-
FHA5-303	M1091T	22	CHD (<45)	-
TD1-363	C1477R	30	MI (51)	-
FHA3-301	Del E,D 1893,94	41	PVD (<54)	smoker, BMI 31.7
FHA3-305	Del E,D 1893,94	41	CHD (44)	ex-smoker
FHA6-201	P2150L	48	CVA (36), fatal MI (58)	-
FHA2-301	R2144X	48	CAD (42), PTCA (47), femoral angioplasty (48), CABG (<50)	hypertensive
Unaffected family members				
FHA5-212	none	-	AP (62)	-
TD3-109	none	-	TIA (80)	diabetic
FHA2-315	none	-	MI (51)	BMI 37
TD1-205	none	-	MI (62)	-
TD1-216	none	-	AP (47)	-

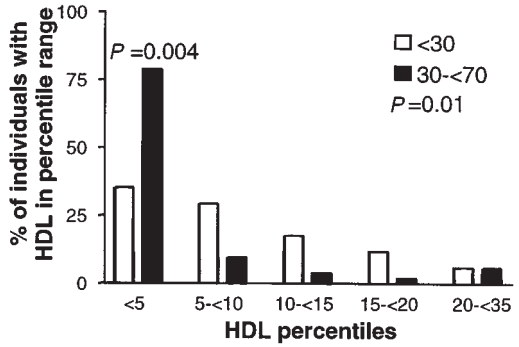


Figure 4
The percentage of individuals less than 30 years of age and from 30 to less than 70 years of age with HDL-C levels in a given percentile range are plotted. Younger individuals have a far broader distribution of HDL-C levels, clearly indicating that the impact of ABCA1 on HDL-C levels is influenced by age.

females (0.70 ± 0.24 vs. 1.21 ± 0.29; $P < 0.0001$, and 0.76 ± 0.25 vs. 1.41 ± 0.38; $P < 0.0001$, respectively). This was reflected in decreased apoAI (0.92 ± 0.27 vs. 1.36 ± 0.22; $P < 0.0001$, and 0.92 ± 0.36 vs. 1.49 ± 0.28; $P < 0.0001$ in males and females, respectively) and a trend toward a mild decrease in apoAII in both males and females compared with unaffected family members (0.35 ± 0.08 vs. 0.40 ± 0.09; $P = 0.08$, and 0.35 ± 0.09 vs. 0.39 ± 0.07; $P = 0.06$, respectively). TG are higher in both male (2.07 ± 2.16 vs. 1.30 ± 1.30; $P = 0.02$) and female (1.34 ± 0.86 vs. 1.09 ± 0.63; $P = 0.08$) heterozygotes compared with unaffected family members. Interestingly, the difference in HDL-C between males and females was reduced in heterozygotes compared with controls ($P = 0.11$), whereas the difference in TG was increased compared with controls ($P = 0.13$).

Another factor known to influence HDL-C and TG levels is BMI (29). The entire cohort was divided into tertiles of BMI, and the mean HDL-C and TG levels of heterozygotes and unaffected individuals by BMI tertile are shown in Figure 6. BMI had a significant effect on both HDL-C and TG in both heterozygotes and controls ($P = 0.0001$). The effect of BMI on HDL-C and TG was more severe in heterozygotes than in controls, being evident at lower BMIs (mid-tertile) in heterozygotes. A raised BMI was more obviously associated with changes in HDL-C and TG in heterozygotes compared with controls. However, neither effect reached statistical significance. HDL-C was reduced in heterozygotes compared with controls in all BMI tertiles ($P < 0.0001$ in each tertile). Although TG were increased in all BMI tertiles in heterozygotes compared with unaffected family members, this difference was only significant in the middle BMI tertile ($P = 0.009$).

Discussion

The reverse transport of cholesterol from peripheral cells to sites of catabolism, first described by Glomset and Norum (4), has been hypothesized to be the primary mechanism whereby HDL-C is antiatherogenic. However, there has been little direct evidence that changes in this pathway are associated with changes in HDL-C levels and susceptibility to CAD (30). Specifically, there has been no direct evidence linking efflux of cholesterol from peripheral cells, the initial step of the reverse cholesterol transport pathway, to CAD.

With the identification of the ABCA1 protein as a key initiator of the efflux pathway, it has now been possible to relate directly cholesterol efflux, HDL-C levels, and CAD. For the first time, we have been able to describe the phenotype in heterozygotes for different mutations in the *ABCA1* gene in a large cohort in which diagnosis has been made by mutation identification. Furthermore, we have been able to compare this with a cohort of unaffected family members, enabling us to control, at least in part, for other genetic and environmental influences.

Here, we have shown that *ABCA1* heterozygotes have an approximate 50% decrease in HDL-C and apoAI and a mild but significant decrease in apoAII. In addition, heterozygotes have increased TG, but in contrast to patients with TD, have no significant change in total or LDL cholesterol. The changes in HDL-C, apoAI, and TG were gene-dose dependent, suggesting they are directly related to ABCA1 function. Furthermore, heterozygotes have a more than threefold increased risk of developing CAD and a younger aver-

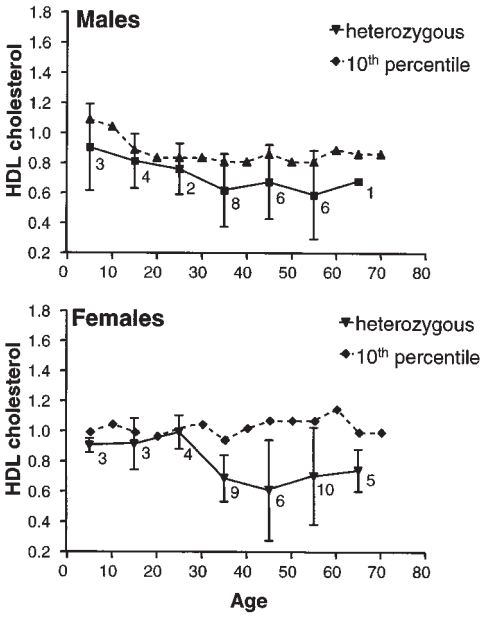


Figure 5
The mean HDL-C in heterozygous males and females (solid line) in 10-year age groups (plotted at the halfway point) are shown compared with the tenth percentile distribution in the Lipid Research Clinics population (dashed line) (25). Error bars represent the SD of each mean. The number of individuals in each group is shown under each data point. Beyond the age of 30 years, mean HDL-C levels in heterozygotes fall much lower than the tenth percentile distribution, whereas at less than 30 years of age, mean HDL-C levels in the heterozygotes more closely approximate the tenth percentile distribution.

Table 4
Mean HDL-C and TG by age in heterozygotes

	Heterozygotes: mean ± SD (*n*)	Unaffected: mean ± SD (*n*)	*P* value, heterozygotes vs. unaffected
HDL-C (mmol/l)			
<30 years	0.91 ± 0.16 (17)	1.26 ± 0.29 (51)	<0.0001
30–<70 years	0.66 ± 0.24 (52)	1.32 ± 0.36 (90)	<0.0001
Change	–0.25	+0.06	0.21
P value <30 vs. ≥30	0.0002	0.23	
TG (mmol/l)			
<30 years	1.07 ± 0.96 (16)	0.88 ± 0.45 (51)	0.26
30–<70 years	1.84 ± 1.79 (52)	1.36 ± 1.24 (87)	*0.07*
Change	+0.77	+0.48	0.97
P value <30 vs. 30–<70	0.03	0.001	

HDL-C and TG in individuals less than 30 and from 30 to less than 70 years of age.

age age of onset compared with unaffected individuals. Further, those heterozygotes with most severe deficiency in efflux had a higher frequency of CAD. It should be noted, however, that the absolute number of CAD cases is small and two of the 62 adult heterozygotes were identified on the basis of their CAD. Thus additional studies examining the extent of CAD in a randomly ascertained heterozygote population will be important to confirm these findings.

Interestingly, the severity of the phenotype observed in the heterozygotes appeared to be mutation dependent, but there was no obvious relationship between the site of mutation and the phenotype. There was a trend toward lower HDL-C in carriers of the severe mutations, causing truncations or null alleles, compared with carriers of missense mutations. One notable exception is the M1091T missense mutation, which had the most severe phenotype, with marked reductions in HDL-C and efflux in affected family members, suggesting that this mutation may act in a dominant-negative fashion, downregulating the function of the wild-type allele. Another interesting finding is the small cluster of mutations at the very COOH-terminal region of the protein, which suggests that this region must be critical to ABCA1 function.

The severe HDL deficiency in *ABCA1* heterozygotes suggests that residual cholesterol efflux is the major determinant of HDL-C levels. While this manuscript was being prepared for submission, a report has appeared, correlating efflux with HDL-C levels in a small number ($n = 9$) of heterozygotes from one family (31). Our results extend these findings to multiple populations, directly linking residual ABCA1 efflux activity to HDL-C levels and now also to the risk of CAD. From the regression equation of mean HDL-C on efflux, we predict that each 8% increase in relative efflux is associated with a 0.1 mmol/l increase in HDL-C levels. Alternatively, a 50% increase in ABCA1-mediated cholesterol efflux would be predicted to result in a 30% increase in HDL-C in a normal 40-year-old male. Although these numbers may not directly extrapolate to what is observed in a general population in which other genetic and environmental factors have not been controlled for, these data nonetheless suggest that relatively small changes in ABCA1 function may have a significant impact on plasma HDL-C levels. Furthermore, the data presented here suggest that variations in efflux due to variations in ABCA1 function directly reflect not only plasma HDL-C levels but also CAD susceptibility, thus providing direct validation of the reverse cholesterol transport hypothesis and validation of ABCA1 as a therapeutic target to raise HDL-C and protect against atherosclerosis.

We have also shown that the phenotype in *ABCA1* heterozygotes is age modulated. Beginning at 20 years of age, there is a small but definite increase in HDL-C with advancing age that is obviously absent in the heterozygotes. One explanation for this finding is that there is normally an age-related increase in ABCA1 function, which is not seen in heterozygotes, perhaps because the remaining functioning allele has already been maximally upregulated secondary to an increase in intracellular cholesterol. This would exaggerate the phenotype in older age groups. There is some evidence for an age-modulated increase of the ABC transporters (32). Further evidence of a potential age-related increase in ABCA1 function comes from the observation that the percentage of apoAI found in the preβ_1 subfraction of HDL, the predominant cholesterol acceptors, decreases with age (33), suggesting increased formation of mature α-migrating HDL particles with age. Clearly, additional experiments directly assessing the impact of age on ABCA1 function are needed to address this.

Here we have shown that heterozygotes for *ABCA1* mutations have age-modulated decreases in HDL-C with significantly increased risk for CAD. Furthermore, this phenotype was highly correlated with efflux, clearly demonstrating that impairment of reverse cholesterol transport is associated with

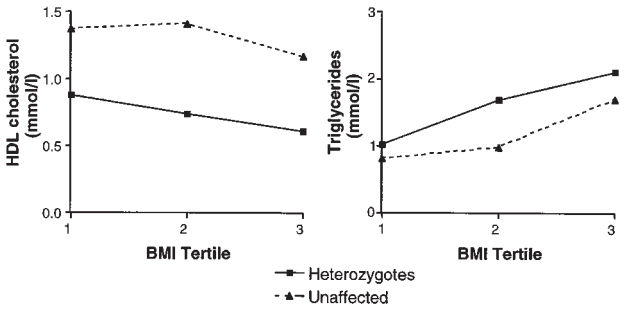


Figure 6
The mean HDL-C and TG levels in heterozygotes and unaffected family members falling within each tertile of BMI are shown. The tertiles of BMI correspond to the following values: 1: BMI < 21.4; 2: 21.4 ≤ BMI ≤ 25.1; 3: BMI > 25.1.

decreased plasma HDL-C and increased atherogenesis. In conclusion, our data suggest that therapies designed specifically to increase ABCA1 function should be associated with increased plasma HDL-C and protection against atherosclerosis.

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