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Do Mutations Causing Low HDL-C Promote Increased Carotid Intima-Media Thickness?

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Abstract

Background—Although observational data support an inverse relationship between high-density lipoprotein (HDL) cholesterol and coronary heart disease (CHD), genetic HDL deficiency states often do not correlate with premature CHD.

Methods—Carotid intima-media thickness (cIMT) measurements were obtained in cases comprising 10 different mutations in *LCAT*, *ABCA1* and *APOA1* to further evaluate the relationship between low HDL resulting from genetic variation and early atherosclerosis.

Results—In a 1:2 case-control study of sex and age-related (± 5 y) subjects ($n=114$), cIMT was nearly identical between cases (0.66 ± 0.17 cm) and controls (0.65 ± 0.18 cm) despite significantly lower HDL cholesterol (0.67 vs 1.58 mmol/l) and apolipoprotein A-I levels (96.7 vs. 151.4 mg/dl) ($P < 0.05$).

Conclusions—Genetic variants identified in the present study may be insufficient to promote early carotid atherosclerosis.

Keywords

HDL cholesterol; genetic mutations; LCAT; ABCA1; APOAI; carotid IMT

Introduction

Low concentrations of HDL cholesterol are highly prevalent in patients with coronary heart disease (CHD) (1) and genetic variation in HDL candidate genes accounts for a sizeable proportion of low HDL cholesterol in the general population (2). What has been less well established however, is the extent to which genetically low HDL cholesterol states may be associated with early atherosclerosis. That is, while complete absence of apolipoprotein AI (*APOA1*) resulting from chromosomal aberration has been associated with premature CHD in some pedigrees (3,4) other mutations causing low HDL cholesterol have not been associated with CHD (5). Therefore, to further investigate the potential impact of low HDL variants on early atherosclerosis, β -mode ultrasound was used to assess carotid intima-media thickness (cIMT) (6) in pedigrees affected by mutations in the primary HDL candidate genes, ATP

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binding cassette protein A1 (*ABCA1*), lecithin-cholesterol acyl transferase (*LCAT*) and apolipoprotein A1 (*APOA1*).

Materials and Methods

We previously identified mutations in *LCAT* [T321M, C-deletion (codon 168) P260X (7,8), *ABCA1* [D1099Y, F2009S, P85L, R1851Q, IVS46: del T-39...-46] (9–11) and *APOA1* [L159P] (12). In addition, we studied another pedigree with a previously reported variant in *LCAT* [I178T] (13). Demographic and CHD risk factor information for each of these kindreds has also been described (7–13). Affected family members with identified genetic variants and sex-matched and age-related controls underwent carotid β -mode ultrasound as previously described (14). Controls were selected as either family members without the mutation or acquaintances of cases. The study was approved by the University of Maryland Institutional Review Board. All images were interpreted at the Wake Forest School of Medicine by readers who were blinded to HDL variant status. Each value represents the mean of up to 20 mean IMT measurements obtained from multiple interrogation angles. Power calculations were performed to determine the number of participants required to find a difference between the groups. Using a 1:2 case control study design, the sample size calculations were based upon estimated differences in population means (0.15) and within group standard deviation (0.22) (15) to provide 90% power for detecting significant differences in cIMT between 35 cases and 70 controls at an α of 0.05. Student's *t*-test was used to compare differences between lipid, lipoprotein, apolipoprotein and cIMT measurements in variant and control subjects using $P < 0.05$ as significant.

Results

There were 10 different variants identified in *LCAT*, *ABCA1* and *APOA1* among 41 cases (50% female) (Table 1). All mutations were heterozygous and 3 subjects were genetic compounds (e.g., IVS46: del T-39...-46/R1851Q and 238X/T321M). For each case, we used 2 sex matched and age related (± 5 y) controls except in selected instances where only 1 control was available. Table 2 evaluates mean lipids, lipoproteins, apolipoproteins and cIMT measurements in the 2 groups. Compared to controls, there were no significant differences in the prevalence of traditional CHD risk factors other than dyslipidemia, where cases evidenced significantly reduced concentrations of HDL-C and apoA1 in association with higher TG and apoB concentrations ($P < 0.05$). However, cIMT concentrations were not different between cases and controls. Further evaluation of the CHD cases ($n=7$) (Table 3), demonstrated a high prevalence of accompanying risk factors and higher cIMT compared to the non-CHD cases ($n=34$) (0.77 ± 0.28 vs. 0.63 ± 0.14 mm; $P < 0.01$ by F-test).

Discussion

In evaluating subjects with genetic variation in *LCAT*, *ABCA1* and *APOA1*, we were unable to find differences in cIMT measurements compared to sex matched and age related controls. These data extend previous observations where increased cIMT was not associated with the *ABCA1* variant, W590L (14). In contrast to results obtained in the present study, cIMT has been reported to be lower in *APOA1*_{Milano} (16) but increased in association with molecular variation in *ABCA1* (17) and *LCAT* (18). In the latter 2 studies, however, there are methodologic concerns including a lack of sex and age related controls, which may have biased the results in favor of increased cIMT among low HDL-C cases. That is, cases trended either toward older age (e.g., mean difference > 5 yrs) (17) or with a greater predominance of men (18). In addition, CHD risk factors (e.g., smoking) were either significantly greater (18) in cases or not reported (17). Because small differences in these parameters may impact cIMT

measurements (19,20), they should have been more rigorously controlled for to minimize these potentially important confounding issues (20).

In the present study, the mean cIMT in the 7 CHD cases (mean age, 60.9 ± 18.9 y) was higher (0.77 ± 0.28 mm) than the 34 non-CHD cases (0.63 ± 0.14 mm). Based on the ARIC study (19) where the mean cIMT in 55 y-old men and women was 0.70 and 0.64 mm respectively, in association with an annual cross-sectional change of ~ 0.008 mm/y, 4 low HDL cases with CHD had increased cIMT (*APOA1* L159P, *LCAT* c-deletion (codon 168) and *LCAT* I178T), 2 had age predicted cIMT (*ABCA1* mutations) and only one had a lower cIMT than predicted (*LCAT* P260X). However, each of these cases exhibited other risk factors, notably combined hyperlipidemia (n=5) smoking history (n=4), hypertension (n=2) or hypertriglyceridemia (n=2), which may have contributed to the premature CHD (< 50 yrs) identified in 5 subjects. Therefore, while we cannot exclude the possibility of an increased prevalence of coronary atherosclerosis in the low-HDL-C group, the contribution solely based upon isolated low HDL-C remains to be determined. Consistent with these observations are the rarity of cases of premature CHD in patients with Tangier Disease or *LCAT* deficiency (21) when other traditional risk factors are lacking and the low likelihood of CHD in the setting of normocholesterolemic low HDL-C (22). This is in contrast to hyperlipidemic states (e.g., familial hypercholesterolemia) where increased cIMT has been consistently observed (23–25). Thus, while our data do not preclude the importance of low HDL-C (or its associated genetic variants) in augmenting CHD risk, they temper these suggestions and raise the possibility that the genetic mutations causing low HDL-C as reported herein, may be insufficient to cause appreciable carotid atherosclerosis as assessed by β -mode ultrasound imaging.

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Table 1

Genetic Variants causing low HDL-C

Gene	Mutation	Number Affected	Reference
<i>LCAT</i>	c-deletion (codon 168)	2	7
	T321M	5	7
	P260X	3	8
	I178T	6	13
<i>ABCA1</i>	D1099Y	5	9
	F2009S	1	9
	P85L	4	10
	R1851Q	6	11
	IVS46: del T-39...-46	6	11
<i>APOAI</i>	L159P	6	12
Total		41 cases (includes 3 compound heterozygotes)	

Table 2

Selected demographic factors, risk factor prevalence, medication use and biochemical measurements (mean and sd) and cIMT in genetic variant HDL-C cases and controls.

	Cases (n=41)	Controls (n=73)
Age (y)	44.8 (20.7)	44.8 (19.1)
BMI (kg/m ²)	28.0 (4.3)	26.4 (4.9)
Hypertension (%)	10.8%	15.9%
Diabetes Mellitus (%)	2.7%	0%
Smoking History (%)	24.3%	31.7%
Antiplatelet therapy (%)	18.9%	9.7%
Lipid lowering therapy (%)	21.6%	12.9%
cIMT (mm)	0.66 (0.17)	0.65 (0.18)
TC (mmol/l)	4.92 (1.52) *	5.03 (1.06)
TG (mmol/l)	2.10 (1.72) *	1.36 (0.90)
HDL-C (mmol/l)	0.67 (0.36) *	1.58 (0.75)
LDL-C (mmol/l)	3.28 (1.31)	2.85 (0.91)
APOAI (mg/dl)	96.7 (37.9) *	151.4 (34.9)
APOB (mg/dl)	123.6 (44.8) *	89.9 (26.6)

* P < 0.05 cases vs. controls

Table 3
Gene mutations, cIMT, onset of CHD and risk factors in 7 cases with low HDL-C

Gene	Mutation	cIMT	Age Onset (y)	CHD Risk Factors
<i>ABCA1</i> P85L	R1851Q	0.64	48	Smoker, HTN, HTG FCHL
<i>ABCA1</i> IVS46: del T-39...-46/ <i>APOA1</i> L159P		0.59	44	
<i>APOA1</i> L159P	c-deletion (codon 168) P260X I178T	0.71	41	Smoker, FCHL FCHL
<i>LCAT</i>		0.82	35	
<i>LCAT</i>		1.37	76	Smoker, HTN, HTG Smoker, FCHL FCHL
<i>LCAT</i>		0.59	57	
<i>LCAT</i>		0.69	39	
Mean		0.77 ± 0.28	60.9 ± 18.9	

Abbreviations: FCHL, familial combined hyperlipidemia; HTN, hypertension; HTG, hypertriglyceridemia