Genetic and secondary causes of severe HDL deficiency and cardiovascular disease[1]

Andrew S. Geller,*,† Eliana Y. Polisecki,[2],* Margaret R. Diffenderfer,* Bela F. Asztalos,† Sotirios K. Karathanasis,§ Robert A. Hegele,†† and Ernst J. Schaefer[3],*,†

Boston Heart Diagnostics,* Framingham, MA 01702; Cardiovascular Nutrition Laboratory,† Human Nutrition Research Center on Aging at Tufts University and Tufts University School of Medicine, Boston, MA 02111; MedImmune,§ Gaithersburg, MD 20878; and Robarts Research Institute,†† Western University, London, ON N6A 5B7 Canada

Abstract **We assessed secondary and genetic causes of severe HDL deficiency in 258,252 subjects, of whom 370 men (0.33%) and 144 women (0.099%) had HDL cholesterol levels <20 mg/dl. We excluded 206 subjects (40.1%) with significant elevations of triglycerides, C-reactive protein, glycosylated hemoglobin, myeloperoxidase, or liver enzymes and men receiving testosterone. We sequenced 23 lipid-related genes in 201 (65.3%) of 308 eligible subjects. Mutations (23 novel) and selected variants were found at the following gene loci: *1*) *ABCA1* (26.9%): 2 homozygotes, 7 compound or double heterozygotes, 30 heterozygotes, and 2 homozygotes and 13 heterozygotes with variants rs9282541/p.R230C or rs111292742/c.-279C>G; *2*) *LCAT* (12.4%): 1 homozygote, 3 compound heterozygotes, 13 heterozygotes, and 8 heterozygotes with variant rs4986970/p.S232T; *3*) *APOA1* (5.0%): 1 homozygote and 9 heterozygotes; and *4*) *LPL* (4.5%): 1 heterozygote and 8 heterozygotes with variant rs268/p.N318S. In addition, 4.5% had other mutations, and 46.8% had no mutations. Atherosclerotic cardiovascular disease (ASCVD) prevalence rates in the *ABCA1*, *LCAT*, *APOA1*, *LPL*, and mutation-negative groups were 37.0%, 4.0%, 40.0%, 11.1%, and 6.4%, respectively. Severe HDL deficiency is uncommon, with 40.1% having secondary causes and 48.8% of the subjects sequenced having *ABCA1*, *LCAT*, *APOA1*, or *LPL* mutations or variants, with the highest ASCVD prevalence rates being observed in the *ABCA1* and *APOA1* groups.**—Geller, A. S., E. Y. Polisecki, M. R. Diffenderfer, B. F. Asztalos, S. K. Karathanasis, R. A. Hegele, and E. J. Schaefer. **Genetic and secondary causes of severe HDL deficiency and cardiovascular disease. *J. Lipid Res.* 2018.** 59: **2421–2435.**

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It has been concluded that genetic HDL deficiency is not causative for atherosclerotic cardiovascular disease (ASCVD), in contrast to genetic hypercholesterolemia associated with elevated levels of LDL cholesterol (LDL-C) or genetic hypertriglyceridemia (1, 2). This assessment has been reported in high-impact journals by a very large number of authors, representing some of the most prominent and well-known scientists in our field. The data generated stem from a very large number of subjects studied with and without ASCVD. The analyses, however, were based entirely on SNPs, mainly in intronic DNA regions, and excluded the four most important genes that regulate HDL cholesterol (HDL-C) levels: *ABCA1*, *LCAT*, *APOA1*, and *LPL*. The exclusions were justified by study findings that *1*) genetic variations at the *ABCA1*, *LCAT*, *APOA1*, and *LPL* gene loci also affect TG and/or LDL-C levels and *2*) an *LIPC* variant was not associated with ASCVD (1, 2). In our view this justification is flawed. Our recent review of severe HDL deficiency has documented that patients with HDL-C levels <20 mg/dl in the absence of secondary causes may have premature ASCVD, especially if such patients are homozygous or compound heterozygous for mutations in the *APOA1* or *ABCA1* genes (3).

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Abbreviations: ABCA1, ATP binding cassette transporter 1; ASCVD, atherosclerotic cardiovascular disease; FED, fish-eye disease; FLD, familial LCAT deficiency; HbA1c, hemoglobin A1c; HDL-C, HDL cholesterol; HOMA-β, homeostasis model assessment of insulin production; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; LCAT, lecithin-cholesterol acyltransferase; LDL-C, LDL cholesterol; MPO, myeloperoxidase; sdLDL-C, small dense LDL cholesterol.

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[2] Present address of E. Y. Polisecki: Foundation Medicine, 150 Second St., Cambridge, MA 02141.

[3] To whom correspondence should be addressed.
e-mail: eschaefer@bostonheartdx.com

Although many studies have examined potential genetic causes of low HDL-C, there is no prior study to our knowledge that has carefully examined both secondary and genetic causes of severe HDL-C deficiency in a population of more than 250,000 subjects and related such findings to ASCVD (3). Our purpose in this investigation was to assess the prevalence of severe HDL deficiency in a large reference laboratory population and to determine potential underlying secondary and genetic causes by direct DNA sequencing of genes that are germane for lipoprotein metabolism, including *APOA1*, *ABCA1*, *LCAT*, and *LPL*, as well as to ascertain the prevalence of ASCVD in the various genetic HDL deficiency states.

Decreased levels of serum HDL-C (<40 mg/dl in men and <50 mg/dl in women) have been found to be a very significant independent risk factor for ASCVD and in this regard were superior to serum apoA-I levels in the Framingham Offspring Study (4, 5). An even better marker of ASCVD risk may be decreased HDL function as assessed by cellular cholesterol efflux (6, 7). Pre-β1 HDL, the smallest HDL subparticle separated by two-dimensional gel electrophoresis, serves as the acceptor of cellular free cholesterol and phospholipids via the ATP binding cassette transporter 1 (ABCA1) and, in the process, is converted to larger α HDL particles, while α1 HDL serves as both a donor and acceptor of cholesterol in its interaction with liver cells via scavenger receptor B1 (8–10). In patients with low HDL and premature ASCVD, compared with control subjects, there are significantly lower apoA-I levels in α1 HDL and higher apoA-I levels in pre-β1 HDL than in other HDL particles (11–14). Hypertriglyceridemia is also associated with increased levels of pre-β1 HDL (15). Increasing apoA-I levels in α1 HDL by means of treatment with the simvastatin/niacin combination has been associated with decreased coronary atherosclerosis (16).

In prior studies of families with premature ASCVD, we reported that 19% had lipoprotein (a) excess, 15% had familial dyslipidemia (high TGs and low HDL-C), 14% had familial combined hyperlipidemia (high LDL-C, high TGs, and usually low HDL-C), 4% had isolated low HDL (hypoalphalipoproteinemia), and 1% had familial hypercholesterolemia, according to the 10th and 90th percentile values from the Framingham Offspring Study (17, 18). Our data indicate that low HDL-C in families with premature ASCVD is frequently associated with either elevated TGs or elevations of both TGs and LDL-C. In the general population, we have also documented that low HDL-C levels are significantly associated with hypertriglyceridemia, obesity, diabetes, men, sedentary lifestyle, and cigarette smoking (19). Patients with markedly low plasma concentrations of HDL-C (<20 mg/dl) in the absence of marked hypertriglyceridemia, marked inflammation, diabetes, liver disease, or the use of anabolic steroids are therefore uncommon (3) and are most likely to have homozygous, compound heterozygous, or heterozygous defects involving the *APOA1*, *ABCA1*, or *LCAT* genes (1).

The overall data presented in this investigation are consistent with the concept that approximately 40% of patients with severe HDL deficiency have secondary causes, including severe hypertriglyceridemia, increased inflammation, and uncontrolled diabetes. In the absence of such secondary causes, about half of patients with markedly low HDL-C have mutations or variants at the *ABCA1*, *LCAT*, or *APOA1* gene loci as determined by DNA sequencing. Moreover, compared with nonaffected control subjects, patients with *ABCA1* or *APOA1* mutations or variants have an increased prevalence of ASCVD. In contrast to the conclusions of some investigators, the data indicate that genetic causes of severe HDL deficiency can, in turn, cause premature ASCVD (1–3).

METHODS

The subject population consisted of 112,776 men and 145,476 women referred to our reference laboratory over a 3-year period (2014–2016) that had the following parameters assessed in serum or plasma after an overnight fast using methods as previously described (20): total cholesterol, TGs, direct LDL-C, small dense LDL-C (sdLDL-C), HDL-C, apoA-I, apoB, HDL particle analysis, glucose, insulin, adiponectin, high-sensitivity C-reactive protein (hsCRP), fibrinogen, myeloperoxidase (MPO), and liver transaminases. We utilized results from only the first laboratory sample if a subject was sampled more than once. The median age of this population, the gender percentage, and the fasting lipid concentrations were quite similar to values previously obtained from subjects participating in cycle 6 of the Framingham Offspring Study (11). However, because our subjects were not randomly selected but were rather selected by their healthcare providers to have their serum and plasma biochemistries characterized by a reference laboratory, they may not be representative of middle-aged and elderly subjects in the general US population.

The lipid assays were standardized by the Lipid Standardization Program of the Centers for Disease Control (Atlanta, GA), and the apolipoprotein assays were standardized by the Northwest Lipid Research Clinics Program of the University of Washington (Seattle, WA). All assays except the HDL particle analyses and the MPO assays were performed using automated enzymatic analyses on Roche COBAS automated analyzers with reagents obtained from Roche Diagnostics (Indianapolis, IN). The within- and between-run coefficients of variation for these assays were <4%. The MPO measurements were performed using a chemiluminescence assay obtained from Siemens (Malvern, PA) on a Siemens Dimension 200 EXL platform, with within- and between-run coefficients of variation of <5%. The apoA-I content of HDL particles was analyzed by two-dimensional gel electrophoresis followed by immunoblotting as previously described, with within- and between-run coefficients of variation of <10.0% for all particles except for pre-β1 HDL, which was <15.0% (11).

In this population, 0.33% of men (n = 370) and 0.099% of women (n = 144) had fasting HDL-C concentrations <20 mg/dl. From this population, we excluded all subjects with hsCRP concentrations >10.0 mg/l, fasting TG concentrations >600 mg/dl, glycosylated hemoglobin (HbA1c) levels >8.0%, and liver transaminase levels >120 U/l, as well as men receiving testosterone replacement. These cutoffs for TGs, MPO, and liver enzymes were chosen because they represent values greater than the 99th percentile of the values in our population, while the remaining cutoffs were chosen because they represent significant inflammation, uncontrolled diabetes mellitus, and/or hormonal effects that are known to lower HDL-C levels (21).

Under a protocol approved by the Schulman Investigational Review Board (Cincinnati, OH), a total of 201 of 308 (65.3%)

eligible subjects were studied, provided that their healthcare providers consented to an anonymized genetic analysis at no cost to either the patient or the healthcare provider. The remaining 107 subjects were not tested because *1*) we never received a sample for DNA analysis (the main reason), *2*) the healthcare provider did not give consent because he/she was no longer following the patient or did not want to participate in the study, or *3*) the healthcare provider did not respond to our letter or phone calls. Data on these 107 subjects are provided in the Results section.

Clinical information from the study subjects was obtained from the healthcare provider by telephone interview using a questionnaire and was placed in an anonymized database. The questionnaire asked about the subject's history of angina pectoris, myocardial infarction, coronary artery bypass grafting or angioplasty, stroke, transient ischemic attacks, carotid or peripheral artery disease, and/or surgery for these latter conditions. If any of these responses was positive, the subject was deemed to have ASCVD. The questionnaire also asked about the subject's history of any form of kidney disease, decreased kidney function, proteinuria, neuropathy or neurological disease, seizures, hepatosplenomegaly, and/or corneal opacification or visual impairment.

DNA was isolated from whole blood of the 201 eligible subjects and analyzed by next-generation sequencing at 23 gene loci (*APOA1*, *ABCA1*, *LCAT*, *LPL*, *LIPC*, *LDLR*, *APOB*, *PCSK9*, *LDLRAP1*, *STAP1*, *ABCG5*, *ABCG8*, *APOE*, *LIPA*, *GPIHBP1*, *APOA5*, *APOC2*, *APOC3*, *CETP*, *SCARB1*, *CYP27A1*, *DHCR24*, and *LIPG*) on an Illumina MiSeqDx platform using 2 × 150 paired-end reads as previously described (22). With the exception of *CYP27A1*, these genes were selected by Johansen et al. (22) because of their known role in causing monogenic lipid disorders. We added the gene *CYP27A1* because of its role in causing cerebrotendinous xanthomatosis. For each gene, sequencing was performed on all known exons plus an additional 25 base pair padding at each intron-exon border. The median read depth was 517×, with 100% sensitivity and specificity for SNPs and 100% sensitivity and 91% specificity for insertions/deletions.

FASTQ files were processed using a custom workflow in CLC Biomedical Genomics Workbench version 3.2 (Qiagen, Valencia, CA). All mapping, variant calling and primary annotation, and quality control were performed using validated protocols. Variant call format files containing all identified variants in targeted regions were annotated and further analyzed using Ingenuity Variant Analysis (Qiagen) with a customized filtering cascade designed to identify potentially disease-causing variants. The analysis incorporated the classification guidelines described by the American College of Medical Genetics and Genomics (23). In addition, copy number variations and large structural variants were assessed using built-in tools from Biomedical Genomics Workbench (Qiagen) and VarSeq copy number variant analysis (Golden Helix, Bozeman, MT). Bioinformatic analysis of the sequencing results was confirmed by Dron et al. (24) as previously described. Our genetic analysis focused on three gene loci directly involved in HDL particle metabolism (*APOA1*, *ABCA1*, and *LCAT*). We also selected four common SNPs (*ABCA1* rs111292742, *ABCA1* rs9282541, *LCAT* rs4986970, and *LPL* rs268) known to be associated with HDL-C and TG plasma concentrations (discussed below) and analyzed their prevalence and minor allele frequency in our study population of 201 subjects versus the reported global population (25). Results of the analysis were given to the healthcare provider if requested.

Further analysis was required for variants that were novel or of uncertain significance to ascertain the potential pathogenicity. Prediction scores used for this assessment included *1*) Genomic Evolutionary Rate Profiling scores to evaluate conservation; *2*) DANN pathogenicity scores, which use computational deep-learning methodologies to classify variants; and *3*) dbNSFP, which aggregates several informatics prediction algorithms and uses a proportion of these algorithms to indicate the number of scores predicting the mutation to be deleterious versus the number predicting low functional impact. These scores were assessed using Varsome (Saphetor, Lausanne, Switzerland) and other computational and literature-based tools in Ingenuity Variant Analysis.

RESULTS

Prevalence of severe HDL deficiency

Data on our population are presented in **Tables 1** and **2**. Of the men in our population, 74.3% had normal HDL-C levels (≥40 mg/dl), 25.4% had low HDL-C levels (20–39 mg/dl), and 0.3% had severe HDL deficiency (<20 mg/dl) (Table 1). Men with severe HDL deficiency, by definition, had significantly ($P < 0.001$) lower levels of HDL-C (−66.7%), as well as significantly ($P < 0.001$) lower levels of total apoA-I (−47.0%) and apoA-I in very large α1 HDL (−77.2%), large α2 HDL (−51.3%), medium α3 HDL (−17.9%), small α4 HDL (−28.1%), and very small pre-β1 HDL (−36.9%) compared with men with normal HDL-C levels. They had 37% lower median HDL-C/apoA-I ratios at 0.214 versus 0.341 in controls.

Men with severe HDL deficiency were also significantly ($P < 0.001$) younger (−10.7%) and had significantly ($P < 0.001$) higher BMI (+14.3%), body weight (+10.3%), sdLDL-C (+46.2%), TGs (+242.5%), HbA1c (+9.4%), glucose (+7.1%), insulin (+80.0%), calculated homeostasis model assessment of insulin resistance (HOMA-IR) (+108%), calculated homeostasis model of insulin production (HOMA-β) (+42.6%), hsCRP (+160.0%), fibrinogen (+36.1%), and MPO (+25.1%) than controls. They also had significantly ($P < 0.001$) lower levels of direct LDL-C (−25.9%). Men with low HDL-C (20–39 mg/dl) generally had values for all of these parameters that were intermediate between the men with normal HDL-C and those with severe HDL deficiency (Table 1). The largest percentage difference between men with severe HDL deficiency and normal men was for elevated TG and hsCRP levels.

Of the women in our population (Table 2), 76.1% had normal HDL-C concentrations (≥50 mg/dl), 23.8% had low HDL-C concentrations (20–49 mg/dl), and 0.1% had severe HDL deficiency (<20 mg/dl). Women with severe HDL deficiency, by definition, had significantly ($P < 0.001$) lower levels of HDL-C (−74.2%), as well as significantly ($P < 0.001$) lower levels of total apoA-I (−51.0%) and apoA-I in very large α1 HDL (−81.3%), large α2 HDL (−55.5%), medium α3 HDL (−15.8%), small α4 HDL (−24.2%), and very small pre-β1 HDL (−26.4%) compared with women with normal HDL-C levels. They also had 47% lower median HDL-C/apoA-I ratios at 0.197 versus 0.374 in controls.

In addition, women with severe HDL deficiency had significantly ($P < 0.001$) higher sdLDL-C (+29.2%), TGs (+228.4%), HbA1c (+5.5%), glucose (+21.7%), insulin (+150.0%), HOMA-IR (+278.9%) and HOMA-β (+41.3%) scores, hsCRP (+153.8%), fibrinogen (+31.9%), and MPO

TABLE 1. Men in the Boston Heart Diagnostics population grouped by HDL-C levels (n = 112,776)

Parameter	Normal HDL-C ⩾40 mg/dl (n = 83,812; 74.32%)	Low HDL-C 20–39 mg/dl (n = 28,594; 25.35%)	Severe[a] HDL-C <20 mg/dl (n = 370; 0.33%)	% Difference: Severe vs. Normal
Age (years)	56 (21)	54 (20)	50 (21)	−10.7
BMI (kg/m^2)	28 (7)	31 (7)	32 (9)	+14.3
Weight (lbs.)	195 (48)	218 (56)	215 (60)	+10.3
Lipids and apolipoproteins (mg/dl)				
HDL-C[b]	51 (16)	34 (5)	17 (4)	−66.7
apoA-I[b]	149.5 (29.8)	118.1 (17.7)	79.3 (31.0)	−47.0
TGs	101 (67)	167 (124)	245 (348)	+242.5
LDL-C	116 (54)	113 (54)	86 (60)	−25.9
sdLDL-C	26 (19)	34 (27)	38 (29)	+46.2
apoB	95 (38)	100 (40)	97 (45)	+2.1
HDL subpopulations (mg/dl)				
α1 apoA-I	26.3 (13.6)	14.1 (5.9)	6.0 (4.9)	−77.2
α2 apoA-I	59.8 (13.7)	45.5 (8.6)	29.1 (10.0)	−51.3
α3 apoA-I	22.9 (6.0)	23.2 (6.1)	18.8 (7.1)	−17.9
α4 apoA-I	19.2 (5.7)	18.2 (5.2)	13.8 (10.0)	−28.1
Pre-β1 apoA-I	8.4 (5.4)	6.9 (4.6)	5.3 (1.7)	−36.9
Glucose metabolism				
Glucose (mg/dl)	98 (16)	102 (27)	105 (43)	+7.1
HbA1c (%)	5.6 (0.6)	5.8 (1.0)	5.8 (1.7)	+9.4
Insulin (μU/ml)	10.0 (10)	16.0 (16.0)	18.0 (18.0)	+80.0
Adiponectin (μg/dl)	9.9 (6.6)	6.8 (4.1)	6.0 (4.2)	−39.4
HOMA-IR	2.4 (2.6)	4.4 (4.9)	5.0 (4.9)	+108.0
HOMA-β	98.2 (90.8)	140.5 (139.3)	140.0 (167.2)	+42.6
Inflammation				
hsCRP (mg/l)	1.0 (1.9)	1.9 (3.2)	2.6 (6.2)	+160.0
Fibrinogen (mg/dl)	355 (105)	422 (143)	483 (341)	+36.1
MPO (pmol/l)	259 (139)	287 (160)	324 (222)	+25.1

Data are expressed as median (interquartile range).

[a] $P < 0.001$ for trend across HDL-C groups for all parameters, as determined by the nonparametric Kruskal-Wallis test.

[b] HDL-C/apoA-I ratio medians in men for the normal HDL-C, low HDL-C, and severely deficient HDL-C groups are 0.341, 0.288, and 0.214, respectively.

(+22.0%). They also had significantly ($P < 0.001$) lower levels of LDL-C (−25.9%) and adiponectin (−55.6%). Women with low HDL-C (20–49 mg/dl) in general had values for all of these parameters that were intermediate between the women with normal HDL-C and those with severe HDL deficiency (Table 2). As with men, there were large percentage differences between women with severe HDL deficiency and normal women for elevated TG and hsCRP levels.

Prevalence of secondary causes of severe HDL deficiency

As shown in **Table 3**, among men with severe HDL deficiency, 19.1% had fasting TG values >600 mg/dl versus 0.1% of normal controls; 18.9% had hsCRP values >10.0 mg/l versus 3.7%; 14.2% had HbA1c values >8.0% versus 3.1%; 4.1% had MPO values >1,000 pmol/l versus 0.6%; and 3.3% had elevated liver transaminase levels >120 U/l versus 0.5% (all $P < 0.001$). These cutoffs for TGs, MPO, and liver enzymes were chosen because they represent values greater than the 99th percentile of the values in our population, while the remaining cutoffs were chosen because they represent significant inflammation and/or uncontrolled diabetes mellitus. Each of these parameters is known to be associated with HDL deficiency (3). Men with low HDL-C (20–39 mg/dl) generally had prevalence rates for these abnormalities that were intermediate between men with severe HDL deficiency and normal controls. All men with these abnormalities were excluded from the genetic studies, as were 38 men (10.2%) with an HDL-C value <20 mg/dl who were receiving testosterone replacement, which is known to lower HDL-C levels significantly (21).

Table 3 also shows that among women with severe HDL deficiency, 24.5% had hsCRP values >10.0 mg/l versus 5.4% of controls, 21.9% had fasting TG values >600 mg/dl versus 0.0%, 11.2% had HbA1c values >8.0% versus 1.6%, 5.1% had MPO values >1,000 pmol/l versus 0.7%, and 1.6% had elevated liver transaminase levels >120 U/l versus 0.3% (all $P < 0.001$). Women with low HDL-C (20–49 mg/dl) generally had prevalence rates for these abnormalities that were intermediate between women with severe HDL deficiency and those with normal HDL-C. All women with these abnormalities were excluded from the genetic studies. In both men and women the major reasons for exclusion were because of severe hypertriglyceridemia, markedly increased inflammation (markedly elevated hsCRP and/or MPO levels), and/or uncontrolled diabetes mellitus.

Genetic causes of severe HDL deficiency

Of a total of 308 eligible subjects, there were 107 (72% men) for whom no DNA was available. Their mean age was 55 years, and their mean lipid and lipoprotein concentrations were as follows: non-HDL-C, 154 mg/dl; direct LDL-C, 107 mg/dl; TGs, 198 mg/dl; HDL-C, 18 mg/dl; and apoA-I, 87 mg/dl, with an HDL-C/apoA-I ratio of 0.207. Of these subjects, 5 (4.7%; all men) were reported by their healthcare provider to have established ASCVD, with a mean age

TABLE 2. Women in the Boston Heart Diagnostics population grouped by HDL-C levels (n = 145,476)

Parameter	Normal: HDL-C ≥50 mg/dl (n = 110,774; 76.15%)	Low: HDL-C 20–49 mg/dl (n = 34,558; 23.76%)	Severe[a]: HDL-C <20 mg/dl (n = 144; 0.099%)	% Difference: Severe vs. Normal
Age (years)	55 (20)	53 (23)	56 (21)	+1.8
BMI (kg/m^2)	26 (8)	32 (10)	27 (6)	+3.8
Weight (lbs.)	154 (47)	185 (62)	173 (46)	+12.3
Lipids and apolipoproteins (mg/dl)				
HDL-C[b]	66.0 (21)	42.0 (8)	17.0 (5)	−74.2
apoA-I[b]	176.3 (37.2)	135.3 (21.5)	86.3 (32.3)	−51.0
TGs	88 (57)	148 (99)	289 (338)	+228.4
LDL-C	118 (50)	121 (53)	73 (50)	−25.9
sdLDL-C	24 (14)	30 (24)	31 (30)	+29.2
apoB	94 (34)	103 (40)	90 (42)	+2.1
HDL subpopulations (mg/dl)				
α1 apoA-I	39.1 (18.0)	20.7 (8.1)	7.3 (5.0)	−81.3
α2 apoA-I	71.5 (16.4)	54.7 (11.0)	31.8 (13.9)	−55.5
α3 apoA-I	22.8 (5.9)	23.0 (6.2)	19.2 (9.3)	−15.8
α4 apoA-I	19.0 (6.7)	18.4 (6.1)	14.4 (8.7)	−24.2
Pre-β1 apoA-I	8.7 (6.0)	7.1 (5.2)	6.4 (6.2)	−26.4
Glucose metabolism				
Glucose (mg/dl)	92 (14)	98 (21)	112 (62)	+21.7
HbA1c (%)	5.5 (0.5)	5.7 (0.7)	5.8 (1.6)	+5.5
Insulin (μU/ml)	8.0 (8.0)	15.0 (14.0)	20.0 (22.2)	+150.0
Adiponectin (μg/dl)	15.3 (9.7)	9.2 (5.7)	6.8 (8.5)	−55.6
HOMA-IR	1.9 (1.9)	3.9 (4.2)	7.2 (10.0)	+278.9
HOMA-β	98.2 (83.7)	152.1 (137.3)	138.8 (142.0)	+41.3
Inflammation				
hsCRP (mg/l)	1.3 (2.8)	3.1 (5.1)	3.3 (7.8)	+153.8
Fibrinogen (mg/dl)	357 (105)	415 (125)	471 (230)	+31.9
MPO (pmol/l)	273 (151)	315 (175)	333 (313)	+22.0

Data are expressed as median (interquartile range).

[a]$P < 0.001$ for trend across HDL cholesterol groups for all parameters, as determined by the nonparametric Kruskal-Wallis test.

[b]HDL-C/apoA-I ratio medians in women for the normal HDL-C, low HDL-C, and severely deficient HDL-C groups are 0.374, 0.310, and 0.197, respectively.

of onset of 61 years. There were 201 subjects (65.3%) with HDL-C levels <20 mg/dl and without known secondary causes of low HDL-C who underwent DNA analysis. Of these subjects, 172 (85.6%) were Caucasian, 16 (8.0%) were Hispanic, 10 (5.0%) were African American, and 3 (1.5%) were Asian. Of 94 of the 201 subjects sequenced (46.8%; 72 men and 22 women), no *ABCA1*, *LCAT*, or *APOA1* mutations, selected variants (*ABCA1* rs111292742, *ABCA1* rs9282541, *LCAT* rs4986970, *LPL* rs268), or other mutations were found (mutation-negative group). The

TABLE 3. Prevalence of abnormal parameters used for excluding subjects from genetic analysis

Parameter	Normal: HDL-C ≥40 mg/dl (n = 83,812; 74.32%)	Low: HDL-C 20–39 mg/dl (n = 28,594; 25.35%)	Severe[a]: HDL-C <20 mg/dl (n = 370; 0.33%)	% Difference: Severe vs. Normal
Men (n = 112,776)				
TG >600 mg/dl	84 (0.1)	772 (2.7)	71 (19.1)	+19,000.0
HbA1c >8%	2,598 (3.1)	2,430 (8.5)	53 (14.2)	+358.0
hsCRP >10 mg/dl	3,101 (3.7)	1,973 (6.9)	70 (18.9)	+410.8
MPO >1,000 pmol/l	503 (0.6)	286 (1.0)	15 (4.1)	+583.3
ALT >120 U/l	19 (0.5)	229 (0.8)	12 (3.3)	+560.0
AST >120 U/l	251 (0.3)	114 (0.4)	12 (3.3)	+1,000.0
Parameter	**Normal: HDL-C ≥50 mg/dl (n = 110,774; 76.15%)**	**Low: HDL-C 20–49 mg/dl (n = 34,558; 23.76%)**	**Severe[a]: HDL-C <20 mg/dl (n = 144; 0.099%)**	**% Difference: Severe vs. Normal**
Women (n = 145,476)				
TG >600 mg/dl	0 (0.0)	276 (0.8)	32 (21.9)	NC
HbA1c >8%	1,772 (1.6)	2,143 (6.2)	16 (11.2)	+600.0
hsCRP >10 mg/dl	5,982 (5.4)	4,527 (13.1)	35 (24.5)	+353.7
MPO >1,000 pmol/l	775 (0.7)	415 (1.2)	7 (5.1)	+628.6
ALT >120 U/l	332 (0.3)	207 (0.6)	2 (1.6)	+433.3
AST >120 U/l	222 (0.2)	104 (0.3)	0 (0.0)	−100.0

Data are expressed as the number (%) of subjects in the HDL-C group. ALT, alanine aminotransferase; AST, aspartate aminotransferase; NC, not calculated.

[a]$P < 0.001$ for trend across HDL-C groups for all parameters except AST in women ($P = 0.002$), as determined by a chi-squared test.

data on these subjects were similar to the data on the subjects for whom no DNA was available for sequencing. The mean age of the mutation-negative group was 53 years; the mean lipid and lipoprotein concentrations were as follows: non-HDL-C, 142 mg/dl; direct LDL-C, 99 mg/dl; TGs, 237 mg/dl; HDL-C, 16 mg/dl; and apoA-I, 81 mg/dl, with an HDL-C/apoA-I ratio of 0.198. Of these 94 subjects, 6 (6.4%; 4 men and 2 women) were reported to have established ASCVD by their healthcare provider, with a mean age of disease onset of 64 years.

ABCA1 mutations and variants

Of the 201 subjects sequenced, 54 (26.9%; 38 men and 16 women) were noted to have *ABCA1* mutations (**Tables 4** and **5**) or SNPs (**Table 6**); 18 of the mutations were determined to be novel. Among the 39 subjects (19.4%) having *ABCA1* mutations, 2 men, subjects ABCA1-01 and ABCA1-02, were homozygous for the novel mutations *ABCA1* p.G851R and p.P1412R, respectively. Their plasma concentrations of HDL-C, apoA-I, nonHDL-C, LDL-C, and TGs were 5 and 6 mg/dl, 10 and 24 mg/dl, 35 and 91 mg/dl, 5 and 77 mg/dl, and 312 and 67 mg/dl, respectively. ABCA1-01 was also heterozygous for *LCAT* rs4986970 (p.S232T) (see below) and at the age of 56 years had evidence of splenomegaly, anemia, and peripheral neuropathy (numbness and tingling of his right arm) but no evidence of ASCVD. At the age of 47 years, he had undergone coronary angiography at the National Institutes of Health that showed no evidence of coronary atherosclerosis. It should be noted that his direct LDL-C value was very low (5 mg/dl). HDL particle analysis indicated that almost all of his apoA-I was in pre-β1 HDL. ABCA1-02 was recently reported, after we supplied the healthcare provider with the genetic information; unfortunately, in the published report the mutation was mislabeled as *ABCA1* p.P1312R (26). At the age of 38 years, this patient was asymptomatic with no evidence of ASCVD and had a marked decrease in apoA-I in all HDL particles, especially in very large α1 HDL, which was approximately 2% of normal.

As shown in Table 4, there were 5 *ABCA1* compound heterozygotes (2 men, 3 women) with a mean age of 46 years and mean lipid and lipoprotein concentrations as follows: HDL-C, 9 mg/dl; apoA-I, 30 mg/dl; non-HDL-C, 118 mg/dl; LDL-C, 94 mg/dl; and TG, 180 mg/dl. Three of the mutations found in these subjects were novel. Subjects ABCA1-03 and ABCA1-06 had established ASCVD (with the age of disease onset of 46 and 58 years, respectively), with non-HDL-C concentrations >80 mg/dl. When analyzed together, the 7 subjects (4 men, 3 women; mean age: 46 years) who were either homozygous or compound heterozygous for mutations in *ABCA1* (Table 4) had mean plasma lipid values as follows: HDL-C, 8 mg/dl; apoA-I, 26 mg/dl; nonHDL-C, 102 mg/dl; direct LDL-C, 79 mg/dl; and TG, 182 mg/dl, with an HDL-C/apoA-I ratio of 0.37. Of these subjects, 29% had evidence of ASCVD.

Subjects ABCA1-08 and ABCA1-09 were double heterozygous, having one mutation in *ABCA1* and the other in *LCAT* and *APOA1*, respectively (Table 4). They were men, aged 35 and 38 years, with no evidence of ASCVD and with the following respective values: HDL-C, 16 and 17 mg/dl; apoA-I, 79 and 95 mg/dl; non-HDL-C, 164 and 116 mg/dl; direct LDL-C, 136 and 75 mg/dl; and TG, 111 and 260 mg/dl. Their HDL-C/apoA-I ratios were 0.20 and 0.18, respectively. All 9 *ABCA1* homozygotes, compound heterozygotes, and double heterozygotes had a marked deficiency of apoA-I in very large α1 and large α2 HDL (<10% of normal; data not shown).

Table 5 shows that there were 20 men and 10 women heterozygous for *ABCA1* (includes 13 novel mutations) with a mean age of 53 years and mean values as follows: HDL-C, 15 mg/dl; apoA-I, 68 mg/dl; non-HDL-C, 143 mg/dl;

TABLE 4. Subjects with homozygous, compound heterozygous, and double heterozygous mutations in *ABCA1* ($n = 9$)

Case	Gender	Age (years)	HDL-C (mg/dl)	apoA-I (mg/dl)	HDL-C/apoA-I Ratio	Non-HDL-C (mg/dl)	LDL-C (mg/dl)	TGs (mg/dl)	CVD	*ABCA1* Mutation	
										Gene	Protein
Homozygous											
ABCA1-01[a]	Male	56	5	10	0.50	35	5	312	—	c.2551G>A[b]	p.G851R
ABCA1-02	Male	38	6	24	0.25	91	77	67	—	c.4235C>G[b]	p.P1412R
Compound heterozygous											
ABCA1-03	Male	49	19	18	1.06	98	57	375	+	c.1770G>C[b]	p.W590C
										c.1769G>T	p.W590L
ABCA1-04	Female	42	5	30	0.16	192	146	291	—	c.720+6T>C	—
										c.4465-1G>C	—
ABCA1-05	Female	49	5	30	0.17	98	91	73	—	c.4430G>T	p.C1477F
										c.1447A>C[b]	p.T483P
ABCA1-06	Female	60	10	53	0.19	96	76	92	+	c.6204G>C	p.L2068L
										c.5604_5605insG[b]	p.L1869fs*22
ABCA1-07	Male	30	5	17	0.29	106	102	67	—	c.4175+1G>T	—
										c.4037G>A	p.G1346E
Mean	2 males/ 3 females	46	9	30	0.37	118	94	180	2/5		
Double heterozygous											
ABCA1-08	Male	35	16	79	0.20	164	136	111	—	*ABCA1* c.2311G>C	*ABCA1* p.V771L
										LCAT c.475C>T	*LCAT* p.R159W
ABCA1-09	Male	38	17	95	0.18	116	75	260	—	*ABCA1* c.2328G>C	*ABCA1* p.K776N
										APOA1 c.327G>A	*APOA1* p.E109E

[a]Subject was also heterozygous for the *LCAT* rs4986970 (p.S232T) SNP.
[b]Novel mutation ($n = 5$).

TABLE 5. Subjects with heterozygous mutations in *ABCA1* ($n = 30$)

Case	Gender	Age (years)	HDL-C (mg/dl)	apoA-I (mg/dl)	HDL-C/ apoA-I Ratio	Non-HDL-C (mg/dl)	LDL-C (mg/dl)	TGs (mg/dl)	CVD	*ABCA1* Mutation	
										Gene	Protein
ABCA1-10	Female	56	17	69	0.25	165	126	259	+	c.1510-1G>T[a]	—
ABCA1-11	Female	39	11	51	0.22	83	65	70	—	c.811G>T[a]	p.E271*
ABCA1-12[b]	Female	66	18	62	0.29	152	104	69	+	c.4492C>T[a]	p.Q1498*
ABCA1-13	Female	59	16	75	0.21	137	111	113	+	c.5577delA[a]	p.V1861fs*9
ABCA1-14	Female	34	13	56	0.23	138	119	125	—	c.2338-3_2338-2delCA	—
ABCA1-15	Male	34	13	61	0.21	156	118	255	+	c.2568delG[a]	p.W856fs*40
ABCA1-16	Female	61	5	21	0.24	112	70	225	+	c.4465-1G>C	—
ABCA1-17	Male	75	29	96	0.30	116	102	102	+	c.4175+1G>T	—
ABCA1-18	Male	39	17	76	0.22	129	110	189	—	c.1529T>G[a]	p.L510R
ABCA1-19[c]	Male	36	16	75	0.21	75	57	176	—	c.1699A>T[a]	p.N567Y
ABCA1-20[d]	Male	64	12	67	0.18	106	70	272	+	c.916C>T[a]	p.R306C
ABCA1-21	Female	58	17	100	0.17	101	58	263	—	c.5477A>T[a]	p.Q1826L
ABCA1-22	Male	52	14	64	0.22	167	131	209	—	c.1862A>G[a]	p.Q621R
ABCA1-23	Male	76	18	82	0.22	67	49	159	+	c.3098T>C[a]	p.L1033P
ABCA1-24	Female	44	19	97	0.20	107	83	202	—	c.1759C>T	p.R587W
ABCA1-25[d]	Male	55	5	36	0.14	161	103	320	—	c.1774G>T[a]	p.G592C
ABCA1-26	Female	36	16	73	0.22	122	89	282	—	c.6218C>T[a]	p.T2073I
ABCA1-27	Female	26	5	16	0.31	151	133	143	—	c.1769G>T	p.W590L
ABCA1-28	Male	44	6	43	0.14	133	89	297	—	c.5376C>T	p.T1792T
ABCA1-29[b]	Male	47	18	94	0.19	177	110	587	+	c.254C>T	p.P85L
ABCA1-30	Male	49	19	114	0.17	184	140	288	+	c.5398A>C	p.N1800H
ABCA1-31	Male	43	16	71	0.23	85	63	115	+	c.360C>T	p.T120T
ABCA1-32	Male	45	6	19	0.32	400	317	388	—	c.474G>C	p.L158L
ABCA1-33	Male	40	19	89	0.21	241	188	424	—	c.720+6T>C	—
ABCA1-34	Male	78	14	104	0.13	148	60	500	+	c.4037G>A	p.G1346E
ABCA1-35	Male	43	10	34	0.29	193	166	130	—	c.254C>T	p.P85L
ABCA1-36	Male	77	18	75	0.24	132	105	102	+	c.4518G>A	p.S1506S
ABCA1-37	Male	73	19	84	0.23	133	90	303	+	c.720+6T>C	—
ABCA1-38	Male	63	18	78	0.23	90	68	101	—	c.5376C>T	p.T1792T
ABCA1-39	Male	65	15	62	0.24	125	103	143	+	c.32T>C	p.L11P
Mean	20 males/ 10 females	53	15	68	0.22	143	107	227	15/30		

[a]Novel mutation ($n = 13$).
[b]Subject was also heterozygous for the *LPL* rs268 (p.N318S) SNP.
[c]Subject was also heterozygous for a previously reported *ABCG8* c.550C>T (p.R184C) mutation.
[d]Subject was also heterozygous for the *LCAT* rs4986970 (p.S232T) SNP.

direct LDL-C, 107 mg/dl; and TG, 227 mg/dl, with an HDL-C/apoA-I ratio of 0.22. Among these 30 subjects, 15 (50.0%) had evidence of ASCVD (11 men, 4 women; mean age of disease onset: 58 years); none had evidence of neuropathy, splenomegaly, or anemia. ABCA1-20 and ABCA1-25 were also found to be heterozygous for *LCAT* rs4986970 (see below), while ABCA1-12 and ABCA1-29 were also heterozygous for *LPL* rs268 (see below).

Data regarding the study subjects having only *ABCA1* variant rs9282541 (p.R230C) and/or rs111292742 (c.-279C>G) and no *ABCA1*, *APOA1*, or *LCAT* mutations are provided in Table 6. An 80-year-old Hispanic man (SNP-01) and a 34-year-old unrelated Hispanic woman (SNP-02) were found to be homozygous for the *ABCA1* rs9282541 variant, which has been reported in the Mexican population to be associated with low HDL-C levels (27). Their respective values were as follows: HDL-C, 7 and 19 mg/dl; apoA-I, 44 and 75 mg/dl; non-HDL-C, 108 and 120 mg/dl; direct LDL-C, 58 and 91 mg/dl; and TG, 155 and 204 mg/dl. SNP-01 also had evidence of ASCVD (postmyocardial infarction at the age of 71 years). SNP-03, SNP-04, and SNP-05 were heterozygous for the *ABCA1* rs9282541 variant. None of these subjects had ASCVD. Their mean values were as follows: HDL-C, 15 mg/dl; apoA-I, 66 mg/dl; non-HDL-C, 149 mg/dl; direct LDL-C, 105 mg/dl; and TG, 244 mg/dl. This allele was found in 1.74% of our subjects versus 1.10% in the global population (not significant).

The *ABCA1* rs111292742 (*ABCA1* c.-279C>G) variant was found in 10 subjects, one of whom (SNP-31) was also heterozygous for *LPL* rs268. *ABCA1* rs111292742 has previously been associated with low HDL-C and hypertension in a Japanese population, while *LPL* rs268 has previously been reported to be associated with hypertriglyceridemia and low HDL-C levels (28, 29). These 10 subjects (all men; mean age: 51 years) had the following mean values: HDL-C, 16 mg/dl; apoA-I, 86 mg/dl; non-HDL-C, 143 mg/dl; direct LDL-C, 92 mg/dl; and TG, 318 mg/dl, with an HDL-C/apoA-I ratio of 0.19. The *ABCA1* rs111292742 allele was found in 3.48% of our subjects versus 2.46% in the global population (not significant). Evidence of ASCVD was noted in 3 of the 15 subjects with *ABCA1* variants (SNP-01, SNP-06, and SNP-10). Overall, 20 of the 54 subjects (37.0%) with *ABCA1* mutations or variants had evidence of ASCVD (mean age of disease onset: 59 years), significantly higher than the prevalence (6.4%) observed in our mutation-negative group ($P < 0.05$). Three of these subjects had evidence of neuropathy: ABCA1-01 with right-arm neuropathy, and ABCA1 SNP-01 and SNP-10 both with numbness and tingling in their lower extremities. SNP-01 and SNP-10 also had diabetes mellitus, which is well-known to cause neuropathy.

TABLE 6. Subjects with selected SNPs at the *ABCA1*, *LCAT*, and/or *LPL* gene loci ($n = 31$)

Case	Gender	Age (years)	HDL-C (mg/dl)	apoA-I (mg/dl)	HDL-C/apoA-I Ratio	Non-HDL-C (mg/dl)	LDL-C (mg/dl)	TGs (mg/dl)	CVD	SNP[a]
ABCA1										
SNP-01	Male	80	7	44	0.16	108	58	155	+	rs9282541/p.R230C[b]
SNP-02[c]	Female	34	19	75	0.25	120	91	204	—	rs9282541/p.R230C[b]
SNP-03	Female	53	19	95	0.20	126	86	287	—	rs9282541/p.R230C
SNP-04	Female	46	18	66	0.27	151	120	144	—	rs9282541/p.R230C
SNP-05	Male	19	7	38	0.18	170	108	302	—	rs9282541/p.R230C
SNP-06	Male	68	17	97	0.18	99	32	394	+	rs111292742/c.-279C>G
SNP-07	Male	36	19	115	0.17	176	113	341	—	rs111292742/c.-279C>G
SNP-08	Male	41	13	60	0.22	179	144	161	—	rs111292742/c.-279C>G
SNP-09	Male	54	18	116	0.16	161	111	342	—	rs111292742/c.-279C>G
SNP-10	Male	64	19	110	0.17	150	91	451	+	rs111292742/c.-279C>G
SNP-11	Male	61	19	86	0.22	66	48	125	—	rs111292742/c.-279C>G
SNP-12	Male	31	18	92	0.20	140	71	462	—	rs111292742/c.-279C>G
SNP-13	Male	43	19	103	0.18	106	66	372	—	rs111292742/c.-279C>G
SNP-14	Male	50	9	45	0.20	153	125	127	—	rs111292742/c.-279C>G
Mean	11 males/3 females	49	16	82	0.20	136	90	276	3/14	
LCAT										
SNP-15	Male	53	14	96	0.15	145	86	361	—	rs4986970/p.S232T
SNP-16	Female	64	20	95	0.21	112	74	220	—	rs4986970/p.S232T
SNP-17	Male	29	17	77	0.22	123	90	174	—	rs4986970/p.S232T
SNP-18	Male	28	10	42	0.24	262	215	184	—	rs4986970/p.S232T
SNP-19	Female	66	6	37	0.16	164	100	318	—	rs4986970/p.S232T
SNP-20	Male	24	10	40	0.25	232	210	169	—	rs4986970/p.S232T
SNP-21	Female	44	13	73	0.18	69	40	162	—	rs4986970/p.S232T
SNP-22	Female	76	5	24	0.21	190	102	338	+	rs4986970/p.S232T
Mean	4 males/4 females	48	12	61	0.20	162	115	241	1/8	
LPL										
SNP-23	Male	53	17	101	0.17	190	135	440	—	rs268/p.N318S[b]
SNP-24	Male	49	16	67	0.24	311	269	327	—	rs268/p.N318S
SNP-25	Female	78	10	68	0.15	81	29	169	+	rs268/p.N318S
SNP-26	Male	71	16	94	0.17	69	39	218	—	rs268/p.N318S
SNP-27	Male	33	17	67	0.25	176	130	239	—	rs268/p.N318S
SNP-28	Male	33	19	77	0.25	201	152	296	—	rs268/p.N318S
SNP-29	Male	43	18	105	0.17	130	51	504	—	rs268/p.N318S
SNP-30	Male	55	17	97	0.18	79	51	198	—	rs268/p.N318S
Mean	7 males/1 females	52	16	85	0.20	155	107	299	1/8	
Double										
SNP-31	Male	63	6	35	0.16	199	116	407	—	*ABCA1* rs111292742 *LPL* rs268

[a]Global minor allele frequencies for the identified SNPs are as follows: *ABCA1* rs9282541 (p.R230C), 1.10%; *ABCA1* rs111292742 (c.-279C>G), 2.46%; *LCAT* rs4986970 (p.S232T), 1.76%; and *LPL* rs268 (p.N318S), 1.29%. In our study population, including subjects with *ABCA1*, *LCAT*, and *APOA1* mutations, the corresponding minor allele frequencies were 1.74%, 3.48%, 2.74%, and 2.99%, respectively.

[b]Homozygous SNP.

[c]Subject was also heterozygous for a novel *APOA5* c.1028G>C (p.R343P) mutation.

LCAT mutations and variants

LCAT mutations or selected variants were found in 25 of the subjects (12.4%); 4 of the mutations were novel (**Table 7**). Subject LCAT-01, a 52-year-old man, was found to have a homozygous *LCAT* c.950T>G (p.M317R) mutation with the following values: HDL-C, 7 mg/dl; apoA-I, 44 mg/dl; non-HDL-C 80 mg/dl; direct LDL-C, <5 mg/dl; and TGs, 145 mg/dl, with an HDL-C/apoA-I ratio of 0.16. The direct LDL-C value of <5 mg/dl was below the detectable limits of the direct LDL-C assay, presumably due to the lack of a cholesteryl ester core in the LDL particles. As reported previously, this subject had marked corneal opacification and renal insufficiency, subsequently requiring hemodialysis (30). He was the first lecithin-cholesterol acyltransferase (LCAT)-deficient patient to receive LCAT enzyme replacement therapy, which transiently stabilized his renal disease over approximately a 4-month period, until such therapy was no longer available (31). HDL particle analysis indicated that his apoA-I was mainly in very small pre-β1 and small α4 HDL, with irregularly sized and poorly lipidated large HDL particles (30). After enzyme replacement, his apoA-I-containing particle distribution was transiently normalized (31). He had no evidence of ASCVD.

Three subjects were found to be *LCAT* compound heterozygotes. LCAT-02 and LCAT-03, with heterozygous *LCAT* c.110C>T, p.T37M and *LCAT* c.101C>T, p.P34L mutations, respectively, were siblings and have been previously reported (32). They were aged 23 and 26 years with the following respective values: HDL-C, 6 and 3 mg/dl; apoA-I, 36 and 14 mg/dl; non-HDL-C, 190 and 214 mg/dl; direct LDL-C, 157 and 156 mg/dl; and TGs, 158 and 290 mg/dl, with HDL-C/apoA-I ratios of 0.17 and 0.21. They presented with peripheral corneal opacification, normal kidney function, and no evidence of ASCVD (32). However, because of their elevated LDL-C and a strong family history of ASCVD, they were placed on statin therapy. LCAT-02 had an excellent response to the therapy; her LDL-C concentration decreased from 157 to 62 mg/dl (32). The diagnosis of

TABLE 7. Subjects with mutations in *LCAT* ($n = 17$)

Case	Gender	Age (years)	HDL-C (mg/dl)	apoA-I (mg/dl)	HDL-C/apoA-I Ratio	Non-HDL-C (mg/dl)	LDL-C (mg/dl)	TGs (mg/dl)	CVD	*LCAT* Mutation	
										Gene	Protein
Homozygous											
LCAT-01	Male	52	7	44	0.16	80	<5	145	—	c.950T>G	p.M317R
Compound heterozygous											
LCAT-02	Female	23	6	36	0.17	190	157	158	—	c.110C>T	p.T37M
										c.101C>T	p.P34L
LCAT-03	Male	26	3	14	0.21	214	156	290	—	c.110C>T	p.T37M
										c.101C>T	p.P34L
LCAT-04	Male	69	13	79	0.16	96	54	216	—	c.491G>A	p.R164H
										c.382G>A	p.G128S
Mean	2 males/1 female	39	7	43	0.18	167	122	221	0/3		
Heterozygous											
LCAT-05	Female	40	19	104	0.18	108	79	176	—	c.491G>A	p.R164H
LCAT-06	Male	53	15	80	0.19	162	114	270	—	c.397delG[a]	p.V133fs*131
LCAT-07	Male	75	15	77	0.19	99	57	236	—	c.911G>A[a]	p.R304H
LCAT-08[b]	Male	72	18	99	0.18	191	137	316	—	c.154+5G>A[a]	—
LCAT-09	Male	34	16	72	0.22	267	249	103	—	c.110C>T	p.T37M
LCAT-10	Female	17	19	90	0.21	133	100	244	—	c.301G>A	p.D101N
LCAT-11	Female	47	19	113	0.17	167	117	304	—	c.1112C>T	p.T371M
LCAT-12	Male	40	17	78	0.22	153	115	168	—	c.1123C>T	p.R375C
LCAT-13	Male	44	17	85	0.20	143	107	226	—	c.101dupC	p.H35fs*7
LCAT-14	Female	75	18	66	0.27	114	92	112	—	c.1139G>A	p.C380Y
LCAT-15[c]	Male	32	13	78	0.17	151	108	292	—	c.512G>A[a]	p.R171Q
LCAT-16	Male	60	10	69	0.14	98	77	90	—	c.101C>T	p.P34L
LCAT-17	Male	49	19	97	0.20	159	116	340	—	c.169G>C	p.G57R
Mean	9 males/4 females	49	17	85	0.20	150	113	221	0/13		

[a]Novel mutation ($n = 4$).
[b]Subject was also homozygous for the *ABCA1* rs111293742 (c.-279C>G) SNP.
[c]Subject was also heterozygous for a previously reported *LPL* c.-241G>C mutation.

the siblings was consistent with fish-eye disease (FED) (32). LCAT-04, the third compound heterozygote (*LCAT* c.491G>A, p.R164H and *LCAT* c.382G>A, p.G128S), was a 69-year-old man with the following values: HDL-C, 13 mg/dl; apoA-I, 79 mg/dl; non-HDL-C, 96 mg/dl; direct LDL-C, 54 mg/dl; and TGs, 216 mg/dl, with a HDL-C/apoA-I ratio of 0.16. He had no evidence of ASCVD or kidney disease but did have mild corneal opacification.

As also shown in Table 7, heterozygous *LCAT* mutations were noted in 13 subjects (9 men and 4 women; mean age: 49 years), with the following mean values: HDL-C, 17 mg/dl; apoA-I, 85 mg/dl; non-HDL-C, 150 mg/dl; direct LDL-C, 113 mg/dl; and TGs, 221 mg/dl, with an HDL-C/apoA-I ratio of 0.20. One of these subjects (LCAT-08) was also homozygous for *ABCA1* rs111292742, while another (LCAT-15) was heterozygous for a previously reported *LPL* mutation (29). None of these subjects had ASCVD or kidney failure, and none was reported to have corneal opacification. Eight additional subjects (4 men, 4 women; mean age: 48 years) were found to be heterozygotes for the *LCAT* rs4986970 (p.S232T) variant (Table 6). Their mean values were as follows: HDL-C, 12 mg/dl; apoA-I, 61 mg/dl; non-HDL-C, 162 mg/dl; direct LDL-C, 115 mg/dl; and TGs, 241 mg/dl. None of these 8 subjects was noted to have kidney disease, and only subject SNP-22, aged 76 years, was noted to have ASCVD (age of onset of 75 years with transient ischemic attacks). The *LCAT* rs4986970 variant has previously been associated with low HDL-C values in an Iranian population (33). We noted that the minor allele frequency for this variant in our severely HDL-deficient study population was 2.74% compared with a reported population frequency of 1.76% (not significant), but we had no data regarding Iranian descent.

APOA1 mutations and variants

Mutations in the *APOA1* gene were found in 10 subjects (6 men, 4 women; 5.0% of the total study population) (**Table 8**). APOA1-01, a 30-year-old man, with an HDL-C of 2 mg/dl and an apoA-I of 15 mg/dl, was found to be homozygous for the novel *APOA1* c.409G>T, p.E137* mutation and the *ABCA1* rs111292742 SNP. He had a truncated form of apoA-I and was asymptomatic, with no evidence of ASCVD. The remaining 9 subjects (mean age: 59 years) had heterozygous *APOA1* mutations and the following mean values: HDL-C, 18 mg/dl; apoA-I, 96 mg/dl; non-HDL-C, 127 mg/dl; direct LDL-C, 90 mg/dl; and TGs, 260 mg/dl, with an HDL-C/apoA-I ratio of 0.20. Subjects APOA1-02, APOA1-03, and APOA-04 were from a kindred with the *APOA1* c.718C>T (p.Q240*) mutation, which also results in a truncated form of apoA-I. They all had ASCVD, as previously reported (34). APOA1-05, a 67-year-old woman who was heterozygous for *APOA1* c.517C>A (p.R149S), had no evidence of ASCVD, but she did have a positive family history. In addition to the HDL deficiency, her plasma had a decreased ability to esterify cholesterol. She and her kindred have been previously reported (35). An *APOA1* in-frame deletion c.391_393delAAG (p.K131del) was found in four subjects, one of whom (APOA1-07), a man aged 71 years, had ASCVD. Subject APOA1-10 with the *APOA1* c.178T>G, p.S60A mutation had no evidence of ASCVD. Therefore, in our study population of 201 subjects with severe HDL deficiency, we found 10 subjects with

TABLE 8. Subjects with mutations in *APOA1* ($n = 10$)

Case	Gender	Age (years)	HDL-C (mg/dl)	apoA-I (mg/dl)	HDL-C/apoA-I Ratio	Non-HDL-C (mg/dl)	LDL-C (mg/dl)	TGs (mg/dl)	CVD	*APOA1* Mutation	
										Gene	Protein
Homozygous											
APOA1-0[a]	Male	30	2	15	0.13	61	54	67	—	c.409G>T[b]	p.E137*
Heterozygous											
APOA1-02	Male	70	19	67	0.28	94	69	140	+	c.718C>T	p.Q240*
APOA1-03	Female	88	19	77	0.25	131	109	121	+	c.718C>T	p.Q240*
APOA1-04	Male	61	19	101	0.19	128	108	154	+	c.718C>T	p.Q240*
APOA1-05	Female	67	12	67	0.18	187	148	235	—	c.517C>A	p.R173S
APOA1-06	Male	49	17	93	0.18	189	135	411	—	c.391_393delAAG	p.K131del
APOA1-07	Male	71	19	103	0.18	108	76	206	+	c.391_393delAAG	p.K131del
APOA1-08	Male	55	18	97	0.19	53	38	116	—	c.391_393delAAG	p.K131del
APOA1-09	Female	18	19	170	0.11	163	99	442	—	c.391_393delAAG	p.K131del
APOA1-10	Male	54	19	85	0.22	92	25	513	—	c.178T>G	p.S60A
Mean	6 males/ 3 females	59	18	96	0.20	127	90	260	4/9		

[a]Subject was also homozygous for the *ABCA1* rs111292742 (c.-279C>G) SNP.
[b]Novel mutation ($n = 1$).

APOA1 mutations that were mainly either truncations or in-frame deletions. Of these 10 subjects, 4 (40%) were noted to have ASCVD (3 men, 1 women; mean age of disease onset: 62 years).

LPL variants

Table 6 indicates that 8 subjects with no other mutations (7 men, 1 woman; 4.0%) had the *LPL* rs268 (p.N318S) variant, one of whom (SNP-23) was homozygous. These subjects had a mean age of 52 years and the following mean values: HDL-C, 16 mg/dl; apoA-I, 85 mg/dl; non-HDL-C, 155 mg/dl; direct LDL-C, 107 mg/dl; and TGs, 299 mg/dl. SNP-25 was a 78-year-old woman who developed ASCVD at the age of 71 years (coronary angioplasty and stent placement). SNP-31, who was heterozygous for *LPL* rs268 and *ABCA1* rs111292742 (as noted above), was a 63-year-old man with no evidence of ASCVD. He had the following values: HDL, 6 mg/dl; apoA-I, 35 mg/dl; non-HDL-C, 199 mg/dl; direct LDL-C, 116 mg/dl; and TGs, 407 mg/dl, with an HDL-C/apoA-I ratio of 0.16. The *LPL* rs268 variant has previously been reported to be associated with hypertriglyceridemia and low HDL-C levels (29). In total, 11 subjects, including the 3 subjects who also had a mutation or another variant (SNP-31, ABCA1-12, and ABCA1-29), carried the *LPL* rs268 variant. The frequency of this allele was significantly more prevalent in our study population (2.99%) than in the reported global population (1.29%; $P < 0.01$).

Other lipid-related mutations

As shown in **Table 9**, 10 subjects with severe HDL deficiency had mutations in genes related to monogenic lipid disorders characterized by elevated LDL-C and/or TG concentrations and/or by sitosterolemia. OTHER-01, found to be homozygous for an *ABCG8* mutation, had markedly elevated β-sitosterol levels, consistent with sitosterolemia (36). Two subjects (OTHER-02 and OTHER-03) were compound heterozygous for *LDLR* mutations and *APOB* mutations, and five subjects were heterozygous for *LDLR* or *APOB* mutations. Some of these subjects had LDL-C values >190 mg/dl, and those that did not were aggressively being treated with lipid-lowering agents (combination statin/ezetimibe). OTHER-05, aged 65 years, had established ASCVD (age of onset: 60 years) and was being aggressively managed with lipid-lowering therapy. OTHER-09, a 62-year-old man who was heterozygous for an *LIPC* mutation resulting in an amino acid substitution in hepatic lipase (p.T405M), had a TG concentration of 483 mg/dl. He had evidence of ASCVD, with angioplasty and stent placement at the age of 61 years. OTHER-10, a 46-year-old man who was heterozygous for the *LPL* p.T45N mutation, had moderate TG elevation (284 mg/dl) and no evidence of ASCVD.

ASCVD prevalence rates

The ASCVD prevalence in the 107 subjects (72% men) without secondary causes and for whom no DNA was available was 4.7%, with a mean age of onset of 61 years. The ASCVD prevalence in the 94 subjects (73% men) without secondary causes who did have DNA sequencing and in whom no mutations were found was 6.4%, with a mean age of onset of 64 years. Therefore, in these 201 pooled subjects with severe HDL deficiency with no secondary causes (72% men), the prevalence of ASCVD was 5.5%, with a mean age of onset of 62 years. In the 25 subjects with severe HDL deficiency with *LCAT* mutations or variants, only 1 subject, a 76-year woman, developed transient ischemic attacks (age of onset: 75 years). In this subject group the ASCVD prevalence was 4.0%. In contrast, in the 54 subjects with severe HDL deficiency and *ABCA1* mutations or variants, the ASCVD prevalence was 37.0%, with a mean age of disease onset of 59 years (significantly higher than in the mutation-negative group; $P < 0.05$). Similarly, in the subjects with severe HDL deficiency and *APOA1* mutations or variants, the ASCVD prevalence was 40%, with a mean age of disease onset of 62 years. Pooling the *ABCA1* and *APOA1* subjects ($n = 64$), the ASCVD prevalence was 37.5%, with a mean age of disease onset of 60 years. The ASCVD prevalence in these 64 subjects was 7-fold greater than in the 201 subjects with severe HDL deficiency who were not found to have any mutations or were never sequenced (5.5%; $P < 0.01$).

TABLE 9. Subjects with mutations in other genes associated with lipid metabolism ($n = 10$)

Case	Gender	Age (years)	HDL-C (mg/dl)	apoA-I (mg/dl)	HDL-C/ apoA-I Ratio	Non-HDL-C (mg/dl)	LDL-C (mg/dl)	TGs (mg/dl)	CVD	Mutation[a] Gene	Mutation[a] Protein
Homozygous											
OTHER-01	Female	26	10	42	0.24	136	111	98	—	*ABCG8* c.154C>G	ABCG8 p.L52V
Compound heterozygous											
OTHER-02	Male	36	5	13	0.38	346	270	409	—	*LDLR* c.1977C>A	LDLr p.T659T
										LDLR c.148G>T	LDLr p.A50S
OTHER-03	Female	62	10	55	0.18	208	147	458	—	*APOB* c.7612C>T	apoB p.L2538L
										APOB c.8112G>A	apoB p.A2704A
Heterozygous											
OTHER-04	Female	63	17	ND	NC	65	44	55	—	*LDLR* c.682G>T	LDLr p.E228*
OTHER-05[b]	Male	65	13	72	0.18	87	53	212	+	*LDLR* c.292G>A	LDLr p.G98S
OTHER-06	Female	27	16	62	0.26	261	243	72	—	*LDLR* c.67+1075G>A	
OTHER-07	Male	41	18	66	0.27	211	190	77	—	*APOB* c.2630C>T	apoB p.P877L
OTHER-08	Male	39	13	75	0.17	317	266	140	—	*APOB* c.3279C>G	apoB p.T1093T
OTHER-09	Male	62	19	104	0.18	141	83	483	+	*LIPC* c.1214C>T	HL p.T405M
OTHER-10	Male	46	16	82	0.20	88	40	284	—	*LPL* c.134C>A	LPL p.T45N
Mean	6 males/ 4 females	47	14	63	0.23	186	145	229	2/10		

HL, hepatic lipase; LDLr, LDL receptor; NC, not calculated; ND, no data.
[a]None of the mutations were novel.
[b]Subject was also heterozygous for the *ABCA1* rs111292742 (c.-279C>G) SNP.

DISCUSSION

The first form of severe HDL deficiency to be described was Tangier disease by Fredrickson in 1964 (37). Homozygotes with the disease, named after the Chesapeake Bay island where the first two cases originated, were noted to have diffuse cholesteryl ester accumulation in macrophages in their tonsils, liver, spleen, and other tissues, as well as rapid catabolism of their HDL proteins, apoA-I, and apoA-II (38–40). Homozygotes were found to have HDL-C levels <10 mg/dl, moderate hypertriglyceridemia, decreased LDL-C, occasional peripheral neuropathy, mild corneal opacification as seen on slit-lamp examination, and an increased risk of premature ASCVD (41–44). The defect in these patients was found to be an inability to efflux cholesterol onto HDL particles from their cells due to defects in the *ABCA1* gene and its gene product ABCA1 (45–52). On HDL particle analysis, homozygotes were found to have apoA-I mainly in pre-β1 HDL (53). Heterozygotes were found to have a 50% reduction in cellular cholesterol efflux, very low levels of very large α1 and large α2 HDL particles, and approximately 50% of normal HDL-C levels (53, 54).

Population studies indicated that three common *ABCA1* variants (p.G596A, p.A2589G, and p.G3456C) were more common in men with low HDL-C (<40 mg/dl) and ASCVD than in control subjects (55). Cohen et al. (56) sequenced the *APOA1*, *ABCA1*, and *LCAT* genes in 284 subjects with HDL-C concentrations below the 5th percentile (<30 mg/dl) and in 236 subjects with HDL-C levels above the 95th percentile (>75 mg/dl). They noted that nonsynonymous mutations or variants were significantly more common in subjects with low HDL-C than in those with high HDL-C (12% vs. 2%). They found that 9.9% of the subjects with low HDL-C had *ABCA1* mutations, 2.2% had *LCAT* mutations, and 0.2% had *APOA1* mutations. Therefore, mutations in *ABCA1* were more common in patients with low HDL-C than mutations in *LCAT* or *APOA1*, in agreement with our findings; however, no clinical or other laboratory information was provided in their study (56).

Fricke-Schmidt et al. (57, 58) did *ABCA1* genotyping in 9,259 Danish subjects followed for 25 years and noted that 5 of 6 SNPs (*ABCA1* p.V771M, p.V825I, p.I883M, p.E1172D, and p.R1587K) predicted increased risk of ASCVD, with *ABCA1* p.V771M, p.I883M, and p.E1172D being the most important risk predictors. They compared ASCVD risk in heterozygotes versus noncarriers for four *ABCA1* variants (p.P1065S, p.G1216V, p.N1800H, and p.R2144X) and noted that these variants reduced HDL-C levels by 17 mg/dl, associated with an adjusted hazard ratio for ASCVD of 1.70 ($P < 0.001$) (57, 58). Akao et al. (59) assessed the *ABCA1* p.R219K variant in 5,414 subjects in a statin trial for the elderly and noted an increased ASCVD event rate for those with the variant.

Abdel-Razek et al. (60) assessed potential differences in clinical and subclinical atherosclerosis (history of ASCVD or positive cardiac calcium score) and plasma cholesterol efflux capacity in 72 patients (mean age: 54 years) with HDL-C concentrations below the 10th percentile based on whether they had *ABCA1* or *APOA1* mutations. Disease-causing or likely pathogenic *ABCA1* mutations were found in 13 patients, and likely pathogenic *APOA1* mutations were found in 3 patients ($n = 16$; 22%), with 83% of these patients having evidence of atherosclerosis compared with 38.6% in patients with low HDL-C without such mutations or variants ($P < 0.01$). Patients with mutations also had lower cellular cholesterol efflux capacity (60). Dron et al. (61) recently reported 4 patients, aged 34–59 years, with low HDL-C associated with large-scale deletions within the *ABCA1* gene. Three of these patients had some evidence of ASCVD. The above data, like ours, indicate an increased risk of ASCVD in patients with low HDL-C and *ABCA1* mutations.

The next form of severe HDL deficiency, known as familial LCAT deficiency (FLD), was first described in 1967 by Norum and Gjone (62). These patients presented with marked corneal opacification, hyperlipidemia, anemia, proteinuria, and marked HDL deficiency (62). In contrast to normal subjects, almost all plasma cholesterol in these patients was unesterified due to a deficiency in LCAT activity. FLD patients were subsequently noted to have homozygous or compound heterozygous mutations in the *LCAT* gene and to develop kidney failure in the fourth or fifth decade of life (1, 62). We have documented that FLD homozygotes generally have apoA-I in plasma present only in very small pre-β1 and small α4 discoidal HDL particles (63). When the LCAT-deficient homozygote in the present study was treated with LCAT enzyme replacement therapy, there was transient appearance of α-HDL particles in the normal HDL-size range and stabilization of the patient's compromised kidney function, as reported previously (29). However, the patient ended up on dialysis when enzyme replacement was no longer available. In our view, the treatment of choice in homozygous FLD patients will be enzyme replacement or gene therapy.

A variant of FLD known as FED was first described by Carlson and Philipson in 1979 in a Norwegian man and his three daughters, all of whom had marked corneal opacification (64). They had normal concentrations of total cholesterol but elevated concentrations of TG, VLDL cholesterol, and LDL-C and marked HDL deficiency (1, 64). The disease was characterized further as being associated with ASCVD in later life, visual impairment, and dense corneal opacification. The two compound heterozygotes we report here as part of this study have previously been reported (32). In our view, the treatment of choice in FED patients is to optimize their LDL-C levels with statin therapy to prevent ASCVD.

Haase et al. (65) examined the effects of low HDL-C on ASCVD causality in 10,281 Danish subjects based on the presence of an *LCAT* variant associated with low HDL-C (*LCAT* rs4986970, p.S208T; seen in 4% of their population). They found that low HDL-C was robustly associated with increased ASCVD risk, but genetically decreased HDL-C due to impaired LCAT activity was not (65). Similar observations were reported in a recent head-to-head comparison of FLD- and FED-associated *LCAT* mutations: FLD mutations were associated with decreased atherosclerosis, while FED mutations were associated with increased atherosclerosis (66). The difference appears to relate to the capacity of LCAT to modulate the cholesteryl ester content of apoB-containing lipoproteins. In FLD this capacity is lost. Overall, the data indicate that FLD causes kidney failure, while FED causes ASCVD (3, 66). In our study we did not see an increased ASCVD prevalence in subjects with severe HDL deficiency associated with *LCAT* mutations.

A third form of severe HDL deficiency, familial apoA-I deficiency, was first described by Schaefer et al. (67, 68) in 1982 in a woman with severe HDL deficiency, undetectable plasma apoA-I, and death at the age of 42 years due to severe premature ASCVD. In 1983, Karathanasis, Zannis, and Breslow (69) became the first investigators to clone the *APO1* gene. This kindred was subsequently noted by Ordovas et al. (70) to be affected with a large deletion of the entire *APOA1/C3/A4* gene complex, with heterozygotes having about 50% of normal HDL-C, apoA-I, apoC-III, and apoA-IV levels in plasma. The disorder is now known as familial apoAI/CIII/AIV deficiency. In addition, in 1982 Norum et al. (71) described two sisters with severe HDL deficiency, undetectable plasma apoA-I and apoC-III levels, and premature ASCVD at ages 28 and 29 years. They were subsequently shown by Karathanasis, Ferris, and Haddad (72) to have a DNA rearrangement affecting the adjacent *APOA1* and *APOC3* genes. This disorder is now known as familial apoAI/CIII deficiency (69). Both forms of severe HDL deficiency are characterized by low TG and normal LDL-C concentrations.

Familial apoA-I deficiency (undetectable plasma apoA-I) with premature ASCVD due to an *APOA1* codon 84 nonsense mutation was first described by Matsunaga et al. (73) in 1991. Other investigators have subsequently described patients with premature ASCVD that had this disorder caused by a homozygous codon 2 nonsense mutation (74, 75). Haase et al. (76) sequenced the *APOA1* gene in 10,330 subjects and found that 0.27% of 10,330 subjects were heterozygous for *APOA1* nonsynonymous variants associated with reductions in apoA-I and/or HDL-C levels and a hazard ratio of 1.72 ($P < 0.01$) for myocardial infarction, largely driven by the *APOA1* p.A164S variant. The data presented herein are in agreement with prior studies indicating that mutations in *APOA1* are less common than those in *ABCA1* and *LCAT* in patients with HDL deficiency and are associated with premature ASCVD.

Genetic variations at the *LPL* gene locus are often associated with both hypertriglyceridemia and low HDL-C levels. The frequencies of three *LPL* polymorphisms (p.D9N, p.N291S, and p.S447X) were noted to be increased in men with low HDL-C levels (<40 mg/dl) and ASCVD versus control subjects (77), with the *LPL* N9 and S291 alleles, which raise TG levels, being more frequent, and the S477X allele, which lowers TG levels, being less frequent in the low HDL-C subjects than in control subjects (65). In the present study we observed that the *LPL* p.N318S allele occurred in 3.0% of our severely HDL-deficient population compared with a reported population frequency of 1.3%. We have carried out extensive genotyping (>1,000 SNPs) in 699 men with low HDL-C (<40 mg/dl) and ASCVD and 705 age-matched ASCVD-free men (78). After adjusting for multiple testing within each gene, SNPs at gene loci significantly associated with ASCVD and low HDL-C were *1*) *LIPC* (hepatic lipase) rs4775065 ($P < 0.0001$ vs. control subjects), *2*) *CETP* rs5882 ($P = 0.0002$), *3*) *ABCA1* rs2249891 ($P = 0.0126$), and *4*) *CUBN* rs7893395 ($P = 0.0246$) (78).

Dron et al. (24) examined the effects of polygenic determinants on extremes of HDL-C in three population cohorts (Western University Lipid Genetics Clinic, Montreal Heart Institute Biobank, and the University of Pennsylvania) that included a total of 686 subjects with low HDL-C levels (≤30.9 mg/dl in men and ≤38.7 mg/dl in women) and 1,165 subjects with high HDL-C (≥54.1 mg/dl in men

and ⩾69.6 mg/dl in women). The prevalence of CVD by history was highest in the Montreal Heart Institute Biobank cohort (ranging from 31.3% to 67.1%) and lowest in the University of Pennsylvania group (ranging from 4.1% to 12.9%) but was always highest in the low HDL groups (24). They reported that 18.7% of the low HDL group had rare heterozygous large-effect variants, mainly in the *ABCA1*, *LCAT*, and *APOA1* genes, with an additional 12.8% having an extreme polygenic trait score (24). The nine SNPs used in their polygenic trait score in order of effect size on HDL-C were *CETP* rs3764261, *LIPC* rs1532085, *ABCA1* rs1883025, *SCARB1* rs838880, *GALNT2* rs48469914, *MVK* rs7134594, *CMIP* rs2925979, *ZNF648* rs1689800, and *ANGPTL4* rs7255436, with an r^2 value of 0.8996 ($P < 0.0001$) (24).

The data presented herein, as well as prior data based on DNA sequencing, would indicate that HDL deficiency due to mutations or variants at the *ABCA1* or *APOA1* gene locus are associated with premature ASCVD (37–78). There may be, however, significant heterogeneity with regard to ASCVD risk in such patients depending on their LDL-C levels. In patients with Tangier disease and hypersplenism, there may be markedly increased catabolism of LDL apoB with very low LDL-C levels, which may protect such patients from ASCVD, in contrast to Tangier patients with normal LDL-C (3). There may also be heterogeneity in patients with HDL deficiency due to *APOA1* variants, with variants associated with decreased LCAT activation, such as ApoA-I_{Milano}, having no increased ASCVD risk, while other variants that do not affect LCAT activation having increased ASCVD risk (3).

As indicated earlier, we consider the HDL Mendelian randomization studies by Voight et al. (1) and Do et al. (2) as being highly flawed. Voight et al. reported no association between the intronic variant *ABCA1* rs3890182 and ASCVD, while Do et al. found no association between the intronic variant *ABCA1* rs1883025 and ASCVD (1, 2). Both studies did see significant relationships between the intronic variants *APOA1* rs6589566 and *APOA1* rs10790162 and ASCVD risk (1, 2); however, the *APOA1* variants were excluded from their analysis because of effects on other lipoproteins. Voight et al. (1) also relied almost entirely on the *LIPC* variant rs61755018 (p.N396S), which had no significant relationship with ASCVD. In our view, relying on intronic SNPs to assess the genetic contributions of a given gene to disease causation is flawed. Instead, investigators are relying increasingly on high-throughput next-generation DNA sequencing, which allows for more precise assessments of genetic variation at any given gene locus with disease, including ASCVD.

It should be stated that our study has its limitations in that the number of subjects that were sequenced was relatively small, and the reference laboratory population may not be representative of the general population. In our view, the measurement of HDL-C and apoA-I levels is helpful in identifying HDL-deficient patients, and the quantification of the distribution of apoA-I in HDL subpopulations by two-dimensional gel electrophoresis and immunoblotting analysis is helpful in making the diagnosis (3). Nevertheless, the definitive diagnosis of the various forms of severe HDL deficiency must rely on DNA sequencing of the *ABCA1*, *LCAT*, and *APOA1* genes (3). Another limitation of our study is the lack of data concerning the known polygenic causes of HDL deficiency, as documented recently by Dron et al (24).

The data presented herein are consistent with the following concepts with regard to severe HDL deficiency (HDL-C <20 mg/dl): this condition is *1*) uncommon in the general population and is significantly more frequent in men than in women; *2*) frequently associated with severe hypertriglyceridemia, increased inflammation, diabetes, and in men with testosterone replacement; *3*) most commonly caused by mutations in *ABCA1*, which can increase ASCVD risk depending on the underlying LDL-C levels; *4*) may be caused by mutations in *LCAT* that can lead to renal failure; and *5*) least commonly caused by mutations in *APOA1* that can lead to premature ASCVD.

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