SCARB1 Gene Variants Are Associated With the Phenotype of Combined High High-Density Lipoprotein Cholesterol and High Lipoprotein (a)

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Background—SR-B1 (scavenger receptor class B type 1), encoded by the gene SCARB1, is a lipoprotein receptor that binds both high-density lipoprotein (HDL) and low-density lipoprotein. We reported that SR-B1 is also a receptor for lipoprotein (a) (Lp(a)), mediating cellular uptake of Lp(a) in vitro and promoting clearance of Lp(a) in vivo. Although genetic variants in SCARB1 are associated with variations in HDL level, no SCARB1 variants affecting Lp(a) have been reported.

Methods and Results—In an index subject with high levels of HDL cholesterol and Lp(a), SCARB1 was sequenced and demonstrated a missense mutation resulting in an S129L substitution in exon 3. To follow up, 2 cohorts (GeneSTAR, the family-based Genetic Study of Atherosclerosis Risk [n=543], and CCHS, the population-based Copenhagen City Heart Study [n=5835]) were screened for combined HDL cholesterol and Lp(a) elevations. Subjects with the extreme phenotype (HDL >80 mg/dL and Lp(a) >100 nmol/L in GeneSTAR, n=8, and >100 mg/dL in CCHS, n=9) underwent sequencing of SCARB1 exons; 15 of 18 from the combined population demonstrated genetic variants, including rare or uncommon missense or splice site mutations in 9 and homozygous synonymous variants in 6. Functional studies with 4 of the SCARB1 variants (c.386C>T, c.631-14T>G, c.4G>A, and c.631-53mC>T & c.726+55mCG>CA) showed decreased receptor function in vitro.

Conclusions—Human SCARB1 gene variants are associated with a new lipid phenotype, characterized by high levels of both HDL cholesterol and Lp(a). SCARB1 exonic variants often result in diminished function of translated SR-B1 via reduced binding/intracellular transport of Lp(a). (Circ Cardiovasc Genet. 2016;9:408-418. DOI: 10.1161/CIRCGENETICS.116.001402.)

Key Words: Copenhagen City Heart Study ■ GeneSTAR ■ high-density lipoprotein cholesterol ■ lipids and lipoproteins ■ lipoprotein (a) ■ SCARB1 gene variants ■ SR-B1 receptor

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Clinical Perspective on p 418

Lp(a) is a proatherogenic lipoprotein particle containing one mole of apolipoprotein(a) (apo(a)) and a mole of LDL.15,16 Found in primates, it is not present in most other animals.15,16 Lp(a) levels are largely under apo(a) genetic
control. Although structurally and compositionally similar to LDL, Lp(a) is a relatively poor ligand for the LDL receptor and the lipoprotein receptor–related protein. Lp(a) can bind to the very low–density lipoprotein receptor, but receptor-defective mice showed only a modest delay in catabolism of Lp(a), suggesting other unidentified receptor(s).

We found that SR-B1 may be a receptor for Lp(a). In vitro, SR-B1 can bind Lp(a) and mediate intracellular transport of Lp(a). Cells overexpressing SR-B1 exposed to dual labeled Lp(a) took up the lipid moiety of the Lp(a) particle much more than the protein, supporting selective lipid transport. Transgenic mice overexpressing human SR-B1 in liver had greater plasma clearance of [1H] cholesteryl ether–labeled Lp(a), whereas Scarb1 knockout mice had less clearance. Because SR-B1 mediates lipid uptake from both HDL and Lp(a), we hypothesized that human SR-B1 deficiency may be characterized by a combined increase in plasma levels of HDL cholesterol (HDLC) and Lp(a). To test this, 2 cohorts were screened for combined elevations of HDL and Lp(a) to examine its association with genetic variants in the SCARB1 gene and with diminished function of the translated SR-B1 protein.

Materials and Methods

Study Populations

An index subject with high levels of HDLC and Lp(a) was identified in the National Institutes of Health (NIH) Lipid Clinic. SCARB1 was sequenced using methods described below. To further investigate the relationship between SCARB1 mutations and the high HDLC/high Lp(a) phenotype, 2 cohorts (Johns Hopkins GeneSTAR [Genetic Study of Atherosclerosis Risk] and the CCHS [Copenhagen City Heart Study]) were studied.

GeneSTAR is a prospective study of families identified from probands hospitalized with early-onset acute coronary syndromes <60 years of age. The cohort included 543 healthy non-coronary artery disease (CAD) siblings, aged 30 to 75 years. The study was approved by the Johns Hopkins Medicine Institutional Review Board, and all participants gave written informed consent. Subjects underwent risk factor screening and measurement of plasma Lp(a) after a 12-hour overnight fast. Blood pressure and blood lipids (total cholesterol, HDLC, triglycerides, and LDL cholesterol (LDLC)) were assessed as previously described. LDLC was estimated using the Friedewald formula. Lipoprotein subclass assays were performed by LipoScience Inc (Raleigh, NC) using nuclear magnetic resonance spectroscopy (see Methods in the Data Supplement). Type 2 diabetes mellitus was defined as a physician-diagnosed history, a measured fasting glucose level ≥ 6.99 mmol/L (126 mg/dL), and use of prescribed hypoglycemic medications. Hypertension was defined as systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg and use of prescribed antihypertensive medications.

CCHS is a prospective study of CAD risk factors in the Copenhagen Central Person Register, with the subjects drawn randomly to represent the adult general population. The cohort included 5835 non-CAD participants with measurements of fasting lipids and Lp(a). Total cholesterol, HDLC, and triglycerides were measured using colorimetric and turbidimetric assays. LDLC was calculated using the Friedewald formula. Hypertension was defined as use of antihypertensive medication, a systolic blood pressure > 140 mm Hg, or use of prescribed antihypertensive medication. Because SR-B1 mediates lipid uptake from both HDL and Lp(a), we hypothesized that human SR-B1 deficiency may be characterized by a combined increase in plasma levels of HDL cholesterol (HDLC) and Lp(a). To test this, 2 cohorts were screened for combined elevations of HDL and Lp(a) to examine its association with genetic variants in the SCARB1 gene and with diminished function of the translated SR-B1 protein.

Measurement of Lp(a)

In GeneSTAR, plasma Lp(a) concentration was measured in nmol/L in the Northwest Lipid Research Laboratory (Seattle, WA) by ELISA using an antibody against the apo(a) component of Lp(a). In CCHS, Lp(a) concentration was measured by a turbidimetric assay (Technicon Axon autoanalyzer, Miles Inc, Tarrytown, NY) using an antibody against the apo(a) component. For additional details, see Methods in the Data Supplement.

Sequencing of the SCARB1 Gene

Sequencing of the SCARB1 gene was performed in 18 subjects (1 from the NIH, 8 from GeneSTAR, and 9 from CCHS) with combined high levels of HDLC (>80 mg/dL) and Lp(a) (>100 nmol/L in GeneSTAR and >100 mg/dL in CCHS and the NIH patient). Genomic DNA was extracted from white blood cells (Qiagen kit, Valencia, CA) and amplified into 4 large fragments with SCARB1 primers (Table I in the Data Supplement) by high fidelity polymerase chain reaction (PCR; Roche Applied Science, Indianapolis, IN). The PCR fragments were inserted into a Topo TA-Clone vector (Invitrogen, Carlsbad, CA) and sequenced (BigDye-terminator chemistry; Perkin–Elmer/Applied Biosystems, Foster City, CA) with vector and intronic primers (Table II in the Data Supplement). For the NIH patient and GeneSTAR subjects, sequencing included all 13 exons with 40 bp of each intron–exon junction in both SCARB1 splice variants 1(v1) and 2 (v2). Additionally, a direct PCR sequence method was used for sequencing of the proximal promoter (400 bp). For the CCHS cohort, the promoter and exon 1 were not sequenced. For DNA methylation screening, bisulfite was used to convert cytosine to uracil, while 5-methylcytosine remained non-reacted. Converted genomic DNA was amplified by PCR and sequenced (Methods in the Data Supplement). The predicted effect of genetic variants on SR-B1 function was calculated by profile extraction from sequence alignments using position-specific independent counts.

Table 1. Characteristics of the GeneSTAR and CCHS Cohorts

| Characteristic | GeneSTAR (n=543) | CCHS (n=5835) |
|---------------|-----------------|---------------|
| Age, y        | 53.9 (7.7)      | 59.2 (16.5)   |
| Male sex, %   | 47.2            | 42.6          |
| Blacks, %     | 20.4            | 1.3           |
| Hypertensive, %| 57.8            | 18.4          |
| Diabetic, %   | 12.5            | 4.8           |
| Current smoking, %| 22.4  | 32.7          |
| BMI, kg/m²    | 29.6 (6.1)      | 25.8 (4.4)    |
| HDLC, mg/dL   | 132.5 (37.7)    | 134.5 (40.6)  |
| TG, mg/dL     | 160.4 (119.9)   | 135.9 (84.4)  |
| Lp(a)         | 69.3 (77.7)*    | 32 (33)†      |
| HDLC ≥80, %   | 4.8             | 11.7          |
| Lp(a) ≥100, % | 28.6            | 5.5           |
| HDLC ≥80 and Lp(a) ≥100, % | 1.5 | 0.15          |

The GeneSTAR cohort has 218 white males, 214 white females, 38 black males, and 73 black females. Values are mean (SD). BMI indicates body mass index; CCHS, Copenhagen City Heart Study; GeneSTAR, Genetic Study of Atherosclerosis Risk; HDLC, high-density lipoprotein cholesterol; LDLC, low-density lipoprotein cholesterol; Lp(a), lipoprotein (a); and TG, triglycerides.

* Lp(a) in nmol/L.
† Lp(a) in mg/dL.
In Vitro Mutagenesis and Cellular Experiments of Lipid Uptake

Wild-type human SCARB1 cDNA was generated as described. Site-directed mutagenesis by overlap PCR was used to produce all mutations in SCARB1 cDNA using mutagenesis primers (Tables II and III in the Data Supplement). All SCARB1 cDNA constructs were cloned into pcDNA3.1 (Invitrogen, Carlsbad, CA). For 2 intronic mutations (the intron 4 acceptor [-14] T/G and the compound mutation intron 4 -53 C>T and intron 5 +54, 55CG>CA), minigene constructs consisting of the 4.4 kb fragment containing exons 4, 5, 6 and introns 4 and 5 were generated by PCR using the subject’s own genomic DNA for the wild-type and each mutation. All SCARB1 gene constructs were confirmed by sequencing. SCARB1 constructs were transfected into 80% confluent HEK293 cells using Effectene transfection reagent (Qiagen, Valencia, CA). For stable transfection, cell clones were grown in DMEM growth medium containing 2 mg/mL G418 for 2 weeks and maintained thereafter with 0.25 mg/mL G418.

\[\text{[3H]} \text{cholesteryl ether–labeled HDL 3 and [3H] cholesteryl ether–labeled Lp(a) were prepared as described.}^5,28 \]

After cells grew to 95% confluence, [3H]-labeled lipoproteins were added to the culture medium at different concentrations and time points. Cells were washed 4× with PBS and harvested with 0.01 N NaOH and 0.1% SDS solution. The cell lysates were used to measure cell-associated lipoproteins, expressed as nanogram-labeled lipoprotein per milligram cell protein.

Statistics
Quantitative functional assays were analyzed with t tests and represent at least 3 independent experiments. Regressions using the general estimating equation\(^29\) to adjust for nonindependence of families were used for comparison of lipids in the siblings with or without SCARB1 variants.

Measurement of Apo(a) Isoforms, Northern and Western Analyses, DNA Methylation Assay, and Plasmid Construction
See Data Supplement.

Results

Prevalence of High HDLC/High Lp(a) Phenotype
The prevalence of the combined phenotype was determined in GeneSTAR (n=543) and CCHS (n=5835) and was defined as the co-occurrence of high plasma levels of HDLC (>80 mg/dL) and Lp(a) (>100 nmol/L in GeneSTAR and >100 mg/dL in CCHS). Characteristics of the populations are shown in Table 1.

In GeneSTAR, all 8 subjects with the phenotype were female, with 4 whites and 4 blacks (Table 2). All subjects had relatively high total cholesterol, but mostly normal triglyceride levels. Only 1 subject had a significantly elevated LDLC. Apo(a) isoforms ranged in size from 500 to 750 kDa (Table 2), and none had small apo(a) isoforms typically associated with high Lp(a).\(^{16,17}\) Only 2 subjects had a body mass index >30, and none had diabetes mellitus.

Table 2. Clinical Data and SCARB1 Variants in NIH Patient and GeneSTAR Cohort With High HDLC/High Lp(a) Phenotype

| Subject ID | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|------------|---|---|---|---|---|---|---|---|---|
| Age, y     | 49 | 50 | 67 | 37 | 44 | 64 | 53 | 63 | 49 |
| Sex        | M  | F  | F  | F  | F  | F  | F  | F  | F  |
| Race       | White | White | White | Black | Black | White | Black | Black | Black |
| BMI, kg/m² | 27 | 22 | 26 | 28 | 31 | 26 | 32 | 28 | 22 |
| Smoking    | –  | –  | –  | –  | –  | +  | –  | +  | –  |
| HTN        | –  | –  | +  | –  | –  | –  | –  | –  | +  |
| Diabetes mellitus | –  | –  | –  | –  | –  | –  | –  | –  | –  |
| TC, mg/dL  | 205 | 222 | 225 | 267 | 236 | 228 | 268 | 302 | 216 |
| TG, mg/dL  | 85 | 87 | 69 | 158 | 60 | 107 | 128 | 44 | 93 |
| LDLC, mg/dL | 86 | 123 | 105 | 150 | 132 | 124 | 155 | 199 | 114 |
| HDLC, mg/dL | 81 | 82 | 106 | 85 | 92 | 83 | 87 | 94 | 83 |
| Lp(a), nmol/L | 128† | 214 | 149 | 271 | 175 | 106 | 163 | 110 | 161 |
| Apo(a) isofrom 1 | 560 | 560 | 630 | 590 | 580 | 520 | 560 | 530 | 500 |
| Apo(a) isofrom 2 | 560 | 560 | 750 | 630 | 620 | 520 | 560 | 600 | 560 |
| SCARB1 variant(s) | c.386C>T | c.4G>A | c.4G>A,1050C>T | c.631-14T>G | IVS4,5 | c.1050C>T | c.1050C>T | 1495G>A | 1495G>A |
| Amino acid changes | S129L | G2S | G2S | delE5 | delE5 | A350A | A350A | G499R | G499R |
| Genotype | Het | Hom | Het | Het | Het | Hom | Hom | Het | Het |
| Siblings (N) | 0 | 1 | 2 | 5 | 1 | 2 | 0 | 1 | 0 |
| Carriers in siblings (N) | 0 | 0 | 1 | 2 | 1 | 1 | 0 | 0 | 0 |

Subjects 2–9, Lp(a) levels are in nmol/L (see Methods). The number of siblings available and sequenced for SCARB1 variants is indicated for each subject. Carriers indicate the number of siblings carrying the same genetic variant as the subject (all heterozygotes). Apo(a) indicates apolipoprotein (a); BMI, body mass index; GeneSTAR, Genetic Study of Atherosclerosis Risk; HDLC, high-density lipoprotein cholesterol; HTN, hypertension; LDLC, low-density lipoprotein cholesterol; Lp(a), lipoprotein (a); NIH, National Institutes of Health; TC, total cholesterol; and TG, triglycerides.

*Subject 1 is a patient from the NIH Lipid Clinic, all others are GeneSTAR participants.
†Lp(a) in mg/dL.
In CCHS, 8 of the 9 subjects with the phenotype were female, and all were white (Table 3). The lipid values were similar to those of GeneSTAR subjects, except that the HDLC values were somewhat higher and the triglyceride values lower, in keeping with the overall CCHS population. One subject had a significantly elevated LDLC, one had a body mass index >30 kg/m², and none had diabetes mellitus.

In GeneSTAR, the HDLC level of 80 mg/dL corresponded to the 95th percentile of values for the population, whereas the Lp(a) level of 100 nmol/L represented the 71st percentile, taking into account sex and race. Only 8 subjects of the 543 had combined elevations of high HDLC/Lp(a) (1.5%; Tables 1 and 2); none of whom were related. In CCHS, the HDLC level of 80 mg/dL represented the 88th percentile for the population, whereas the Lp(a) level of 100 mg/dL represented the 94th percentile. Of the 5835 subjects, only 9 (0.15%) had combined elevations of HDLC/Lp(a) of this magnitude (Tables 1 and 3). To further examine the striking female predominance for the high HDLC/high Lp(a) phenotype, we applied sex- and race-specific 90th and 95th percentile thresholds for both HDLC and Lp(a) to identify subjects. In GeneSTAR, 10/13 subjects exceeding the 90th percentile for both HDLC and Lp(a) were female, whereas in CCHS, 3/4 subjects exceeding the 95th percentile were female, strongly supporting the female predominance for this phenotype.

### Sequencing of SCARB1 in Subjects With the High HDLC/High Lp(a) Phenotype

In the NIH patient and the GeneSTAR cohort (Table 2), 5 rare (expected frequency <1%) or uncommon (expected frequency 1%–10%) SCARB1 variants were found in 7 subjects, resulting in functional coding changes: a c.389C>T base change in exon 3 (subject 1, resulting in a missense serine to leucine substitution at amino acid 129), a c.4G>A base change in exon 1 (subjects 2 and 3, resulting in a missense glycine to serine substitution at amino acid 2), a c.631-14T>G base change in intron 4 near the exon 5 acceptor splice site (subject 4, resulting in deletion of exon 5), a compound mutation at c.631-53mC>T and c.726 +54,55mCG>CA (subject 5, intron 4 and intron 5 variants, resulting in a deletion of exon 5), and a relatively uncommon c.1495G>A polymorphism of SCARB1_v2, an alternatively spliced form of the SCARB1 gene, resulting in a G499R substitution in the carboxy-terminal tail (subjects 8 and 9). All of these missense/deletion variants were heterozygous. The c.631-14T>G base change in subject 4 is located within a polypyrimidine sequence, which may affect mRNA splicing. The base change in subjects 2 and 3 is located just after the ATG initiation codon and was previously found to be important for efficient protein translation. The base change in subjects 8 and 9 is predicted to impair function of the translated protein.

In CCHS, variants were found in 6 of the 9 subjects (Table 3). Subjects 11 and 12 were heterozygous for a novel mutation, c.389A>G (exon 3 variant, resulting in a missense substitution of glutamic acid to glycine at position 130) in the extracellular loop of SR-B1, predicted

| Subject ID | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|------------|----|----|----|----|----|----|----|----|----|
| Age, y     | 60 | 49 | 53 | 55 | 44 | 83 | 63 | 69 | 80 |
| Sex        | F  | F  | F  | M  | F  | F  | F  | F  | F  |
| Race       | White | White | White | White | White | White | White | White | White |
| BMI        | 23 | 23 | 20 | 35 | 22 | 23 | 21 | 28 | 24 |
| Smoking    | −  | −  | −  | +  | −  | +  | −  | −  | −  |
| Diabetes mellitus | −  | −  | −  | −  | −  | −  | −  | −  | −  |
| TC, mg/dL  | 232 | 437 | 213 | 251 | 224 | 201 | 282 | 255 | 247 |
| TG, mg/dL  | 71 | 58 | 43 | 86 | 71 | 71 | 63 | 166 | 101 |
| LDLC, mg/dL | 131 | 294 | 101 | 131 | 135 | 116 | 170 | 124 | 147 |
| HDLC, mg/dL | 94 | 132 | 104 | 97 | 95 | 95 | 101 | 106 | 97 |
| Lp(a), mg/dL | 118 | 187 | 134 | 168 | 200 | 169 | 112 | 148 | 153 |
| SCARB1 Variant(s)* | None | c.389A>G | c.389A>G | c.1050C>T | c.1050C>T | c.1050C>T | c.1050C>T | None | None |
| Amino acid change | None | E130G | E130G | A350A | A350A | A350A | A350A | None | None |
| Genotype   | NA | Het | Het | Hom | Hom | Hom | NA | NA |

BMI indicates body mass index; CCHS, Copenhagen City Heart Study; HDLC, high-density lipoprotein cholesterol; LDLC, low-density lipoprotein cholesterol; Lp(a), lipoprotein (a); TC, total cholesterol; and TG, triglyceride.

*SCARB1 variants were identified by sequencing of 12 exons (Exon 2–12, v-12) of the SCARB1 gene.
to impair SR-B1 function (Table 4). Four additional subjects (13, 14, 15, and 16) were found to be homozygous for the common c.1050C>T polymorphism (Tables 3 and 4). The variabilities in Lp(a) and HDL levels in subjects with this polymorphism (Tables 2 and 3) suggest that other unidentified factors contribute to the high Lp(a)/high HDL phenotype.

### Relation Between SCARB1 Variants and Lp(a) and HDLC Levels in GeneSTAR Families

The GeneSTAR subjects in Table 2, all of whom had variants in the *SCARB1* gene, had 12 related siblings. Five of them shared one of the *SCARB1* variants and were, therefore, carriers without the full high HDLC/Lp(a) phenotype. Comparing the 5 sibling carriers of *SCARB1* variants (all heterozygous) with the 7 noncarriers, carriers had significantly higher levels of Lp(a) (160±47 versus 76±63 nmol/L; *P* =0.0097) and large HDL particles (HDL5+4, 17±5 vs 10±6 mg/dL; *P* =0.0001). Total HDLC level was not significantly different in carriers (52±8 versus 45±13 mg/dL; *P* =0.59).

### Table 4. Identified SCARB1 Variants

| Variant | ID  | Chr 12 Position | rs ID | Exon/Intron | Amino Acid Δ | Subject Prevalence | Variant Effect* | Strand and Ref →Alt allele† | Minor Allele, MAF, and Ref Populations |
|---------|-----|----------------|-------|-------------|--------------|------------------|-----------------|--------------------------|-------------------------------------|
| c.4G>A  | 2,3 | 125348263      | 4238001 | Exon 1      | G2S          | 0.22†            | Protein translation and receptor function* | Fwd:C→T                        | T=0.1084 (ExAC) T=0.0643 (1000 Genomes) T=0.0831 (GO-ESP) |
| c.386C>T| 1   | 125299559      | 15022965 | Exon 3      | S129L        | 0.11             | Uptake of lipoprotein*  | Fwd:A→G                      | A=0.0028 (ExAC) A=0.0020 (1000 Genomes) A=0.0017 (GO-ESP) |
| c.389A>G| 11,12| 124815010     |       | Exon 3      | E130G        | 0.22             | Damaging, PSIC=2.28 | Novel mutation |
| c.631-14T>G| 4 | 125296525 | 1139013 | Intron 4    | delExon 5    | 0.11             | Truncated protein, defective lipoprotein uptake* | Fwd:A→C                      | C=0.0006 (ExAC) C=0.0022 (1000 Genomes) C=0.0018 (GO-ESP) |
| c.631–53°C>T| 5 | 125296564 | 7774046 | Introns 4,5 | delExon 5    | 0.11             | Truncated protein, defective lipoprotein uptake* | Fwd:A→G Fwd:C→T              | A=0.0144 (1000 Genomes) A=0.0107 (GO-ESP) A=0.0114 (1000 Genomes) |
| c.1050C>T| 3,6,7,13,14,15,16 | 125284748 | 5888 | Exon 8      | A350A        | 0.33†            | Synonymous        | Rev:C→T                    | A=0.4148 (ExAC) A=0.3229 (1000 Genomes) A=0.3816 (GO-ESP) |
| c.1495G>A| 8,9 | 125263039 | 701103 | Exon 12 (v2) | G499R        | 0.22             | Damaging, PSIC=2.03 | Fwd:C→T | T=0.0669 (ExAC) T=0.0690 (1000 Genomes) T=0.0535 (GO-ESP) |

ExAC indicates Exome Aggregation Consortium; GO-ESP, NHLBI Grand Opportunity Exome Sequencing Project; PSIC, position-specific independent count; and SR-B1, scavenger receptor class B type 1.

††Accounts for the frequency of homozygotes. The predicted effect of genetic variants on SR-B1 function is calculated by the position-specific independent count (PSIC).

*Variant effect=actual in vitro test result. The function of the following variants were not tested: E130G (immediately adjacent to S129L), G499R (located in splice variant 2, role uncertain), and A350A (previously studied).36

†In dbSNP.

There were no significant differences in other lipids or apo(a) isoforms between the groups.

Results from the 5 siblings of GeneSTAR subject 4, who had a *SCARB1* splice site mutation (c.631-14T>G), are shown in more detail in Figure 1. By PCR-restriction enzyme analysis, 2 of the siblings were found to be heterozygous for the mutation, evident by the presence of 2 bands (lanes 3 and 4), and 3 were found to be homozygous for the wild-type sequence. Subjects with the mutation (lanes 3, 4, and 5) had higher Lp(a) levels and tended to have higher HDLC levels than subjects without the mutation. Two different apo(a) size isoforms of 580 and 620 kDa were found in the family, but the isoform size did not seem to correlate with Lp(a) levels.

### Functional Assessment of SCARB1 Variants

We tested the effect of 4 of the identified *SCARB1* variants on the uptake of cholesteryl ester from HDL and Lp(a) following expression in 293 cells. The c.386C>T (S129L) mutation (subject 1) showed a similar level of mRNA and
protein expression compared with the wild-type sequence (Figure 2A), but the mutation resulted in an ≈60% reduction in cholesteryl ester uptake from HDL and Lp(a) (Figure 2B). The methylation pattern of the mutant sequence was altered compared with the wild-type sequence (Figure 1 in the Data Supplement). Interestingly, wild-type SR-B1 demonstrated greater uptake of cholesteryl ester from Lp(a) than from HDL, suggesting that SR-B1 may be a more important clearance pathway for Lp(a) than HDL.

The c.4G>A polymorphism found in subjects 2 and 3 was also expressed in 293 cells and, as recently described, demonstrated reduced translation efficiency and decreased receptor function (Figure 4). The common c.1050C>T polymorphism, which was found to be homozygous in 6 subjects and heterozygous in 1 (Tables 2 and 3), has been previously associated with increased HDLC, but a possible relationship with Lp(a) has not been reported.

Discussion

Our major finding was the association of variants in the SCARB1 gene with a previously unrecognized dyslipidemic phenotype of combined increases in HDLC and Lp(a) levels. Co-occurrence of elevated HDLC and Lp(a) levels was uncommon, found in only 1.5% of apparently healthy family subjects in GeneSTAR and 0.15% of the Copenhagen City Heart Study population. There was a striking preponderance of women with this phenotype, which persisted when sex-specific 90th and 95th percentiles for HDL and Lp(a) were used. Women have higher HDL and Lp(a) levels and may have a higher rate of synthesis of both lipid particles, allowing expression of this phenotype in the presence of a functional variant in the SCARB1 gene.

Of the 18 subjects with the high HDLC/high Lp(a) phenotype, 15 had a genetic variant in SCARB1. Rare or uncommon missense or splice variants were found in 9 subjects, a much higher frequency than reported in a population with high HDLC alone or in the general population (Table 4). Using cellular studies, we examined the function of the transcribed protein for 4 of the missense/splice variants and found decreased function in all (Figures 2–4). The healthy siblings of GeneSTAR subjects in Table 2 who were identified as carrying a SCARB1 mutation or polymorphism had higher Lp(a) levels and an increase in large HDL subfractions compared with their siblings without the variants. Concordance of these genetic variants with increased Lp(a) levels within families provides additional support for a role for SR-B1 in the uptake of Lp(a), as well as HDLC. Recently, a genome-wide association study found that the LPA gene locus encoding Lp(a) (chromosome 6, rs1084651) and a SCARB1 locus (chromosome 12, rs8388880) are associated with increased HDLC.

Our finding of an association of SCARB1 variants with elevated HDLC is consistent with previous reports and also with findings in Scarb1 knockout mice. Recently, among subjects with a high HDL, an individual was reported homozygous for the P376L loss of function variant in SCARB1. Lp(a) was not elevated in this person, nor in others heterozygous for the P376L variant. The
explanation may involve the location of this variant in the C-terminal portion of the extracellular domain, which may be less important for Lp(a) binding than the N-terminal domain, where almost all of our variants were located.42

Our finding that SCARB1 variants are associated with both elevated HDL and elevated Lp(a) is novel and consistent with our recent finding that SR-B1 mediates cellular uptake of Lp(a) particles in vitro and the clearance of Lp(a)–cholesteryl ester in vivo in mice.5 Compared with wild-type mice, transgenic mice overexpressing human SR-B1 in liver showed ≈4-fold increased plasma clearance of [3H] cholesteryl ether–labeled Lp(a), whereas Sr-b1 knockout mice had ≈2.5-fold decreased plasma clearance.5 Although SR-B1 appears to bind both HDL and Lp(a) and mediate intracellular transport of lipid, the precise mechanism of binding is unknown. The binding of Lp(a) can be competed away by excess HDL,5 and based on the selective transport of cholesteryl ester from Lp(a),5 the binding probably occurs through the lipid rather than the protein moiety. SR-B1 probably does not have specific domains for either Lp(a) or HDL, but the 2 may compete based on the molar concentration of each lipid moiety.

Four SCARB1 mutations (c.386C>T and c.389A>G and the 2 mutations causing exon 5 deletions) were in the extracellular loop of SR-B1, which is known to be involved in ligand binding.42,45 Serine 129, located in this region,
is conserved in several species and, when tested by site-directed mutagenesis, was found to decrease cholesteryl ester uptake (Figure 2). The truncated protein missing exon 5 did not show any residual cholesteryl ester uptake activity (Figure 3C).

In addition, we found 2 uncommon SCARB1 variants and 1 common polymorphism previously associated with increased HDLC. Six subjects with combined elevations of HDLC and Lp(a) were homozygous for c.1050C>T (2 in GeneSTAR and 4 in CCHS), and 1 was heterozygous for both the c.1050C>T and the c.G>A polymorphism, with no single heterozygotes identified. Homozygosity for these polymorphisms may be required to produce the high HDLC/high Lp(a) phenotype. One subject with
the phenotype was homozygous for the c.G>A polymorphism in Exon 1 (N-terminal intracellular domain). Two subjects were heterozygous for the uncommon c.1495G>A polymorphism, which is present only in subjects of African descent. This polymorphism encodes an alternative spliced form of SCARB1 containing a different carboxy-terminal cytoplasmic tail\textsuperscript{44} and would be predicted to also affect SR-B1 function; however, we did not test this.

The effect of the high HDLC/high Lp(a) phenotype on human cardiovascular disease is not known. High HDLC may reduce cardiovascular risk; however, not all conditions that raise HDL are protective. Scarb1 null mice, for example, have increased atherosclerosis,\textsuperscript{5,9} despite increased HDLC levels.\textsuperscript{39,40} Zanoni et al\textsuperscript{41} found that elevated HDL related to heterozygosity of the P376L SCARB1 variant was associated with an increased risk of CAD. Raising HDL with drugs has shown no benefit.\textsuperscript{45} On the other hand, Lp(a) is generally viewed as proatherogenic.\textsuperscript{16,24,46} A large Mendelian randomization study demonstrated that a genetically determined doubling of Lp(a) was associated with a 22% increase in risk.\textsuperscript{47} Additional studies may determine whether variants in SCARB1 have an impact on atherosclerosis.

There remains a lack of specific Lp(a)-lowering therapies to test whether Lp(a) should be reduced in patients at risk for CAD. For example, nicotinic acid produces a 20% to 40% lowering of Lp(a), but also reduces LDL and triglycerides and raises HDLC.\textsuperscript{48} The mechanism seems to be a reduction in Lp(a) synthesis, without an effect on catabolism or clearance, so it is likely to be independent of SR-B1.\textsuperscript{49} Inhibitors of PCSK9 (proprotein convertase subtilisin/kexin type 9) lower Lp(a) by 20% to 30% in addition to their primary effect on LDL.\textsuperscript{49} The mechanism seems to be a reduction in Lp(a) synthesis, without an effect on catabolism or clearance, so it is likely to be independent of SR-B1.\textsuperscript{49} The mechanism may be related to reduced synthesis caused by a reduction in apolipoprotein B synthesis or availability,\textsuperscript{49} but is probably independent of the SR-B1 receptor.\textsuperscript{50} Lp(a) could be reduced by increasing function of SR-B1, although HDLC might also be reduced. Understanding whether, how, and under what circumstances to lower Lp(a) is important and will require future well-designed clinical studies.

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**Disclosures**

None.

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SR-B1 (scavenger receptor class B type 1), encoded by the gene *SCARB1*, binds high-density lipoprotein (HDL). We previously showed that SR-B1 also binds lipoprotein (a) (Lp(a)) and mediates intracellular transport of lipid from Lp(a). In the current study, we found an association between variants in the *SCARB1* gene and an unrecognized dyslipidemic phenotype, combined high HDL cholesterol (HDLC) and Lp(a) levels. In its severe form, this combination was found in only 1.5% of healthy subjects in GeneSTAR (Genetic Study of Atherosclerosis Risk) and in 0.15% of the CCHS (Copenhagen City Heart Study) population, with a striking preponderance in women. Of the 18 subjects with the phenotype, 15 had a genetic variant in *SCARB1*. Rare or uncommon missense or splice variants were found in 9 subjects, far more prevalent than in the general population or in populations with only high HDLC. Using cellular studies, we examined the function of the transcribed protein for 4 of the missense/splice variants and found decreased function in all. Thus, patients with combined marked elevations of HDLC and Lp(a) should be suspected of having a genetic variant in *SCARB1*. Although the clinical impact of the phenotype is not yet known, elevated Lp(a) and very high levels of HDLC each have been shown to be independently atherogenic. Available lipid-lowering drugs virtually all function independently of SR-B1. Better defining the proatherogenic impact of combined Lp(a) or HDLC elevations and any attendant therapy require future adequately powered population studies and well-designed clinical studies.