Rare variant in scavenger receptor BI raises HDL cholesterol and increases risk of coronary heart disease

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1

Scavenger receptor BI (SR-BI) is the major receptor for high-density lipoprotein (HDL) cholesterol (HDL-C). In humans, high amounts of HDL-C in plasma are associated with a lower risk of coronary heart disease (CHD). Mice that have depleted Scarb1 (SR-BI knockout mice) have markedly elevated HDL-C levels but, paradoxically, increased atherosclerosis. The impact of SR-BI on HDL metabolism and CHD risk in humans remains unclear. Through targeted sequencing of coding regions of lipid-modifying genes in 328 individuals with extremely high plasma HDL-C levels, we identified a homozygote for a loss-of-function variant, in which leucine replaces proline 376 (P376L), in individuals with extremely high plasma HDL-C levels, we identified a homozygote for a loss-of-function variant, in which leucine replaces proline 376 (P376L), in

Identification of SCARB1 P376L homozygote and association with extremely high HDL-C

We hypothesized that humans with extremely high levels of HDL-C may harbor loss-of-function variants in SCARB1 and undertook a targeted resequencing discovery experiment in 328 participants with very high HDL-C (>95th percentile, mean HDL-C of 106.8 mg/dl) and a control group of 398 subjects with low HDL-C (<25th percentile, mean HDL-C of 30.4 mg/dl). In this cohort, we sequenced the exons of ~990 genes located within 300 kb of each of the 95 loci with significant associations (P < 5 × 10^-8) with plasma lipid levels identified by the Global Lipids Genetics Consortium as of 2010 (22). Among the high HDL-C subjects, we identified a homozygote for SCARB1 P376L (g.125234671 G>A, c.1127 C>T, p.P376L, rs74830677), a 67-year-old female with an HDL-C of 152 mg/dl, and confirmed this finding by Sanger sequencing. This subject harbored no mutations in other high HDL-C genes such as CETP and LIPC. In addition to this homozygote, four P376L heterozygotes were identified by targeted sequencing in the high HDL-C group; no heterozygotes were found in the low HDL-C group (P = 0.008, Fisher’s exact test).

To identify additional P376L carriers, we genotyped an expanded cohort of very high versus low HDL-C subjects. Among 524 additional subjects with very high HDL-C (mean HDL-C 95.0 mg/dl), we identified 11 heterozygotes for P376L whereas among 758 subjects with low HDL-C (mean HDL-C 33.5 mg/dl), we identified 3 heterozygotes. In total, our combined sequencing and genotyping for discovery of the P376L variant showed that this variant is significantly overrepresented in subjects with high HDL-C [minor allele frequency (MAF) = 0.010 in high HDL-C versus 0.003 in low HDL-C controls, P = 0.000127, Fisher’s exact test, Table 1].

Because this variant is present on the exome array, we expanded our analysis to the Global Lipid Genetics Consortium exome array data in >300,000 individuals. The P376L variant was very rare in this population (MAF of ~0.00002). It was significantly associated with higher HDL-C (odds ratio = 1.79, which is statistically significant). Rare variant in scavenger receptor BI raises HDL cholesterol and increases risk of coronary heart disease

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he strong inverse association between amounts of high-density lipoprotein (HDL) cholesterol (HDL-C) and coronary heart disease (CHD) risk has generated interest in a potential causal relationship between HDL metabolism and CHD. However, clinical trials with drugs that raise HDL-C levels, niacin and heterozygotes

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European ancestry, almost exclusively of Ashkenazi Jewish descent. Clinical characteristics and lipid profiles of the subjects are reported in Table 2. Fast protein liquid chromatography (FPLC) analysis of plasma lipoproteins confirmed the increase in large HDL particles in the homozygote (Fig. 1A). Cholesterol and apolipoprotein A-I (apoA-I) levels in HDL were significantly increased in the homozygote and heterozygotes.

These authors contributed equally to this work.

Table 1. Association of SCARB1 P376L with HDL-C in high versus low HDL-C cohorts. Carriers of the P376L variant were ascertained from the Penn High HDL Study through two approaches, targeted sequencing of the SCARB1 gene in a total of 726 subjects (328 high HDL-C and 398 low HDL-C subjects) and genotyping on the exome array (Illumina) in an additional 1282 subjects (524 high HDL-C subjects and 758 low HDL-C subjects). The association of the P376L variant with the high HDL-C cohort from both approaches individually and combined together was tested using Fisher’s exact test.

<table>
<thead>
<tr>
<th>Discovery cohort</th>
<th>High HDL-C (&gt;95th percentile) (N)</th>
<th>Low HDL-C (&lt;25th percentile) (N)</th>
<th>Association (P)</th>
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<td>328 323 4 1 398 398 0 0</td>
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<td>Exome array genotyping</td>
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<td>Combined</td>
<td>852 836 15 1 1156 1153 3 0</td>
<td>0.000127</td>
<td></td>
</tr>
</tbody>
</table>

For each consortium and study, authors and affiliations are listed in the supplementary materials.
lysates, as well as mouse liver lysates expressing WT or mutant SR-BI (Fig. 2, F and G). Higher-molecular-weight forms represent N-glycosylation modified Endo-H-resistant and partially sensitive forms at the cell surface after modification by alpha-mannosidase II in the Golgi apparatus (28).

In the iPSC-derived differentiated HCLs from the P376L homozygote (Fig. 2F), we found much less total cellular SR-BI in the mutant cell lines relative to that of WT cells, despite comparable SCARB1 gene expression (fig. S3C). After Endo-H treatment, the SR-BI from SCARB1 WT cell and liver lysates across models was predominantly the partially sensitive form, along with small amounts of the fully resistant form. In contrast, the SR-BI from cell and tissue lysates across P376L-expressing groups was all the immature, fully Endo-H-sensitive form (Fig. 2, F and G, and fig. S4F). Together, these data

![Graphs and images](image_url)

**Fig. 1.** HDL composition and functionality in a SCARB1 P376L homozygote, heterozygous carriers, and controls. (A) FPLC fractionation of plasma lipoproteins from the P376L homozygote subject (red) and from a control with normal HDL-C. (B) Cholesterol, apoA-I, and apoA-II content in total HDL. (C) Free cholesterol (FC) and esterified cholesterol (CE) in total HDL (left) and the FC/CE ratio in total HDL (right). (D) HDL subclass concentrations after separation by density-gradient ultracentrifugation. (E) ApoA-I content in the same HDL subclasses. (F) ApoC-III content in the same HDL subclasses. (G) Cholesterol efflux capacity from macrophages of the THP-1 cell line. All data are reported as means ± SD.

**Table 2. Characteristics of SCARB1 P376L carriers and controls recruited for deep phenotyping.** Demographic, plasma lipid, and apolipoprotein traits measured from one P376L homozygote, eight heterozygotes, and noncarrier controls from subjects identified from sequencing or genotyping of the Penn High HDL Study cohort for deep phenotyping. Lipid measurements from plasma were performed using an autoanalyzer. Where applicable, data are presented as means ± SD. Numbers correspond to groups for comparison. Group 1, normal HDL-C controls; group 2, high HDL-C controls; group 3, SCARB1 P376L heterozygotes. Tested: ANOVA or chi-square. Groups: Comparison between groups by number with Tukey’s multiple comparison. *Significant at P < 0.05. **Significant at P < 0.01 by chi-square but not ANOVA. Dash indicates no significant comparison. BMI, body mass index; PTA, phosphotungstate precipitation method; VLDDL, very low density lipoprotein; Lp(a), lipoprotein a.
are consistent with a model in which the P376L sequence variant alters the endogenous post-translational N-glycosylation of SR-BI to prevent either transit from the ER to the Golgi or further posttranslational modifications in the Golgi, which ultimately result in reduced cell surface expression.

**SCARB1 P376L is associated with increased risk of CHD in humans**

Despite a profound increase in HDL-C, SR-BI deficiency in mice causes accelerated atherosclerosis (17–20). The relationship of reduced SR-BI function to atherosclerotic cardiovascular disease in humans has not been established. The P376L homozygous subject did not have clinical CHD, but her carotid intimal-medial thickness (cIMT) was 0.789 mm (left-right average), which is in the >75th percentile for females of her age; in addition, she had detectable plaque throughout the left internal carotid artery and at the bifurcation of her right internal carotid artery. cIMT measurements were not significantly different in the P376L heterozygotes compared with both groups of controls (fig. S8), but because of small sample size, the statistical power is limited.

To achieve greater statistical power to address this question, we performed a meta-analysis of large exome array genotyping studies of CHD cases and healthy controls to determine the relationship of the P376L variant with risk of CHD (Table 3). Among 16 sample sets from two consortia [the CARDIoGRAM Exome Consortium and the CHD Exome+ Consortium], we tested the association between P376L carrier status and CHD in 137,995 individuals. Across 49,846 CHD cases and 88,149 CHD controls, we found that P376L carriers had a significantly higher risk of CHD compared with noncarriers [odds ratio for disease among carriers = 1.79; \( P = 0.018 \)] (Table 3). Thus, carriers of this SCARB1 P376L variant have significantly increased HDL-C levels and a significantly increased risk of CHD.

**Discussion**

Studies of mice have provided important insights into the effects of SR-BI on HDL metabolism, RCT, and atherosclerosis. These studies revealed that overexpression of SR-BI reduces HDL-C (7–10) and reduces atherosclerosis (14–16), whereas gene deletion of SR-BI increases HDL-C (11–13) and accelerates atherosclerosis (17–20). The clinical relevance of these findings has remained uncertain, however. Studies of injected labeled HDL-CE in humans suggested that the majority of the HDL-CE was transported to the liver via
CETP-mediated exchange to apoB-containing lipoproteins rather than by direct uptake from HDL by the liver (30), which brings into question the importance of hepatic SR-BI in human physiology. Common genetic variants near the SCARB1 locus were found to be significantly associated with plasma HDL-C levels, which suggests that SR-BI may play a role in HDL metabolism in humans (22, 31). A family with a rare SCARB1 variant in which serine replaces proline 297 (P297S) was reported in which the heterozygous carriers of the variant had modestly elevated HDL-C levels (32). However, the variant retains substantial SR-BI activity, no homozygotes were identified, the apparent effect on HDL-C was modest, and there was insufficient power to address its effects on atherosclerosis.

Through sequencing of subjects with extremely high plasma levels of HDL-C, we identified a homozygote for a P376L variant in SR-BI. Our complementary approaches consistently demonstrated that this variant confers virtually complete loss of function of SR-BI. Our results demonstrate many similarities in the consequences of SR-BI deficiency on HDL composition between mice and humans, including a shift toward large, buoyant HDL particles and a significant increase in apoA-I, but not apoA-II, in plasma and HDL (22, 32, 33). The homozygote is a woman who had two healthy children without fertility issues or delivery complications, which suggests that, in humans, SR-BI deficiency may not impair reproductive function in the same manner as it does in mice (18, 34). In mice, SR-BI-mediated CE uptake from HDL is a critical process underlying steroid hormone synthesis in adrenal and gonadal tissues, and SR-BI deficiency alters adrenal cholesterol content, impairs adrenal glucocorticoid response under stress, and can lead to fasting-induced hypoglycemia (6, 35, 36). We did not observe any differences in fasting glucose, serum cortisol, adrenocorticotropic hormone, or female gonadal hormones in P376L heterozygous subjects versus controls, and we saw only a modest increase in testosterone in male P376L heterozygotes relative to noncarriers. We postulate that differences in expression or capacity for up-regulation of apoB-containing lipoprotein receptors relative to SR-BI between mouse models and humans in steroidogenic tissues may account, at least partially, for the lack of recapitulation of some of the phenotypes of SR-BI deficiency in mice. We also observed no differences in platelet levels, cholesterol content, and activation from the P376L carriers, despite reports of thrombocytopenia and altered platelet activity in Scabri KO mice (31). These results suggest a relatively different contribution of SR-BI to platelet function between mice and humans. Note that the phenotypes of human SCARB1 P376L homozygote (elevated HDL-C and large HDL particles but relatively normal steroidogenesis, reproductive viability, and platelet function) are comparable to those observed in mice lacking PDZ domain containing 1 (PDZK1), an adaptor protein for SR-BI (37).

Perhaps the most important finding of our study is that, despite the elevation in HDL-C, P376L carriers exhibit increased risk of CHD, as do Scabri KO mice. Our results are consistent with a growing theme in HDL biology that steady-state concentrations of HDL-C are not causally protective against CHD and that HDL function and cholesterol flux may be more important than absolute levels. Using an in vivo assay of macrophage RCT, we previously showed that Scabri KO mice have impaired macrophage RCT even though they have elevated HDL-C levels (21). Our results suggest that reduced hepatic SR-BI function in humans causes impaired RCT, which leads to increased risk of CHD despite elevation in HDL-C levels. However, SR-BI is also expressed in vascular cell types, including endothelial cells, vascular smooth muscle cells, and macrophages, where it could have protective effects against atherosclerosis as well (38, 39). Our results are also consistent with the previously suggested concept (39) that up-regulation or enhancement of SR-BI

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**Table 3. Meta-analysis of association of SCARB1 P376L variant with CHD.** CHD cases and healthy controls across the CARDioGRAM Exome Consortium and CHD Exome+ Consortium were genotyped for the SCARB1 P376L variant by using the exome array. BioVU, Vanderbilt University Medical Center Biorepository; BHF, British Heart Foundation; GoDARTS-CAD, Genetics of Diabetes and Audit Research Tayside Study; MHI, Montreal Heart Institute; North German, German North Coronary Artery Disease Study; Ottawa, Ottawa Heart Study; PAS, Premature Atherosclerosis Study—Academic Medical Center—Amsterdam; Penn, University of Pennsylvania CHD Cohort; South German, German South Coronary Artery Disease Study; WHI-EA, Women’s Health Initiative—European American Cohort; CCHS, Copenhagen City Heart Study; CHD5/CGPS, Copenhagen Ischemic Heart Disease Study/Copenhagen General Population Study; EPIC-CVD, European Prospective Investigation into Cancer and Nutrition—Cardiovascular Disease Study; MORGAM, MONica Risk, Genetics, and Monograph Project; PROSPER, Prospective Study of Pravastatin in the Elderly at Risk Study; WOSCOPS, West of Scotland Coronary Prevention Study. The association of the P376L variant with CHD cases was determined using a Mantel-Haenszel fixed-effects meta-analysis; results were odds ratio = 1.79; P = 0.018.
Wavelike charge density fluctuations and van der Waals interactions at the nanoscale

Alberto Ambrosetti, Nicola Ferri, Robert A. DiStasio Jr., Alexandre Tkatchenko

Recent experiments on noncovalent interactions at the nanoscale have challenged the basic assumptions of commonly used particle- or fragment-based models for describing van der Waals (vdW) or dispersion forces. We demonstrate that a qualitatively correct description of the vdW interactions between polarizable nanostructures over a wide range of finite distances can only be attained by accounting for the wavelike nature of charge density fluctuations. By considering a diverse set of materials and biological systems with markedly different dimensionalities, topologies, and polarizabilities, we find a visible enhancement in the nonlinearity of the charge density response in the range of 10 to 20 nanometers. These collective wavelike fluctuations are responsible for the emergence of nontrivial modifications of the power laws that govern noncovalent interactions at the nanoscale.

does not yield a sound theoretical description.

Even a slight variation in these power laws can have a profound impact on observed properties and therefore demands an accurate, physically sound theoretical description.

Thus far, both our conceptual understanding of vdW interactions and the quantitative models widely used for describing such quantum mechanical phenomena are primarily rooted in low-order intermolecular perturbation theory (IPT), wherein vdW binding originates from the interactions between transient local multipoles (9), and macroscopic Lifshitz theory (10). Although IPT-based approaches have had enormous success in describing vdW binding in (small) gas-phase molecular systems (11, 12), recent advanced experimental techniques have produced several findings that are challenging the basic assumptions of IPT and macroscopic approaches for nanomaterials, and are strongly indicative that even our qualitative understanding of these interactions is incomplete and needs to be substantially revised (13). Examples of such experimental observations include (i) ultra-long-range vdW interactions extending up to tens of nanometers into heterogeneous dielectric interfaces (14, 15), (ii) complete screening of the vdW interaction between an atomic force microscope (AFM) tip and a SiO2 surface by the presence of one or more layers of graphene adsorbed on the surface (16), (iii) super-linear sticking power laws for the self-assembly of metallic clusters on carbon nanotubes with increasing surface area (17), and (iv) nonlinear increases in the vdW attraction between homologous molecules and an Au(111) surface as a function of molecular size (18). Satisfactory theoretical explanations for these experimental findings either require ad hoc modifications to IPT ((ii) and (iv)) or are inherently outside the domain of applicability of IPT ((i) and (ii)).

To address these issues, we note that the spatial extent of the instantaneous charge density fluctuations responsible for vdW interactions depends rather sensitively on the nature and character of the occupied-to-virtual transitions of the
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