Rare Genetic Variants and High-Density Lipoprotein

Marching to a Different Drum

Bernardo L. Trigatti, Robert A. Hegele

Although static plasma concentration of high-density lipoprotein cholesterol (HDL-C), usually measured in the fasting state, is an excellent marker of atherosclerosis risk, its direct causal role in atherogenesis has lately been called into question.1 Strong evidence refuting a causal protective role for HDL has come from studies of rare genetic variants that affect plasma HDL-C concentrations. Initially, >3 decades ago, deletion of the adjacent APOA1 and APOC3 genes was found to be associated with reduced HDL-C and premature atherosclerosis in 2 sisters, consistent with an atheroprotective role for HDL.2 But since then observations in families with very low HDL-C because of rare loss-of-function variants in either APOA1, LCAT, or ABCA1 genes have shown variable associations with increased atherosclerosis risk.3–6 Other families with markedly elevated HDL-C because of rare loss-of-function variants in LIPC (hepatic lipase) paradoxically had increased atherosclerosis risk,7 whereas risk was variable in families with rare loss-of-function variants in CETP (Figure 1).8 Heterozygotes for the ultrarare p.P376L variant in SCARB1 (scavenger receptor SR-B1) had increased HDL-C, reduced cholesterol efflux, altered platelet function, decreased adrenal steroidogenesis but no reduction in atherosclerosis risk.9 Large Mendelian randomization studies in unrelated subjects similarly showed no relationship between common variants in several loci that modulated HDL-C levels and atherosclerosis risk.10–12

A new chapter of the HDL genetics story was recently written when Zanoni et al13 reported patients with a rare loss-of-function mutation in the SCARB1 gene encoding SR-B1. The multi-institutional effort led by Daniel Rader, Chair of the Department of Genetics at the University of Pennsylvania, set out to discover rare human DNA variants among large numbers of individuals with extreme levels of plasma lipoproteins.13 The team used targeted next generation sequencing of genomic DNA from 328 patients with high HDL-C (overall mean 106.8 mg/dL or 2.76 mmol/L). Their contrast group consisted of 398 subjects with low HDL-C (overall mean 30.4 mg/dL or 0.79 mmol/L). They interrogated ≈1000 genes at 95 loci that were previously shown with microarrays to be significantly associated with plasma lipoproteins.14

A fascinating pearl emerged with identification of a single female proband whose plasma HDL-C was tremendously elevated at 152 mg/dL or 3.93 mmol/L.13 This woman was the sole individual in this cohort who was homozygous for a loss-of-function variant in the SCARB1 gene, which resulted in substitution of leucine for proline at amino acid residue 376 (p.P376L). Interestingly, this patient, who was of Ashkenazi Jewish background, while asymptomatic, had increased mean carotid intima media thickness on ultrasound, suggesting that she was not protected against atherosclerosis despite her extremely high HDL-C.

Because the homozygous SCARB1 individual was so rare, the authors gathered heterozygotes for p.P376L, expanding their screening into independent study samples. They found a total of 15 heterozygotes for the mutation among a total of 852 individuals with high HDL-C, which was significantly >3 heterozygotes seen among 1156 individuals with low HDL-C (although the presence of these 3 individuals in the pool of low HDL-C subjects was itself interesting, and reflects the complexities of the genetics of lipoproteins). Heterozygotes for SCARB1 p.P376L had significantly increased mean HDL-C of ≈87 mg/dL or 2.24 mmol/L, which was intermediate between those of normolipidemic individuals and the homozygote. The heterozygotes and homozygote also had 3- and 6-fold increases in the large HDL-2b subfraction, increased apolipoprotein A-I (apo A-I) and apo C-III in large HDL particles, but normal cholesterol efflux capacity. There were no defects in steroidogenesis or hematologic abnormalities, as seen in heterozygotes for the SCARB1 p.P297S loss-of-function variant.9 Finally, because p.P376L was part of the Illumina exome array, the investigators could test for its association with coronary heart disease (CHD) in a meta-analysis of 16 studies. They found that 34 of 49846 cases and 52 of 88149 CHD-free controls were heterozygotes, which translated into an increased CHD odds ratio of 1.79 (P=0.018). A possible caveat is that the p.P376L variant may have been an indirect marker for a substratum of the population in whom other unmeasured genetic or nongenetic factors were acting to increase atherosclerosis risk.

This recent report by Zanoni et al13 together with 2 previous reports15 of 3 other rare point mutations in the human SCARB1 gene confirm that rare mutations that inactivate SR-B1 in humans result in dramatically increased HDL-C. The functional studies of the SCARB1 p.P376L variant are informative. In particular, Zanoni et al13 showed that the mutation drastically diminished cell surface SR-B1 protein, possibly

From the Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada (B.L.T.); Thrombosis and Atherosclerosis Research Institute, McMaster University and Hamilton Health Sciences, Hamilton, Ontario, Canada (B.L.T.); and Department of Medicine and Robarts Research Institute, Schulich School of Medicine and Dentistry, Western University, London, Ontario, Canada (R.A.H.).

Correspondence to Robert A. Hegele, MD, Robarts Research Institute, 4288A-1151, Richmond St N, London, Ontario N6A 5B7, Canada.
E-mail hegele@robarts.ca

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because of defective glycosylation or trafficking through the secretory pathway. The variant SR-B1 exhibited defective selective HDL-C uptake in hepatocyte-like cells differentiated from induced pluripotent stem cells, derived from the homozygous individual, as well as in a transfected mammalian cell line. Consistent with this, adenov-associated virus–mediated gene delivery into SR-B1–deficient mice revealed that while wild-type SR-B1 restored defective selective HDL-C clearance from blood, the p.P376L variant could not. Thus, defective hepatic selective HDL-C clearance because of reduced cell surface levels of the p.P376L variant seems to be the likely mechanism that explains the abnormally high level of plasma HDL-C in the homozygote, whereas a partial defect in SR-B1–mediated hepatic clearance would explain the moderately increased HDL-C levels in heterozygotes. These effects are strikingly similar to the effects of inactivating SR-B1 expression/activity either globally or in livers of mice, either by targeted or tissue-specific SR-B1 knockout, chemical mutagenesis, or knockout of PDZK1, an adapter protein required for SR-B1 protein expression in liver. That is, SR-B1 inactivation leads to defective hepatic HDL-C clearance, increased levels of HDL-C in plasma and reduced cholesterol in bile, with stronger effects seen in homozygous Scarb1–null and more moderate effects seen in heterozygous Scarb1–null mice. Studies in atherosclerosis-susceptible mice showed that inactivating SR-B1 globally or in a liver-specific manner increased atherosclerosis, whereas overexpressing SR-B1 in liver protected against atherosclerosis. Disrupting SR-B1 activity thus blocks hepatic HDL-selective uptake and back up hepatic RCT, triggering an increase in HDL-C and impairs HDL-dependent cholesterol removal from atherosclerotic plaques. Therefore, despite the build-up of HDL in plasma, the flux of cholesterol through HDL via RCT is impaired, thwarting HDL’s role in atheroprotection. SR-B1 may also mediate atheroprotective HDL signaling responses in endothelial cells. Therefore, global inactivation of SR-B1 would render such cells incapable of normal signaling responses to HDL, despite the context of increased HDL levels.

The most likely explanation for the increased CHD risk in heterozygous carriers of p.P376L is that in humans, as in mice, SR-B1 plays a key role in driving hepatic RCT. Inactivation of SR-B1 thus triggers a back-up in the flow of cholesterol from the artery wall, through the RCT pathway, resulting in increased atherosclerosis development, despite the increased HDL-C levels. The extent to which inactivation of SR-B1 in humans may also impair atheroprotective HDL signaling responses, and to what extent this may contribute to atherosclerosis development in the p.P376L mutant individuals remains to be tested.

Not all phenotypes seen in the global Scarb1-null mice were recapitulated by the human SR-B1 p.P376L homozygote: these include female infertility, adrenal insufficiency, abnormally high lipoprotein–unesterified cholesterol content and platelet abnormalities, although some of these have been associated with the p.P297S variant. These phenotypes likewise do not seem to be seen in mice with residual SR-B1 protein levels in liver or normal SR-B1 in other tissues such as steroidogenic tissues, suggesting that residual SR-B1 activity in liver or potential tissue specificity of the effects of the p.P397L variant may account for this, although this remains to be tested.

The results of Zanoni et al amplify the drumbeat of evidence in the HDL field that steady-state fasting plasma HDL-C concentrations do not play a direct causal role in atherosclerosis. But the iconoclasm is taken a step further: elevated HDL-C levels are not just neutral with respect to increased CHD risk, but in the context of genetically compromised SR-B1 function, they may actually increase risk. An extrapolation of this understanding is that enhancement of SR-B1 function could represent a novel therapeutic approach to reduce CHD risk in the general population, although there are still many caveats to this suggestion, including the relatively small odds ratios observed and the overall complexity of the HDL metabolic network. Although the functional data seem to fit into the emerging narrative that HDL function and cholesterol flux are more important than absolute levels of HDL-C, the findings from the SR-B1–deficient subjects do not allow for a definitive answer in this regard; more work is needed. Nonetheless, the ongoing story of the human genetics of HDL continues to undergo more twists and turns than a double helix.

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**Disclosures**

None.

**References**


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