HDL cholesterol: atherosclerosis and beyond

Bochem, A.E.

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Novel Mutations in Scavenger Receptor BI Associated with High HDL Cholesterol in Humans

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Abstract

The scavenger receptor class B, member 1 is a key cellular receptor for high density lipoprotein (HDL) in mice, but its relevance to human physiology has not been well established. Recently a family was reported with a mutation in SR-BI and high HDL cholesterol (HDL-c). Here we report two additional individuals with extremely high HDL-c (greater than the 90th percentile for age and gender) with rare mutations in the gene encoding SR-BI. These mutations segregate with high HDL-c in family members of each proband and are associated with a 37% increase in plasma HDL-c in heterozygous individuals carrying them. Both mutations occur at highly conserved positions in the large extracellular loop region of SR-BI and are predicted to impair the function of the SR-BI protein. Our findings, combined with the prior report of a single mutation in the gene encoding SR-BI further validate that mutations in SR-BI are a rare but recurring cause of elevated HDL-c in humans.
Introduction
The scavenger receptor class B, member 1 (SR-BI) is a high-affinity HDL receptor in mice that is highly expressed in the liver and steroidogenic tissues and is essential for HDL cholesterol uptake and excretion.\(^1,2\) In mice, targeted deletion of the *Scarb1* gene that encodes SR-BI leads to significantly elevated levels of plasma HDL-c\(^3\) and impaired selective uptake of HDL-c by the liver and adrenal glands\(^4\). Conversely, overexpression of SR-BI in mice results in the disappearance of HDL-c from plasma.\(^5,6\) These and other data have established SR-BI as the major HDL receptor in the liver and other tissues and a key regulator of the reverse cholesterol transport pathway by which excess cholesterol is removed by HDL particles and delivered to the liver for excretion into bile. Consistent with its importance in reverse cholesterol transport,\(^7\) deletion of *Scarb1* leads to increased atherosclerosis in mice deficient for ApoE or fed a Western-type diet.\(^8,9\)

Despite its established importance in mice, the role of SR-BI in HDL metabolism in humans is less clear. Common polymorphisms in SR-BI have been described with a mild effect on HDL-c levels.\(^10-15\) In addition, in a large genome wide association study involving over 100,000 individuals, the minor allele of the rs838880 variant, located approximately 400 bp downstream of the gene encoding SR-BI, was associated with a small but statistically significant 0.016 mmol/L (~1.4%) elevation in HDL-c.\(^16\) However, such association studies do not identify causality between a variant and the phenotype of interest, and the variants identified have only very small effects on plasma HDL-c, leaving unanswered the question of the potential quantitative importance of SR-BI in human HDL metabolism.

Recently, Vergeer and colleagues identified a single loss-of-function mutation, P297S, in the gene encoding SR-BI that was associated with elevated HDL-c, platelet dysfunction, and adrenal steroid hormone deficiency.\(^17\) It is unknown if this mutation represents a unique event, or if there are other mutations in SR-BI that cause extreme high HDL-c. Additionally, the frequency of SR-BI mutations in individuals with high HDL-c is unknown. In this study we evaluated the hypothesis that rare sequence variants in the gene encoding SR-BI are a cause of high HDL-c levels in humans. We report two novel mutations in SR-BI associated with high HDL-c.

Material and methods
Patients
We identified 120 unrelated probands of Caucasian ancestry with plasma HDL-c concentrations greater than or equal to the 90th percentile adjusted for age and gender. We also identified 80 individuals of Caucasian ancestry with HDL-c below the 10th percentile and no other lipid abnormalities. The study protocol was approved by the Ethics Committees of the Academic Medical Center, Amsterdam and the University of British Columbia, Vancouver. All subjects provided written informed consent.
Lipoprotein Analysis

Lipoprotein measurement on fresh plasma was performed as described. Cholesterol and triglyceride levels were determined in total plasma and plasma at density d<1.006 g/mL obtained after preparative ultracentrifugation, before and after precipitation with dextran manganese.

Sequencing and Mutation Detection

The SR-BI gene was sequenced from genomic DNA in all probands using either standard fluorescent dye terminator chemistry (Seqwright, Houston TX) or next generation paired-end read sequencing (Illumina, San Diego CA). For standard sequencing, DNA primers were designed to flank SR-BI exons and adjacent intron and UTR sequence as defined in human genome hg18. APOA1, LCAT and ABCA1 coding exons were sequenced using similarly designed primer sets. Sequence changes were identified from data using Sequencher 4.7 (Ann Arbor, MI) and confirmed in dbSNP build 130 and 1000Genomes November 2010 data release. For next generation sequencing, sequence changes were identified by alignment of sequence data to the human genome (NCBI Build 36.1) using CASAVA v.1.7 software (Illumina, San Diego CA) and confirmed by standard sequencing. Nucleotide and amino acid positions are with respect to Ensemble transcript ENST00000415380. Mutations were genotyped in the family members of probands that carry mutations using standard sequencing techniques. Multiple sequence alignment was performed using ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/).

Statistical Analysis

Differences in lipid and other values between mutation carriers and related controls were compared using a two-tailed student t-test and are reported as mean plus or minus standard deviation. A p value of 0.05 or less was considered statistically significant.

Results

We sequenced the gene encoding SR-BI in 120 unrelated probands with HDL-c levels greater than or equal to the 90th percentile for gender and age. We identified two novel missense mutations, S112F (nucleotide C588T) and T175A (nucleotide A776T), occurring in 1 separate individual each, for an overall rate of SR-BI mutations in individuals with high HDL-c of 1.2% in this cohort. In contrast, no novel SR-BI variants were found in 80 individuals with HDL-c less than the 10th percentile. Neither S112F nor T175A were identified in dbSNP or in the 1000genomes databases, indicating that these are novel, rare mutations.

Both S112F and T175A occur in the large extracellular loop of the SR-BI protein and affect residues highly conserved across multiple species (figure 1). The serine residue at position 112 is completely conserved in all mammalian species as well as the zebra fish, Danio rerio (figure 1B). The threonine residue at position 175 is conserved across mammals, but differs in Danio rerio where the orthologous position aligns to proline. Both mutations are predicted to be damaging by Polyphen. In addition, the substitution position specific evolutionary conservation score for T175A is −2.28, indicating a moderate prediction of a deleterious effect on protein function.
Figure 1. A) The location of mutations identified in this study and in the study by Vergeer and colleagues (17) are shown in a schematic of the SR-BI protein. All mutations identified to date occur in the large extracellular loop corresponding to the CD36 superfamily domain indicating that this is a critical functional domain.

B) Multiple sequence alignment of the SR-BI protein from different species. The residues at which the S112F and T175A occur are highlighted. S112 is completely conserved across all available vertebrate sequences. T175 is conserved across all mammalian species and differs only in the zebra fish, Danio rerio.
We next genotyped the family members of these two probands to assess segregation of these mutations with high HDL-c. The T175A mutation segregates with the high HDL-c phenotype in the pedigree of this proband (figure 2A), such that individuals carrying the T175A allele have HDL-c levels greater than the 95th percentile (3 individuals) whereas no individuals without T175A have HDL-c levels above the 90th percentile (11 individuals). One individual heterozygous for the T175A mutation (ID II:04 in Figure 2A) had HDL-c at the 71st percentile. This individual has a history of other cardiovascular risk factors including hypertension and is on medication that may influence this phenotype (supplementary table 1). Similarly in the pedigree of the proband heterozygous for S112F, no individual without this mutation had elevated HDL-c (figure 2B) suggesting that this mutation is causal for high HDL-c in this family.

Interestingly, the mother of the proband in figure 2B (individual I:02) was found to carry S112F but has low HDL-c (15th percentile). This led us to hypothesize that this individual might carry a second mutation leading to low HDL-c. We therefore sequenced the LCAT, APOA1 and ABCA1 genes in this individual, three common genetic causes of low HDL-c.21 We identified a novel V2091I mutation in ABCA1 that occurs in the C-terminal region of the encoded protein – a region known to be highly susceptible to deleterious mutations.22 Moreover, this mutation segregates with low HDL-c in this pedigree, such that both individuals with V2091I had HDL-c less than the 20th percentile. Importantly, individual I:02 in figure 2B who is heterozygous for both the S112F mutation in SR-BI and the V2091I mutation in ABCA1 has low HDL-c, suggesting that ABCA1 mutations may be dominant to SR-BI mutations with respect to HDL-c levels. This individual also has a history of coronary artery disease and cerebrovascular disease.

Compared to family member controls, individuals heterozygous for mutations in SR-BI had a 37% increase in HDL-c (1.80 +/- 0.3 mmol/L vs. 1.31 +/- 0.2 mmol/L, p=0.002; table 1). No significant differences in triglycerides, LDL-c or body mass index (BMI) were observed between carriers and controls. Apart from individual I:02 in Figure 2B who carries both the SR-BI S112F and ABCA1 V2091I mutations, no mutation carrier or control had a history of coronary artery disease, cerebrovascular disease or peripheral vascular disease (supplementary table 1).

Discussion

The study of familial disorders of lipoprotein metabolism has led to significant advances in our understanding of HDL metabolism, including the identification of key proteins involved in HDL biogenesis, transport and modification such as ABCA1,23 APOA1,24 LCAT,25 and CETP.26 SR-BI plays a key role in hepatic HDL uptake in mice, but until recently the role of SR-BI in HDL metabolism in humans had not been well established. This is a crucial question, as strategies to therapeutically augment HDL levels or function will depend on an accurate understanding of the pathways of clearance of HDL from plasma.

Recently, Vergeer and colleagues reported a family in which a mutation in SR-BI, P279S, segregated with a high HDL-c phenotype.17 These authors also showed that carriers of P279S had reduced platelet function as well as evidence of adrenal insufficiency, suggesting a role for SR-BI-mediated uptake of cholesterol in these cell types. We used
Figure 2. A) Pedigree of the proband with T175A mutation in SR-BI. Below each individual is listed age in years, total plasma cholesterol (TC), triglycerides, HDL-c (and percentile) – all in mmol/L, body mass index and genotype with respect to T175A. B) Pedigree of the proband with S112F mutation in SR-BI. Below each individual is listed age in years, total plasma cholesterol (TC), triglycerides, HDL-c (and percentile) – all in mmol/L, body mass index, genotype with respect to SR-BI S112F and genotype with respect to ABCA1 V2091I mutation. Squares, Males; Circles, Females; Filled shape, HDL-c ≥90th percentile; empty shape, HDL-c <90th percentile; Arrow, proband.
a similar strategy of screening unrelated probands with very high levels of HDL-c (>90\textsuperscript{th} percentile) and identified two novel mutations (S112F and T175A) in the gene encoding SR-BI, representing the 2\textsuperscript{nd} and 3\textsuperscript{rd} mutations described in this gene that underlie elevated HDL-c in humans. Together with the prior report\textsuperscript{17} there are now three different mutations reported in the gene encoding SR-BI in 23 individuals all of whom have extremely high HDL-c. In our cohort, the frequency of SR-BI mutations was 1.2%, indicating that SR-BI is an important, though infrequent, cause of high HDL-c.

We showed that both the S112F and T175A mutations segregate with the high HDL-c phenotype in the families of the probands, providing strong evidence that these mutations are causally related to high HDL-c. Moreover, no novel SR-BI variants were detected in 80 individuals with very low HDL-c, which additionally supports the interpretation that S112F and T175A are functional mutations that contribute to high HDL-c.

One individual (ID I:02) in the pedigree in figure 2B was heterozygous for both the S112F mutation in SR-BI as well as the V2091I mutation in ABCA1. This patient’s phenotype of low HDL-c is consistent with heterozygous deficiency for ABCA1.\textsuperscript{27} This suggests that ABCA1 mutations may be dominant to SR-BI mutations with respect to HDL-c. Although this notion requires testing in a larger number of individuals, this would be consistent with the roles of these proteins in HDL metabolism, namely ABCA1 acting on the initial step in HDL biogenesis and SR-BI acting on the downstream step of hepatic HDL uptake.\textsuperscript{28} This also highlights the importance of further investigation of individuals carrying a mutation but who manifest an unexpected phenotype.

We did not observe clinical evidence of accelerated atherosclerosis in SR-BI mutation carriers, consistent with prior reports.\textsuperscript{17} In mice, targeted deletion of SR-BI does not

<p>| Table 1. Characteristics of SR-BI Mutation Carriers and Family Member Controls |
|-------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
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Lipid values are in mmol/L. Statistically significant differences are bolded. Individuals also carrying mutations in ABCA1 were excluded.
result in accelerated atherosclerosis in chow fed animals at 5-7 weeks of age. However in the context of apoE deficiency or feeding of a western-type diet, loss of SR-BI results in dramatically accelerated atherosclerosis. Thus it is possible that in humans, SR-BI mutations may result in accelerated atherosclerosis only when combined with a second atherogenic factor. Interestingly, the one individual we identified with an SR-BI mutation as well as a mutation in ABCA1 indeed had premature atherosclerotic disease manifesting as cerebrovascular disease at age 55 and coronary artery disease later in life (supplementary table 1). Consistent with this, combined deletion of macrophage SR-BI and ABCA1 in mice is associated with greater atherosclerotic burden than deletion of SR-BI alone.

Previous studies of SR-BI polymorphisms with small effects on protein function have suggested an association between SR-BI polymorphisms and BMI as well as LDL-c. We observed no effect of the S112F and T175A mutations on either BMI or LDL-c consistent, with data of Vergeer and colleagues. Overall these data suggest that SR-BI does not have a major influence on non-HDL lipid parameters or BMI.

Both of the mutations we describe as well the P279S mutation reported previously occur in the large extracellular loop corresponding to the CD36 cell adhesion superfamily domain. Whether mutations in other regions of this protein, such as the transmembrane domains, will also lead to impaired HDL uptake is unknown. In other proteins with multiple transmembrane and extracellular domains, such as ABCA1, mutations in the transmembrane regions occur very infrequently compared to mutations in the extracellular domains, and mutations in the transmembrane regions of SR-BI may be similarly infrequent. In mice, deletion of the C-terminal region of SR-BI required for PDZK1 binding leads to unstable expression of SR-BI at the cell surface, suggesting that mutations that effect this region of the protein may also be pathogenic.

In summary, we provide evidence for two novel mutations in the gene encoding SR-BI associated with elevated HDL-c in humans. Our data expand the number of documented human mutations in this gene to three and add further support for the concept of SR-BI as a physiologically relevant HDL receptor in humans.

Acknowledgements
This work was supported by Xenon Pharmaceuticals and the Dutch Heart Foundation (to JJK). MRH holds a Canadian Research Chair in Human Genetics and is a Killam Professor at the University of British Columbia. JJK is a recipient of the Lifetime Achievement Award (2010 T082) of the Dutch Heart Foundation.
Reference List

### Supplementary Table S1.

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All lipid values in mmol/L. HTN, hypertension. CABG, coronary artery bypass grafting. T2D, type 2 diabetes. TIA, transient ischemic attack. ASA, acetylsalicylic acid. nd, no data.