Rare SCARB1 mutations associate with high-density lipoprotein cholesterol but not with coronary artery disease

Anna Helgadottir¹, Patrick Sulem¹, Gudmundur Thorgeirsson¹,²,³, Solveig Gretarsdottir¹, Gudmar Thorleifsson¹, Brynjar Ö. Jansson¹, Gudny A. Arnadottir¹, Isleifur Olafsson⁴, Gudmundur I. Eyjolfsson⁵, Olof Sigurdardottir⁶, Unnur Thorsteinsdottir¹,², Daniel F. Gudbjartsson¹,⁷, Hilma Holm¹*, and Kari Stefansson¹,²*

¹deCODE Genetics/Amgen, Inc., Sturlugata 8, 101 Reykjavik, Iceland; ²Faculty of Medicine, Department of Medicine, University of Iceland, Saemundargata 2, 101 Reykjavik, Iceland; ³Division of Cardiology, Department of Internal Medicine, Landspítali, National University Hospital of Iceland, Hringbraut, 101 Reykjavik, Iceland; ⁴Department of Clinical Biochemistry, Landspítali, National University Hospital, Hringbraut, 101 Reykjavik, Iceland; ⁵The Laboratory in Mjöld, RAM, 109 Reykjavik, Iceland; ⁶Department of Clinical Biochemistry, Akureyri Hospital, 600 Akureyri, Iceland; and ⁷School of Engineering and Natural Sciences, University of Iceland, 101 Reykjavik, Iceland

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Aims
Scavenger receptor Class B Type 1 (SR-BI) is a major receptor for high-density lipoprotein (HDL) that promotes hepatic uptake of cholesterol from HDL. A rare mutation p.P376L, in the gene encoding SR-BI, SCARB1, was recently reported to associate with elevated HDL cholesterol (HDL-C) and increased risk of coronary artery disease (CAD), suggesting that increased HDL-C caused by SR-BI impairment might be an independent marker of cardiovascular risk. We tested the hypothesis that alleles in or close to SCARB1 that associate with elevated levels of HDL-C also associate with increased risk of CAD in the relatively homogeneous population of Iceland.

Methods and results
Using a large resource of whole-genome sequenced Icelanders, we identified thirteen SCARB1 coding mutations that we examined for association with HDL-C (n = 136 672). Three rare SCARB1 mutations, encoding p.G319V, p.V111M, and p.V32M (combined allelic frequency = 0.2%) associate with elevated levels of HDL-C (p.G319V: β = 11.1 mg/dL, P = 8.0 × 10⁻⁷; p.V111M: β = 8.3 mg/dL, P = 1.1 × 10⁻⁶; p.V32M: β = 10.2 mg/dL, P = 8.1 × 10⁻⁴). These mutations do not associate with CAD (36 886 cases/306 268 controls) (odds ratio = 0.90, 95% confidence interval 0.67–1.22, P = 0.49), despite effects on HDL-C comparable to that reported for p.P376L, both in terms of direction and magnitude. Furthermore, HDL-C raising alleles of three common SCARB1 non-coding variants, including one previously unreported (rs61941676-C: β = 1.25 mg/dL, P = 1.7 × 10⁻¹⁸), and of one low frequency coding variant (p.V135I) that independently associate with higher HDL-C, do not confer increased risk of CAD.

Conclusion
Elevated HDL-C due to genetically compromised SR-BI function is not a marker of CAD risk.

Keywords
SR-BI • HDL cholesterol • Mutation • Coronary artery disease
Introduction

Despite marked improvements in treatment and prevention, cardiovascular diseases remain the most common cause of death in Iceland like in other European countries. Epidemiological studies consistently show an inverse relationship between levels of high-density lipoprotein cholesterol (HDL-C) and the risk of coronary artery disease (CAD). This relationship has been explained by a potential antiatherogenic properties of HDL, including its role in reverse cholesterol transport, in which cholesterol from peripheral tissues is returned to the liver for excretion in bile. However, neither Mendelian randomization studies, nor interventional studies support the notion that HDL-C directly protects against CAD. This implies that HDL-C is not antiatherogenic itself, but rather a marker of other antiatherogenic factors. Accordingly, the most recent European Society of Cardiology and European Atherosclerosis Society Guidelines for the management of dyslipidaemias do not recommend HDL-C as a target for treatment.

A recent study reported that a rare missense mutation p.P376L in SCARB1 encoding the scavenger receptor Class B Type 1 (SR-BI), associates with impaired function of the encoded protein and elevated HDL-C levels. The mutation was also found to associate with CAD in a meta-analysis of 16 studies with an odds ratio (OR) of 1.79 and \( P = 0.018 \). The investigators concluded that reduced hepatic SR-BI function in humans causes impaired reverse cholesterol transport, leading to increased risk of CAD. This would suggest that high HDL-C might in some cases be an independent marker of increased risk of cardiovascular disease. However, given the extremely low and variable carrier rate of the mutation between study groups (overall 86 carriers in 137,995 or 1 in 1600 individuals), the possibility has been raised, that the p.P376L variant may be an indirect marker for a substratum of the population.

Scavenger receptor Class B Type 1, an integral membrane protein expressed most abundantly in the liver and endocrine organs that make steroids, is a receptor for HDL. Mouse studies have demonstrated that SR-BI plays a key role in reverse cholesterol transport, promoting the hepatic selective uptake of cholesterol from HDL, and facilitating the secretion of cholesterol into bile. In SR-BI deficient mice biliary cholesterol is decreased, but HDL-C levels in blood are elevated, and there is acceleration of atherosclerosis. Hepatic overexpression of SR-BI in mice has the opposite effect, enhanced hepatocellular cholesterol uptake and increased cholesterol secretion into bile, lower levels of circulating HDL-C, and attenuated atherosclerosis. Scavenger receptor Class B Type 1 up-regulation in mouse models is also associated with biliary cholesterol hypersecretion and increased gallstone formation.

The effects of SR-BI mutations on HDL-C levels in humans have been clearly demonstrated. Three rare missense mutations in SCARB1, other than the mutation encoding p.P376L, were reported to have HDL-C increasing effects comparable to that of p.P376L (8.4–18.9 mg/dL). In addition, genome wide association studies (GWAS) have found two common non-coding variants within the SCARB1 locus that associate with HDL-C levels, both with small effects relative to those of the rare coding ones. Neither the rare variants (encoding p.S297S, p.S112F, and p.T175A), nor the common HDL-C associating variants have been reported to associate with CAD, although, we note that the sample sizes in the studies describing the rare mutations may have been too small to detect such an association.

In view of the contradictory results of previous studies, assessing the effects of rare SCARB1 variants on the risk of CAD, a further inquiry is called for. Herein, we use the relatively homogeneous population of Iceland to test the hypothesis that alleles in or close to SCARB1 that associate with elevated levels of HDL-C also associate with increased risk of CAD. Further, we examine whether SCARB1 variants that associate with HDL-C in humans alter the susceptibility to gallstone formation, as suggested by mouse models.

Methods

The study was approved by The National Bioethics Committee in Iceland (Approval no. 07–085, with amendments) and the Data Protection Authority of Iceland (Approval no.2007060474ThS—, with amendments). All participating subjects donating samples signed informed consents. Personal identities of the phenotypes and biological samples were encrypted by a third party system provided by the Icelandic Data Protection Authority.

Enrolment of participants, the phenotypic definitions for CAD, information on lipid measurements, genotyping, imputation methods, and association analysis have previously been described in detail (see also Supplementary material online, Note). Briefly, lipid measurements were obtained from three of the largest clinical laboratories in Iceland. We used HDL-C measurements from 136,672 Icelanders, 93,169 were chip-typed and directly imputed, and 43,503 were first and second degree relatives of chip-typed individuals and had their genotypes inferred based on a third party system provided by the Icelandic Data Protection Authority. The study was approved by The National Bioethics Committee in Iceland (Approval no. 07–085, with amendments) and the Data Protection Authority of Iceland (Approval no.2007060474ThS—, with amendments). All participating subjects donating samples signed informed consents. Personal identities of the phenotypes and biological samples were encrypted by a third party system provided by the Icelandic Data Protection Authority.

Results and discussion

Using our population-based resource of 8453 whole-genome sequenced Icelanders, we identified thirteen SCARB1 coding variants...
and one splice region variant (Supplementary material online, Table S1) that we imputed into chip-genotyped Icelanders and their close relatives and tested for association with HDL-C (n = 136 672). Three very rare SCARB1 missense variants that never occur together on the same chromosome, p.G319V, p.V111M, and p.V32M (allelic frequency 0.056%, 0.111%, and 0.026%, respectively) associate with elevated levels of HDL-C (p.G319V: β = 11.1 mg/dL, P = 8.0 × 10⁻⁶; p.V111M: β = 8.3 mg/dL, P = 1.1 × 10⁻⁶; p.V32M: β = 10.2 mg/dL, P = 8.1 × 10⁻⁴) (Table 1). The associations of these variants with HDL-C have not been reported before. Overall, one in 250 Icelanders carries one of these three variants and none of them associates with other lipid fractions (Supplementary material online, Table S3). Although the missense variants p.P376L, p.S297S, p.S511F, and p.T175A previously reported to associate with increased HDL-C11,24,25 (reported effects: 8.4–18.9 mg/dL) were not observed in Iceland, the three rare missense variants identified have effects in the same direction and of comparable magnitude (8–11 mg/dL) as the published ones. Similar to all previously described HDL-C increasing variants in SCARB1,11,24,25 the variants encoding p.G319V and p.V111M occur in the large extracellular loop of the SR-BI protein, within highly conserved regions and are predicted to be damaging (Supplementary material online, Table S1). The missense variant p.V32M is predicted to be benign, and is in a region less conserved between species (Supplementary material online, Table S1). In addition to the rare variants, we observed one low frequency missense variant p.V135I (frequency 1.23%) that associates with increased HDL-C, albeit with considerably less effect (β = 2.1 mg/dL, P = 6.4 × 10⁻⁶) than the rare ones (Table 1). Two of the rare coding sequence variants (p.G319V and p.V111M) are reported in the Genome Aggregation Database (gnomAD at http://gnomad.broadinstitute.org, assessed March 2018) in European populations, but at much lower frequencies than in Iceland.

We tested the missense variants encoding p.G319V, p.V111M, p.V32M, and p.V135I for association with CAD among 36 886 cases and 306 268 controls (Table 1). None of the variants associates with CAD risk (P > 0.05). To increase power to detect association we aggregated the three rare large impact variants p.G319V, p.V111M, and p.V32M (combined allelic frequency = 0.2%) and tested for association with increased risk of CAD. This aggregate test gives an ORCAD = 0.90, 95% confidence interval (CI) 0.67–1.22; P = 0.49 (Supplementary material online, Table S2).

We further tested three common non-coding variants that independently associate with HDL-C, for association with CAD in Iceland and in the publicly available CARDIOGRAM/C4D 1000G data (Table 2). Of these common HDL-C associating variants one is novel (rs61941676) and two represent previously reported6 GWAS signals (rs838876 and rs838909) (Supplementary material online, Note and Table S3). In the combined results from the Icelandic and CARDIOGRAM/C4D datasets, two of the three common variants show weak evidence for association with CAD (rs61941676-C: OR = 0.97, 95% CI 0.95–1.00; P = 0.03 and rs838876-A: OR = 0.98, 95% CI 0.96–0.99; P = 0.0026) (Table 2), with the HDL-C increasing allele tending towards reduced risk of CAD.

In light of the seemingly discrepant effects of rare SCARB1 variants on the risk of CAD, it could be argued that the three Icelandic rare variants that associate with raised HDL-C could do so without inhibiting the hepatocellular trafficking of cholesterol to bile; thus explaining the lack of association with CAD. In this scenario, enhancement of cholesteryl ester transfer protein (CETP)-mediated exchange of cholesteryl esters from HDL to apoB containing lipoproteins, would counteract the genetically compromised SR-B1, resulting in minimal or no net effect on the hepatic cholesterol removal in carriers of the Icelandic variants. These effects would contrast the hindered hepatic cholesterol uptake observed in the SR-B1 deficient mice (mice do not express CETP) and in hepatocytes derived from human induced pluripotent stem cells, carrying the p.P376L mutation.11 To test the impact of the Icelandic SCARB1 mutations, and other HDL-C associating variants at the locus, on transhepatic cholesterol flux, we used gallstone risk as a proxy. It has been shown that gallstone formation largely results from cholesterol hypersecretion from HDL-C, albeit with considerably less effect (β = 2.1 mg/dL, P = 6.4 × 10⁻⁶) than the rare ones (Table 1). Two of the rare coding sequence variants (p.G319V and p.V111M) are reported in the Genome Aggregation Database (gnomAD at http://gnomad.broadinstitute.org, assessed March 2018) in European populations, but at much lower frequencies than in Iceland.

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variants showed nominally significant association with gallstones (Supplementary material online, Table S3). A genetic risk score for HDL-C, constructed on the basis of seven SCARB1 HDL-C associating variants, associates with gallstones. For each standard deviation (SD), increase in HDL-C due to the genetic risk score, the risk of gallstones decreases by 61% (OR = 0.39, 95% CI 0.24–0.63; P = 1.0 ⋅ 10⁻³) (Supplementary material online, Table S2). This finding supports the conclusion that SCARB1 variants associating with increased HDL-C in humans impair cholesterol excretion through bile, thus playing a role in the late stages of reverse cholesterol transport, as described in the mouse and for other SCARB1 mutations.1 124

However, in concordance with the results for individual variants, the SCARB1 HDL-C genetic risk score does not associate with CAD risk (for one SD of genetically elevated HDL-C: OR = 0.84, 95% CI 0.58–1.22; P = 0.36, Supplementary material online, Table S2) further tilting the scale against the hypothesis that hindered flux of HDL-C to the liver due to SR-BI impairment increases CAD susceptibility in humans.

Although we have demonstrated that SCARB1 variants leading to decreased flux of HDL-C to the liver do not increase CAD risk (Take home figure), other SR-BI functions may still do so. In the Icelandic data a common SCARB1 intronic variant rs11057837-T (allele frequency = 9.5%) associates with CAD (OR = 1.11, P = 1.2 ⋅ 10⁻³) (Table 2), but not with HDL-C or gallstones after adjusting for HDL-C variants in the region (Supplementary material online, Table S3 and Note). Rs11057837-T also associates with CAD in the public 1000G data from CARDIOGRAM/C4D (OR = 1.08 and P = 1.9 ⋅ 10⁻⁸ for Iceland and CARDIOGRAM/C4D combined) (Table 2). The
rs11057837 correlates (R² = 0.7) with other intrinsic variants (rs11057841, rs11057830, and rs10846744) that have previously been found to associate with Lp-PLA2 activity and mass,
15 vitamin E levels,
16 subclinical atherosclerosis,
17 and with CAD.
18,19 The association of correlated variants with vitamin E levels support the notion that rs11057837 mediates its effect through SCARB1, rather than other genes in the region, since in vitro studies have demonstrated the influence of SR-BI on tissue antioxidant uptake (vitamin E and carotenoids).
20,21 These effects, or other functions that have been linked to SR-BI, such as the effect on endothelial cell nitric oxide metabolism,
22 bacterial or viral recognition and degradation,
23,24 or induction of apoptosis,
25 are mechanisms that could explain the association of rs11057837 with CAD. Further, effects on other genes in the region cannot be ruled out.

To summarize, the HDL-C increasing effects (8–11 mg/dL) of the three rare SCARB1 missense variants described in our study, encoding p.G319V, p.V111M, and p.V32M, are comparable to the HDL-C of apoptosis,
44 are mechanisms that could explain the association of similar degree as the one reported.
11 Specifically, this striking difference in carrier frequency, to the influence of SR-BI on tissue antioxidant uptake (vitamin E and carotenoids).
38,39 These effects, or other functions that have been linked to SR-BI, such as the effect on endothelial cell nitric oxide metabolism,
40 bacterial or viral recognition and degradation,
41–43 or induction of apoptosis,
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11 Importantly the 95% CI indicates that OR above 1.22 is unlikely. Assuming a true association between the HDL-C raising variants and increased risk of CAD, we have 90% power to detect variant association with OR = 1.29 at P-value <0.05. It is conceivable that the mutation encoding p.P376L has CAD susceptibility effects that are not shared by other HDL-C-raising variants. However, given that it is relatively specific to Ashkenazi Jews (carried by about 1 in 20 Ashkenazi Jews vs. about 1 in 10 000 Europeans that are not Ashkenazi Jews)
45,46 it is more likely that differences in population substructure between subjects and controls is the main explanation of the reported association of p. P376L with CAD. Specifically, this striking difference in carrier frequency, together with a relatively small imbalance in the number of Ashkenazi Jews between CAD cases and controls, could introduce a false association of similar degree as the one reported.
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In conclusion, our results do not support a relationship between elevated HDL-C levels due to genetically compromised SR-BI function and increased risk of CAD. These findings are in keeping with recent genetic and interventional studies,
4–9 failing to show causal relationship between HDL-C levels and atherosclerosis and support current dyslipidaemia guidelines.

### Supplementary material
Supplementary material is available at European Heart Journal online.

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### Conflict of interest
The authors A.H., P.S., G.T., G.T., B.O. & C. are employed by deCODE genetics/Amgen, Inc. and are employed by the company.

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