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Inherited Variants in *SCARB1* Cause Severe Early-Onset Coronary Artery Disease

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Abstract

Rationale: Coronary artery disease (CAD) is a pervasive and critical healthcare problem. Elevated high density lipoprotein-associated cholesterol (HDL-C) is associated with improved atherosclerotic cardiovascular disease (ASCVD) outcomes on a population level, but clinical trials aimed at HDL-C elevation have not succeeded in improving ASCVD event risk. Nevertheless, human variants in the HDL receptor, encoded by *SCARB1*, are associated with dyslipidemia, suggesting that HDL metabolism, not HDL-C, is a suitable target for therapy. However, variants in *SCARB1* have never been directly attributed to CAD by Mendelian inheritance.

Objective: To determine if compound heterozygous variants in *SCARB1* cause disease in two brothers with severe, early-onset CAD.

Methods and Results: Using whole exome sequencing, we have identified rare, compound heterozygous variants in *SCARB1* that segregate with severe, premature CAD, following patterns of Mendelian inheritance. Using induced pluripotent stem cell-derived hepatocyte-like cells (iPSC-

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DISCLOSURES
None.

SUPPLEMENTAL MATERIALS
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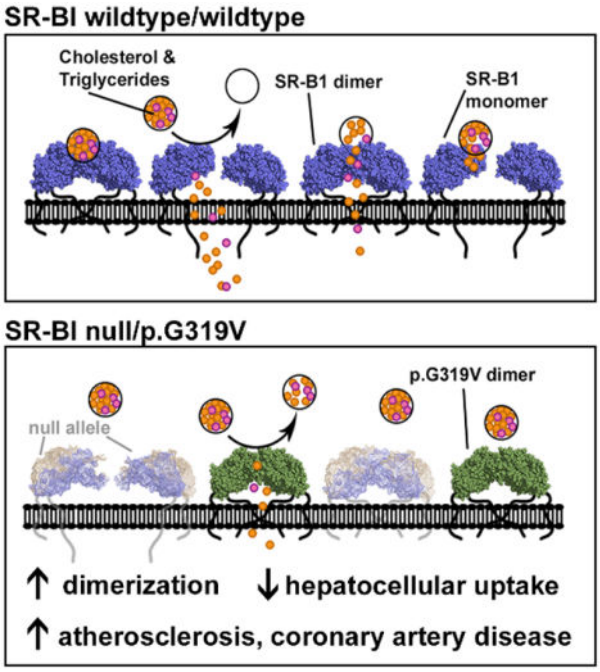
HLCs) from the proband, we discovered the maternal variant (c.754_755delinsC) to be the first identified *SCARB1* null allele, characterized by the absence of RNA and protein expression. Further, we demonstrate that the variant on the paternal allele (c.956G>T (p.G319V)) results in decreased cholesterol uptake, decreased SR-BI:HDL binding, and increased affinity for SR-BI dimerization. Finally, we generated a p.G319V knock-in mouse model that displays nearly 100% homozygous lethality and elevated plasma cholesterol in heterozygous animals, confirming pathogenicity of this variant.

Conclusions: In summary, our data provide the first molecular mechanism to show the Mendelian inheritance of CAD as a result of human *SCARB1* variants. The rarity of these variants supports pathogenicity in this family. Furthermore, SR-BI p.G319V, which has previously been reported benign in the context of heterozygosity, was uniquely presented alongside a null allele, demonstrating the disease-contributing capability of loss-of-function *SCARB1* variants within the population.

Graphical Abstract



Therapeutic options for dyslipidemia and atherosclerotic disease are largely limited to statins. Although generally considered effective, additional therapeutics are necessary to improve patient outcomes by addressing inter-individual efficacy variability and offsetting statin discontinuance as a result of statin-associated symptoms. CETP inhibition was trialed as an HDL-mediated therapy, but failed to improve outcomes of associated cardiovascular events. However, HDL has not been fully leveraged to optimize cholesterol clearance, as other related mechanisms remain to be investigated. *SCARB1* polymorphisms are associated with elevated HDL-C, dyslipidemia, and CAD, and genetic deletion of *Scarb1* accelerates atherosclerosis in mice. However, this report is the first to demonstrate the influence of inherited *SCARB1* variants on human disease. We demonstrate that compound heterozygous *SCARB1* variants segregate with severe, early-onset coronary artery disease. Through use of hepatocyte-like cells from patient derived iPSCs, we

establish one variant as a genetic null. This model is further used in conjunction with *in vitro* assays and a novel knock-in mouse model to demonstrate functional defects of the second variant, SR-BI p.G319V, including dimerization, HDL binding, and cholesterol uptake. Overall, our findings show that variants in SCARB1 directly cause CAD and support investigation into SR-BI as a pathway for HDL-mediated therapy.

Keywords

SCARB1; dyslipidemia; genetics; human; genetics; animal models; physiology; high density lipoprotein cholesterol; coronary heart disease; coronary artery disease

Subject Terms:

Coronary Artery Disease; Genetics; Lipids and Cholesterol; Translational Studies

INTRODUCTION

Cardiovascular disease is the leading cause of death in the United States, with approximately 43% attributed to coronary heart disease (CHD).[1] Elevated plasma cholesterol associated with low density lipoprotein (LDL-C) increases the risk of plaque formation via deposition of cholesterol in the arterial wall. Treatment with hydroxymethylglutaryl CoA reductase inhibitors (statins) for inhibition of cholesterol biosynthesis is indicated in patients with high cholesterol and atherosclerotic cardiovascular disease (ASCVD), but this treatment option has variable inter-individual success and tolerance.[2, 3] Recent advances in PCSK9 inhibition therapy, which lowers plasma LDL-C by approximately 60%, have provided an additional treatment option for the approximate half of individuals with atherosclerotic disease who qualify for treatment.[4, 5] However, the price of this therapy has led to suboptimal treatment persistence, ultimately resulting in medical disparity with increased risk of cardiovascular disease disproportionally affecting women, racial minorities, and low-income groups.[6]

Reverse cholesterol transport (RCT) is the mechanism by which excess cholesterol is cleared from tissue via high density lipoprotein (HDL) and processed through hepatocellular uptake for resecretion into the plasma or excretion into bile. HDL-associated cholesterol (HDL-C) has historically been considered cardio-protective, as elevated levels in plasma correlate with a decreased risk of ASCVD, including coronary artery disease (CAD).[7] Several clinical trials have been successful in raising HDL-C and lowering LDL-C with cholesteryl ester transfer protein (CETP) inhibition, yet they were not successful in altering clinical outcomes of ASCVD, such as associated cardiovascular events and mortality.[8–11] However, there remain additional targets of HDL-mediated RCT that are suitable for therapy. In fact, multiple population studies support a protective role of HDL cholesterol efflux capacity in ASCVD[12–14], suggesting that improvement to ASCVD medical management may be achieved through targeted therapy of alternative pathways in cholesterol metabolism, such as enhancement of RCT efficiency via modulation of HDL functions.

SR-BI, encoded by *SCARB1*, is a transmembrane receptor that facilitates cholesterol efflux and selective cholesterol uptake.[15] Murine studies demonstrate that SR-BI plays a critical

role in RCT via hepatocellular cholesterol absorption and excretion of cholesterol through bile.[16–19] In addition, SR-BI dosage may modulate atherosclerosis in murine models, illustrating the importance of RCT in regulation of cholesterol and prevention of atherosclerosis in mice.[20–22]

In several human genetic studies, *SCARB1* polymorphisms have been correlated with elevated HDL-C, dyslipidemia, and CAD.[23–28] However, variants in *SCARB1* have not previously been reported to directly cause atherosclerotic disease through Mendelian inheritance. Variants in *SCARB1* have previously been associated with a modest and significant elevation in HDL-C (p.G319V, β = 11mg/dl), but they have never been shown to *directly* contribute to CAD in humans. In fact, conflicting reports have been published on the pathogenicity of *SCARB1* variants, including one we present here (p.G319V).[24, 25]

In the present study, we identify compound heterozygous variants in *SCARB1* that segregate with early-onset, severe human CAD. We define one of the variants as a genetic null, and functional assays of the expressed variant, SR-BI p.G319V, revealed decreased cholesterol uptake, decreased SR-BI:HDL binding, and increased affinity for dimerization. Furthermore, a knock-in mouse model of the p.G319V variant displays homozygous lethality, as well as increased plasma cholesterol levels in heterozygous animals. Based on standards set by the American College of Medical Genetics (ACMG)[29], our data demonstrate that SR-BI p.G319V is a likely pathogenic variant that causes severe CAD in humans in the absence of a wildtype allele. Our work functionally demonstrates the first causative relationship between variants in *SCARB1* and severe, early-onset CAD, and our data support the exploration of HDL metabolism for targeted cholesterol-lowering therapy. Further, this work supports the exploration of HDL metabolism as a therapeutic target for dyslipidemia and ASCVD.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Human subjects.

This study complies with the Declaration of Helsinki. All human subjects research was approved by the Institutional Review Board at The Ohio State University under protocol numbers 2004H0136, 2017H0292 and 2018H0290. Human subject demographics are listed in Table I of the Online Supplement.

Additional methods in SI Appendix

Please see the Major Resources Table in the Supplemental Materials

RESULTS

Identification of family with early-onset, severe coronary artery disease.

A 28 year-old African American male tri-athlete with no history of tobacco or substance abuse and 8 years of controlled essential hypertension presented to the local emergency

department with intermittent substernal chest pain that started after a 10-mile run and continued for approximately 24 hours. His heart rate and blood pressure were normal at presentation and his exam revealed normal heart sounds and no evidence of congestion. His initial electrocardiogram demonstrated normal sinus rhythm with left ventricular hypertrophy and non-specific ST/T wave changes (Figure I in the Data Supplement). Serum troponin I was elevated at 2.59 ng/dL. He was diagnosed with a non-ST elevation myocardial infarction (NSTEMI) and coronary angiography demonstrated severe, diffuse 3-vessel CAD with preserved left ventricular systolic function (Figure 1A). His initial lipid panel included elevated TC, LDL-C and HDL-C (Figure 1A, Table II in the Data Supplement). Given the extent and diffuse nature of atherosclerosis, his disease was managed with daily medical therapy including aspirin (81mg), atorvastatin (40mg), clopidogrel (75mg), atenolol (25mg) and lisinopril (40mg).

Approximately four years later, the patient was admitted with recurrent NSTEMI, with angiographically evident progression of his CAD including a 99% stenosis in the mid right coronary artery (RCA) and a subtotal occlusion of the ramus intermedius artery (Figure 1A). These lesions were treated with percutaneous coronary intervention (PCI), and his residual CAD was medically managed. Four years later, he underwent a nuclear stress test for unstable angina. Subsequent coronary angiogram revealed high-grade in-stent-restenosis of the ramus, requiring repeat PCI. Left ventricular systolic function remained normal. Genetic testing was performed given the presence of accelerated early CAD and progressive atherosclerosis despite maximal medical therapy, but did not identify gene variants previously linked with familial hypercholesterolemia (FH).

The proband's younger brother (II-4) was also diagnosed with severe CAD at 30 years of age when he presented with exertional chest pain and dyspnea (Figure 1B). In addition, the proband's mother had severe CAD at the age of 55 (Table III in the Data Supplement). His immediate family also included his father and an older brother who was healthy and free of known atherosclerotic cardiovascular disease. However, medical history on each of these family members was not available.

Identification of compound heterozygous disease-causing variants in SCARB1.

NMR-based lipoprotein analysis of the proband revealed an atheroprotective large HDL particle size (10.0 nm; >75th percentile for males of this age) (Table IV in the Data Supplement).[30, 31] Mild LDL particle size discordance (LDL-P exceeds approximate expected [LDL-C × 10]) was also observed and is considered a pro-atherosclerotic risk factor,[32] but the degree of elevation was not expected to account for the severe, early-onset CAD phenotype. To determine possible genetic origins of disease in this family, whole exome sequencing was performed on two brothers with premature, severe coronary artery disease (II-3 and II-4) and their mother (I-2), who has partial expressivity of the phenotype (Figure 2A). No candidate variants in genes associated with familial hypercholesterolemia were identified as potentially disease-causing. Instead, the proband and his affected brother were found to harbor compound heterozygous variants in the gene encoding scavenger receptor class B, member 1 (SR-BI, *SCARB1*) (c.754_755delinsC; c.956G>T) (Figure 2). In these two affected patients, the maternally-inherited allele harbors a deletion of two adenines

and insertion of a cytosine at *SCARB1* c.754–755 (c.754_755delinsC) that has not previously been observed in the population (ExAC[33] minor allele frequency (MAF) = 0). This variant causes a frameshift in a highly conserved region, resulting in a premature stop codon at p.253 (p.Asn252Profs*2) (Figure 2C), and is classified as pathogenic according to the ACMG guidelines (Table V in the Data Supplement).[29] The paternal allele harbors a substitution of guanine to thymine at c.956 (rs150728540; c.956G>T; ExAC MAF = 0 in individuals of African ancestry and 0.00006594 in all ancestries), resulting in a glycine to valine substitution at p.319 (p.G319V), another highly conserved region of the extracellular domain (Figure 2C; Figure 4D–E). This variant was originally reported by Helgadottir et al, however in the context of a normal second allele,[24] and is classified as likely pathogenic according to ACMG guidelines (Table V in the Data Supplement).[29] Sanger sequencing confirmed the presence of these variants (Figure 2B).

SCARB1 c.754_755delinsC is a genetic null, resulting in negligible gene and protein expression.

To define the potential contribution of the identified variants to disease mechanism, *in vivo* gene and protein expression of *SCARB1* was measured in hepatocyte-like cells differentiated from proband-derived induced pluripotent stem cells (iPSC-HLCs) (Figure 3A–B). RNA-sequencing revealed 97% allelic expression of the c.956G>T variant (paternal allele), suggesting the maternal allele is a genetic null (Figure 3C–D, Figure II in the Data Supplement). Digital droplet PCR confirmed minimal expression (<1%) of the c.754_755delinsC variant (Figure 3E). Furthermore, SR-BI protein isolated from the proband's iPSC-HLCs was only observed at 75kD (Figure 3F). Protein was not observed at a lower molecular weight (29kDa; ExPASy, Swiss Institute of Bioinformatics), as would be expected if the maternal allele was translated (Figure 3F). These findings indicated that the maternal variant results in the first *SCARB1* null allele identified. Thus, the compound heterozygous brothers expressed the p.G319V allele, but not the maternal allele (c.754_755delinsC). Furthermore, these data explained partial expressivity of the phenotype in the mother, who was diagnosed with severe CAD decades after her sons, likely due to the presence of only a single wildtype allele.

SCARB1 p.G319V displays pathogenicity in vitro.

To gain a better understanding of potential functional deficits of p.G319V, we performed computational modeling based on the solved SR-BI C-terminus and the homologous LIMP-2 protein.[34] Our model defines that amino acid residue 319 is located in the extracellular region that is critical for SR-BI function, including HDL binding and CE uptake (Figure 4A). As SR-BI is critical for normal RCT,[35–38] we sought to determine if RCT was inhibited in this family via a cholesterol uptake assay. After treatment with media containing CE-BODIPY, iPSC-HLCs from our proband contained significantly less CE than control (Figure 4C). We have demonstrated that p.G319V is expressed at normal levels in the proband (Figure 3F), but to determine if p.G319V is properly localized to the cell membrane, immuno-staining was performed. No differences were observed in membrane localization between GFP-tagged wildtype and p.G319V proteins via TEM (Figure IIIA in the Data Supplement). Furthermore, there was no detectable difference in SR-BI localization between iPSC-HLCs derived from the proband or control (Figure IIIB in the Data

Supplement), suggesting that diminished CE-uptake was not a result of reduced SR-BI at the cell membrane.

To assess the function of the p.G319V protein, wildtype and mutant SR-BI were overexpressed in a cell line with minimal endogenous HDL binding (HEK293) after treatment with DiI-HDL (Figure 4D; Figure IV in the Data Supplement). Overexpression of wildtype SR-BI increases HDL binding by ~142% (GFP = 1.452 ± 0.514; SR-BI = 3.521 ± 1.020; $p=8.44\times10^{-3}$), whereas overexpression of p.G319V had no effect. Furthermore, co-overexpression of wildtype and mutant SR-BI did not increase HDL binding, suggesting that p.G319V may function via a dominant-negative mechanism (Figure 4D).

Homodimerization of SR-BI has been suggested to increase receptor activity, but the specific dimerization domain is unclear and has been reported to be localized to both the N-terminus and C-terminus.[34, 39, 40] Our SR-BI model suggested that G319 is located in a dimerization interface and that this putative dimeric structure is stabilized through hydrophobic interactions (Figure 4B). Thus, we predicted that the p.G319V substitution results in more robust dimer formation via stabilization of hydrophobic interactions with two amphipathic alpha-helices on the opposite dimer (p.152–167 and p.186–195) (Figure 4C). We tested this hypothesis using an *in vitro* binding assay and illustrated that the mutant protein has increased binding affinity relative to wildtype (Figure 4F). Specifically, the heterodimer G319V:WT displayed a trend towards a relatively weaker interaction than G319V:G319V (Figure 4F). Thus, our findings identify p.G319V as a pathogenic variant in SR-BI (Figure 4G–H). However, the ability of this variant to impact clinical phenotype will likely vary by individual, as the majority of carriers will also harbor a wildtype allele.

SCARB1 p.G319V variant is pathogenic in vivo.

To further determine the effects of the p.G319V variant *in vivo*, a knock-in mouse model was created on a C57Bl/6 background at the orthologous site in *Scarb1* (c.956G>T; p.G319V). A single nucleotide change was confirmed in first and second generation offspring by sanger sequencing (Figure 5A–B). SR-BI protein expression was similar in heterozygous knock-in mice ($SRBI^{G319V/wt}$) and wildtype littermates ($SRBI^{wt/wt}$; wildtype; Figure 5C–D). Surprisingly, homozygous knock-in mice ($SRBI^{G319V/G319V}$) suffered >95% lethality by weaning, a phenotype that we observed and has been reported for SR-BI knock-out mice (Table VII in the Data Supplement; The Jackson Laboratory, 003379). Lethality of $SRBI^{G319V/G319V}$ supported pathogenicity of this variant. The human variant has been associated with elevated HDL-C.[24] To determine if this variant has an effect on mouse plasma cholesterol in a heterozygous setting, we measured total cholesterol, HDL-C, LDL-C and triglycerides from $SRBI^{G319V/wt}$ mice in a fasted state (Figure 5E). Consistent with the impact of p.G319V as a loss-of-function variant, we observed an elevation of total cholesterol and LDL-C in $SRBI^{G319V/wt}$ mice compared with littermate controls (Figure 5E). After 4 weeks of a modified western diet (21% fat, 1.25% cholesterol), we observed significantly elevated HDL-C in female $SRBI^{G319V/wt}$ mice compared with littermate controls and a trend toward elevated HDL-C in heterozygous male mice (p=0.0635) (Figure 5E). A similar trend of cholesterol elevation in *Scarb1* knock-out mice has previously been

reported and was observed in our lab (Figure V in the Data Supplement).[15] Together, these data supported pathogenicity of *SCARB1* p.G319V *in vivo*.

DISCUSSION

Here we provide the first report of a family with minimal ASCVD risk factors who presented with severe atherosclerosis and premature CAD caused by variants in *SCARB1*. The c.754_755delinsC variant is the first identified *SCARB1* null, resulting in minimal RNA expression and no detectable protein expression. This genetic null allele supports partial expressivity of the phenotype in the proband's mother, who was diagnosed with CAD at the age of 55 years (disease onset decades after her two sons). Functional studies of SR-BI from the paternal allele, p.G319V, revealed a deleterious variant protein, with increased dimerization affinity, decreased HDL binding, and decreased cholesterol uptake activity. Furthermore, we have reported the first computational model of SR-BI, based on the crystal structure of homologous protein LIMP-2, and showed that amino acid 319 is located at a possible dimerization interface. Previous studies have reported conflicting data with respect to the dimerization domain.[34, 39, 40] Finally, a knock-in mouse model harboring the p.G319V variant displays homozygous lethality and elevated plasma cholesterol in the heterozygous animals, confirming pathogenicity of this variant *in vivo*.

Variants in *SCARB1* have previously been found to cause a modest and significant elevation in HDL-C (p.G319V, β = 11mg/dl); yet, we cannot confirm that our patients' HDL-C levels correspond with previously published data, as baseline levels of HDL-C were not reported.[24, 25] While not common in the population (ExAC MAF = 0.00006594), p.G319V was reported by Helgadottir et al with a MAF of 0.00056 in the Icelandic population.[24] Although they found heterozygous p.G319V associated with modestly elevated HDL-C, there was no association with atherosclerosis or CAD in Icelanders. However, our functional data support a loss-of-function molecular mechanism where the p.G319V allele decreases SRBI:HDL binding and CE uptake. Additionally, our computational model suggests localization of G319 adjacent to amphipathic alpha-helices at the dimerization interface. We predict that substitution of valine at this site would stabilize this dimeric interaction through increased hydrophobicity, which is supported by our *in vitro* data demonstrating that p.G319V results in increased dimer binding affinity and suggests a deleterious effect in the setting of a wildtype allele. Furthermore, our *in vivo* data shows that in the absence of a wildtype allele, p.G319V results in >95% lethality in mice, a phenotype that is similarly observed in the *Scarb1* knock-out mouse model. The ACMG has outlined a variant classification system to determine pathogenicity of identified variants based on population frequency, previously published data, *in silico* analysis. Based on this classification system, our *in vitro* and *in vivo* analysis results in scores of pathogenic (null allele) and likely pathogenic (p.G319V) (SI Appendix Table V).

Helgadottir et al cite the homogeneous nature of the Icelandic population as a strength of their study.[24] However, ancestry and genetic background may have a role in the human phenotype discrepancy of p.G319V in addition to its heterozygous presentation in the Icelandic study. Homogenous populations are a critical and important tool for understanding genotype-phenotype relationships, however it is important to consider ancestry, genetic

diversity, and epidemiology when interpreting these studies. American males of African ancestry are affected by an increased incidence of myocardial infarction (MI) and fatal CHD throughout the lifespan in comparison to males of European ancestry and females.[1] In the setting of a null allele, as seen in our patients, p.G319V is not sufficient to prevent dyslipidemia and severe, early-onset CAD. Considering the entirety of this data, we predict that *heterozygous* individuals for p.G319V may have an increased risk of developing atherosclerosis with respect to other *modifiable and non-modifiable risk factors* that may not have been captured in the Icelandic population.

The data presented in this manuscript raises additional important questions concerning regulation and function of SR-BI. Our computation model predicts a putative dimerization domain adjacent to G319. Data from our *in vitro* binding assay demonstrates increased binding preference for SR-BIp.G319V in comparison to wildtype SR-BI (Figure 4E–F). Future experiments will explore the requirement of this proposed dimerization domain (p.152–167 and p.186–195) through mutational analysis. Increased binding affinity and decreased function of the receptor is indicative of a dominant-negative mechanism. Additional *in vivo* experimentation with the SRBIp.G319V and SRBI knock-out mouse lines after their transition to a homogeneous background (>95% backcrossed) will shed additional light on this potential mechanism.

Our findings (one null allele, one likely pathogenic allele) substantiate a causative role for SR-BI in severe atherosclerotic disease whereby 1) HDL-C is ineffectively absorbed and excreted by hepatocytes and 2) elevated HDL-C via inadequate hepatocellular uptake increases the plasma bioavailability of cholesteryl esters (Figure 4G–H). This work reveals clinical implications for HDL and RCT regulation in atherosclerotic disease. HDL has three key functions to consider for targeted therapies: 1) cholesteryl ester transfer to intermediate density lipoprotein via CETP in exchange for triglycerides, 2) cholesterol efflux from foam cells, and 3) hepatocellular cholesterol uptake (Figure VI in the Data Supplement). Clinical trials did not have success with improving outcomes of cardiovascular events with CETP inhibition, likely because this mechanism does not have an effect on cholesterol efflux or excretion. Future studies should consider investigating the therapeutic potential of augmenting RCT to improve these functions.[8–11]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Nonstandard Abbreviations and Acronyms:

CAD	coronary artery disease
ASCVD	atherosclerotic cardiovascular disease
LDL	low density lipoprotein
HDL	high density lipoprotein
RCT	reverse cholesterol transport
CETP	cholesteryl ester transfer protein
ACMG	American College of Medical Genetics and Genomics
RCA	right coronary artery
PCI	percutaneous coronary intervention
NSTEMI	non-ST elevation myocardial infarction
SR-BI	scavenger receptor class B type I

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NOVELTY AND SIGNIFICANCE

What Is Known?

- Coronary artery disease (CAD) is the most common type of heart disease, with an estimated economic burden of over $200 billion per year in the United States, affecting ~18.2 million adults over the age 20 years.
- Several human genetic studies demonstrate *SCARB1* as a disease modifier of CAD through correlation of *SCARB1* polymorphisms with elevated HDL-C, dyslipidemia, and CAD.
- Elevated high density lipoprotein-associated cholesterol (HDL-C) is correlated with improved outcomes in CAD patients, but improved outcomes of associated cardiovascular events are not observed when HDL-C is elevated due to inhibition of cholesteryl ester transfer protein (CETP).

What New Information Does This Article Contribute?

- This report is the first to show that Mendelian inheritance of *SCARB1* variants can cause severe, early-onset CAD.
- We have identified a *SCARB1* null allele, and our data suggests that its presence escalates the pathogenicity of SR-BI p.G319V, a variant that is clinically benign in a heterozygous setting in the Icelandic population.
- In vitro assays and a novel knock-in mouse model support pathogenicity of SR-BI p.G319V.
- Our results support further investigation into SR-BI as an alternative pathway for HDL-mediated therapy.

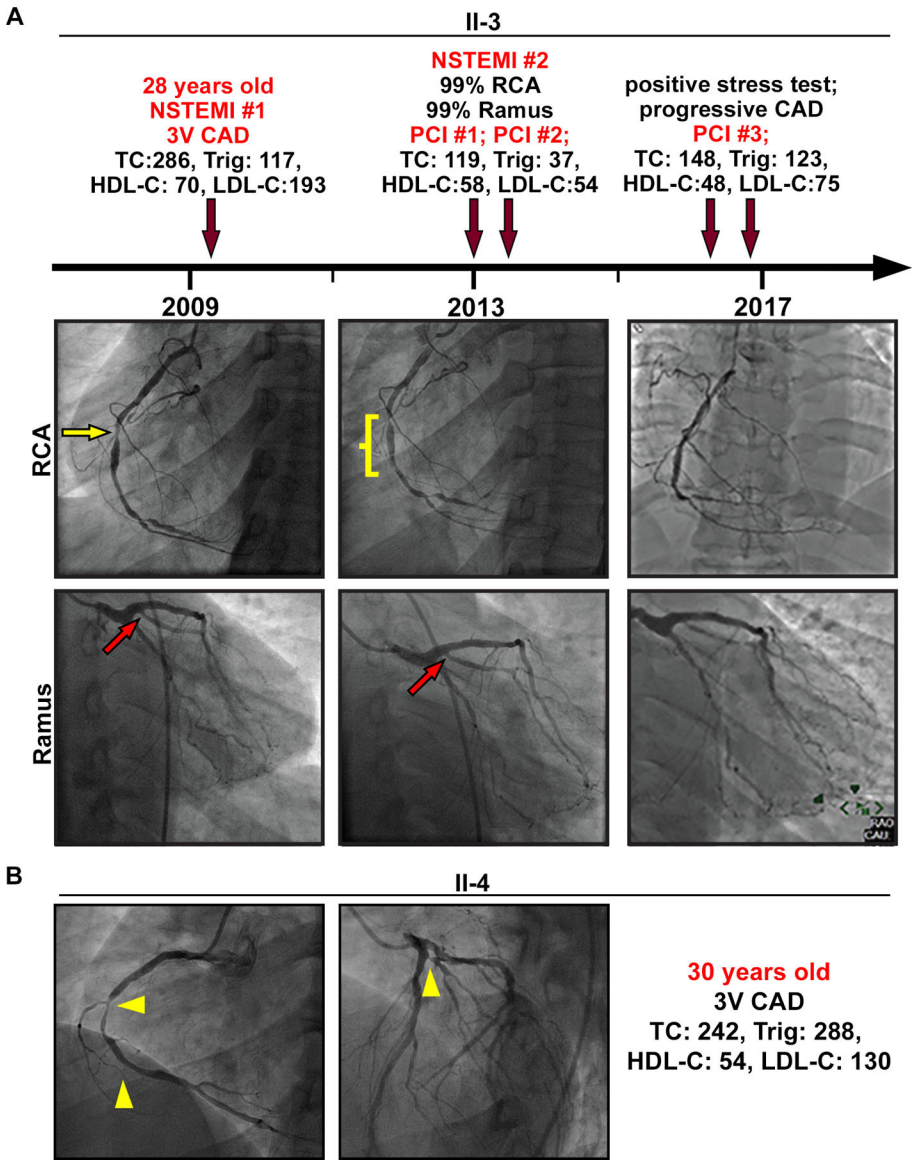

Figure 1. A familial case of severe, 3-vessel coronary artery disease.

A) Clinical history of proband demonstrating initial presentation in 2009 with a non-ST elevation myocardial infarction (NSTEMI) and severe 3-vessel coronary artery disease (CAD). The proband presented again in 2013 with a second NSTEMI for which he received percutaneous intervention (PCI #1) with a drug-eluting stent to the right coronary artery (RCA, yellow arrow), followed by a second stent 3 months later (yellow bracket indicates overlapping stents) for progressive CAD (PCI #2). In 2017, the proband had a stress test positive for ischemia, and follow-up angiogram revealed progressive disease in the ramus intermedius artery (red arrows) for which he received PCI #3. Lipid profiles are highlighted above the timeline. HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, TC: total cholesterol, Trig: triglycerides. **B)** Coronary angiograms from the proband's younger brother (II-4, Figure 1B) at diagnosis of severe 3-vessel coronary artery disease involving the RCA, left circumflex artery and a small diagonal

branch. Left panel: severe coronary artery disease (CAD) in the right coronary artery (yellow arrows). Right panel: severe CAD in the first diagonal branch (yellow arrow).

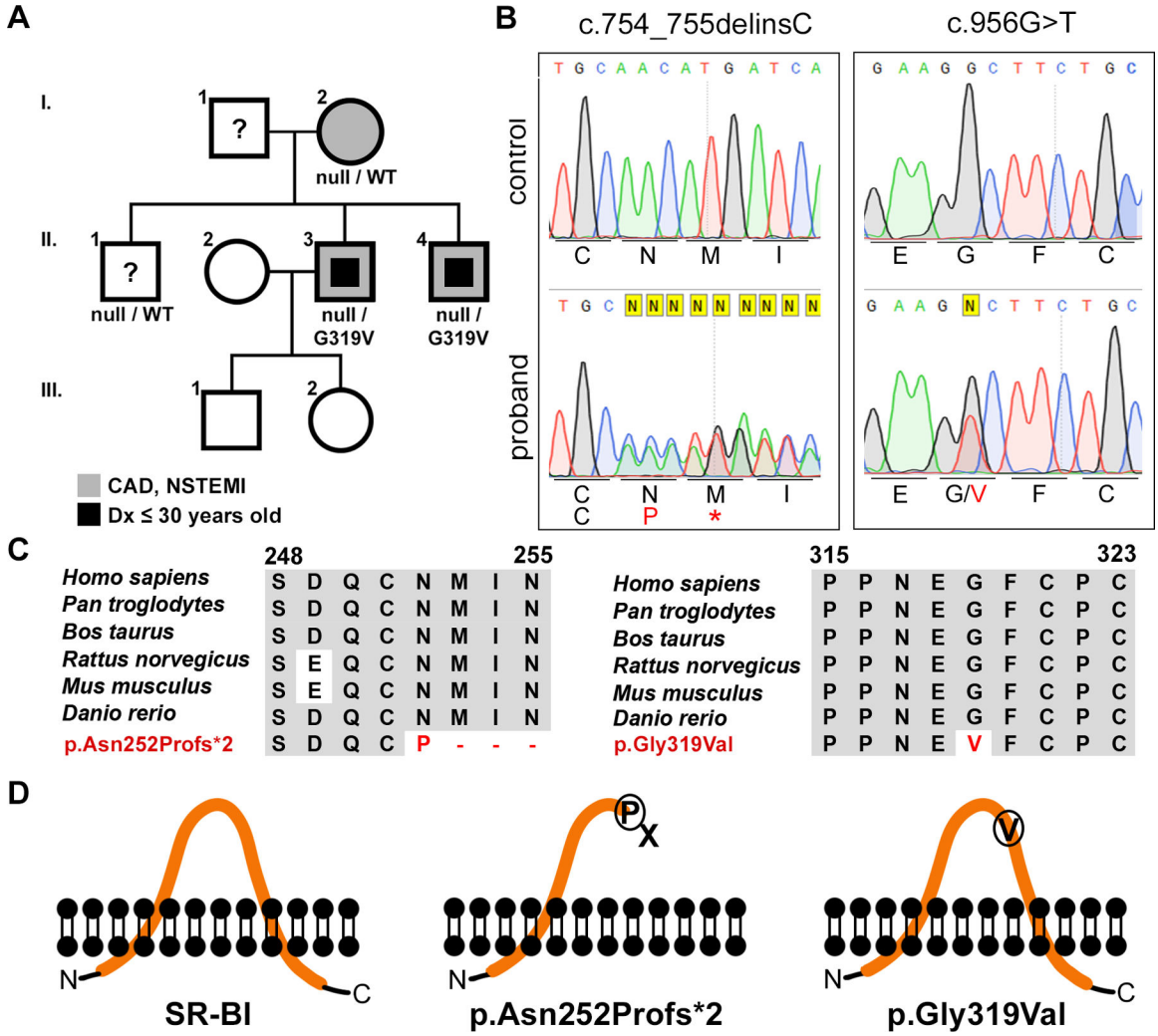

Figure 2. Variants in *SCARB1* segregate with disease in family with severe coronary artery disease.

A) Pedigree of family with severe CAD shows that the proband (II-3) and his younger brother (II-4) inherited compound heterozygous *SCARB1* variants and were diagnosed with CAD at or before 30 years of age. Question mark denotes an unknown phenotype, as medical records were not available. **B)** Sanger sequencing chromatograms confirm whole exome sequencing results. **C)** Orthologous regions of SR-BI are evolutionarily conserved across species at the location of p.Asn252Profs*2 and p.Gly319Val. **D)** Schematic showing location of animo acid changes in the predicted variant proteins.

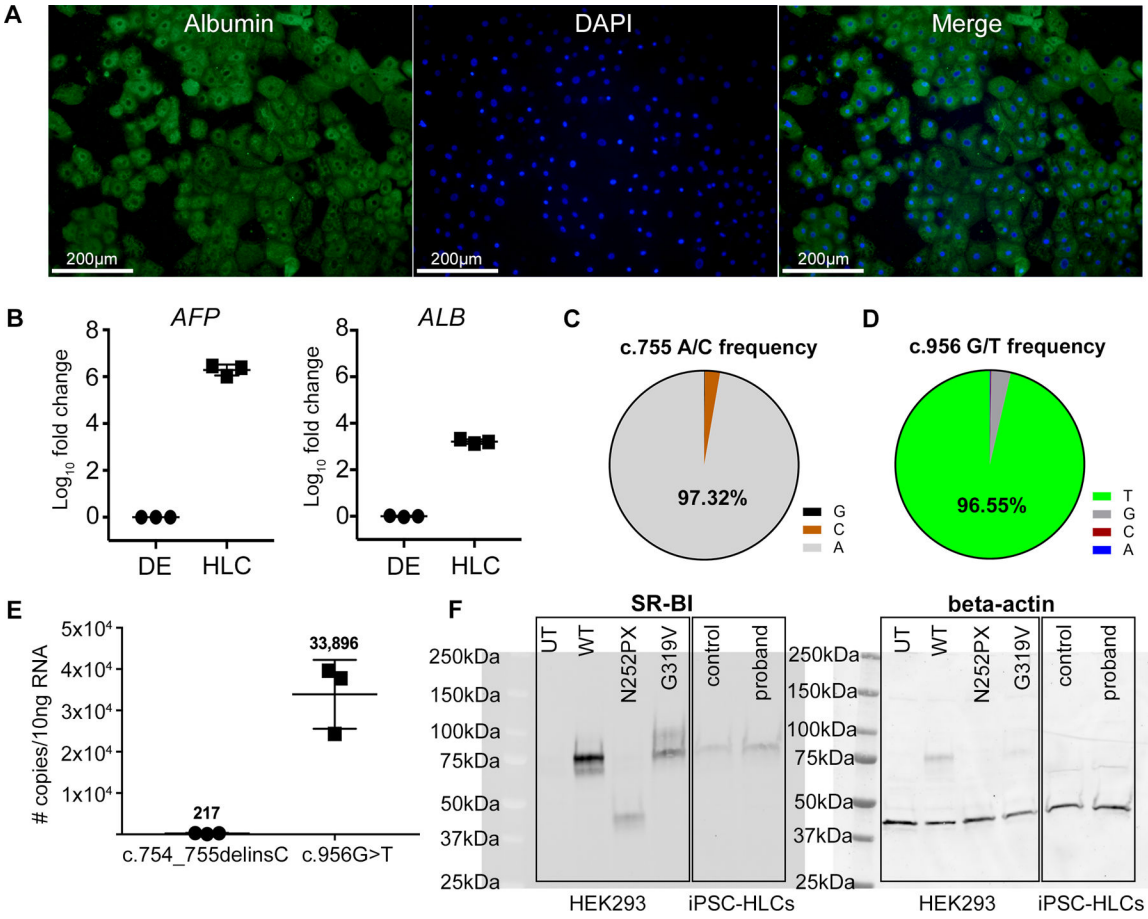

Figure 3. The c.754_755delinsC variant is a genetic null.

A) Hepatocyte like cells differentiated from proband derived induced pluripotent stem cells (iPSC-HLC) express maker of mature hepatocytes, albumin; 20x magnification. Scale bar represents 200µm. n=4 **B)** Gene expression of mature hepatocyte markers in iPSC-HLCs and definitive endoderm intermediate cells (DE) is shown in log_{10} fold change. AFP, p=0.1, Mann-Whitney test, n=3; ALB, p=0.1; Mann-Whitney test, n=3. **C-D)** Next generation sequencing data of RNA isolated from iPSC-HLCs show that the maternal allele is transcribed at a very low frequency compared to the paternal allele (n=3). Pie charts depict **(C)** the percentage of cytosine (brown; maternal allele) and adenine (gray; reference nucleotide) at cDNA position 755 and **(D)** the percentage of thymine (green; paternal allele) and guanine (gray; reference nucleotide) at cDNA position 956. **E)** Transcript counts of proband iPSC-HLCs were measured by digital droplet PCR. At baseline, ~99% of the transcripts expressed were from the paternal allele. p=0.1, Mann-Whitney test, n=3. **F)** SR-BI in the proband's iPSC-HLCs is only expressed at ~75kD, representing protein from the paternal allele (p.G319V). A lower molecular weight band from the predicted p.N252P*fs is not observed. HEK293 cells untransfected (UT) or overexpressing wildtype (WT), SR-BI p.N252PX, or SR-BI p.G319V were used as molecular weight controls. Control iPSCs (Thermo Fisher) were differentiated to iPSC-HLCs as the experimental control. Beta-actin was used as loading control. n=3.

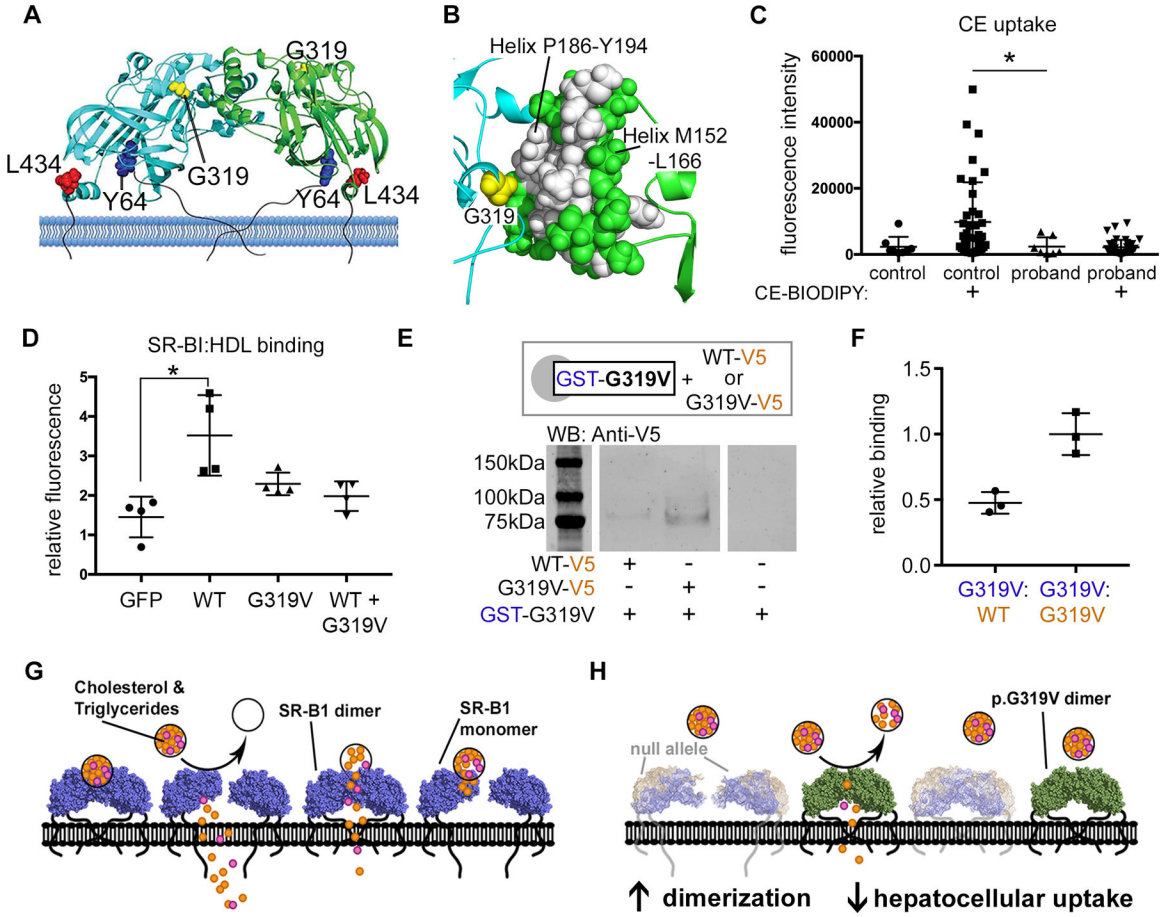

Figure 4. The p.G319V variant is functionally deleterious.

A) Computational model of the SR-BI homodimer (monomers represented by light blue and green) demonstrate that G319 is located in the extracellular domain near a predicted dimerization domain. Red, C-terminal L434; dark blue, N-terminal Y64; yellow, G319. **B)** Close-up of amphipathic pocket and G319. White, hydrophobic residues in close proximity to G319. We predict that substitution of glycine to valine at 319 would stabilize this dimeric interaction through increased hydrophobicity. **C)** Cholesterol ester (CE) uptake assay demonstrates pathogenicity in proband iPSC-HLCs in comparison to a control line (Thermo Fisher). CE-BIODIPY expression was normalized to albumin gene expression. *$p=3.45\times10^{-4}$; Kruskal-Wallis test with Dunn's multiple comparisons; control untreated, n=8; control treated, n = 39; proband untreated, n=7; control treated, n=38. **D)** HDL binding assay demonstrates that overexpression of wildtype SR-BI-GFP promotes HDL binding in HEK cells, and this effect is attenuated in the presence of p.G319V. DiI-HDL fluorescence was normalized to GFP within each group. *GFP vs WT $p=8.44\times10^{-3}$; Kruskal-Wallis test with Dunn's multiple comparisons; n=4. **E-F)** *In vitro* binding assay with GST-tagged (blue) SR-BIp.G319V and V5-tagged (orange) WT or G319V indicates that there is a stronger binding preference for G319V (homodimer; G319V:G319V) than WT (heterodimer; G319V:WT). **F)** Quantification shows diminished affinity for G319V:WT in comparison to G319V:G319V. When GST-tagged SR-BIp.G319V was used as bait, we observed more p.G319V in the output than wildtype SR-BI *p=0.1; Mann-Whitney test, n=3. **G-H)**

Schematic model of wildtype SR-BI **(G)** and SR-BI in our proband **(H)**, showing decreased SR-BI expression due to the null allele, increased homodimerization and decreased receptor activity in the proband. The faded molecule represents the null allele. Blue, WT SR-BI; Green, p.G319

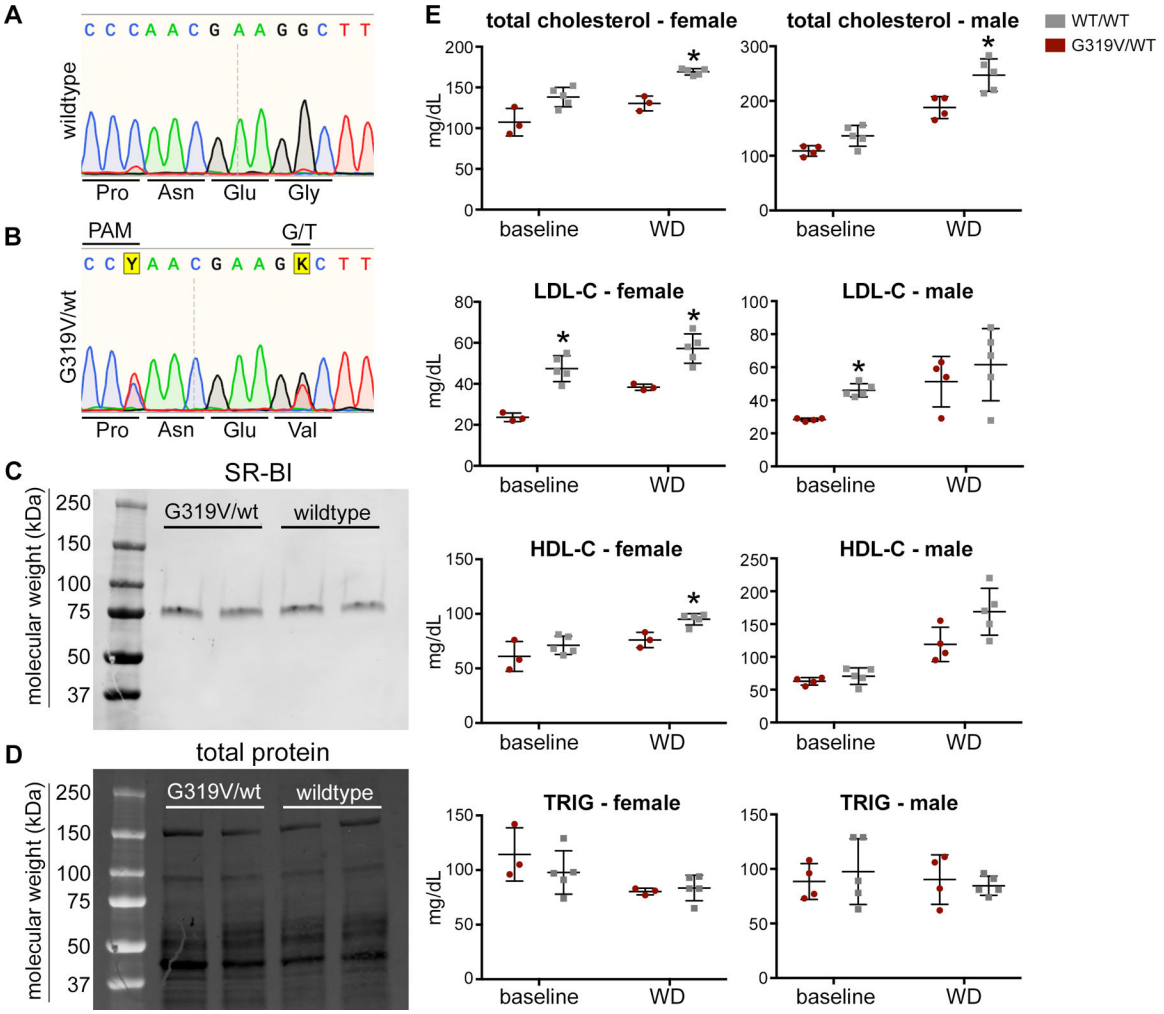

Figure 5. Knock-in mouse model harboring the p.G319V variant demonstrates pathogenicity *in vivo*.

A-B) Representative sanger sequencing chromatogram from the F1 generation confirming base pair change from guanine to thymine at c.956, the orthologous site to the human c.956G>T variant. This change results in a glycine to valine substitution at p.319. The PAM sequence also harbors a silent mutation (c.948C>T) to prevent re-cutting during genome editing. **C-D)** Normal expression of SR-BI in mice that are heterozygous for the knock-in mutation (G319V/wt). n=4. **E)** Plasma levels of total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C) and triglycerides (TRIG) in the p.G319V knock-in mouse. Cholesterol was measured at baseline and after 4 weeks of a modified western diet (WD; 21% milk fat, 1.25% cholesterol). *p<0.05 (10 weeks old at baseline; SR-BI$^{WT/WT}$, N = 7 [3 females, 4 males]; SR-BI$^{G319V/WT}$, N = 10 [5 females, 5 males]; Welch's t-tests were used to compare G319V heterozygous mice to wildtype littermates of the same sex. Statistical summary and individual p-values listed in Table VI of the Online Supplement. Mouse demographics are listed in Table VIII of the Online Supplement.

Table 1.

SR-BI p.G319V homozygous knock-in mice display postnatal lethality by P21.

p.G319V knock-in mouse lethality			
Alleles	**wt/wt**	**G319V/wt**	**G319V/G319V**
Expected	18 (25%)	36 (50%)	18 (25%)
Observed	18 (41.8%)	23 (53.4%)	**2 (4.7%)**