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ORIGINAL ARTICLE

Heterozygous *ABCG5* Gene Deficiency and Risk of Coronary Artery Disease

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BACKGROUND: Familial sitosterolemia is a rare Mendelian disorder characterized by hyperabsorption and decreased biliary excretion of dietary sterols. Affected individuals typically have complete genetic deficiency—homozygous loss-of-function (LoF) variants—in the *ABCG5* or *ABCG8* genes and have substantially elevated plasma sitosterol and LDL (low-density lipoprotein) cholesterol (LDL-C) levels. The impact of partial genetic deficiency of *ABCG5* or *ABCG8*—as occurs in heterozygous carriers of LoF variants—on LDL-C and risk of coronary artery disease (CAD) has remained uncertain.

METHODS: We first recruited 9 sitosterolemia families, identified causative LoF variants in *ABCG5* or *ABCG8*, and evaluated the associations of these *ABCG5* or *ABCG8* LoF variants with plasma phytosterols and lipid levels. We next assessed for LoF variants in *ABCG5* or *ABCG8* in CAD cases (n=29 321) versus controls (n=357 326). We tested the association of rare LoF variants in *ABCG5* or *ABCG8* with blood lipids and risk for CAD. Rare LoF variants were defined as protein-truncating variants with minor allele frequency <0.1% in *ABCG5* or *ABCG8*.

RESULTS: In sitosterolemia families, 7 pedigrees harbored causative LoF variants in *ABCG5* and 2 pedigrees in *ABCG8*. Homozygous LoF variants in either *ABCG5* or *ABCG8* led to marked elevations in sitosterol and LDL-C. Of those sitosterolemia families, heterozygous carriers of *ABCG5* LoF variants exhibited increased sitosterol and LDL-C levels compared with noncarriers. Within large-scale CAD case-control cohorts, prevalence of rare LoF variants in *ABCG5* and in *ABCG8* was ≈0.1% each. *ABCG5* heterozygous LoF variant carriers had significantly elevated LDL-C levels (25 mg/dL [95% CI, 14–35]; $P=1.1\times10^{-6}$) and were at 2-fold increased risk of CAD (odds ratio, 2.06 [95% CI, 1.27–3.35]; $P=0.004$). By contrast, *ABCG8* heterozygous LoF carrier status was not associated with increased LDL-C or risk of CAD.

CONCLUSIONS: Although familial sitosterolemia is traditionally considered as a recessive disorder, we observed that heterozygous carriers of an LoF variant in *ABCG5* had significantly increased sitosterol and LDL-C levels and a 2-fold increase in risk of CAD.

Key Words: coronary artery disease ■ lipids ■ odds ratio ■ pedigree ■ prevalence

Familial sitosterolemia (OMIM No. 210250) is a rare Mendelian disorder characterized by tendonous xanthomas, high plasma plant sterols and cholesterol levels, and increased risk of premature myocardial infarction.[1–4] The *ABCG5* and *ABCG8* are the primary causal genes of familial sitosterolemia. *ABCG5*, *ABCG8*, and *NPC1L1* determine the efflux and absorption of sterols on the surface of intestine and bile duct.[5–8] *NPC1L1*

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Nonstandard Abbreviations and Acronyms

CAD	coronary artery disease
LDL	low-density lipoprotein
LDL-C	low-density lipoprotein cholesterol
LoF	loss of function
TSCA	TruSeq Custom Amplicon target resequencing studies

regulates sterol absorption, whereas *ABCG5* and *ABCG8* form obligate heterodimers[9] and coordinately control the excretion at both the brush border membrane of enterocyte and the apical membrane of hepatocytes.[5,10–12]

Complete deficiency due to homozygous or compound heterozygous loss-of-function (LoF) variants in *ABCG5* or *ABCG8* causes markedly increased sitosterolemia and cholesterol levels and potentially accelerated atherosclerotic disease as well.[1–4] Genome-wide association studies also demonstrated that common genetic variants in the *ABCG5-ABCG8* gene region were associated with phytosterols, LDL (low-density lipoprotein) cholesterol (LDL-C),[13] and risk of coronary artery disease (CAD).[14] However, it is uncertain whether partial deficiency of *ABCG5* or *ABCG8* as conferred by LoF variants in the heterozygous state is also associated with higher cholesterol levels and an increased risk of CAD.

Here, we explored the metabolic and clinical consequences of *ABCG5* or *ABCG8* deficiency. We recruited probands and relatives in sitosterolemia families and assessed whether observed *ABCG5* or *ABCG8* causative LoF variants were associated with increased plasma phytosterols and LDL-C. We then analyzed exome sequences from 93 513 participants and genotype data from an additional 293 134 individuals to test whether carriers of rare heterozygous LoF variants in *ABCG5* or *ABCG8* had elevated blood lipids and risk of CAD.

METHODS

The detailed methods of this study are available in Materials in the Data Supplement. The data that support the findings of this study are available from the corresponding author upon reasonable request. All participants in each study provided written informed consent for genetic studies. The Institutional Review Board at Partners HealthCare (Boston, MA) and each participating institution approved the study protocol. Analyses conducted using the UK Biobank Resource were conducted under application number 7089.

RESULTS

ABCG5 or *ABCG8* Causative LoF Variants, Blood Phytosterol, and Cholesterol Levels in Sitosterolemia Families

We recruited 9 Japanese families with sitosterolemia and sequenced the exons of the *ABCG5* and *ABCG8* genes in 47 individuals from these families (Figure I in the Data Supplement). Among the individuals within these families, 9 carried a homozygous or compound

Table 1. Clinical Characteristics by *ABCG5* and *ABCG8* Variant Carrier Status in Sitosterolemia Families

		ABCG5 LoF variant		*ABCG8 LoF* Variant	
	Noncarrier	Heterozygote	Homozygote	Heterozygote	Homozygote
n	12	21	8	5	1
Age, y; mean (SD)	42.1 (19)	40.2 (21)	12.9 (20)	45.2 (19)	1
Male sex, n (%)	3 (25)	12 (57)	3 (38)	2 (40)	0
Lipid profile					
Total cholesterol, mg/dL; median (IQR)	181 (166–207)	217 (185–276)*	539 (247–700)	293 (223–307)	968
LDL cholesterol, mg/dL; median (IQR)	100 (84–143)	145 (126–176)*	408 (166–594)	169 (121–169)	832
HDL cholesterol, mg/dL; median (IQR)	58 (52–76)	50 (40–71)	47 (40–54)	65 (46–65)	46
Triglycerides, mg/dL; median (IQR)	85 (65–95)	91 (55–151)	188 (140–248)*	154 (73–154)	71
Lipoproteins					
Apolipoprotein A1, mg/dL; median (IQR)	148 (139–164)	139 (126–150)	106 (97–129)	NA	NA
Apolipoprotein B, mg/dL; median (IQR)	73 (63–109)	104 (90–118)*	262 (198–303)	NA	NA
Noncholesterol sterols					
Sitosterol, μg/mL; median (IQR)	2.3 (1.8–2.8)	7.8 (6.0–11)*	102 (74–125)*	9.9 (8.2–12)*	36.5
Campesterol, μg/mL; median (IQR)	3.7 (3.3–5.2)	13 (11–14)*	70 (65–95)*	NA	NA
Sitosterol/TC, μg/mg; median (IQR)	1.3 (1.1–1.4)	3.5 (2.7–4.7)*	22 (14–30)*	4.4 (3.9–5.3)*	3.8
Campesterol/TC, μg/mg; median (IQR)	2.6 (1.8–2.6)	5.4 (4.8–6.6)*	13 (7.5–18)	NA	NA

HDL indicates high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; NA, not available; SD, standard deviation; and TC, total cholesterol.
*$P<0.025$ compared with noncarrier controls. *P* values were calculated by linear regression adjusted by kinship matrix within each family using the log-transformed values.

heterozygous *ABCG5* or *ABCG8* causative LoF variants while 26 carried a heterozygous *ABCG5* or *ABCG8* LoF causative variants. Of those, 10 of 11 LoF variants were classified as pathogenic protein-truncating or missense variants and one as likely pathogenic according to the American College of Medical Genetics variant classification guidelines (Table I in the Data Supplement).[15] As expected, *ABCG5* or *ABCG8* homozygote or compound heterozygous LoF variant carriers showed high sitosterol/total cholesterol ratios and LDL-C levels compared with noncarriers. Regarding heterozygous state, carriers of *ABCG5* or *ABCG8* heterozygous LoF variant exhibited increased sitosterol/total cholesterol ratio compared with noncarriers. Moreover, *ABCG5* heterozygous LoF variant carrier status was associated with an increased LDL-C level (Table 1; Figure 1).

ABCG5 or *ABCG8* Rare Heterozygous LoF Variation, Blood Lipids, and Risk for CAD in Large Cohorts

Next, we examined whether rare heterozygous LoF variant carrier status in *ABCG5* or *ABCG8* associated with higher blood lipids and elevated risk of CAD. We sequenced the protein coding regions of *ABCG5* and *ABCG8* in 93 513 individuals from 3 datasets: 48 576 participants from Myocardial Infarction Genetics consortium, 43 223 participants from UK Biobank, and 1714

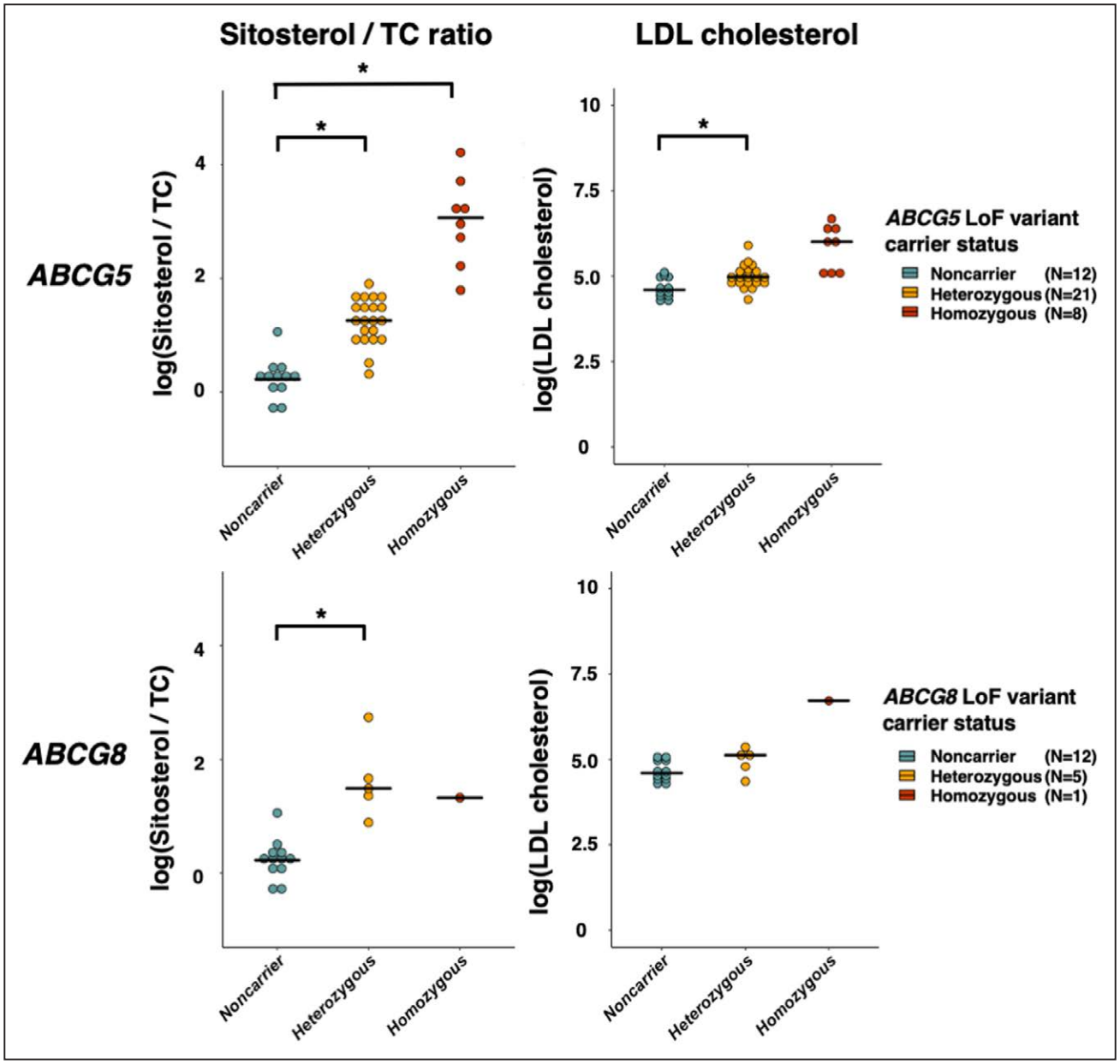

Figure 1. Sitosterol-to-total cholesterol (TC) ratio and LDL (low-density lipoprotein) cholesterol levels among individuals with homozygous or heterozygous sitosterolemia and unaffected controls in sitosterolemia families.
Each dot indicates an individual's value. Each horizontal line represents a mean value for each carrier status. *P<0.025.

participants from TSCA (TruSeq Custom Amplicon target resequencing studies; Table 2). We detected 108 individuals harboring rare *ABCG5* LoF alleles, and the prevalence of *ABCG5* heterozygous carrier status was 0.12% (Table II in the Data Supplement). We also discovered 142 individuals who harbored rare *ABCG8* LoF alleles, a heterozygous carrier prevalence also around 0.15% (Table III in the Data Supplement).

Individuals carrying *ABCG5* LoF variants had significantly increased total cholesterol (17 mg/dL [95% CI, 13–32]; $P=6.9\times10^{-6}$) and LDL-C levels (25 mg/dL [95% CI, 13–32]; $P=1.1\times10^{-6}$; Figure 2).

We investigated the association between rare *ABCG5* heterozygous LoF variant carrier status and CAD risk using >380 000 participants from the 3 sequencing cohorts and additional UK Biobank genotyping array-based cohort. We identified 34 carriers of *ABCG5* heterozygous LoF variants among 29 321 CAD cases (0.12%) and 63 among 357 326 controls (0.018%). In a Cochran-Mantel-Haenszel fixed-effects meta-analysis, individuals carrying *ABCG5* heterozygous LoF variants were at 2-fold risk of CAD (odds ratio, 2.06 [95% CI, 1.27–3.35]; $P=0.004$; Figure 3). A similar effect estimate was noted in a meta-analysis of adjusted odds ratios derived using logistic regression (odds ratio, 2.04 [95% CI, 1.28–3.26]; $P=0.003$).

In contrast to *ABCG5*, carriers of rare *ABCG8* heterozygous LoF variants did not exhibit significant increase in any of blood lipids including LDL-C level ($\beta=0.06$ [95% CI, −0.09 to 0.22]; $P=0.47$; Table IV in the Data Supplement). Moreover, *ABCG8* heterozygous LoF variant carrier status was not at elevated risk for CAD (odds ratio, 0.79 [95% CI, 0.47–1.31]; $P=0.36$; Table IV in the Data Supplement).

We also explored whether the effect size of *ABCG5* LoF variants on CAD risk was consistent with their impact on LDL-C. We observed a linear dose-response relationship between CAD risk and LDL-C change conferred by DNA sequence variants in *LDLR*, *PCSK9*, *ABCG5*, or *ABCG8* (Table V in the Data Supplement). The effect of *ABCG5* LoF variants on CAD (a doubling in risk) was consistent with the estimate based on their impact in LDL-C (25 mg/dL; Figure 4).

DISCUSSION

In this study, we evaluated whether rare heterozygous LoF variations in *ABCG5* or *ABCG8* were associated with blood lipid levels and CAD risk. We used 2 different approaches—sitosterolemia family-based analysis and population-based analysis from over 380 000 individuals—to test whether rare heterozygous LoF variants in *ABCG5* or *ABCG8* associated with phytosterols, lipids, and CAD. We found that when compared with noncarriers, carriers of heterozygous LoF variants in *ABCG5* had higher sitosterol and ≈25 mg/dL higher LDL-C and were at 2-fold risk of CAD.

These results permit several conclusions. First, individuals who carry rare heterozygous LoF variants in *ABCG5* (but not *ABCG8*) have significantly elevated LDL-C levels and are at elevated risk for CAD. Although there have been reports of premature atherosclerosis among sitosterolemia patients with homozygous causative LoF variant carriers,[2–4] it had been unclear whether *ABCG5* or *ABCG8* partial deficiency also increases blood lipid levels and CAD risk. These findings imply that *ABCG5* LoF variant carriers may derive clinical benefit from LDL-C–lowering therapy. Importantly, the *NPC1L1* inhibitor ezetimibe

Table 2. Clinical Characteristics of Participants in MIGen, UK Biobank, and TSCA

	MIGen	UK Biobank (Sequencing and Genotyping)	TSCA
	n=48 576	n=336 357	n=1714
Age, y (SD)	54 (10)	57 (8)	57.2 (13)
Male sex, n (%)	41 203 (71)	156 112 (46)	1140 (67)
BMI (SD), kg/m^2	27 (5)	27 (5)	29 (6)
Current smoker, n (%)	18 173 (33)	25 802 (8)	639 (37)
Medical history			
Coronary artery disease, n (%)	16 106 (33)	12 073 (3)	1184 (69)
Hypertension, n (%)	16 839 (35)	153 535 (46)	798 (47)
Type 2 diabetes mellitus, n (%)	11 245 (22)	15 770 (5)	277 (16)
Lipid profile			
Total cholesterol, mg/dL (SD)	178 (60)	222 (41)	182 (51)
LDL cholesterol, mg/dL (SD)	109 (45)	138 (34)	108 (41)
HDL cholesterol, mg/dL (SD)	36 (14)	56 (15)	43 (7)
Triglycerides, mg/dL (SD)	154 (127)	155 (90)	135 (82)

BMI indicates body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MIGen, Myocardial Infarction Genetics consortium; and TSCA, TruSeq Custom Amplicon target resequencing studies.

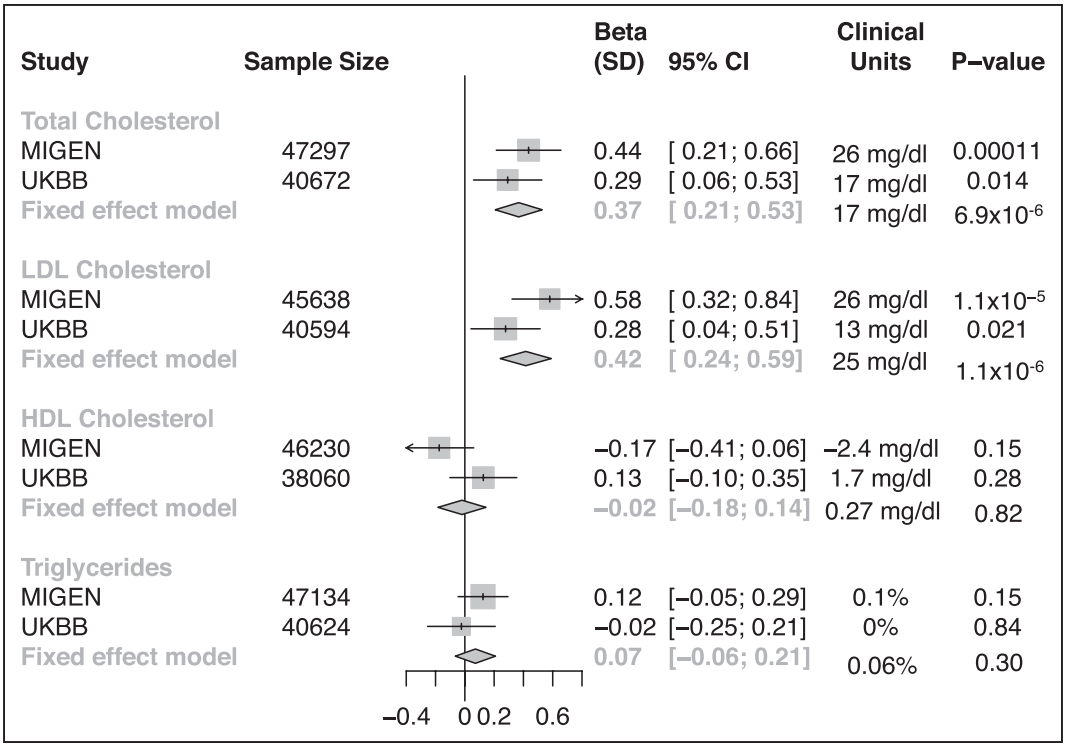

Figure 2. Effects of loss-of-function variants in ABCG5 (ATP-binding cassette transporter G5) on blood lipid profiles from Myocardial Infarction Genetics consortium (MIGen) and UK Biobank (UKBB).
Effect sizes were calculated using linear regression adjusted by age, sex, study, case-control status, and first 5 principal components of ancestry. Triglycerides were natural log-transformed before analysis. Fixed-effects meta-analysis was applied to combine results. HDL indicates high-density lipoprotein; and LDL, low-density lipoprotein.

is known to reduce intestinal cholesterol and phytosterol absorption in patients with sitosterolemia and could have increased efficacy in individuals with partial *ABCG5* deficiency.[16] Although both *ABCG5* and *ABCG8* are part of a heterodimer complex involved in the excretion of sterols from intestine to the lumen and from hepatocytes into the biliary tree, heterozygous *ABCG5* deficiency seems to affect plasma LDL-C and CAD, whereas heterozygous *ABCG8* deficiency does not. Additional functional studies are needed to explain this new finding.[17]

Second, it has been unclear whether elevated plant sterol levels or elevated blood cholesterol levels cause atherosclerosis among patients with sitosterolemia.[14] The impact of heterozygous LoF carriers status on risk of CAD was proportional to the effect on LDL-C elevation, suggesting that LDL-C rather than sitosterol itself is the key driver of the accelerated atherosclerosis. These findings were also consistent with a recent meta-analysis that did not observe a significant association between circulating sitosterol levels and risk of cardiovascular disease.[18]

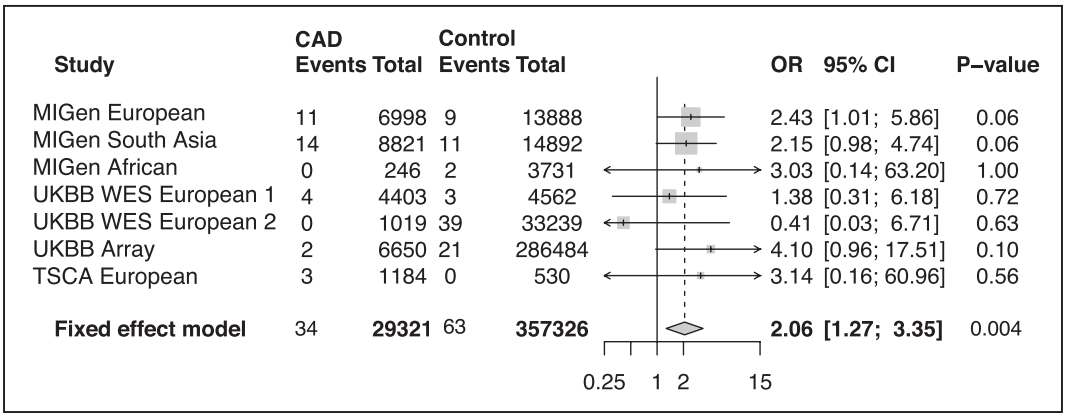

Figure 3. Effect of loss-of-function variants in ABCG5 (ATP-binding cassette transporter G5) on coronary artery disease (CAD).
A meta-analysis across studies was performed using the Cochran-Mantel-Haenszel statistics for stratified 2-by-2 tables. MIGen indicates Myocardial Infarction Genetics consortium; OR, odds ratio; TSCA, TruSeq Custom Amplicon target resequencing studies; UKBB, UK Biobank; and WES, whole exome sequencing.

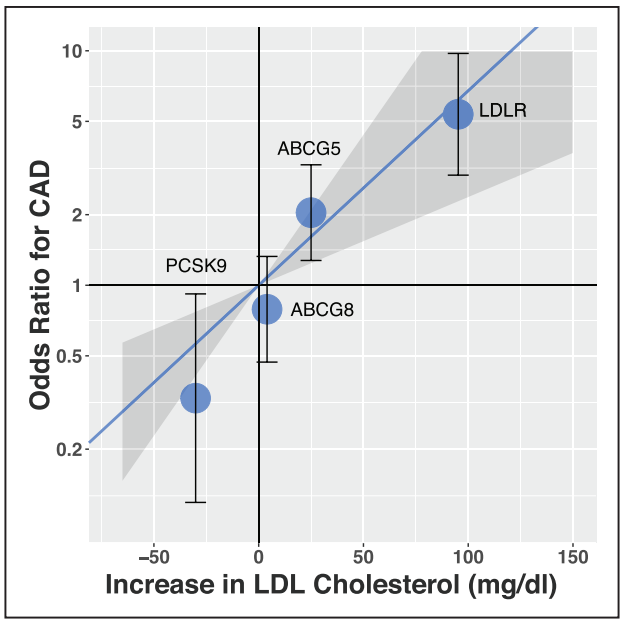

Figure 4. For loss-of-function (LoF) variants at the *ABCG5*, *ABCG8*, *PCSK9*, or *LDLR* genes, relationship between impact on LDL (low-density lipoprotein) cholesterol (LDL-C) levels and coronary artery disease (CAD) risk.
Solid line indicates a dose-response reference line with the 95% CI indicated by shadow. Each dot represents the effects of LoF variants in that gene on both LDL-C and CAD risk with 95% CI.

Moreover, the effect size of *ABCG5* heterozygous LoF variant carrier status on both blood lipids and CAD risk was consistent with predictions based on known familial hypercholesterolemia and hypobetalipoproteinemia variants (Figure 4; Table V in the Data Supplement).

This study has several limitations. First, detailed functional analyses of each observed variant predicted to cause LoF were not performed. Second, the number of *ABCG8* causative LoF variant carriers in sitosterol families was relatively small and thus our statistical power to evaluate an effect of heterozygous *ABCG8* deficiency was more limited. Third, lipid measurements and CAD definition were different among study cohorts. However, the effect direction among studies was largely consistent, and we observed little heterogeneity in the meta-analysis (I^2 of 0% for CAD).

In conclusion, ≈0.1% of population carried rare LoF variants in *ABCG5*, and compared with noncarriers, *ABCG5* heterozygous LoF variant carriers had elevated sitosterol and LDL-C levels and were at 2-fold risk for CAD.

ARTICLE INFORMATION

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Disclosures

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