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Heterozygous ATP-binding Cassette Transporter G5 Gene Deficiency and Risk of Coronary Artery Disease

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Abstract

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 ATP-binding cassette transporter G5 (*ABCG5*) or G8 (*ABCG8*) genes and have substantially elevated plasma sitosterol and 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 nine 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 less than 0.1% in *ABCG5* or *ABCG8*.

Results: In sitosterolemia families, seven pedigrees harbored causative LoF variants in *ABCG5* and two 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 to non-carriers. Within large-scale CAD case-control cohorts, prevalence of rare LoF variants in *ABCG5* and in *ABCG8* were approximately 0.1% each. *ABCG5* heterozygous LoF variant carriers had

significantly elevated LDL-C levels (25 mg/dL; 95% confidence interval [CI] 14 to 35; $P=1.1\times10^{-6}$) and were at two-fold increased risk of CAD (odds ratio 2.06, 95% CI 1.27 to 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 a LoF variant in *ABCG5* had significantly increased sitosterol and LDL-C levels and a two-fold increase in risk of CAD.

Keywords

Lipids and Cholesterol; Cardiovascular Disease; Genetics; coronary artery disease; lipids; cholesterol; sitosterol; population genetics

Introduction

Familial sitosterolemia (OMIM #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 ATP-binding cassette transporters G5 (*ABCG5*) and G8 (*ABCG8*) are the primary causal genes of familial sitosterolemia. *ABCG5, ABCG8,* and N-terminal Niemann-Pick C1 Like 1 (*NPC1L1*) determine the efflux and absorption of sterols on the surface of intestine and bile duct.[5–8] *NPC1L1* 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* and/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, 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 are 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 detail methods of this study are available in the Supplemental Material. 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, USA) 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 nine Japanese families with sitosterolemia and sequenced the exons of the *ABCG5* and *ABCG8* genes in 47 individuals from these families (Supplemental Figure). Among the individuals within these families, 9 carried a homozygous or compound heterozygous *ABCG5* or *ABCG8* causative LoF variants while 26 carried a heterozygous *ABCG5* or *ABCG8* LoF causative variants. Of those, 10 out 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 (Supplemental Table 1).[15] As expected, *ABCG5* or *ABCG8* homozygote or compound heterozygous LoF variant carriers showed very high sitosterol / Total Cholesterol (TC) ratios and LDL-C levels compared to non-carriers. Regarding heterozygous state, carriers of *ABCG5* or *ABCG8* heterozygous LoF variant carriers exhibited increased sitosterol / TC ratio compared with non-carriers. Moreover, *ABCG5* heterozygous LoF variant carrier status was associated with an increased LDL-C level. (Table 1 and 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 three datasets: 48,576 participants from MIGen, 43,223 participants from UK Biobank and 1,714 participants from TSCA (Table 2). We detected 108 individuals harboring rare *ABCG5* LoF alleles and the prevalence of *ABCG5* heterozygous carrier status was 0.12%. (Supplemental Table 2). We also discovered 142 individuals who harbored rare *ABCG8* LoF alleles, a heterozygous carrier prevalence also around 0.15% (Supplemental Table 3).

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

We investigated the association between rare *ABCG5* heterozygous LoF variant carrier status and CAD risk using more than 380,000 participants from the three 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-effect meta-analysis, individuals carrying *ABCG5* heterozygous LoF variants were at two-fold risk of CAD (Odds ratio [OR], 2.06; 95% CI, 1.27 to 3.35; P value = 0.004) (Figure 3). A similar effect estimate

was noted in a meta-analysis of adjusted odds ratios derived using logistic regression (OR 2.04; 95% CI 1.28 to 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) (Supplemental Table 4). Moreover, *ABCG8* heterozygous LoF variant carrier status was not at elevated risk for CAD (OR, 0.79; 95% CI, 0.47 to 1.31; P = 0.36) (Supplemental Table 4).

We also explored whether the effect size of *ABCG5* LoF variants on CAD risk was consistent with the 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* (Supplemental Table 5). The effect of *ABCG5* LoF variants on CAD (a doubling of in risk) was consistent with the estimate based on the 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 two 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 to non-carriers, carriers of heterozygous LoF variants in *ABCG5* had higher sitosterol and ~25 mg/dL higher LDL-C and were at two-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 if *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 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] 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, Supplemental Table 5).

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-squared of 0% for CAD).

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

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Disclosures

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Pharmaceuticals, received grant support from the Novartis Institute for Biomedical Research and IBM Research, and reports a patent related to a genetic risk predictor (20190017119). S.K. is an employee of Verve Therapeutics. He is a founder of Maze Therapeutics, Verve Therapeutics, and San Therapeutics. He holds equity in Catabasis and San Therapeutics. He is a member of the scientific advisory boards for Regeneron Genetics Center and Corvidia Therapeutics; served as a consultant for Acceleron, Eli Lilly, Novartis, Merck, Novo Nordisk, Novo Ventures, Ionis, Alnylam, Aegerion, Huag Partners, Noble Insights, Leerink Partners, Bayer Healthcare, Illumina, Color Genomics, MedGenome, Quest, and Medscape; and reports patents related to a method of identifying and treating a person having a predisposition to or afflicted with cardiometabolic disease (20180010185) and a genetic risk predictor (20190017119). The remaining authors have nothing to disclose.

Non-standard Abbreviations and Acronyms

ABCG5	ATP-binding cassette transporters G5
ABCG8	ATP-binding cassette transporters G8
CAD	coronary artery disease
CI	confidence interval
LDL-C	low-density lipoprotein cholesterol
LoF	Loss-of-function
NPC1L1	N-terminal Niemann-Pick C1 Like 1
OR	odds ratio
TC	total cholesterol

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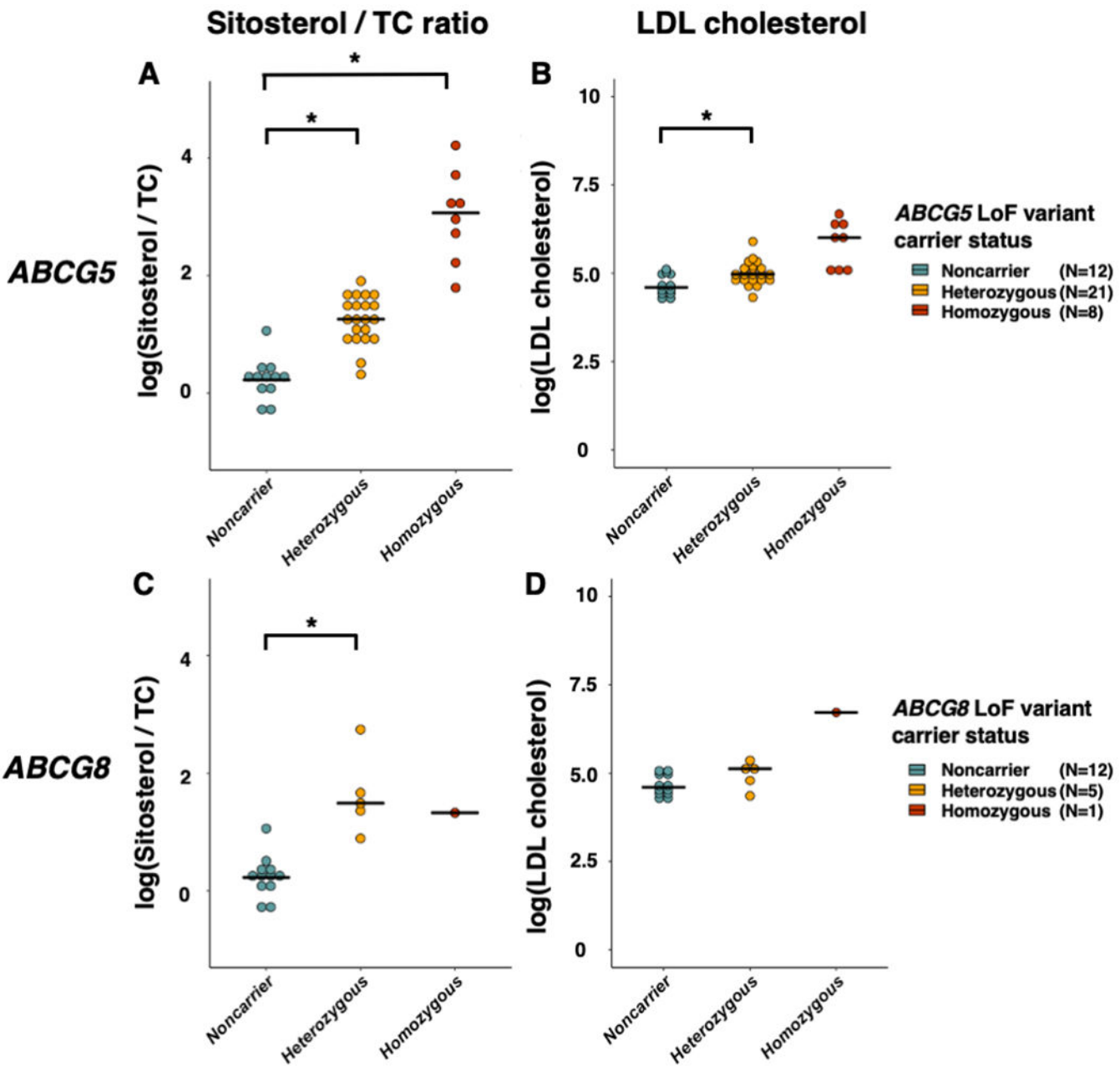

Figure 1. Sitosterol to total cholesterol ratio and LDL-C levels among individuals with homozygous or heterozygous sitosterolemia, and unaffected controls in sitosterolemia families. Each dot indicates an individual's value. Each horizontal line represents a mean value for each carrier status. *: P value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

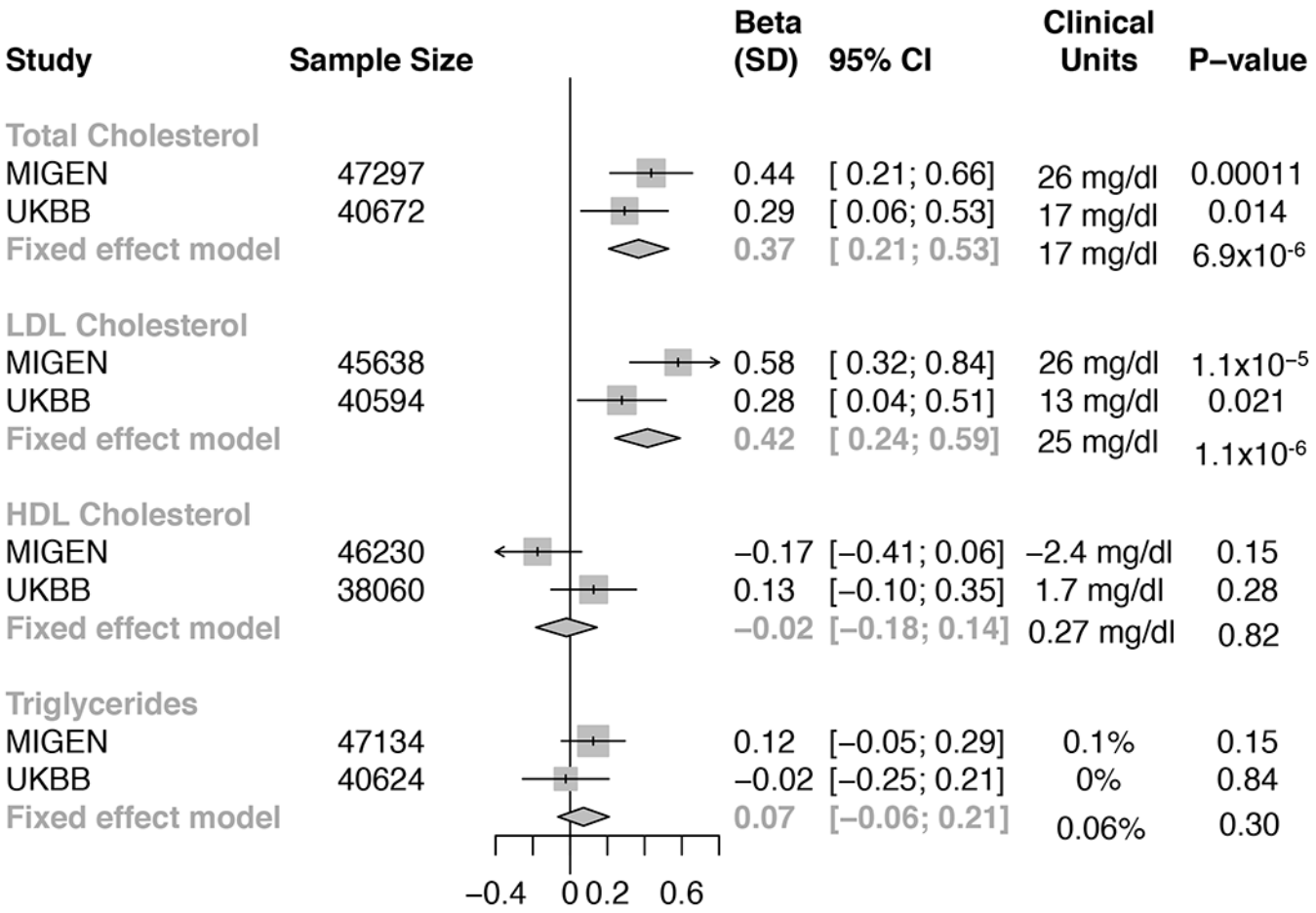

Figure 2. Effects of loss-of-function variants in *ABCG5* on blood lipid profiles from MIGen and UK Biobank.

Effect sizes were calculated using linear regression adjusted by age, gender, study, case-control status, and first five principal components of ancestry. Triglycerides was natural log-transformed before analysis. Fixed-effects meta-analysis was applied to combine results. Abbreviation: UKBB, UK biobank.

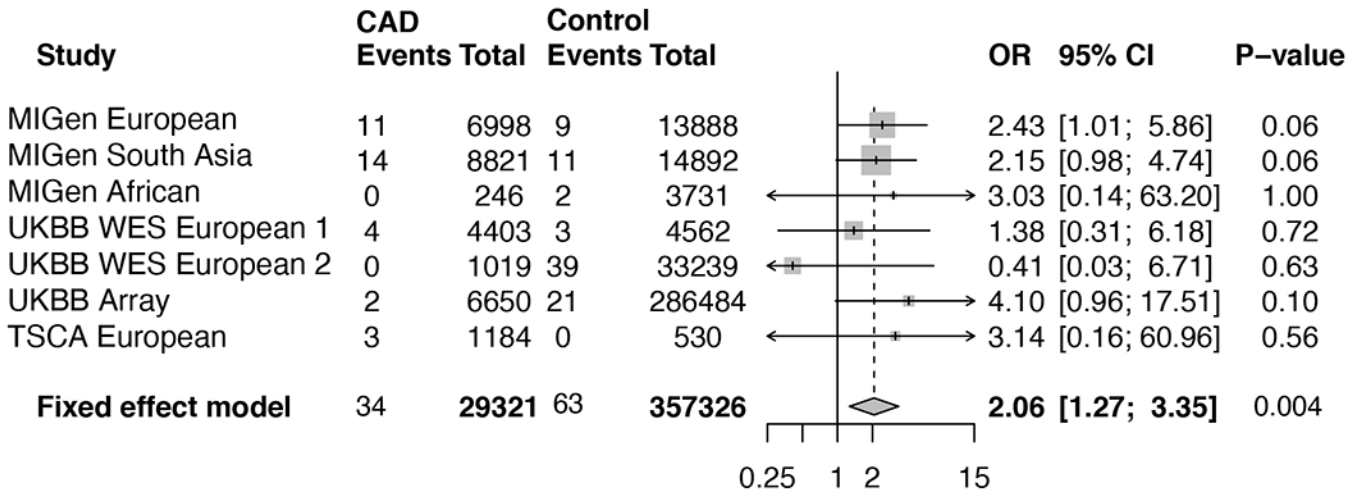

Figure 3. Effect of LoF variants in *ABCG5* on CAD.

A meta-analysis across studies was performed using the Cochran–Mantel–Haenszel statistics for stratified 2-by-2 tables. MIGen, Myocardial Infarction Genetics Consortium.

Abbreviations: CAD, coronary artery disease; CI, confidence interval; LoF, loss-of-function; OR, odds ratio; TSCA, TruSeq Custom Amplicon target resequencing studies; UKBB, UK biobank.

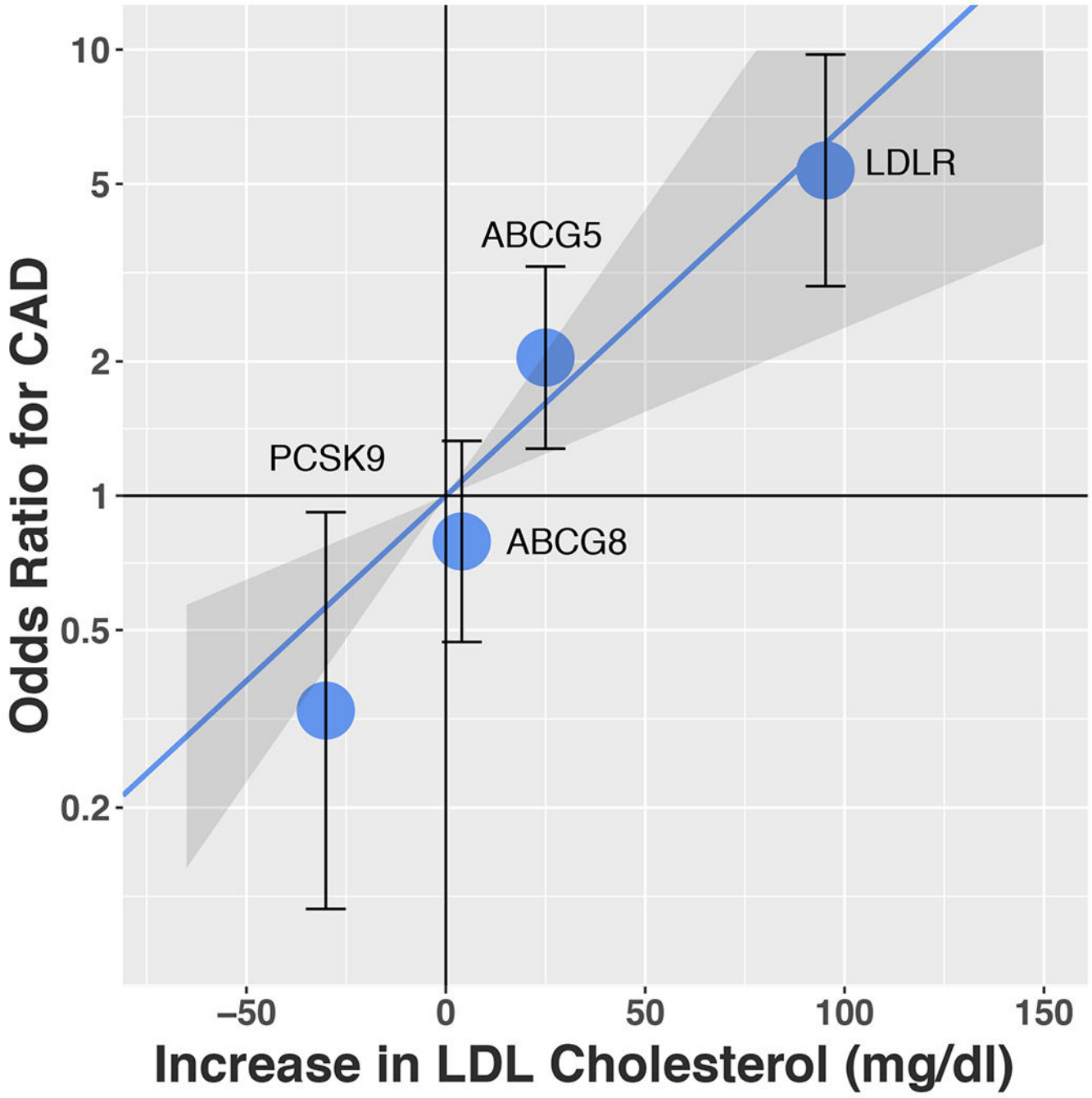

Figure 4. For LoF variants at the *ABCG5*, *ABCG8*, *PCSK9* or *LDLR* genes, relationship between impact on LDL cholesterol levels and CAD risk

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.

Abbreviations; CAD, coronary artery disease; LDL, low-density lipoprotein; LoF, loss-of-function.

Table 1.

Clinical characteristics by *ABCG5* and *ABCG8* variant carrier status in sitosterolemia families.

	Non-carrier	*ABCG5 LoF variant*		*ABCG8 LoF Variant*	
		Heterozygote	**Homozygote**	**Heterozygote**	**Homozygote**
N	12	21	8	5	1
Age, 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
Non–cholesterol 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

* P value < 0.025 compared to non-carrier controls. P values were calculated by linear regression adjusted by kinship matrix within each family using the log-transformed values.

Abbreviations: HDL, high-density lipoprotein; IQR, interquartile range; LDL, low density lipoprotein; SD, standard deviation; TC, total cholesterol.

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 = 1,714**
Age, years (SD)	54 (10)	57 (8)	57.2 (13)
Male gender, n (%)	41,203 (71)	156,112 (46)	1,140 (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)	1,184 (69)
Hypertension, n (%)	16,839 (35)	153,535 (46)	798 (47)
Type 2 diabetes, 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)

Abbreviations: MIGen, Myocardial Infarction Genetics consortium; SD, standard deviation; TSCA, TruSeq Custom Amplicon target resequencing studies.