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# Genetic variability in the absorption of dietary sterols affects the risk of coronary artery disease

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## Aims

To explore whether variability in dietary cholesterol and phytosterol absorption impacts the risk of coronary artery disease (CAD) using as instruments sequence variants in the *ABCG5/8* genes, key regulators of intestinal absorption of dietary sterols.

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## Methods and results

We examined the effects of *ABCG5/8* variants on non-high-density lipoprotein (non-HDL) cholesterol (*N* up to 610 532) and phytosterol levels (*N*=3039) and the risk of CAD in Iceland, Denmark, and the UK Biobank (105 490 cases and 844 025 controls). We used genetic scores for non-HDL cholesterol to determine whether *ABCG5/8* variants confer greater risk of CAD than predicted by their effect on non-HDL cholesterol. We identified nine rare *ABCG5/8* coding variants with substantial impact on non-HDL cholesterol. Carriers have elevated phytosterol levels and are at increased risk of CAD. Consistent with impact on *ABCG5/8* transporter function in hepatocytes, eight rare *ABCG5/8* variants associate with gallstones. A genetic score of *ABCG5/8* variants predicting 1 mmol/L increase in non-HDL cholesterol associates with two-fold increase in CAD risk [odds ratio (OR) = 2.01, 95% confidence interval (CI) 1.75–2.31,  $P = 9.8 \times 10^{-23}$ ] compared with a 54% increase in CAD risk (OR = 1.54, 95% CI 1.49–1.59,  $P = 1.1 \times 10^{-154}$ ) associated with a score of other non-HDL cholesterol variants predicting the same increase in non-HDL cholesterol ( $P$  for difference in effects =  $2.4 \times 10^{-4}$ ).

## Conclusions

Genetic variation in cholesterol absorption affects levels of circulating non-HDL cholesterol and risk of CAD. Our results indicate that both dietary cholesterol and phytosterols contribute directly to atherogenesis.

## Keywords

Dietary cholesterol • Phytosterols • Absorption • Genetics • *ABCG5/8*

## Translational perspective

The importance of dietary cholesterol absorption in the regulation of cholesterol levels in blood and the risk of coronary artery disease (CAD) has been the subject of controversy. We find that sequence variants that decrease the function of the *ABCG5/8* transporter increase absorption of both cholesterol and phytosterols and increase the risk of CAD. The findings provide mechanistic insights indicating harmful effects of dietary cholesterol on cardiovascular disease. We also find that the impact of *ABCG5/8* variants on the risk of CAD is not fully explained by non-HDL cholesterol. Thus, in addition to dietary cholesterol other dietary sterols such as phytosterols may contribute directly to atherogenesis, raising questions about the safety of supplementing food with phytosterols for the purpose of cardiovascular risk reduction.

## Introduction

The *ABCG5* and *ABCG8* genes encode the obligate heterodimers of the ATP-binding cassette (ABC) transporters G5 and G8 (*ABCG5/8*) that have a major role in preventing accumulation of dietary sterols, including cholesterol and sterols derived from plants (phytosterols), in the body.<sup>1</sup> The *ABCG5/8* transporter is mainly expressed in the small intestine on the absorptive surface of enterocytes and in the liver on hepatocytes facing the bile canaliculi (Figure 1).

While the *NPC1L1* transporter, the target of the cholesterol-lowering drug ezetimibe,<sup>2</sup> is responsible for the non-selective uptake of sterols into enterocytes and hepatocytes from the intestinal lumen and bile, respectively, the *ABCG5/8* excretes them back into the intestinal lumen and bile<sup>1</sup> (Figure 1).

Rare inactivating mutations in the *ABCG5/8* genes cause autosomal recessive phytosterolaemia (also termed sitosterolaemia). This rare disorder is characterized by impaired sterol elimination from enterocytes and hepatocytes leading to excessive intestinal absorption of cholesterol and phytosterols, as well as reduced secretion to bile.<sup>3</sup> Although autosomal recessive phytosterolaemia frequently involves hypercholesterolaemia, sometimes to the extreme,<sup>3</sup> this is not always the case and significant premature atherosclerosis has been documented in the absence of substantial hypercholesterolaemia.<sup>3,4</sup>

Common variants at the *ABCG5/8* locus associate with low-density lipoprotein (LDL) cholesterol,<sup>5,6</sup> phytosterol levels, and the risk of coronary artery disease (CAD).<sup>7,8</sup> Alleles that associate with decreased levels of LDL cholesterol also associate with increased risk of gallstones,<sup>7,9,10</sup> likely mediated through an effect on cholesterol

saturation in bile. Furthermore, *NPC1L1* variants associate with LDL cholesterol and CAD,<sup>5,11</sup> and rare *NPC1L1* inactivating variants associate with reduced levels of LDL cholesterol and phytosterols.<sup>12</sup>

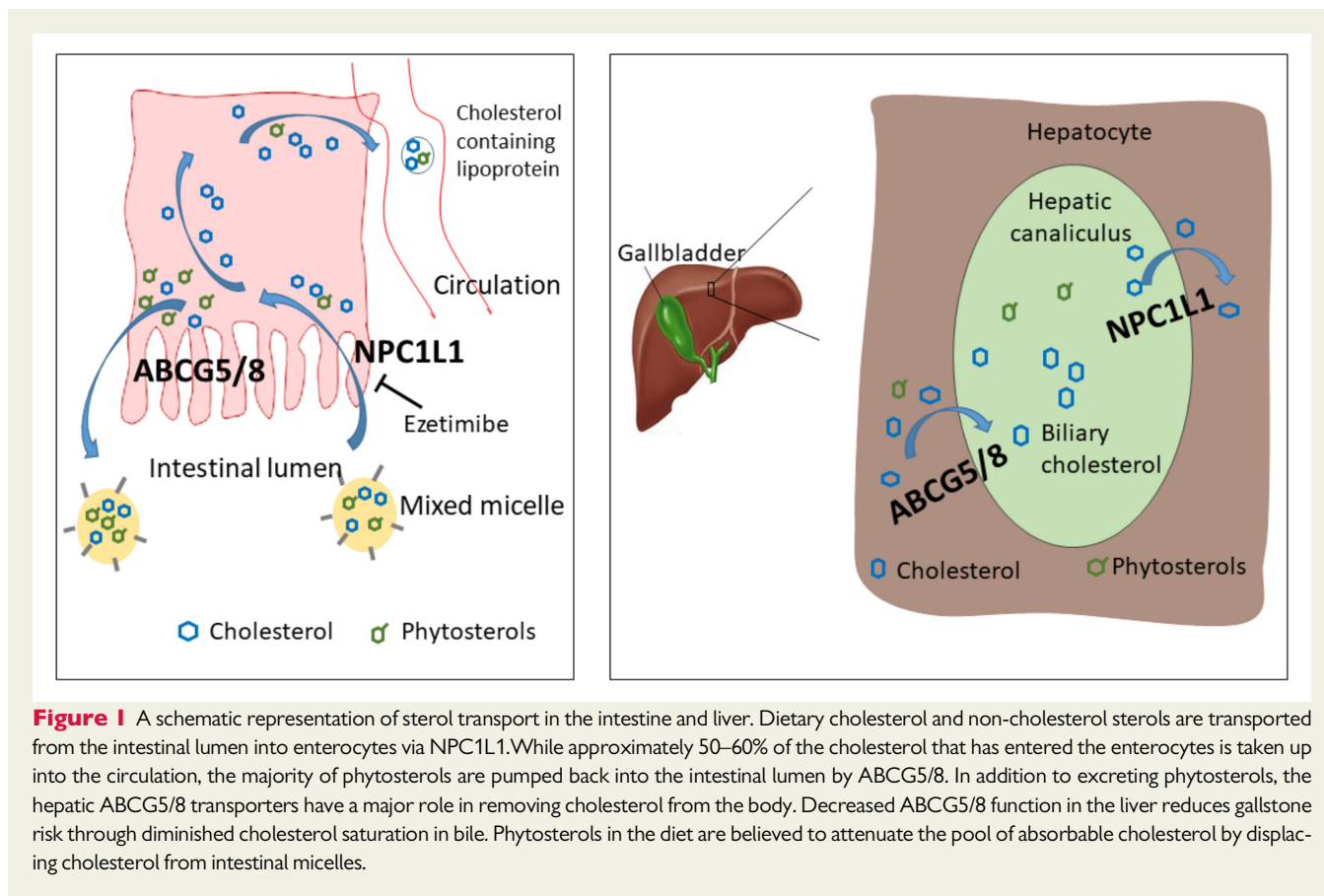
While evidence from genetic studies and randomized clinical trials of cholesterol-lowering drugs demonstrates that the relationship between non-HDL/LDL cholesterol and CAD is causal,<sup>13,14</sup> the contribution of dietary cholesterol to cardiovascular diseases (CVDs) and mortality has been debated for decades.<sup>15</sup> Over the last few years, the importance of dietary cholesterol has been deemphasized in dietary recommendations in many countries.<sup>16,17</sup>

The role of phytosterols in atherosclerotic disease is also a matter of controversy.<sup>18–20</sup> The ESC/EAS Guidelines for the management of dyslipidaemias<sup>17</sup> recommend food enriched with phytosterols as a lifestyle intervention to reduce cholesterol levels by interfering with intestinal cholesterol absorption.<sup>21</sup>

Here, we explore whether variability in dietary cholesterol and phytosterol absorption impacts the risk of CAD, using sequence variants of the *ABCG5/8* genes as instruments.

## Methods

Detailed description of the studies included and methods used is provided in [Supplementary material online, Methods](#). Briefly, we analysed data from three studies of individuals of European origin from Iceland, Denmark, and UK Biobank. We examined association of sequence variants in *ABCG5/8* with non-HDL cholesterol<sup>22</sup> in up to 610 532 individuals, phytosterol levels in 3039 individuals, and the risk of CAD in 105 490



**Figure 1** A schematic representation of sterol transport in the intestine and liver. Dietary cholesterol and non-cholesterol sterols are transported from the intestinal lumen into enterocytes via NPC1L1. While approximately 50–60% of the cholesterol that has entered the enterocytes is taken up into the circulation, the majority of phytosterols are pumped back into the intestinal lumen by ABCG5/8. In addition to excreting phytosterols, the hepatic ABCG5/8 transporters have a major role in removing cholesterol from the body. Decreased ABCG5/8 function in the liver reduces gallstone risk through diminished cholesterol saturation in bile. Phytosterols in the diet are believed to attenuate the pool of absorbable cholesterol by displacing cholesterol from intestinal micelles.

cases and 844 025 controls. Variant associations were also assessed in public data from the Global Lipids Genetics Consortium<sup>23</sup> ( $N$  up to 333 359) and CARDIoGRAM Exome<sup>24</sup> (42 355 cases and 78 240 controls).

Logistic or linear regression, assuming additive models, was used to test for the association of variants with binary or quantitative traits, respectively. Variant association results from the different study groups were combined into meta-analyses assuming fixed effects. All  $P$ -values reported in this study are two-sided.

We constructed individual-level genetic risk scores (GRS) for levels of non-HDL cholesterol<sup>22</sup> and calculated into the study subjects. The GRSs were generated for each individual by summing the product of the allele count and the corresponding non-HDL cholesterol effect size.

## Results

### Coding variants in *ABCG5/8* and association with non-high density lipoprotein cholesterol and coronary artery disease

We identified 35 rare [minor allele frequency (MAF)  $\geq 0.01\%$  and  $< 1\%$ ] coding variants in 28 075 whole-genome sequenced Icelanders that we subsequently imputed into chip-typed Icelanders and their close relatives (Supplementary material online, Methods). Six

common (MAF  $> 5\%$ ) variants (five coding and one intronic) reported<sup>5–10</sup> to associate with LDL cholesterol, CAD, and gallstones were also examined. We tested these *ABCG5/8* variants for association with non-HDL and LDL cholesterol in datasets from Iceland, Denmark, the UK Biobank, and the Global Lipids Genetics Consortium (GLGC),<sup>23</sup> and in a meta-analysis ( $N$  up to 943 891; Supplementary material online, Tables S1 and S2).

Of the 35 rare coding variants, nine associate with non-HDL cholesterol ( $P < 0.05/41 = 1.2 \times 10^{-3}$ ) (Table 1, Figure 2, and Supplementary material online, Table S1). We note that two or more of these nine rare variants never occur on the same haplotype ( $D' = -1$ , pairwise  $R^2 < 3.0 \times 10^{-6}$ ), with the exception of p. Phe624Leu in *ABCG5* that is always observed on the background of p. Gly27Ala in the same gene ( $D' = 1$  and  $R^2 = 0.32$ ).

All six reported common variants associate with non-HDL cholesterol in our dataset (Supplementary material online, Table S1). However, these associations are fully captured by three variants with low pairwise correlation ( $R^2 \leq 0.1$ ; Supplementary material online, Table S3), p. Asp19His (rs11887534), the intronic rs4299376, and p. Thr400Lys (rs4148217) (Figure 2 and Supplementary material online, Table S3).

Next, we examined the association of the 35 rare coding and the 3 common variants with CAD in Iceland (39 020 cases and 319 620 controls) and in a meta-analysis of data from Iceland, Denmark, UK Biobank, and the public CARDIoGRAM Exome<sup>24</sup> (combined up to

**Table 1** The association of ABCG5/8 variants with non-high density lipoprotein cholesterol, coronary artery disease, and gallstones

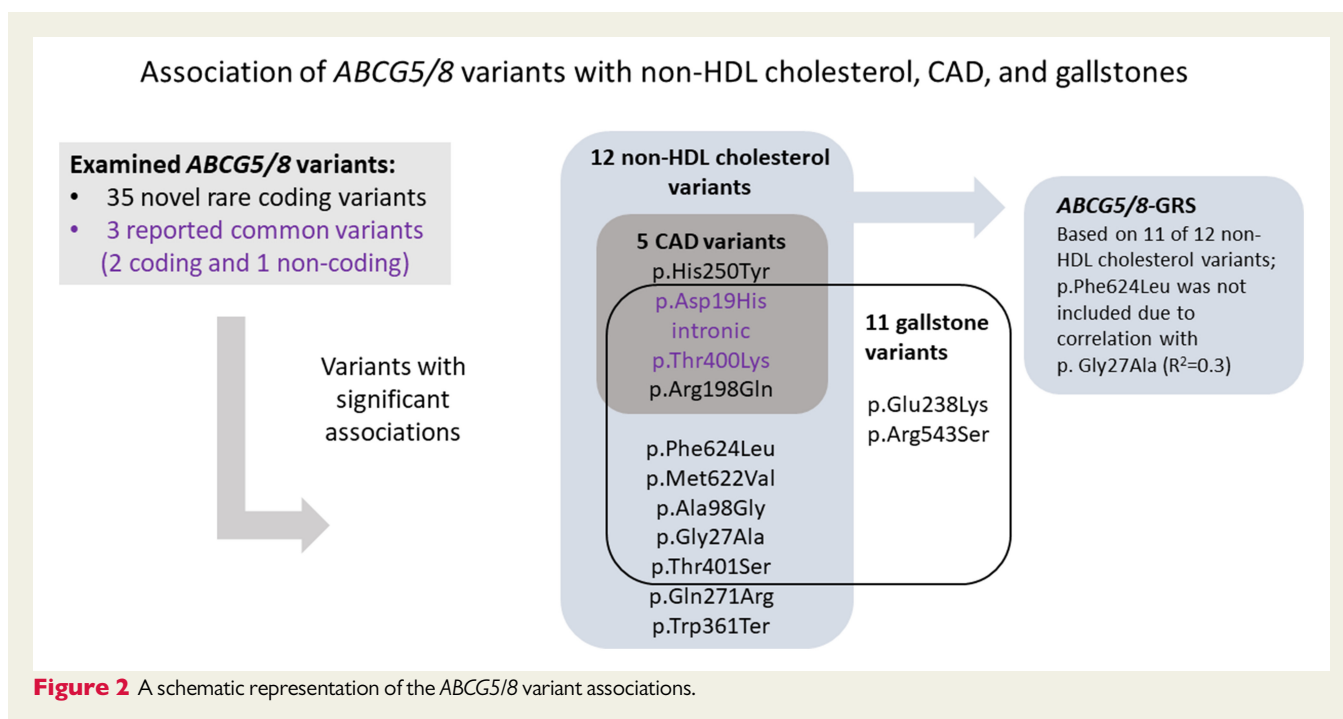
ABCG-[5/8]	Coding change	rsName	EA/non-EA	EA frequency (%)		Non-HDL cholesterol (N up to 943 891) <sup>a</sup>			CAD (N up to 147 825 cases/922 265 controls) <sup>b</sup>			Gallstones (N up to 27 441 cases/738 791 controls) <sup>c</sup>		
				Iceland/Denmark/UK Biobank/GLGC	B	95% CI	P	OR	95% CI	P	OR	95% CI	P	
5	p-Phe24Leu	rs150401285	G/A	0.031/0.138/0.098/0.065	0.19	(0.14, 0.25)	$1.9 \times 10^{-11}$	1.12	(0.96, 1.31)	0.16	0.37	(0.24, 0.57)	$6.3 \times 10^{-6}$	
5	p-Met622Val	rs140374206	C/T	0.104/0.541/0.649/0.503	0.05	(0.02, 0.07)	$8.2 \times 10^{-5}$	1.02	(0.96, 1.09)	0.5	0.70	(0.61, 0.82)	$6.1 \times 10^{-6}$	
5	p-His250Tyr	rs776502883	A/G	0.091/NA/NA/NA	0.58	(0.42, 0.75)	$6.0 \times 10^{-12}$	1.96	(1.35, 2.83)	$3.9 \times 10^{-4}$	0.80	(0.40, 1.61)	0.52	
5	p-Arg198Gln	rs141828689	T/C	0.145/0.157/0.132/0.138	0.16	(0.11, 0.21)	$2.8 \times 10^{-11}$	1.29	(1.11, 1.49)	$6.2 \times 10^{-4}$	0.42	(0.31, 0.59)	$3.3 \times 10^{-7}$	
5	p-Phe125Leu	NA	G/A	0.027/NA/NA/NA	0.41	(0.11, 0.70)	$6.5 \times 10^{-3}$	2.48	(1.30, 4.71)	$5.7 \times 10^{-3}$	0.46	(0.12, 1.73)	0.25	
5	p-Ala98Gly	rs145164937	C/G	0.021/0.231/0.176/NA	0.11	(0.06, 0.17)	$1.6 \times 10^{-4}$	1.11	(0.96, 1.29)	0.16	0.42	(0.29, 0.60)	$1.8 \times 10^{-6}$	
5	p-Gly27Ala	rs56204478	G/C	0.072/0.358/0.354/NA	0.14	(0.10, 0.18)	$2.7 \times 10^{-13}$	1.08	(0.98, 1.19)	0.13	0.50	(0.42, 0.61)	$4.5 \times 10^{-12}$	
8	p-Asp19His	rs11887534	C/G	5.461/6.104/6.49/5.8	-0.11	(-0.11, -0.10)	$3.5 \times 10^{-203}$	0.93	(0.91, 0.95)	$9.4 \times 10^{-13}$	1.94	(1.87, 2.00)	$<1 \times 10^{-300}$	
8	intron	rs4299376	G/T	27.958/29.541/31.667/25.17	0.07	(0.06, 0.07)	$6.6 \times 10^{-266}$	1.05	(1.04, 1.06)	$1.1 \times 10^{-22}$	0.80	(0.79, 0.82)	$1.1 \times 10^{-119}$	
8	p-Glu238Lys	rs34754243	A/G	0.019/0.292/0.162/NA	-0.01	(-0.06, 0.05)	0.83	1.11	(0.97, 1.26)	0.13	1.51	(1.21, 1.89)	$3.1 \times 10^{-4}$	
8	p-Arg263Gln	rs137852990	A/G	0.117/NA/NA/0.013	0.17	(0.05, 0.29)	$5.5 \times 10^{-3}$	1.14	(0.81, 1.60)	0.5	0.41	(0.22, 0.75)	$4.0 \times 10^{-3}$	
8	p-Gln271Arg	rs770309304	G/A	0.106/NA/NA/NA	0.40	(0.25, 0.55)	$2.9 \times 10^{-7}$	1.36	(0.95, 1.96)	0.091	0.39	(0.20, 0.79)	$8.2 \times 10^{-3}$	
8	p-Trp361Ter	rs137852987	A/G	0.147/0.159/0.076/0.111	0.13	(0.08, 0.18)	$9.9 \times 10^{-8}$	1.14	(1.00, 1.31)	0.059	0.66	(0.48, 0.90)	$8.5 \times 10^{-3}$	
8	p-Thr400Lys	rs4148217	A/C	19.076/18.405/18.502/NA	-0.04	(-0.05, -0.04)	$2.8 \times 10^{-48}$	0.97	(0.95, 0.98)	$3.2 \times 10^{-6}$	1.11	(1.09, 1.14)	$6.8 \times 10^{-19}$	
8	p-Thr401Ser	rs144200355	T/A	0.006/0.174/0.233/0.157	-0.09	(-0.14, -0.05)	$5.7 \times 10^{-6}$	0.91	(0.80, 1.03)	0.12	1.55	(1.27, 1.88)	$1.3 \times 10^{-5}$	
8	p-Arg543Ser	rs201690654	T/G	0.032/0.036/0.058/0.029	0.08	(-0.01, 0.16)	0.069	1.13	(0.86, 1.48)	0.39	0.35	(0.20, 0.62)	$3.1 \times 10^{-4}$	

The effect (β) on non-HDL cholesterol is given in standard deviation units. p-Arg263Gln causes phytosterolemia in 2 Icelandic sisters. p-Phe125Leu has borderline significant association with non-HDL cholesterol and CAD. CAD, coronary artery disease; CI, confidence interval; EA, effect allele; HDL, high-density lipoprotein; OR, odds ratio.

<sup>a</sup>Combined Iceland (N = 139 033), Denmark (N = 113 038), UK Biobank (N = 358 461), and/or Global Lipids Genetics Consortium (GLGC) (N up to 333 359).

<sup>b</sup>Combined Iceland (39 020 cases/319 620 controls), Denmark (33 603 cases/148 707 controls), UK Biobank (32 867 cases/375 698 controls), and CARDIOGRAM exome (42 335 cases/78 240 controls).

<sup>c</sup>Combined Iceland (9024 cases/348 643 controls) and UK Biobank (18 417 cases/348 643 controls).



147 825 cases and 922 265 controls) (Table 1 and Supplementary material online, Table S4). Two rare variants associate with CAD ( $P < 0.05/35 = 1.4 \times 10^{-3}$ ), p. His250Tyr (OR = 1.96,  $P = 3.9 \times 10^{-4}$ ), and p. Arg198Gln (OR = 1.29,  $P = 6.2 \times 10^{-4}$ ), and both are predicted to have deleterious impact on the protein (Supplementary material online, Table S5). Furthermore, His250 is located in a highly conserved motive (GERP score = 5.56; top 0.3% genome wide), the histidine loop (H-loop) in the nucleotide-binding domain of ABC transporters (Supplementary material online, Methods). We also replicate the CAD association of the common variants<sup>7,8</sup> (Table 1). The alleles of the five variants that associate with higher risk of CAD all associate with higher levels of non-HDL cholesterol.

The association of the 12 non-HDL cholesterol *ABCG5/8* variants with other atherosclerosis-related phenotypes is shown in Supplementary material online, Table S6. We find several nominally significant associations between rare *ABCG5/8* coding variants and other CVD. For example, p. His250Tyr that has the largest effect on non-HDL cholesterol and phytosterols and associates with CAD, also associates with aortic valve stenosis ( $P = 0.0056$ ), heart failure ( $P = 0.0018$ ), and sudden cardiac death ( $P = 3.1 \times 10^{-5}$ ).

None of the variants associates (at  $P < 8.3 \times 10^{-4}$ , corrected for the number of variants and traits tested) with the atherosclerotic risk factors, hypertension, type 2 diabetes, body mass index, triglyceride, or HDL cholesterol, except the common intronic variant rs4299376 that has small effect on triglyceride levels ( $\beta = 0.0096$ ,  $P = 6.5 \times 10^{-6}$ ) (Supplementary material online, Table S6).

### Variant effects on phytosterol levels

We measured three of the most common phytosterols (sitosterol, campesterol, and stigmasterol) in serum from 3039 Icelanders, enriched for carriers of the rare coding variants in *ABCG5/8* that

associate with non-HDL cholesterol. Sufficiently many serum samples were available from carriers of seven rare variants and of those six associate with phytosterol levels. The variant p. His250Tyr with greatest effect on non-HDL cholesterol also has the greatest effect on all three phytosterols ( $\beta$  for stigmasterol = 1.27 SD,  $P = 2.2 \times 10^{-15}$ ) (Table 2).

In the Icelandic dataset, we identified seven homozygous or compound heterozygous carriers of rare *ABCG5/8* coding variants. Two homozygous carriers of p. Arg263Gln in *ABCG8* have extremely high phytosterol levels consistent with autosomal recessive phytosterolaemia (see Supplementary material online, Note).

In agreement with the role of the *ABCG5/8* transporter in regulating intestinal absorption of both cholesterol and phytosterols, the *ABCG5/8* variant effects on non-HDL cholesterol and phytosterol levels are highly correlated ( $R^2 = 0.97$ , Figure 3 and Supplementary material online, Table S7C and G). In the Icelandic data, 1 mmol/L increase in non-HDL cholesterol driven by the *ABCG5/8* variants associates with 2.56 SD increase in stigmasterol levels ( $P = 1.1 \times 10^{-8}$ ). Two common *NPC1L1* variants measured in our dataset associate with phytosterol levels (Supplementary material online, Table S7), but the phytosterol effect per unit change in non-HDL cholesterol is smaller than that observed for the *ABCG5/8* variants (Figure 3). The apparent difference in the effects of *NPC1L1* and *ABCG5/8* variants on phytosterol levels is consistent with the non-selective uptake of sterols into enterocytes mediated by *NPC1L1*,<sup>1,25</sup> as opposed to the preferential excretion of phytosterols from enterocytes into the intestinal lumen mediated by *ABCG5/8*.<sup>1</sup>

Consistent correlation between effects on non-HDL cholesterol and phytosterol levels is not observed for non-HDL cholesterol associating variants outside the *ABCG5/8* and *NPC1L1* loci ( $R^2 = 0.13$ ,  $P = 0.0012$ , Figure 3 and Supplementary material online, Table S7).

**Table 2** Association of *ABCG5/8* variants with phytoosterol levels

ABCG-[5/8]	Coding change	rsName	EA/non-EA	N carriers measured	EA frequency in measured samples (%)	Stigmasterol (N = 3039)			Sitosterol (N = 3039)			Campesterol (N = 3039)		
						$\beta$ (95% CI)	P	P	$\beta$ (95% CI)	P	P	$\beta$ (95% CI)	P	P
5	p.Phe624Leu	rs150401285	G/A	19	0.31	0.88 (0.37, 1.39)	$6.9 \times 10^{-4}$	$6.9 \times 10^{-4}$	0.85 (0.33, 1.37)	$1.2 \times 10^{-3}$	$1.2 \times 10^{-3}$	0.81 (0.30, 1.32)	$1.7 \times 10^{-3}$	
5	p.His250Tyr	rs776502883	A/G	50	0.82	1.27 (0.96, 1.58)	$2.2 \times 10^{-15}$	$2.2 \times 10^{-15}$	1.17 (0.86, 1.49)	$3.9 \times 10^{-13}$	$3.9 \times 10^{-13}$	1.10 (0.79, 1.41)	$4.4 \times 10^{-12}$	
5	p.Arg198Gln	rs141828689	T/C	54	0.89	0.20 (-0.09, 0.50)	0.18	0.18	0.29 (-0.01, 0.59)	0.056	0.056	0.30 (0.01, 0.59)	0.044	
5	p.Gly27Ala	rs56204478	G/C	51	0.84	0.49 (0.16, 0.82)	$3.7 \times 10^{-3}$	$3.7 \times 10^{-3}$	0.43 (0.09, 0.76)	0.012	0.012	0.40 (0.08, 0.73)	0.016	
8	p.Asp19His	rs11887534	C/G	266	4.38	-0.30 (-0.42, -0.18)	$1.1 \times 10^{-6}$	$1.1 \times 10^{-6}$	-0.32 (-0.45, -0.20)	$1.6 \times 10^{-7}$	$1.6 \times 10^{-7}$	-0.26 (-0.38, -0.13)	$3.5 \times 10^{-5}$	
8	intronic	rs4299376	G/T	1529	25.16	0.24 (0.19, 0.30)	$2.6 \times 10^{-17}$	$2.6 \times 10^{-17}$	0.27 (0.22, 0.33)	$1.6 \times 10^{-21}$	$1.6 \times 10^{-21}$	0.23 (0.18, 0.29)	$3.1 \times 10^{-16}$	
8	p.Gln271Arg	rs770309304	G/A	40	0.66	0.79 (0.46, 1.12)	$2.6 \times 10^{-6}$	$2.6 \times 10^{-6}$	0.90 (0.56, 1.23)	$1.2 \times 10^{-7}$	$1.2 \times 10^{-7}$	0.73 (0.40, 1.06)	$1.3 \times 10^{-5}$	
8	p.Arg263Gln	rs137852990	A/G	72	1.22	0.57 (0.33, 0.82)	$3.6 \times 10^{-6}$	$3.6 \times 10^{-6}$	0.45 (0.21, 0.69)	$3.0 \times 10^{-4}$	$3.0 \times 10^{-4}$	0.40 (0.15, 0.64)	$1.3 \times 10^{-3}$	
8	p.Trp361Ter	rs137852987	A/G	59	0.97	0.19 (-0.09, 0.47)	0.18	0.18	0.17 (-0.11, 0.45)	0.23	0.23	0.16 (-0.11, 0.44)	0.25	
8	p.Thr400Lys	rs4148217	A/C	1049	17.26	-0.15 (-0.22, -0.09)	$3.5 \times 10^{-6}$	$3.5 \times 10^{-6}$	-0.16 (-0.23, -0.10)	$1.6 \times 10^{-7}$	$1.6 \times 10^{-7}$	-0.13 (-0.20, -0.07)	$3.6 \times 10^{-5}$	

The effect ( $\beta$ ) is given in standard deviation units. N carriers measured refers to the number of carriers with phytoosterol measurements. EA frequency is for the phytoosterol measured dataset, enriched with rare variant carriers. CI, confidence interval; EA, effect allele.



**Figure 3** The relationship between variant effects on non-high-density lipoprotein cholesterol and stigmasterol. The crosses show 95% confidence intervals. SD, standard deviation units. The red (for *ABCG5/8* variants) and black (for variants outside *ABCG5/8* locus) lines are the best lines fitting the stigmasterol effects for non-high-density lipoprotein cholesterol variants using weighted regression with one over standard error squared as weights. The grey-shaded area around the line is the 95% confidence interval. *NPC1L1* variants are plotted in blue. See data in [Supplementary material online, Table S7C](#) and [G](#).

**Table 3** Disparate effects of genetic risk scores for non-high density lipoprotein cholesterol on the risk of coronary artery disease

Cases/controls	GRS-other Non-HDL cholesterol variants, outside <i>ABCG5/8</i> and <i>NPC1L1</i> loci			GRS- <i>ABCG5/8</i> Non-HDL cholesterol variants at <i>ABCG5/8</i> locus			GRS- <i>NPC1L1</i> Non-HDL cholesterol variants at <i>NPC1L1</i> locus		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
Iceland 19 074/124 037	1.47	(1.37, 1.59)	$1.3 \times 10^{-23}$	1.96	(1.48, 2.58)	$2.0 \times 10^{-6}$	1.89	(1.18, 3.01)	0.0079
Denmark 33 603/148 707	1.64	(1.54, 1.75)	$7.3 \times 10^{-55}$	2.30	(1.63, 3.26)	$2.5 \times 10^{-6}$	2.94	(1.73, 5.00)	$7.2 \times 10^{-5}$
UK Biobank 32 867/375 698	1.51	(1.45, 1.58)	$3.3 \times 10^{-81}$	1.96	(1.63, 2.35)	$4.9 \times 10^{-13}$	1.64	(1.13, 2.37)	0.0087
Combined 85 544/648 442	1.54	(1.49, 1.59)	$1.1 \times 10^{-154}$	2.01	(1.75, 2.31)	$9.8 \times 10^{-23}$	1.95	(1.51, 2.52)	$2.6 \times 10^{-7}$
GRS- <i>ABCG5/8</i> vs. GRS-other							$P_{het}$ (for difference in effects on CAD)		
GRS- <i>NPC1L1</i> vs. GRS-other							$2.4 \times 10^{-4}$		
							0.067		

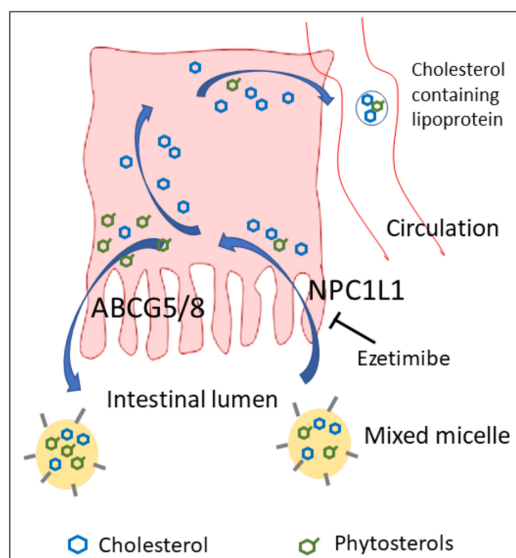
The effects on CAD are given per 1 mmol/L of genetically elevated non-HDL cholesterol levels. CAD, coronary artery disease; CI, confidence interval; GRS, genetic risk score; HDL, high-density lipoprotein; OR, odds ratio.  $P_{het}$ : P-value for heterogeneity in effects.

### Association with coronary artery disease is not fully explained by non-high density lipoprotein cholesterol

We then explored whether *ABCG5/8* impacts the risk of CAD beyond what is expected by their effect on non-HDL cholesterol. We constructed 2 GRS for non-HDL cholesterol, one using *ABCG5/8* variants (GRS-*ABCG5/8*) and another using reported variants outside the *ABCG5/8* locus (GRS-*other*) ([Supplementary material online, Methods](#),

[Table S7 and S8](#)) and compared their effects on CAD in 85 544 cases and 648 442 controls/non-CAD cases from Iceland, Denmark, and UK Biobank ([Table 3](#)). *NPC1L1* variants were not included in these GRSs. We scaled the units of the GRSs to mmol/L of non-HDL cholesterol and the odds ratios (OR) for CAD are calculated per 1 mmol/L of the genetically predicted increase in non-HDL cholesterol. The *ABCG5/8* GRS associates with double the risk of CAD for a 1 mmol increase in non-HDL cholesterol (OR 2.01, 95% CI 1.75–2.31;  $P = 9.8 \times 10^{-23}$ , [Table 3](#)) compared with a 54% increase in CAD

## Genetic variability in intestinal cholesterol and phytosterol absorption affects cardiovascular disease risk



Intestinal absorption of dietary cholesterol and phytosterols is regulated by NPC1L1 and ABCG5/8 transporters

Genetic variants that decrease function of the ABCG5/8 transporter increase uptake of both cholesterol and phytosterols into the circulation and increase the risk of CAD

The risk of CAD conferred by ABCG5/8 variants is significantly greater than expected by their effect on non-HDL cholesterol

- 62% of the effect of on CAD can be explained by non-HDL cholesterol
- 38% has to be explained by other mechanisms - phytosterols are plausible atherogenic candidates

**Take home figure** Genetic analysis using ABCG5/8 variants as instruments indicates that both dietary cholesterol and phytosterols contribute directly to atherogenesis.

risk for GRS-*other* (OR = 1.54, 95% CI 1.49–1.59;  $P = 1.1 \times 10^{-154}$ ,  $P$  for difference in effects =  $2.4 \times 10^{-4}$ ). This greater effect of the GRS-ABCG5/8 on CAD indicates that there are atherogenic effects of ABCG5/8 variants that are not mediated through non-HDL cholesterol.

For comparison, we examined the association of a GRS based on four NPC1L1 variants with CAD. Although the results for GRS-NPC1L1 were similar to GRS-ABCG5/8, there were fewer variants behind this risk score than for the GRS-ABCG5/8 resulting in less accurate CAD risk estimate. The CAD risk conferred by NPC1L1 variants was not significantly different from that expected by non-HDL cholesterol variants at other loci ( $P = 0.067$ ) (Table 3).

### Association with gallstones and haematologic traits

Since the ABCG5/8 transporter is known to affect biliary cholesterol secretion and gallstone formation, we tested the 35 rare coding and 3 common variants for association with gallstone risk in a meta-analysis including data from Iceland and the UK Biobank (27 441 cases and 738 791 controls). We identified associations between eight rare coding variants and gallstones ( $P < 1.4 \times 10^{-3} = 0.05/35$ ) and replicated the association of the common variants<sup>9,10</sup> (Table 1 and Supplementary material online, Table S9). We note that among eight rare variants that associate with gallstone risk, six also associate with non-HDL cholesterol, with the non-HDL cholesterol increasing alleles consistently associating with lower risk of gallstones (Table 1). However, we do not observe a clear dose-response relationship

between variant effects on non-HDL cholesterol and on gallstones (Table 1 and Supplementary material online, Table S9).

Because of the reported macrothrombocytopenia and haemolytic anaemia in some phytosterolaemia patients,<sup>3</sup> we tested ABCG5/8 variants for association with platelet traits and haemoglobin (Supplementary material online, Table S10). The three common variants associate with mean platelet volume (rs4299376:  $P = 2.5 \times 10^{-15}$ , p. Asp19His:  $P = 1.7 \times 10^{-4}$ , p. Thr400Lys:  $P = 1.9 \times 10^{-4}$ ) and with haemoglobin levels (rs4299376:  $P = 0.030$ , p. Asp19His:  $P = 3.2 \times 10^{-8}$ , p. Thr400Lys:  $P = 3.3 \times 10^{-4}$ ). Furthermore, the rare variant p. His250Tyr that has the largest effect on phytosterol levels, associates with greater mean platelet volume ( $P = 5.7 \times 10^{-3}$ ). The directions of the effects on platelet size and haemoglobin levels are consistent with those reported in phytosterolaemia (Supplementary material online, Table S10).

### Discussion

We identified several rare ABCG5/8 coding variants with substantial impact on circulating levels of non-HDL cholesterol and phytosterols, and demonstrate that heterozygous carriers are at increased risk of CAD and other CVD (Take home figure).

The role of dietary cholesterol absorption in the regulation of circulating cholesterol and subsequent CVD is debated.<sup>15</sup> We show that for variants at the ABCG5/8 locus, the effect on non-HDL cholesterol is highly correlated ( $R^2 = 0.97$ ) with the effect on phytosterols that are only derived from the diet. This is consistent with the

common mechanism of intestinal absorption of cholesterol and phytosterols, regulated by NPC1L1 and ABCG5/8 sterol transporters. Indeed, phytosterol levels are frequently used as surrogate markers of intestinal cholesterol absorption.<sup>26</sup> Thus, the results indicate that increased intestinal absorption has a major contribution to the levels of cholesterol, although cholesterol removal through the liver may also play a role. However, less consistent relationship between variant effects on non-HDL cholesterol and on the formation of gallstones, a marker of cholesterol efflux to bile,<sup>27</sup> suggests a smaller role for this mechanism. Furthermore, in carriers of the ABCG5/8 variants that associate with increased non-HDL cholesterol less cholesterol from the enterohepatic circulation is expected to be within the gut than in non-carriers since these variants associate with less secretion of cholesterol to bile. This suggests that the ABCG5/8 variants affect cholesterol levels in blood, mainly through regulation of dietary cholesterol absorption. Our findings thus provide mechanistic insights into how dietary cholesterol may affect CVD. A cautious view towards dietary cholesterol is also proposed by a recent large observational study, finding that higher consumption of dietary cholesterol associates with incident CVD and all-cause mortality in a dose-dependent manner.<sup>28</sup> In line with what other studies have suggested (reviewed in Ref.<sup>29</sup>), our results support the opinion that 'high cholesterol absorbers' might benefit in particular from moderation in cholesterol intake and ezetimibe treatment.

The role of phytosterols in atherosclerotic disease is a matter of an ongoing dispute.<sup>18–20</sup> We demonstrate that the degree of CAD risk conferred by ABCG5/8 variants is greater than predicted by their effect on non-HDL cholesterol levels. Based on the effect of non-HDL cholesterol variants in other genes than ABCG5/8 and NPC1L1 as reflected in GRS-other, non-HDL cholesterol can only explain around 62% of the CAD risk inferred from effect of variants in GRS-ABCG5/8 on CAD, the remaining 38% must be due to other mechanisms. The excess risk is unlikely driven through other traditional risk factors for CAD since the ABCG5/8 variants do not associate with them. In contrast, the rare and common ABCG5/8 variants have a consistent close relationship with phytosterol levels, making elevated phytosterol levels a plausible explanation for the excess CAD risk. The chemical relatedness to cholesterol also makes phytosterols credible atherogenic candidates. Evidence from humans with phytosterolaemia, from animal studies, and *in vitro* experiments further support atherogenic effect of phytosterols.<sup>3,30,31</sup>

While our results indicate that genetic susceptibility to high absorption of cholesterol and phytosterols increases the risk of CAD, the total and relative amount of these dietary components in the gut may also play a role in the net absorption. Thus, high intakes may increase absorption because of more availability. However, phytosterols in the diet may also reduce intraluminal availability of cholesterol, through physicochemical interference.<sup>21</sup>

While our findings raise concerns about the safety of phytosterol-supplemented food, given their propensity to raise phytosterol levels in blood,<sup>21</sup> harmful effects of phytosterol supplementation cannot be concluded based on our data. Ultimately, it needs to be established in clinical trials whether the non-HDL/LDL cholesterol-lowering effects of phytosterol-supplemented food products truly lower cardiovascular risk, or whether swapping the cholesterol with another atherogenic lipid might override this effect, or possibly increase risk.

The main limitation to our study is that we cannot demonstrate directly the dietary origin of the non-HDL cholesterol in blood. Neither was our study equipped to address the effects of various proportions of cholesterol and phytosterols in diet on the amount absorbed, or on the effect on CVD.

In conclusion, we used genetics to demonstrate a role of dietary cholesterol in the regulation of non-HDL cholesterol levels and the risk of CVD. Furthermore, other dietary sterols such as phytosterols may contribute directly to atherogenesis.

## Supplementary material

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

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**Conflict of interest:** The authors affiliated with deCODE genetics/Amgen, Inc. are employed by the company.

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## CARDIOVASCULAR FLASHLIGHT

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### Choriocarcinoma metastasis in the left atrium

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A 36-year-old woman was transferred to our hospital with dizziness and headache for 10 days and had left-side hemiparesis for 1 day. Head computed tomography suggested multiple intracerebral haemorrhages. Digital subtraction angiography showed that the right internal carotid artery was occluded (Panel A, Video S1). During angiography, a mass in the right lung was observed by accident (Panel B). Chest-enhanced computed tomography revealed a solid 35-mm × 32-mm mass in the right lung with intermediate bronchus stenosis (Panel C). Additionally, the left atrium and pulmonary vein showed tumour invasion (Panels C and D). The patient had no respiratory system symptoms. However, she complained of a 6-month history of irregular vaginal bleeding after full-term delivery of a baby. Blood tests revealed a  $\beta$ -human chorionic gonadotropin (HCG) concentration of  $3.5 \times 10^5$  mIU/mL but vaginal ultrasound suggested no abnormalities.

On the basis of the patient's history of irregular vaginal bleeding after delivery, level of  $\beta$ -HCG, right lung mass and multiple intracerebral haemorrhages, the diagnosis of choriocarcinoma with lung and brain metastasis was made. The diagnosis was confirmed by pathological assessment of the chest tumour biopsy (Panel E). Immunohistochemical analysis showed positivity for HCG (Panel F). This diagnosis indicated that the cause of the right internal carotid artery occlusion was tumour embolism.

Choriocarcinoma is a rare and aggressive gynaecological cancer. Early diagnosis and chemotherapy lead to a high long-term survival rate. The main treatment of cases presenting with a cardiac intracavitary mass is surgery to prevent organ embolization.

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

