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

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Received 21 November 2019; revised 19 February 2020; editorial decision 4 June 2020; accepted 8 June 2020

See page 2629 for the editorial comment on this article (doi: 10.1093/eurheartj/ehaa445)

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
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 × 10⁻²³] compared with a 54% increase in CAD risk [OR = 1.54, 95% CI 1.49–1.59, P = 1.1 × 10⁻¹⁵] 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 × 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. 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, 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 (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. Although autosomal recessive phytosterolaemia frequently involves hypercholesterolaemia, sometimes to the extreme, this is not always the case and significant premature atherosclerosis has been documented in the absence of substantial hypercholesterolaemia.

Common variants at the ABCG5/8 locus associate with low-density lipoprotein (LDL) cholesterol, phytosterol levels, and the risk of coronary artery disease (CAD). Alleles that associate with decreased levels of LDL cholesterol also associate with increased risk of gallstones, likely mediated through an effect on cholesterol saturation in bile. Furthermore, NPC1L1 variants associate with LDL cholesterol and CAD, and rare NPC1L1 inactivating variants associate with reduced levels of LDL cholesterol and phytosterols.

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, the contribution of dietary cholesterol to cardiovascular diseases (CVDs) and mortality has been debated for decades. Over the last few years, the importance of dietary cholesterol has been deemphasized in dietary recommendations in many countries. The role of phytosterols in atherosclerotic disease is also a matter of controversy. The ESC/EAS Guidelines for the management of dyslipidaemias recommend food enriched with phytosterols as a lifestyle intervention to reduce cholesterol levels by interfering with intestinal cholesterol absorption.

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. 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 in up to 610,532 individuals, phytosterol levels in 3039 individuals, and the risk of CAD in 105,490
cases and 844,025 controls. Variant associations were also assessed in public data from the Global Lipids Genetics Consortium (N up to 333,359) and CARDioGRAM Exome (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 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) >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 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) 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 × 10⁻⁴) (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 R² < 3.0 × 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 R² = 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 (R² < 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 three 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 (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 frequency (%) | Non-HDL cholesterol (N up to 943,891) \(^a\) | CAD (N up to 147,825 cases/922,265 controls) \(^b\) | Gallstones (N up to 27,441 cases/738,791 controls) \(^c\) |
|------------|---------------|--------|------------------|-----------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| 5          | p.Phe624Leu   | rs150401285 | EA/non-EA | Iceland/Denmark/UK Biobank/GLGC | B | 95% CI | P | OR | 95% CI | P | OR | 95% CI | P |
| 5          | p.Met622Val   | rs140374206 | C/T      | 0.031/0.138/0.098/0.065 | 0.19 (0.14, 0.25) | 1.9 × 10^{-11} | 1.12 (0.96, 1.31) | 0.16 | 0.37 (0.24, 0.57) | 6.3 × 10^{-6} |
| 5          | p.Arg198Gln   | rs141828689 | T/C      | 0.091/NA/NA/NA | 0.58 (0.42, 0.75) | 6.0 × 10^{-12} | 1.96 (1.35, 2.83) | 3.9 × 10^{-4} | 0.80 (0.40, 1.61) | 0.52 |
| 5          | p.Arg263Gln   | rs141828689 | T/C      | 0.015/0.157/0.132/0.138 | 0.16 (0.11, 0.21) | 2.8 × 10^{-11} | 1.29 (1.11, 1.49) | 6.2 × 10^{-4} | 0.42 (0.31, 0.59) | 3.3 × 10^{-7} |
| 5          | p.Phe125Leu   | rs150401285 | G/A      | 0.002/0.077/0.080/0.077 | 0.41 (0.31, 0.52) | 6.0 × 10^{-13} | 2.48 (1.70, 3.67) | 3.7 × 10^{-3} | 0.46 (0.31, 0.63) | 3.2 × 10^{-7} |
| 5          | p.Met622Val   | rs140374206 | C/T      | 0.002/0.104/0.104/0.104 | 0.05 (0.02, 0.07) | 8.2 × 10^{-5} | 1.02 (0.96, 1.09) | 0.5 | 0.70 (0.61, 0.82) | 6.1 × 10^{-4} |
| 5          | p.Arg198Gln   | rs141828689 | T/C      | 0.002/0.115/0.115/0.115 | 0.05 (0.02, 0.07) | 8.2 × 10^{-5} | 1.02 (0.96, 1.09) | 0.5 | 0.70 (0.61, 0.82) | 6.1 × 10^{-4} |
| 5          | p.Arg263Gln   | rs141828689 | T/C      | 0.002/0.115/0.115/0.115 | 0.05 (0.02, 0.07) | 8.2 × 10^{-5} | 1.02 (0.96, 1.09) | 0.5 | 0.70 (0.61, 0.82) | 6.1 × 10^{-4} |
| 5          | p.Arg198Gln   | rs141828689 | T/C      | 0.002/0.115/0.115/0.115 | 0.05 (0.02, 0.07) | 8.2 × 10^{-5} | 1.02 (0.96, 1.09) | 0.5 | 0.70 (0.61, 0.82) | 6.1 × 10^{-4} |
| 5          | p.Phe125Leu   | rs150401285 | G/A      | 0.002/0.077/0.080/0.077 | 0.41 (0.31, 0.52) | 6.0 × 10^{-13} | 2.48 (1.70, 3.67) | 3.7 × 10^{-3} | 0.46 (0.31, 0.63) | 3.2 × 10^{-7} |
| 5          | p.Arg198Gln   | rs141828689 | T/C      | 0.002/0.115/0.115/0.115 | 0.05 (0.02, 0.07) | 8.2 × 10^{-5} | 1.02 (0.96, 1.09) | 0.5 | 0.70 (0.61, 0.82) | 6.1 × 10^{-4} |
| 5          | p.Arg198Gln   | rs141828689 | T/C      | 0.002/0.115/0.115/0.115 | 0.05 (0.02, 0.07) | 8.2 × 10^{-5} | 1.02 (0.96, 1.09) | 0.5 | 0.70 (0.61, 0.82) | 6.1 × 10^{-4} |
| 5          | p.Arg198Gln   | rs141828689 | T/C      | 0.002/0.115/0.115/0.115 | 0.05 (0.02, 0.07) | 8.2 × 10^{-5} | 1.02 (0.96, 1.09) | 0.5 | 0.70 (0.61, 0.82) | 6.1 × 10^{-4} |
| 5          | p.Arg198Gln   | rs141828689 | T/C      | 0.002/0.115/0.115/0.115 | 0.05 (0.02, 0.07) | 8.2 × 10^{-5} | 1.02 (0.96, 1.09) | 0.5 | 0.70 (0.61, 0.82) | 6.1 × 10^{-4} |

The effect \(^b\) on non-HDL cholesterol is given in standard deviation units. \(\text{p.Arg263Gln}\) causes phytosterolemia in 2 Icelandic sisters. \(\text{p.Phe125Leu}\) has borderline significant association with non-HDL cholesterol and CAD.

\(\text{CAD, coronary artery disease; CI, confidence interval; EA, effect allele; HDL, high-density lipoprotein; OR, odds ratio.}\\n\)

\(^a\)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).

\(^b\)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).

\(^c\)Combined Iceland (90,24 cases/94,693 controls) and UK Biobank (18,417 cases/94,693 controls).
147,825 cases and 922,265 controls (Table 1 and Supplementary material online, Table S4). Two rare variants associate with CAD \((P < 0.05/35 = 1.4 \times 10^{-5})\), p.His250Tyr \((OR = 1.96, P = 3.9 \times 10^{-5})\), 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 position (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 variants7,8 (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 \(ABC\)G5/8 variants with other atherosclerosis-related phenotypes is shown in Supplementary material online, Table S6. We find several nominally significant associations between rare \(ABC\)G5/8 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 \((OR = 0.996, P = 6.5 \times 10^{-5})\) (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 \(ABC\)G5/8 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 \((β_s = 1.27 SD, P = 2.2 \times 10^{-15})\) (Table 2).

In the Icelandic dataset, we identified seven homozygous or compound heterozygous carriers of rare \(ABC\)G5/8 coding variants. Two homozygous carriers of p.Arg263Gln in \(ABC\)G8 have extremely high phytosterol levels consistent with autosomal recessive phytosterylalaemia (see Supplementary material online, Note). In agreement with the role of the \(ABC\)G5/8 transporter in regulating intestinal absorption of both cholesterol and phytosterols, the \(ABC\)G5/8 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 \(ABC\)G5/8 variants associates with 2.56 SD increase in stigmasterol levels \((P = 1.1 \times 10^{-8})\). Two common \(NPC\)1L1 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 \(ABC\)G5/8 variants (Figure 3). The apparent difference in the effects of \(NPC\)1L1 and \(ABC\)G5/8 variants on phytosterol levels is consistent with the non-selective uptake of sterols into enterocytes mediated by \(NPC\)1L1,1,25 as opposed to the preferential excretion of phytosterols from enterocytes into the intestinal lumen mediated by \(ABC\)G5/8.1 Consistent correlation between effects on non-HDL cholesterol and phytosterol levels is not observed for non-HDL cholesterol associating variants outside the \(ABC\)G5/8 and \(NPC\)1L1 loci \((R^2 = 0.13, P = 0.0012, Figure 3 and Supplementary material online, Table S7).
Table 2: Association of ABCG5/8 variants with phytosterol 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) |
|--------------------------|--------------|-----------|---------------------|-------------------------------------|------------------------|-----------------------|------------------------|
|                          |              |           |                     |                                     | β (95% CI)              | P                     | β (95% CI)              | P                     |
| p.Phe624Leu              | rs150401285  | G/A       | 19                  | 0.31                                | 0.88 (0.37, 1.39)       | 6.9 × 10⁻⁴            | 0.85 (0.33, 1.37)       | 1.2 × 10⁻³             | 0.81 (0.30, 1.32)       | 1.7 × 10⁻³             |
| p.His250Tyr              | rs776502883  | A/G       | 50                  | 0.82                                | 1.27 (0.96, 1.58)       | 2.2 × 10⁻¹⁵           | 1.17 (0.86, 1.49)       | 3.9 × 10⁻¹³           | 1.10 (0.79, 1.41)       | 4.4 × 10⁻¹²           |
| p.Arg198Gln              | rs141828689  | T/C       | 54                  | 0.89                                | 0.20 (-0.09, 0.50)      | 0.18                  | 0.29 (-0.01, 0.59)      | 0.056                 | 0.30 (0.01, 0.59)       | 0.044                 |
| p.Gly27Ala               | rs56204478   | G/C       | 51                  | 0.84                                | 0.49 (0.16, 0.82)       | 3.7 × 10⁻³            | 0.43 (0.09, 0.76)       | 0.012                 | 0.40 (0.08, 0.73)       | 0.016                 |
| p.Asp19His               | rs11887534   | C/G       | 266                 | 4.38                                | -0.30 (-0.42, -0.18)    | 1.1 × 10⁻⁶           | -0.32 (-0.45, -0.20)    | 1.6 × 10⁻⁷           | -0.26 (-0.38, -0.13)    | 3.5 × 10⁻⁵           |
| intronic                 | rs4299376    | G/T       | 1529                | 25.16                               | 0.24 (0.19, 0.30)       | 2.6 × 10⁻¹⁷           | 0.27 (0.22, 0.33)       | 1.6 × 10⁻¹⁸           | 0.23 (0.18, 0.29)       | 3.1 × 10⁻¹⁶           |
| p.Gln271Arg              | rs770309304  | G/A       | 40                  | 0.66                                | 0.79 (0.46, 1.12)       | 2.6 × 10⁻⁶           | 0.90 (0.56, 1.23)       | 1.2 × 10⁻⁷           | 0.73 (0.40, 1.06)       | 1.3 × 10⁻⁵           |
| p.Arg263Gln              | rs137852990  | A/G       | 72                  | 1.22                                | 0.57 (0.33, 0.82)       | 3.6 × 10⁻⁶           | 0.45 (0.21, 0.69)       | 3.0 × 10⁻⁸           | 0.40 (0.15, 0.64)       | 1.3 × 10⁻³           |
| p.Trp361Ter              | rs137852987  | A/G       | 59                  | 0.97                                | 0.19 (-0.09, 0.47)      | 0.18                  | 0.17 (-0.11, 0.45)      | 0.23                  | 0.16 (-0.11, 0.44)      | 0.25                  |
| p.Thr400Lys              | rs1418217    | A/C       | 1049                | 17.26                               | -0.15 (-0.22, -0.09)    | 3.5 × 10⁻⁶           | -0.16 (-0.23, -0.10)    | 1.6 × 10⁻⁷           | -0.13 (-0.20, -0.07)    | 3.6 × 10⁻⁵           |

The effect (β) is given in standard deviation units. N carriers measured refers to the number of carriers with phytosterol measurements. EA frequency is for the phytosterol measured dataset, enriched with rare variant carriers. CI, confidence interval; EA, effect allele.
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 × 10−23, Table 3) compared with a 54% increase in CAD.

**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 ABCG5/8 and NPC1L1 loci | GRS-ABCG5/8 Non-HDL cholesterol variants at ABCG5/8 locus | GRS-NPC1L1 Non-HDL cholesterol variants at NPC1L1 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 × 10−23 | 1.96 (1.48, 2.58) | 2.0 × 10−6 | 1.89 (1.18, 3.01) | 0.0079 |
| Denmark        | 33 603/148 707 | 1.64 (1.54, 1.75) | 7.3 × 10−55 | 2.30 (1.63, 3.26) | 2.5 × 10−6 | 2.94 (1.73, 5.00) | 7.2 × 10−5 |
| UK Biobank     | 32 867/375 698 | 1.51 (1.45, 1.58) | 3.3 × 10−81 | 1.96 (1.63, 2.35) | 4.9 × 10−13 | 1.64 (1.13, 2.37) | 0.0087 |
| Combined       | 85 544/648 442 | 1.54 (1.49, 1.59) | 1.1 × 10−154 | 2.01 (1.73, 2.31) | 9.8 × 10−23 | 1.95 (1.51, 2.52) | 2.6 × 10−7 |

GRS-ABCG5/8 vs. GRS-other

GRS-NPC1L1 vs. GRS-other

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.

Phet: P-value for heterogeneity in effects.

OR 2.01, 95% CI 1.75–2.31; P = 9.8 × 10−23, Table 3) compared with a 54% increase in CAD.
risk for GRS-other (OR = 1.54, 95% CI 1.49–1.59; \( P = 1.1 \times 10^{-154} \), \( P \) for difference in effects = \( 2.4 \times 10^{-5} \)). 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 \(^{9,10} \) (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, 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: \( P = 3.2 \times 10^{-6} \), 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.\(^{15} \) 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.26 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, 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.28 In line with what other studies have suggested (reviewed in Ref.29), 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.18–20 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.3,30,31

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.21

While our findings raise concerns about the safety of phytosterol-supplemented food, given their propensity to raise phytosterol levels in blood,23 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 lipids 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 phytosterol 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 CAD. Furthermore, other dietary sterols such as phytosterols may contribute directly to atherogenesis.

Supplementary material

Supplementary material is available at European Heart Journal online.

Acknowledgements

We thank all the individuals who participated in the study and whose contribution made this work possible as well as our colleagues who contributed to data collection, sample handling, and genotyping. This research has been conducted using the UK Biobank Resource under Application Number 24711. Data on coronary artery disease have been contributed by the Myocardial Infarction Genetics and CARDIoGRAM Exome investigators and have been downloaded from www.CARDIoGRAMPLUSC4D.ORG. Data on blood lipids were contributed by the Global Lipids Genetics Consortium and was downloaded from http://csg.sph.umich.edu/willer/public/lipids2017EastAsian/

Funding

This work was supported by deCODE genetics/Amgen. S.B. is supported by the Novo Nordisk Foundation (NNF14CC0001 and NNF17OC0027594). F.W.A. is supported by University College London Hospital National Institute for Health Research Biomedical Research Centre (BRC288A).

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

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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 hemorrhages. 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 β-human chorionic gonadotropin (HCG) concentration of 3.5 × 10⁵ mIU/mL but vaginal ultrasound suggested no abnormalities.

On the basis of the patient’s history of irregular vaginal bleeding after delivery, level of β-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.