The ABCG5/8 Cholesterol Transporter and Myocardial Infarction Versus Gallstone Disease

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Objectives
The study sought to test the hypothesis that genetic variation in ABCG5/8, the transporter responsible for intestinal and hepatobiliary cholesterol efflux, may simultaneously influence plasma and biliary cholesterol levels, and hence risk of myocardial infarction (MI) and gallstone disease in opposite directions.

Background
High plasma levels of low-density lipoprotein (LDL) cholesterol are a causal risk factor for MI, whereas high levels of biliary cholesterol promote gallstone formation.

Methods
A total of 60,239 subjects from Copenhagen were included, including 5,647 with MI and 3,174 with symptomatic gallstone disease. Subjects were genotyped for 6 common, nonsynonymous and functional variants in ABCG5/8, and a combined weighted genotype score was calculated.

Results
Combined, weighted genotype scores were associated with stepwise decreases in LDL cholesterol of up to 5.9% (0.20 mmol/l) for individuals with a score ≥8.0 (prevalence = 11%) versus <2.0 (prevalence = 9%; p for trend across 5 groups = 2 × 10⁻35). The cumulative incidences of MI and gallstone disease as a function of age and increasing genotype score were decreased and increased (log-rank ps for trend: 6 × 10⁻4 and 9 × 10⁻45), respectively. The multifactorially adjusted odds ratios were 0.83 (95% confidence interval: 0.73 to 0.94) for MI and 2.85 (95% confidence interval: 2.39 to 3.39) for symptomatic gallstone disease for individuals with a genotype score ≥8.0 versus <2.0.

Conclusions
Genetic variation in ABCG5/8, which associates with decreased levels of plasma LDL cholesterol protects against MI, but increases the risk of symptomatic gallstone disease. These results suggest that MI and gallstones, 2 seemingly unrelated diseases, are intrinsically linked via the function of the ABCG5/8 cholesterol transporter. (J Am Coll Cardiol 2014;63:2121–8) © 2014 by the American College of Cardiology Foundation

Cholesterol plays a pivotal role in the pathogenesis of both myocardial infarction (MI) and gallstone disease, 2 exceedingly common diseases. Elevated plasma levels of low-density lipoprotein (LDL) cholesterol are a well-known causal risk factor for MI, whereas elevated levels of biliary cholesterol promote the formation of cholesterol gallstones, the most common form of gallstones in the West (1,2).

The adenosine triphosphate–binding cassette transporter G5/8 (ABCG5/8) effluxes sterols including cholesterol from enterocytes and hepatocytes into the intestine and bile, respectively, and may therefore simultaneously lower plasma cholesterol levels and increase biliary cholesterol excretion (Fig. 1) (3).

This is clinically important, because drugs such as statins, ezetimibe, and fibrates, which all lower plasma cholesterol and hence protect against ischemic heart disease, may have opposite effects on biliary cholesterol levels, depending on whether they up- or down-regulate biliary cholesterol excretion via ABCG5/8. As a consequence, these drugs either protect against (statins) or may increase (ezetimibe and fibrates) the risk of gallstone disease (4,5).
In the present study, we hypothesized that genetic variation in ABCG5/8, the 2 genes that each encode half of the ABCG5/8 cholesterol transporter, may influence risk of MI and gallstone disease in opposite directions.

We genotyped all common nonsynonymous variants (minor allele frequency >5%) in ABCG5/8 (ABCG8 D19H, Y54C, T400K, A632V; ABCG5 Q604E) and a functional intronic variant (ABCG8 IVS3+981) in 2 prospective studies of the Danish general population, CGPS (Copenhagen General Population Study) and CCHS (Copenhagen City Heart Study), and in a case-control study, CIHDS (Copenhagen Ischemic Heart Disease Study), totaling 60,239 participants, including 5,647 with MI and 3,174 with symptomatic gallstone disease. We subsequently tested whether ABCG5 and ABCG8 genotypes, individually or combined, were associated with plasma levels of LDL cholesterol and other lipids and lipoproteins, and with risk of MI and symptomatic gallstone disease.

### Methods

Studies were approved by institutional review boards and Danish ethical committees, and were conducted according to the Declaration of Helsinki. Written informed consent was obtained from participants. All participants were white and of Danish descent, as determined by the National Danish Person Registration System. There was no overlap of individuals between the studies.

**Participants.** We included participants in 2 studies of the Danish general population, the CGPS and CCHS studies, and a case-control study, CIHDS study (6-9). To achieve maximal statistical power, these 3 studies were combined, yielding a total of 60,239 participants, including 5,647 cases and 3,174 with symptomatic gallstone disease. For a detailed description of the cohorts, see the Online Appendix.

**Diagnoses, genotyping, laboratory analyses, and other covariates.** See the Online Appendix for information on diagnoses, genotyping, laboratory analyses, and other covariates.

**Statistical analyses.** Data were analyzed using STATA/SE 12 (StataCorp, College Station, Texas). Chi-square tests evaluated Hardy-Weinberg equilibrium. Mann-Whitney U test or Pearson chi-square test were used in 2-group comparisons. Tests for trend as a function of genotypes, individually or combined, were by Cuzick’s extension of a Wilcoxon rank sum test with genotypes coded 1, 2, 3, and so forth. Kruskal-Wallis analysis of variance or Pearson chi-square test evaluated the association of genotypes with potential confounders.

From the 5 genotypes that were individually associated with reductions in LDL cholesterol levels, we generated combined, weighted genotype scores based on the percentage reductions in LDL cholesterol compared to the reference genotype: ABCG8 D19H, DD = 0, DH = 2.7, HH = 5.8; IVS3+981 T>C, CC = 0, TC = 2.5, TT = 4.5; Y54C, YY = 0, YC = 0.2, CC = 1.1; T400K, TT = 0, TK = 0.9, KK = 2.9; A632V, VV = 0, AV = 0.9, AA = 1.1. For each participant, a weighted genotype score was then calculated by summation of scores across the 5 genetic variants (range 0.0 to 14.3). The weighted genotype scores were divided into 5 groups: <2.0 (reference), 2.0 to 3.9, 4.0 to 5.9, 6.0 to 7.9, and ≥8.0 (Online Table 1). In sensitivity analyses, we grouped the genotype scores by approximate tertiles, quartiles, or quintiles. A genotype score was also generated for variants in the LDLR (W23X, W66G, W556S), APOB (R3500W, rs5742904), and PCSK9 (rs46L, rs11591147) (6), known to associate with plasma levels of LDL cholesterol, but not with levels of plant sterols (10). In analyses stratified on ABCG8 D19H, a weighted genotype score without D19H was constructed (i.e., including IVS3+981, Y54C, T400K, and A632V).

Kaplan-Meier curves and log-rank tests for trend evaluated the cumulative incidences of MI and gallstone disease as a function of age and weighted genotype scores. Logistic regression analyses, adjusted for age and sex, or multifactorially for age, sex, body mass index, hypertension, diabetes mellitus, physical activity, smoking, alcohol consumption, hormone replacement therapy (women only), triglycerides, and high-density lipoprotein cholesterol, or for all of the previous including lipid lowering therapy, and in addition for LDL cholesterol corrected for lipid-lowering therapy (6), estimated odds ratios (ORs) for MI in the CGPS, CCHS, and CIHDS studies combined, or symptomatic gallstone disease in the CGPS and CCHS studies combined. Hazard ratios for incident MI/gallstone disease as a function of prevalent gallstone disease/MI were estimated by Cox regression. In multifactorially adjusted analyses, missing data on covariates (<3%) were imputed based on age and sex. We used instrumental variable analysis (6-8), to compare the effect on risk of MI of a 1 mmol/l decrease in LDL cholesterol due to either variants in ABCG5/8 or variants in LDLR, APOB, and PCSK9 (see the Online Appendix for details). To test whether the associations were independent of ABCG8 D19H, a known gain-of-function variant strongly associated with increased risk of gallstones, analyses were also stratified on D19H genotype (9,11,12).

### Results

**Clinical characteristics.** Baseline characteristics of individuals with MI or symptomatic gallstone disease are shown in Table 1. Conventional risk factors for MI or gallstone disease were equally distributed among genotypes, although use of lipid-lowering therapy tended to be less frequent among carriers of LDL cholesterol decreasing genotypes versus noncarriers (Online Table 2). Genotype frequencies did not deviate from those predicted by the Hardy-Weinberg equilibrium. ABCG5 R50C and ABCG8 D19H were in almost complete linkage disequilibrium in
the CCHS (D’ = 0.98; r² = 0.94), as previously described (11), and therefore only D19H was genotyped in the CGPS and the CIHDS studies (Online Fig. 1). For additional data on the epidemiological associations between gallstone disease and MI, or between LDL cholesterol and gallstones, see the Online Appendix.

Plasma lipid and lipoprotein levels. Nonfasting plasma levels of lipids and lipoproteins as a function of ABCG8/5 genotypes, individually and combined, are shown in Figure 2. For all genotypes except ABCG5 Q604E, there were stepwise decreases in total and LDL cholesterol levels as a function of genotypes from 0.8% (0.04 mmol/l) to 4.1% (0.24 mmol/l) for total cholesterol and 1.1% (0.04 mmol/l) to 5.8% (0.19 mmol/l) for LDL cholesterol, in homozygotes versus noncarriers (p for trend: 0.08 to 1 × 10⁻²⁸). When combining the 5 LDL-associated genotypes into weighted genotype scores, there were stepwise decreases in total and LDL cholesterol of up to 3.8% (0.22 mmol/l) and 5.9% (0.20 mmol/l), respectively, for individuals with a genotype score of >8.0 versus <2.0 (p for trend: 1 × 10⁻³² and 2 × 10⁻³⁵) (Fig. 2). The relationship between genotype score and LDL cholesterol was approximately linear: A 1-U increase in genotype score was associated with on average 0.025 mmol/l lower LDL cholesterol. None of the variants, individually or combined, were associated with plasma levels of high-density lipoprotein cholesterol or triglycerides (Fig. 2).

Risk of myocardial infarction and symptomatic gallstone disease. In total 5,647 individuals had MI and 3,174 individuals had symptomatic gallstone disease. In individuals with a genotype score <4.0 through 4.0 to 7.9 to >8.0, there was a stepwise decrease in the cumulative incidence of MI (Fig. 3, upper panel) (log-rank trend p = 6 × 10⁻⁴), and a corresponding stepwise increase in risk of gallstone disease (Fig. 3, lower panel) (p = 9 × 10⁻⁴⁵). The relationships between genotype score and risk of MI and gallstone disease were approximately linear, with much stronger effects on symptomatic gallstone disease than on MI. The multifactorially adjusted OR for MI was 0.83 (95% confidence interval [CI]: 0.73 to 0.94) for individuals with a genotype score of ≥8.0 versus <2.0 (p for trend across 5 groups = 2 × 10⁻⁴), and the corresponding OR for symptomatic gallstone disease was 2.85 (95% CI: 2.39 to 3.39; p for trend = 1 × 10⁻⁴⁵) (Fig. 4). Further adjustments for lipid-

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**Table 1 Baseline Characteristics of Participants**

<table>
<thead>
<tr>
<th></th>
<th>No Event</th>
<th>Myocardial Infarction</th>
<th>Symptomatic Gallstone Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>51,654</td>
<td>5,647</td>
<td>3,174</td>
</tr>
<tr>
<td>Women, %</td>
<td>28,537 (55)</td>
<td>1,703 (30)*</td>
<td>2,269 (71)*</td>
</tr>
<tr>
<td>Age, years</td>
<td>56 (46-66)</td>
<td>66 (58-74)*</td>
<td>62 (52-70)*</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25 (23-28)</td>
<td>27 (24-30)*</td>
<td>27 (24-30)*</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>29,132 (56)</td>
<td>2,993 (53)</td>
<td>2,029 (64)*</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>1,689 (3)</td>
<td>618 (11)*</td>
<td>184 (6)*</td>
</tr>
<tr>
<td>Physical activity, %</td>
<td>24,028 (47)</td>
<td>937 (35)*</td>
<td>1,109 (35)*</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>13,217 (26)</td>
<td>1,963 (38)*</td>
<td>927 (29)*</td>
</tr>
<tr>
<td>Alcohol consumption, %</td>
<td>9,227 (18)</td>
<td>419 (15)*</td>
<td>383 (12)*</td>
</tr>
<tr>
<td>Hormone replacement therapy, %</td>
<td>3,775 (13)</td>
<td>143 (15)*</td>
<td>427 (19)*</td>
</tr>
<tr>
<td>Lipid-lowering therapy, %</td>
<td>3,485 (7)</td>
<td>2,014 (36)*</td>
<td>318 (10)*</td>
</tr>
</tbody>
</table>

Values are n (%) or median (interquartile range). *p < 0.001 versus individuals with no event.

Information on physical activity, alcohol consumption, and hormone replacement therapy was only available in ~2,700 participants with myocardial infarction. In women only.
lowering therapy alone, or for LDL cholesterol corrected for lipid-lowering therapy, marginally or partially attenuated the associations with MI, but not with gallstone disease (Online Fig. 2). For the individual genotypes, ORs for MI ranged from 0.83 (95% CI: 0.76 to 0.92) for IVS3\(\text{+981 TT}\) to 1.07 (95% CI: 0.91 to 1.25) for T400K KK versus noncarriers, while ORs for gallstone disease ranged from 3.82 (95% CI: 2.66 to 5.49) for D19H HH to 1.11 (95% CI: 0.99 to 1.23) for Y54C CC versus noncarriers (Online Fig. 3).

In sensitivity analyses, we repeated the analyses using other methods of grouping the genotype score (approximate tertiles, quartiles, or quintiles). This did not change the results substantially for risk of MI or gallstone disease (Online Fig. 4).

Genetically reduced LDL cholesterol and risk of myocardial infarction. In instrumental variable analysis, the risk ratios for MI for a similar 1 mmol/l reduction in LDL cholesterol for variants in \(\text{ABCG5/8}\) (which associate with LDL cholesterol and plant sterols) and for variants in \(\text{LDLR, APOB, and PCSK9}\) (which associate with LDL cholesterol, and not with plant sterols) were nearly identical: 0.42 (95% CI: 0.23 to 0.78) and 0.43 (95% CI: 0.25 to 0.70), respectively (\(p\) for comparison = 0.95) (Fig. 5).

**Stratification on \(\text{ABCG8 D19H genotype}\).** As previously shown in this cohort (9), the H-allele of D19H was a strong risk factor for symptomatic gallstone disease, with stepwise increased ORs of up to 3.82 (95% CI: 2.66 to 5.49) for HH versus DD-homozygotes (\(p\) for trend: 7\(\times\)10\(^{-56}\)) (Online Fig. 3). Therefore, to assess whether the other \(\text{ABCG5/8}\) variants were independently associated with LDL cholesterol, and with risk of MI and/or symptomatic gallstones, we tested this on a D19H DD wild-type background (Fig. 6).

Among \(\text{ABCG8 D19H DD-homozygotes (n = 52,769, or 88% of the population), a genotype score constructed from the remaining LDL-lowering variants was associated with stepwise decreases in LDL cholesterol of up to 4.1% (0.14 mmol/l) for individuals with a genotype score of \(\geq 6.0\) versus \(<2.0\) (\(p\) for trend: 5\(\times\)10\(^{-22}\)) (Fig. 6). The corresponding multifactorially adjusted ORs for MI and symptomatic gallstone disease were 0.88 (95% CI: 0.80 to 0.98), and 1.74 (95% CI: 1.48 to 2.05), respectively (\(p\) for trend
across 4 groups = 0.003 and $3 \times 10^{-15}$) (Fig. 6). Results for the individual genotypes were generally consistent with those presented previously for the genotype score (Online Fig. 5). The power to detect associations was naturally low in the smaller subgroup of D19H DH-heterozygotes and HH-homozygotes combined ($n = 7,470$, or 12% of the population) (Online Fig. 6).

Discussion

The main finding of this study is that genetic variation in ABCG5/8 associated with decreased levels of plasma LDL cholesterol protects against MI, but increases the risk of symptomatic gallstone disease. These results suggest that MI and gallstones, 2 seemingly unrelated diseases, are intrinsically linked via the function of the ABCG5/8 cholesterol/sterol transporter.

This is the first study to simultaneously assess plasma lipid levels, risk of MI, and risk of symptomatic gallstone disease as a function of genetic variation in ABCG5/8, and the first study to use combined genotype scores. Previous studies have reported associations for individual genetic variants with plasma lipid levels, risk of MI, or risk of gallstone disease viewed in isolation: first, common genetic variation in ABCG5/8 consistently associates with plasma levels of LDL cholesterol (14). Second, homozygosity for complete loss-of-function variants in ABCG5/8 causes sitosterolemia, a rare disease characterized by elevated levels of plasma plant sterols, moderately elevated total cholesterol, and increased risk of premature MI (15). Third, recent studies have identified modest associations between common variants in ABCG5/8 and risk of ischemic cardiovascular disease (10,16,17). Fourth, it is firmly established that ABCG8 D19H constitutes a risk factor for gallstone disease (12).

The mechanistic interpretation of the data presented here is straightforward. Gain-of-function genetic variation in ABCG5/8 increases intestinal and hepatobiliary sterol including cholesterol efflux, reduces levels of plasma LDL cholesterol and consequently protects against MI, but at the cost of increasing the risk of gallstone disease due to elevated levels of biliary cholesterol; the latter was naturally not measured in individuals in the present large studies. Conversely, genetic variation associated with reduced activity of ABCG5/8 increases the risk of MI due to elevated plasma LDL cholesterol, but protects against gallstones due to reduced biliary cholesterol. This interpretation is in agreement with results from animal models, in which overexpression of ABCG5/8 reduces plasma sterol including cholesterol levels and protects against atherosclerosis, but increases biliary cholesterol levels (18,19), whereas knockout of ABCG5/8 increases plasma cholesterol levels, but decreases biliary cholesterol levels (20). Moreover, we observed that variants in ABCG5/8, which were associated with plasma levels of LDL cholesterol, had a much stronger effect on risk of gallstone disease than on MI. This fits well with the fact that ABCG5/8 effluxes sterols directly into bile (and the intestinal lumen), whereas the effect on plasma LDL is a secondary phenomenon.

The link between plasma and biliary cholesterol is clinically important, because LDL cholesterol lowering drugs such as statins, ezetimibe, and fibrates may have opposite effects on biliary cholesterol levels, depending on whether they up- or down-regulate biliary cholesterol excretion via ABCG5/8. As a consequence, these drugs either protect against (statins) or may increase (ezetimibe and fibrates) the risk of gallstone disease (4,5).
Another clinically relevant question is whether gallstone disease per se associates with a decreased risk of MI. Previous studies have reported inconsistent epidemiological associations between MI and gallstone disease (21,22). In our study, prevalent gallstone disease did not influence the risk of future MI.

**Myocardial infarction.** LDL cholesterol is a well-known causal risk factor for MI and other ischemic cardiovascular endpoints. Concordantly, we observed that genetic variation in ABCG5/8 that associated with a 5.9% reduction in LDL cholesterol was also associated with an odds ratio for MI of 0.83 (i.e., a 17% lower risk).

The common genetic variants in ABCG5/8 that associate with reduced plasma LDL cholesterol are known to also associate with reduced levels of plasma plant sterols (13). It has been suggested that moderately elevated plasma levels of plant sterols could be atherogenic (23). Therefore, an alternative explanation to the reduced risk of MI observed for carriers of LDL cholesterol lowering variants in our study could be that these individuals also had genetically determined lower plasma levels of plant sterols. To elucidate this, we compared the effect of the LDL lowering ABCG5/8-variants to that of LDL-lowering variants in LDLR, APOB, and PCSK9, which do not associate with plant sterols. The risk of MI for a similar reduction in LDL cholesterol for ABCG5/8 variants was virtually identical to that seen for variants in LDLR, APOB, and PCSK9, suggesting that the ABCG5/8-mediated protection against MI observed in our study was mediated by lower levels of LDL cholesterol, and not by lower levels of plant sterols. In agreement with this, 2 recent studies suggested that elevated plant sterol levels, at least within the normal range, are not a causal risk factor for the development of atherosclerosis. First, a large systematic review and meta-analysis did not report an association between serum concentrations of plant sterols and risk of cardiovascular disease (24). Second, heterozygotes for a rare loss-of-function variant in ABCG8 had 36% elevated levels of plasma plant sterols, no change in LDL cholesterol, and slightly decreased carotid intima-media wall thickness (a proxy for atherosclerosis) (25). Taken together, it therefore seems unlikely that reduced plant sterols played a major role in the protection against MI seen in carriers of LDL cholesterol lowering ABCG5/8 variants in our study.

Common genetic variants in ABCG5/8 may be useful to include in genetic scores for predicting risk of MI and other cardiovascular endpoints. For instance, a common intronic ABCG8 variant was recently used together with...
11 other common LDL-related variants to construct a gene score (26). This gene score was strongly associated with increased LDL cholesterol, and consequently also with an increase in risk of familial hypercholesterolemia, demonstrating the potential clinical use of gene scores based on multiple common variants in LDL-related genes, including ABCG5/8 (26).

**Symptomatic gallstone disease.** The H-allele of ABCG8 D19H is a strong risk factor for gallstone disease, comparable in magnitude to well-known risk factors such as female sex and obesity (7,9,12). The H-allele of D19H increases the transport activity of ABCG5/8 approximately 3-fold in vitro, indicating that the variant likely confers a gain of function that increases cholesterol efflux from the liver into the gallbladder (11). Cholesterol supersaturation in the bile is a key event in the formation of cholesterol gallstones (2). Apart from D19H, other genetic variants in ABCG5/8 have not been consistently associated with risk of gallstones. Although 3 smaller studies (<287 gallstone cases) have reported associations for ABCG5 Q604E and ABCG8 T400K with risk of gallstone disease, the large genome-wide association study (n = 2,280 cases) that initially identified ABCG8 D19H did not report other risk variants in ABCG5/8 (27–30). However, re-evaluating the thorough fine-mapping of variants in the ABCG5/8-region performed in this genome-wide association study revealed that ABCG8 IVS+981 and T400K, as well as ABCG5 Q604E, were indeed associated with gallstone disease, but that the associations did not reach the stringent requirements for statistical significance and/or replication (30). We found that ABCG8 IVS+981 and T400K, as well as ABCG5 Q604E, were individually associated with risk of symptomatic gallstone disease independent of D19H genotype. In other words, these data, and the results from the studies mentioned previously (27–30), suggest that D19H is not the only lithogenic variant at the ABCG5/8 locus.

By which mechanisms might the non-D19H variants promote the formation of gallstones? For ABCG8 IVS+981 and T400K, the gallstone risk alleles were also associated with decreased levels of plasma LDL cholesterol. This resembles the association seen for D19H, suggesting that gain-of-function effects similar to the H-allele of D19H might underlie the associations for IVS+981 and T400K. Indeed, the gallstone-associated T-allele of IVS+981 has previously been associated with an increased expression of ABCG8 mRNA (i.e., a gain-of-function effect) (10). ABCG5 Q604E was associated with an increased risk of symptomatic gallstone disease in heterozygotes, but in contrast to the ABCG8 alleles that increased the risk of gallstones in the present study, Q604E did not associate with low LDL cholesterol. The reason for this discrepancy remains to be elucidated.

**Study limitations and strengths.** There are naturally potential limitations as well as strengths to our study. A strength of our genetic study is that we studied only white individuals from an ethnically homogeneous population. Although our results may therefore not necessarily apply to other ethnicities, we are not aware of data to suggest that our results should not be applicable to all humans. We defined symptomatic gallstone disease by International Classification of Diseases-codes for cholelithiasis or cholecystitis diagnosed in hospital. This definition most likely captured symptomatic gallstones verified by ultrasound. Accordingly, approximately 68% of participants with “symptomatic gallstone disease” underwent cholecystectomy in this cohort (9). However, we cannot rule out that a small part of gallstone cases had asymptomatic gallstones diagnosed incidentally. Because diagnoses of MI were extensively validated by reviewing medical records, and because MI is a hard clinical endpoint with well-defined diagnostic criteria, major misclassification of the MI endpoint was very unlikely. We hypothesized that the LDL-lowering ABCG5/8-variants conferred a gain of function. However, this has only been shown experimentally for the H-allele of D19H (11). Plant sterols were not measured in our cohorts. As discussed previously, we provided indirect evidence that plant sterols were not likely to have influenced the risk of MI. However, other cohorts with measurements of both LDL cholesterol and plant sterols may be better suited to directly assess the LDL cholesterol.

### Table: Risk of Myocardial Infarction and Symptomatic Gallstone Disease as a Function of ABCG5/8 Genotype Score on a D19H DD Background

<table>
<thead>
<tr>
<th>ABCG5/8 genotype</th>
<th>D19H Combined score</th>
<th>N total</th>
<th>Mean</th>
<th>Mean (%)</th>
<th>P trend</th>
<th>Risk of myocardial infarction</th>
<th>Risk of symptomatic gallstone disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N events</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>DD</td>
<td>IVS3</td>
<td>-2.0</td>
<td>5,386</td>
<td>0%</td>
<td></td>
<td>543</td>
<td>1 (reference)</td>
</tr>
<tr>
<td>DD</td>
<td>T400K</td>
<td>2.0-3.9</td>
<td>17,184</td>
<td>-1.5%</td>
<td></td>
<td>1,701</td>
<td>0.97 (0.67-1.08)</td>
</tr>
<tr>
<td>DD</td>
<td>Y54C</td>
<td>4.0-5.9</td>
<td>9,897</td>
<td>-2.5%</td>
<td></td>
<td>882</td>
<td>0.89 (0.79-1.00)</td>
</tr>
<tr>
<td>DD</td>
<td>A632V</td>
<td>6.0</td>
<td>20,302</td>
<td>-4.1%</td>
<td></td>
<td>1,854</td>
<td>0.88 (0.80-0.98)</td>
</tr>
</tbody>
</table>

For statistical significance most likely captured symptomatic gallstones diagnosed in hospital. Accordingly, approximately 68% of participants with “symptomatic gallstone disease” underwent cholecystectomy in this cohort (9). However, we cannot rule out that a small part of gallstone cases had asymptomatic gallstones diagnosed incidentally. Because diagnoses of MI were extensively validated by reviewing medical records, and because MI is a hard clinical endpoint with well-defined diagnostic criteria, major misclassification of the MI endpoint was very unlikely. We hypothesized that the LDL-lowering ABCG5/8-variants conferred a gain of function. However, this has only been shown experimentally for the H-allele of D19H (11). Plant sterols were not measured in our cohorts. As discussed previously, we provided indirect evidence that plant sterols were not likely to have influenced the risk of MI. However, other cohorts with measurements of both LDL cholesterol and plant sterols may be better suited to directly assess the LDL cholesterol.

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**Figure 6**

Risk of Myocardial Infarction and Symptomatic Gallstone Disease as a Function of ABCG5/8 Genotype Score on a D19H DD Background

The genotype score was constructed by summation of weighted low-density lipoprotein (LDL) cholesterol lowering alleles of adenosine triphosphate–binding cassette transporter G8 (ABCG8) IVS+981, T400K, Y54C, and A632V. The p values are for trend tests by Cuzick’s extension of a Wilcoxon rank sum test, or for trend tests of odds ratios (ORs). CI = confidence interval.
versus plant sterol effect on risk of MI (10,17). Finally, strengths of our study also include the large sample sizes, the large number of endpoints mainly from individuals in the general population, and no losses to follow-up.

Conclusions

Common genetic variation in ABCG5/8 that associates with decreased levels of plasma LDL cholesterol protects against MI, but increases the risk of symptomatic gallstone disease in the general population.

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REFERENCES


Key Words: ABCG5 • ABCG8 • cholesterol • gallstones • myocardial infarction.

APPENDIX

For expanded Methods, Results, and References sections, as well as supplemental tables and figures, please see the online version of this article.