Pharmacogenetic meta-analysis of genome-wide association studies of LDL cholesterol response to statins.

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Statins effectively lower cholesterol levels in large studies and the observed interindividual response variability may be partially explained by genetic variation. Here we perform a pharmacogenetic meta-analysis of genome-wide association studies (GWAS) in studies addressing the LDL cholesterol response to statins, including up to 18,596 statin-treated subjects. We validate the most promising signals in a further 22,318 statin-treated participants and identify two loci, SORT1/CELSR2/PSRC1, not previously identified in GWAS. Moreover, we confirm the previously described associations with SLCO1B1 and LPA.

Our findings advance the understanding of the pharmacogenetic architecture of statin response.

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The 3-hydroxyethyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, also known as statins, are widely prescribed and are highly effective in the management and prevention of cardiovascular disease. Statin therapy results in a lowering of low-density lipoprotein cholesterol (LDL-C) levels by up to 55% and a 20–30% reduction of cardiovascular events. Despite the clinical efficacy of statins in a wide range of patients, interindividual variability exists with regard to LDL-C-lowering response as well as efficacy in reducing major cardiovascular events. The suggestion that some of this variability may be due, in part, to common pharmacogenetic variation is supported by previous studies that have identified genetic variants associated with differential LDL-C response to statin therapy.

A small number of genome-wide association studies (GWAS) have previously identified loci associated with statin response on a genome-wide level. A GWAS in the JUPITER trial identified three genetic loci, ABCG2 (rs2199936), LPA (rs10455872) and APOE (rs7412), that were associated with percentage LDL-C and off-treatment as the response variable (see Methods). Differences between the natural log-transformed LDL-C levels on- and off-treatment indicate a 5.9% smaller LDL-C lowering per minor allele for carriers of the SNP compared with non-carriers. Associations at both loci have previously been described. A third genome-wide significant association was found with a SNP at RICTOR on chromosome 5 (rs13166647, MAF = 0.230, β = −0.253, s.e. = 0.046, \( P = 4.50 \times 10^{-8} \)), although genotypes for this SNP were only available in two studies within the first stage (\( n = 2,144 \)).

The first-stage meta-analysis identified three loci, including 13 SNPs with \( P < 5 \times 10^{-8} \) from 158 loci for further investigation in three additional studies comprising up to 22,318 statin-treated subjects (see Methods; Supplementary Tables 1 and 5; Supplementary Note 3). This second stage confirmed the genome-wide significant associations between variations within the APOE and LPA loci and LDL-C response, as observed in the first stage (Table 1; Supplementary Fig. 2; Supplementary Table 5). In addition, SNPs at two new loci with \( P \) values between 6.70 × 10^{-7} and 2.26 × 10^{-6} in the first phase were shown to be significantly associated with statin-induced LDL-C lowering after statin treatment in the total combined meta-analysis at a genome-wide level: SORT1/CERS2/PSRC1 (rs646776, \( \beta = −0.013 \), s.e. = 0.002, \( P = 1.05 \times 10^{-9} \) and rs12740374, \( \beta = −0.013 \), s.e. = 0.002, \( P = 1.05 \times 10^{-9} \)).

The six next-ranked SNPs with \( P \) values just below 5 × 10^{-8} in the combined meta-analysis, including the two SNPs at RICTOR (rs13166647 and rs13172966), were selected for additional genotyping in the Scandinavian ASCOT participants (see Methods). None of these six SNPs reached genome-wide significance after this additional genotyping (Supplementary Table 6). Therefore, our overall genome-wide significant findings were the SNPs at APOE, LPA, SORT1/CERS2/PSRC1 and SLCO1B1.

Subfraction analyses. To extend our results for the novel GWAS finding SORT1/CERS2/PSRC1, we performed additional association analyses, using measurements of cholesterol levels in four LDL subfractions (large, medium, small and very small) from two of the trials in GIST, CAP and PRINCE (Table 2; see Methods). The minor allele of SORT1 rs6466776 was associated with greater statin-induced reductions in levels of all LDL subfractions, and there was a nonsignificant trend for larger effect sizes and greater statistical significance for lowering of small and very small LDL (Table 2). In contrast, the APOE SNP associated with greater
LDL-C response to statins (rs445925) showed a small and non-significant association with change in very small LDL (Table 2). For the minor allele of rs2900478 (SLCO1B1), the borderline significant association with smaller magnitude of LDL-C reduction showed a trend for preferential association with larger versus smaller LDL subfractions. The lack of association of rs10455872 (LPA) with changes in LDL subfractions is consistent with evidence discussed below that this locus affects levels of lipoprotein(a) (Lp(a)) and not LDL particles. Using generalized estimating equations, we tested the association of log change in each of the LDL subfractions with interactions of the four SNPs. For very small LDL, the association with the rs646776 minor allele was significantly different from that of the other minor alleles ($P = 0.03$ after adjustment for multiple testing).

**Effects of off-treatment LDL-C.** To demonstrate that our findings for LDL-C response to statin treatment are unlikely to be explained through associations with baseline LDL-C levels, we performed a number of additional analyses (see Methods). First, Supplementary Table 7 shows regression coefficients for baseline- and modelling measurement noise at baseline reduced the effect size estimate for all the markers, suggesting that genetic effects on baseline LDL-C as manifested in the CARD data set. Correcting our effect size estimate further, a positive beta a worse statin response. Betas and $P$ values were generated using linear regression analysis.

**Previous findings.** In Supplementary Table 10, we performed a look-up in our GWAS meta-analysis for SNPs previously described in the literature (NHGRI Catalogue11 of Published GWAS and Candidate gene studies) to be associated with statin response, besides the loci associated at a genome-wide level in the current study. None of these SNPs was associated with statin response in our GWAS after correcting for multiple testing.

**Functional analyses.** Functional characterization of the 246 SNPs selected for the second stage was performed using a range of bioinformatics tools (see Methods). A total of 420 expression quantitative trait loci (eQTL) associations were identified across a wide range of tissues (Supplementary Data 1), which comprised 67 independent gene eQTL associations. Eleven genes, including APOE, SORT1, CELSR2 and PSRC1, showed eQTLs in liver, which considering its primary role in mediating statin-induced LDL reduction may be particularly relevant to statin response. Putative gene eQTLs were combined with genes annotated to variants in linkage disequilibrium (LD) with LDL-C response-associated variants, resulting in a list of 185 candidate gene loci, defined by 2,681 SNPs (Supplementary Data 2 and 3). To identify statin responsive genes among the candidate loci, gene expression data measured in response to statin treatment in a range of cell lines was retrieved from the Connectivity Map resource (see Methods). Five genes (APOE, BRCA1, GRPEL1, ADRB2 and ETV1) showed convincing evidence of statin responsiveness on the basis of greater than twofold differential expression in response to statin treatment. Eight genes showed suggestive evidence (1.5- to 2-fold change; TOMM40, SREBP1, PSRC1, BCL3, BCAM, ANK3, SIVA1 and RANBP9; Supplementary Data 3).

**Genome-Wide Conditional Analysis.** To investigate whether there were multiple SNPs within any gene and multiple loci associated with differential LDL-C lowering to statin therapy, we performed a conditional analysis across the genome using the summary statistics of the combined meta-analysis. The results of

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Table 1 | Genome-wide significant associations in stage 1, stage 2 and combined meta-analysis.

<table>
<thead>
<tr>
<th>Chr</th>
<th>Position</th>
<th>Lead SNP</th>
<th>Gene</th>
<th>Coding allele</th>
<th>Noncoding allele</th>
<th>Phase</th>
<th>N</th>
<th>Frequency-coding allele</th>
<th>Beta*</th>
<th>s.e.</th>
<th>% Extra reduction</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>109620053</td>
<td>rs646776</td>
<td>SOR/T</td>
<td>C</td>
<td>T</td>
<td>Stage 1</td>
<td>16,697</td>
<td>0.230</td>
<td>– 0.015</td>
<td>0.003</td>
<td>1.5</td>
<td>$6.70 \times 10^{-7}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CELSR2/PSRC1</td>
<td></td>
<td></td>
<td>Combine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>160930108</td>
<td>rs10455872</td>
<td>LPA</td>
<td>G</td>
<td>A</td>
<td>Stage 1</td>
<td>12,981</td>
<td>0.069</td>
<td>0.041</td>
<td>0.006</td>
<td>– 4.1</td>
<td>$1.95 \times 10^{-11}$</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Combine</td>
<td></td>
<td></td>
<td>0.013</td>
<td>0.002</td>
<td>1.3</td>
<td>$1.05 \times 10^{-9}$</td>
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<tr>
<td>12</td>
<td>21260064</td>
<td>rs2900478</td>
<td>SLCO1B1</td>
<td>A</td>
<td>T</td>
<td>Stage 1</td>
<td>16,749</td>
<td>0.165</td>
<td>0.016</td>
<td>0.003</td>
<td>– 1.6</td>
<td>$2.26 \times 10^{-6}$</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Combine</td>
<td></td>
<td></td>
<td>0.052</td>
<td>0.004</td>
<td>5.2</td>
<td>$7.41 \times 10^{-4}$</td>
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<tr>
<td>19</td>
<td>50107480</td>
<td>rs445925</td>
<td>APOE</td>
<td>A</td>
<td>G</td>
<td>Stage 1</td>
<td>13,909</td>
<td>0.098</td>
<td>0.043</td>
<td>0.005</td>
<td>4.3</td>
<td>$1.58 \times 10^{-18}$</td>
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<td></td>
<td></td>
<td>Combine</td>
<td></td>
<td></td>
<td>0.016</td>
<td>0.003</td>
<td>1.6</td>
<td>$1.22 \times 10^{-9}$</td>
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<td></td>
<td></td>
<td>Combine</td>
<td></td>
<td></td>
<td>0.016</td>
<td>0.003</td>
<td>3.4</td>
<td>$1.41 \times 10^{-15}$</td>
</tr>
</tbody>
</table>

Chr, chromosome; SNP, single nucleotide polymorphism. *Beta for difference between the natural log-transformed on- and off-treatment low-density lipoprotein cholesterol (LDL-C) levels adjusted for natural log-transformed off-treatment LDL-C-, age-, sex- and study-specific covariates. The beta reflects the fraction of differential LDL-C lowering in carriers versus non-carriers of the SNP; a negative beta indicates a better statin response (stronger LDL-C reduction), a positive beta a worse statin response. Betas and $P$ values were generated using linear regression analysis.

This percentage reflects the % extra LDL-C lowering in carriers versus non-carriers of the SNP.
A conservative network of direct interactions was constructed between query genes (Supplementary Data 4). The network included 24 genes located in the LDL-C-associated loci (Supplementary Fig. 4). Collectively, our functional and pathway analysis confirms a strong biological and functional role in statin response for several strongly associated gene loci, including APOE/TOMM40/PVRL2 and SORT1/CERS2/PSRC2.

**Discussion**

We have performed a meta-analysis of GWAS including more than 40,000 subjects, investigating genetic variants associated with variation in LDL-C lowering on statin treatment independent from associations with baseline LDL-C. We identified four loci at genome-wide significance, including the previously identified APOE and LPA, and the novel GWAS loci SORT1/CERS2/PSRC1 and SLC01B1.

Nine SNPs in the APOE gene region reached genome-wide significance for LDL-C response. The minor allele of the lead SNP rs445925, which is a proxy for the apoE2 protein variant defining SNP rs7412 (ref. 14), was associated with larger LDL-C-lowering response to statins compared with carriers of the major allele. The magnitude and direction of the effect size was similar to previously reported findings for the rs445925 variant in
the GWAS study performed in CARDS and ASCOT8 and of the SNP rs7412 in JUPITER2. Since the apoE e2 protein results in increased hepatic cholesterol synthesis, it may also predispose to stronger inhibition of cholesterol synthesis by statin treatment23,24.

Three independent SNPs at LPA were significantly associated with LDL-C response to statins. The minor G allele of the lead SNP rs10455872 was associated with smaller LDL-C reduction than the major allele. This result was similar to the previous GWAS findings for this SNP in the JUPITER trial and the combined ASCOT and CARDS study2,8. The rs10455872 SNP was strongly associated with the KIV-2 copy number variant in Lp(a), which encodes variability in apo(a) size and is responsible for ~30% of variance in Lp(a) levels8,15. Furthermore, rs10455872 was shown to be strongly associated with plasma Lp(a) levels15. Standard assays of LDL-C, as well as the Friedewald formula, include cholesterol that resides in Lp(a)8,15. Carriers of this LPA variant are characterized by higher Lp(a) levels and a larger proportion of their measured LDL-C resides in Lp(a) particles8,10. Since statin therapy does not reduce the number of Lp(a) particles12, their presence attenuates the measured LDL-C response to statins.

Two SNPs at SORT1/CERS2/PSRC1 (rs646776 and rs12740374) on chromosome 1p were associated with an enhanced statin LDL-C response. A similar association was previously observed in a large candidate gene study in HPS5; however, we demonstrate this finding now first at a genome-wide significance level. The minor allele of rs12740374 has been shown to generate a binding site for the transcription factor C/EBPa18. Transcription results in upregulation of hepatic expression of three genes at this locus, SORT1, CELSR2 and PSRC1 (ref. 18), which we also showed in our eQTL analysis (Supplementary Data 1). Of these, SORT1 is most notable, in that it encodes the multifunctional intracellular trafficking protein sortilin, which has been shown to bind tightly to apoB19. Sortilin-induced lowering of plasma LDL-C results from two mechanisms: reduced secretion of apoB-containing precursors, and, perhaps of greater importance, increased hepatic LDL uptake via binding to sortilin at the cell surface, with subsequent internalization and lysosomal degradation19. Notably, the minor allele of rs646776 is preferentially associated with lower levels of small and very small LDL (Table 2), suggesting that sortilin is of particular importance for regulating levels of these particles18. Smaller LDL subfractions have been shown to be relatively enriched in particles with reduced LDL receptor binding affinity and cellular uptake20, a property that may contribute to their associations with increased risk for cardiovascular disease21. This property may also underlie the diminished efficacy of statins for reduction of these particles (Supplementary Fig. 3)22, since statins act to reduce LDL-C levels to a large extent by increasing LDL receptor expression as a result of upregulation of the transcription factor SREBP2, whereas SORT1 is not regulated by this mechanism. Hence, the greater statin-mediated reduction of LDL-C among carriers of the rs646776 minor allele could be attributed to relative depletion of LDL particles dependent on sortilin for clearance and hence a residually greater proportion of those LDL particles whose uptake is more dependent on the LDL receptor than on sortilin.

Notably, the strong association of rs646776 with statin-induced reductions in small and very small LDL particles contrasts to the weaker associations of changes in these particles with rs445925, likely the result of differing mechanisms underlying the effects of these SNPs on statin response. As noted above, rs445925 is a proxy for the SNP defining the apoE e2 protein variant that is thought to predispose to heightened statin response as a result of greater statin inhibition of cholesterol synthesis and hence upregulation of SREBP and LDL receptor activity.

The SLCO1B1 rs2900478 minor allele was associated with a smaller LDL-C reduction in response to statin treatment. SLCO1B1 encodes the organic anion-transporting polypeptide OATP1B1 and facilitates the hepatic uptake of statins25. SNP rs2900478 is in strong LD (r² = 0.89) with rs4149056, which represents the Val174Ala substitution resulting in complete loss of function. In the HPS trial, which used simvastatin, this candidate gene SNP was associated with a 1% lower LDL-C reduction per allele25. Single-dose studies have shown that the observed area under the curve of plasma level of active simvastatin after a dose of 40 mg was 221% higher in rs4149056 CC homozygotes compared with rs4149056 TT homozygotes, as compared with atorvastatin 20 mg (144% higher for CC versus TT) and rosuvastatin 40 mg (117% higher for CC versus TT)24. This finding results from the slower hepatic uptake of statins caused by the genetic variant, which would also be expected to result in a reduction in the cholesterol-lowering effect25. In a GWAS of the genetic risk factors for simvastatin-induced myopathy, SLCO1B1 showed the strongest association25. Phenotype carriers of the SLCO1B1 variant had a 16.9 times higher risk for myopathy compared with non-carriers. This might have led to a decrease in study medication adherence, and consequently a decreased effect on LDL-C in carriers of this SNP. In addition, previous analysis in the GoDARTS study showed that the effect of the SLCO1B1 gene on statin efficacy was abolished after removal of individuals who showed signs of intolerance26.

GWCA identified three independent loci in the APOE gene region and two loci in the LPA gene region (Supplementary Table 9). GWCA also showed several other loci with P < 5 × 10⁻⁸ that were not GWAS significant on single-SNP analysis (HGD, RNF175, ISCA1-HTR1A, GLIS3-SLC1A1, LOC100128657, NXX2-3-SLC25A28 and PEL12). These findings will require replication in independent, larger data sets. The significant SNPs in the GWCA analysis explained ~5% of the variation in LDL-C response to statin treatment. Whether this 5% is clinically relevant should be investigated by other studies. For example, it would be of interest to investigate whether this differential LDL-C lowering is also associated with differential event reduction by statin treatment.

In the current study, we combined the results of 6 randomized clinical trials and 10 observational studies in the first stage. This approach resulted also in combining several types of statins, since different statins were studied in the trials and within the observational studies (Supplementary Table 2). This, and the variation in statin dosage during follow-up for an individual, is a limitation of the current study, since, for example, the impact of the SLCO1B1 variant on statin pharmacogenetics is known to be highly dependent on statin type and dose24,27. To overcome this limitation, the individual study analyses were adjusted for statin dose. Dividing the actual statin dose given by the statin-specific dose equivalent (Supplementary Table 3) gives the statin-adjusted LDL lowering, this highlights the thoroughness of our analytical approach, in which the analyses were correctly adjusted for the type and dose of statins used (Supplementary Table 3). Moreover, a comparison of the estimates of the SNPs between the RCTs (where there are no intra-individual differences in dosages) with the estimates of the SNPs in the observational studies showed large homogeneity between the estimates in the various study designs (Supplementary Fig. 2), indicating that our adjustment for dosage seems to be sufficient within this study.
Another possible limitation of the current study is the influence of the identified genetic variants on baseline LDL-C levels. In pharmacogenetic studies investigating the LDL-C-lowering response to statins, it is important to eliminate the effect of association between the genetic variant and baseline LDL-C levels, since those findings may confound the response to treatment associations. Previous large GWAS studies have shown strong associations between baseline LDL-C levels and genetic variants in SORT1/CELSR2/PSRC1, APOE and LPA18. To eliminate those possible confounding effects, our response to treatment analyses were adjusted for baseline LDL-C levels. In addition, additional analysis in CARDS and JUPITER suggests no or little influence of genetic associations with baseline LDL-C on the genetic effects on LDL-C-lowering response.

In conclusion, this study is the largest meta-analysis of GWAS for LDL-C response to statin therapy conducted to date. Our results demonstrate that apart from the previously identified for LDL-C response conducted to date. Our analyses were adjusted for baseline LDL-C levels. In addition, additional analysis in CARDS and JUPITER suggests no or little influence of genetic associations with baseline LDL-C on the genetic effects on LDL-C-lowering response.

Quality control and meta-analysis. Centrally, within each study, SNPs with MAF <1% or imputation quality <0.3 were excluded from the analysis. QQ-plots were assessed by each study to identify between study heterogeneity (Supplementary Fig 1). The software package METAL was used for performing the meta-analysis (http://www.sph.umich.edu/csg/abecasis/Metal/index.html). A fixed effects, inverse variance weighted approach was used. Using an inverse variance weighted meta-analysis will give smaller weights to studies with large s.e. To correct for possible population stratification, genomic control was performed by adjusting the within-study findings and the meta-analysis results for the genomic inflation factor.

Second stage. SNPs with P values <5 × 10^{-8} in the first-stage meta-analysis were selected for further investigation in a second stage. A maximum of two SNPs per locus were selected, based on statistical significance, except for the APOE locus, for which all genome-wide significant associated SNPs were selected for validation. A total of 14 SNPs, within 5 independent loci, were selected for the second stage, which was performed in the JUPITER trial, HPS study and the Rotterdam Study, all which had GWAS data and response to statin treatment available. For 2 of the 246 SNPs, a proxy was used in the JUPITER trial, and 31 SNPs were not available, nor was a proxy SNP. HPS provided data on 151 directly genotyped SNPs from GWAS and IPLEX experiments, including 48 of the second stage proxy SNPS. Six SNPs with P values 5 × 10^{-8} < P < 5 × 10^{-7} in the combined meta-analysis were selected for additional genotyping in the Scandinavian participants of the ASCOT study. Kaspar assays were designed for four of the SNPs using the KBioscience Primmerpick software, and oligos were provided by Integrated DNA Technologies (http://eu.idtdna.com/site). Full Kaspar methodology is available from LGC SNP genotyping (http://www.lgcgenomics.com/genotyping/kasp-genotyping-reagents/). Two SNPs (rs981844 and rs13166647) were genotyped using Taqman assays supplied by Life Technologies (http://www.lifetechnologies.com/uk/en/home.html) using the standard Taqman protocol. Results of the additional genotyping were combined with results from the first and second stages using a fixed effects, inverse variance weighted meta-analysis and analysed by METAL.

Determination of changes in LDL subfractions. LDL subclasses were analysed as described previously25 using non-denaturing gradient gel electrophoresis of fasting plasma samples taken at baseline and after 6 weeks of simvastatin 40 mg per day (CAP study, n = 579) or 12 weeks of pravastatin 40 mg per day (PRINCE study, n = l284). Aliquots (0.1 ml) of whole plasma were mixed 1:1 with a sampling buffer of 20% sucrose and 0.25% bromophenol blue. Electrophoresis of samples and size calibration standards was performed using 2–14% polyacrylamide gradients at 150 V for 3 h following a 15-min pre-run at 75 V. Gels were stained with 0.07% Sudan black for 1 h and stored in a 0.8% acetic acid, 4% methanol solution until they were scanned by computer-assisted densitometry for determination of areas of LDL IVb (22.0–23.2 nm), LDL IVA (23.3–24.1 nm), LDL IIB (24.2–24.6 nm), LDL IIb (24.7–25.5 nm), LDL IIIb (25.6–26.4 nm), LDL IIIa (26.5–27.1 nm) and LDL I (27.2–28.5 nm). The cholesterol concentrations of the subfractions (mg dL^{-1} plasma) were determined by multiplying percent of the total stained LDL area for each subfraction by the LDL-C for that sample. For genetic association analyses, subfractions were grouped into large LDL (LDL I + IIa), medium LDL (LDL IIB, LDL IIb and small LDL (LDL IIIb, LDL IIIa and LDL I)) as described previously25. A generalized estimating equation method was used to test the association of log change with the interaction of the four SNPs by LDL subfraction.

Effect of off-treatment LDL-C. Effects of genetic variation on treatment response as measured by on-treatment LDL-C levels could be mediated through effects on the off-treatment LDL-C. To evaluate whether genetic on-treatment LDL-C likely reflects residual effect on off-treatment LDL-C, it is necessary to adjust for the off-treatment LDL-C levels and to correct the maximum likelihood estimate of the adjusted effect of genotype on on-treatment value for the noise in off-treatment values (the noise is both random measurement error and intra-individual variation in usual LDL-C). This analysis was only carried out in CAPS. Only in CAPS, baseline measurements were available. From the rules of path analysis, we calculated the direct effect of a genotype on an on-treatment trait value by $\beta = \alpha (1 - p)$, where control for possible associations with off-treatment LDL-C levels, analyses were adjusted for the natural log-transformed off-treatment LDL-C level. An additive genetic model was assumed and tested using a linear regression model. The imputed SNPs, regression analysis was performed on expected allele dosage. Analyses were additionally adjusted for age, sex and study-specific covariates (for example, ancestry principal components or country). Analyses in the observational studies were, if available, additionally adjusted for the statin dose by the natural logarithm of the dose equivalent implied in Supplementary Table 3. This table shows the dose equivalent per statin type; dividing the statin dosage of an individual by the dose equivalent shown in Supplementary Table 3 will give the adjusted statin dosage.
β is the coefficient of regression for on-treatment trait value on genotype adjusted for measured off-treatment value, x is the coefficient of regression of baseline LDL on genotype adjusted for measured baseline cholesterol, and δ is the coefficient of regression of on-treatment trait value on observed off-treatment value. For these calculations, we used ρ = 0.8 as a plausible value for the intraclass correlation based on the within-person correlation in LDL-C values taken over two off-treatment visits in CARD6. The interaction of candidate SNPs with statin versus placebo allocation was assessed in the JUPITER trial, since this study was not involved in the first-stage meta-analysis. Regression models were applied to the combined population of statin- and placebo-treated subjects by including extra terms encoding placebo allocation and the product of placebo allocation with SNP minor allele dose.

GWCA using Genome-Complex Trait Analysis. There may be multiple causal variants in a gene and the total variation that could be explained at a locus may be underestimated if only the most significant SNP in the region is selected. To identify independent SNPs, we ideally can perform a conditional analysis, starting with the top associated SNP, across the whole genome followed by a stepwise procedure of selecting additional SNPs, one by one, according to their conditional P values, such a strategy would allow the discovery of more than two associated SNPs at a locus. To identify independent SNPs across the genome-wide data, we used an approximate conditional and joint analysis approach implemented in Genome-Complex Trait Analysis (GCTA) software (http://www.complex-genetomics.com/software/gcta/). We used summary-level statistics from the first-and second-stage combined meta-analysis and LD corrections between SNPs estimated from CARD6 GWAS data. SNPs on different chromosomes or more than 10 Mb distant are assumed to be in linkage equilibrium. The model selection process in GCTA starts with the most significant SNP in the single-SNP meta-analysis across the whole genome with P value < 5 × 10\(^{-8}\). In the next step, it calculates the P values of all the remaining SNPs conditional on the top SNP that have already been selected in the model. To avoid problems due to colinearity, if the squared multiple correlations between a SNP to be tested and the selected SNPs is larger than a cut-off value, such as 0.9, the conditional P value for that SNP will be set to 1. Select the SNPs with minimum conditional P value that is lower than the cut-off P value. Fit all the selected SNPs jointly in a model and drop the SNPs with the P value that is greater than the cut-off P value. This process is repeated until no SNPs can be added or removed from the model.

Pathway analysis and construction of a statin response network. Genes showing evidence of association (based on direct association or LD (HapMap CEU r² > 0.8)) were reviewed for evidence of involvement in statin response at a pathway level using GeneGo MetaCore (Thomson Reuters (portal.genego.com)). A statin response network was constructed in two stages. First, all genes with a literature-reported involvement in statin response (based on Medical Subject Headings (MeSH)) were identified using GeneGo MetaCore (Supplementary Data 3). Second, reported involvement in statin response (based on Medical Subject Headings data set (best eSNP), and the LD between the best eSNP and GIST-selected eSNPs lower than the cut-off.

eQTL analysis. LDL-C-associated index SNPs (246 SNPs) were used to identify 1,443 LD proxy SNPs displaying complete LD (r² = 1) across four HapMap builds in European ancestry samples (CEU) using the SNP tool (http://www.broadinstitute.org/mpg/snap/). The primary index SNPs and LD proxies were selected against a collected database of expression eSNPs (eSNP) results, including the following: those from lymphocytes, leukocytes, lymphoblastoid cell lines (LCL) derived from asthmatic children, leukocyte samples in different cultured human cancer cell lines (MCF7, ssMCF7, HL60, PC3 and SKMELS). We selected (prostate tumour-derived) PC3 cells as they showed the most responsiveness to statins at a genome-wide level. Four statins were included in our analysis, including pravastatin, atorvastatin, simvastatin and rosuvastatin. PC3 Instance reference files for each statin treatment were extracted (as defined by Lamb et al.\(^{13}\)), that is, a treatment associated to its control pair. Transcripts were considered to show evidence of differential expression with a fold change > 2. A fold change > 1.5 was considered to be suggestive of differential expression only.

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Author contributions


Additional information

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