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SLCO1B1 Variants and Statin-Induced Myopathy — A Genomewide Study

The SEARCH Collaborative Group*

ABSTRACT

BACKGROUND

Lowering low-density lipoprotein cholesterol with statin therapy results in substantial reductions in cardiovascular events, and larger reductions in cholesterol may produce larger benefits. In rare cases, myopathy occurs in association with statin therapy, especially when the statins are administered at higher doses and with certain other medications.

METHODS

We carried out a genomewide association study using approximately 300,000 markers (and additional fine-mapping) in 85 subjects with definite or incipient myopathy and 90 controls, all of whom were taking 80 mg of simvastatin daily as part of a trial involving 12,000 participants. Replication was tested in a trial of 40 mg of simvastatin daily involving 20,000 participants.

RESULTS

The genomewide scan yielded a single strong association of myopathy with the rs4363657 single-nucleotide polymorphism (SNP) located within *SLCO1B1* on chromosome 12 ($P=4\times10^{-9}$). *SLCO1B1* encodes the organic anion–transporting polypeptide OATP1B1, which has been shown to regulate the hepatic uptake of statins. The noncoding rs4363657 SNP was in nearly complete linkage disequilibrium with the nonsynonymous rs4149056 SNP ($r^2=0.97$), which has been linked to statin metabolism. The prevalence of the rs4149056 C allele in the population was 15%. The odds ratio for myopathy was 4.5 (95% confidence interval [CI], 2.6 to 7.7) per copy of the C allele, and 16.9 (95% CI, 4.7 to 61.1) in CC as compared with TT homozygotes. More than 60% of these myopathy cases could be attributed to the C variant. The association of rs4149056 with myopathy was replicated in the trial of 40 mg of simvastatin daily, which also showed an association between rs4149056 and the cholesterol-lowering effects of simvastatin. No SNPs in any other region were clearly associated with myopathy.

CONCLUSIONS

We have identified common variants in *SLCO1B1* that are strongly associated with an increased risk of statin-induced myopathy. Genotyping these variants may help to achieve the benefits of statin therapy more safely and effectively. (Current Controlled Trials number, ISRCTN74348595.)

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*The investigators and institutions participating in the Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH) are listed in the Appendix and in the Supplementary Appendix, available with the full text of this article at www.nejm.org.

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Evidence from large-scale, randomized studies shows that statin therapy reduces the incidence of heart attacks, strokes, and revascularization procedures by about one fifth for each reduction of 40 mg per deciliter (1 mmol per liter) in the low-density lipoprotein (LDL) cholesterol level.[1] In rare cases, statins can cause muscle pain or weakness in association with elevated creatine kinase levels (i.e., myopathy), and occasionally, this leads to muscle breakdown and myoglobin release (i.e., rhabdomyolysis), with a risk of renal failure and death.[2] The mechanisms by which statins cause myopathy remain unknown but appear to be related to statin concentrations in the blood. The incidence of myopathy is typically only about 1 case per 10,000 patients per year with standard doses of statins (e.g., 20 to 40 mg of simvastatin daily), but it increases with higher doses (e.g., 80 mg of simvastatin daily) and with concomitant use of certain drugs (e.g., cyclosporine, which can inhibit statin metabolism).[3,4] Although higher doses of statins may well result in larger reductions in the risk of vascular events,[1,5-9] large, long-term, randomized studies comparing different doses are needed to make a reliable assessment of the balance between efficacy and safety.

The ongoing Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH), a randomized trial involving 12,064 participants with prior myocardial infarction, aims to determine whether a daily dose of 80 mg of simvastatin (Zocor, Merck) safely produces greater benefits than does a daily dose of 20 mg of simvastatin.[10] During an average follow-up of about 6 years among the 6031 participants who were assigned to receive 80 mg of simvastatin, there were 98 definite or incipient cases of myopathy; more than half occurred in the first year, and all of the patients had a full recovery. Interim analyses revealed a strong, previously unrecognized, association of myopathy with 80 mg of simvastatin daily and the concomitant use of amiodarone (relative risk of nearly 10). Consequently, participants who were taking amiodarone were given 20 mg of simvastatin daily (irrespective of their original assignment), and treatment with amiodarone is now contraindicated with higher doses of simvastatin.[11] We hypothesized that similarly strong associations might exist between myopathy with high-dose statin regimens and genetic variants, especially those affecting blood statin levels.

Previous studies have considered the relevance to myopathy of various candidate genes, such as *CYP3A4*, which is involved in the metabolism of certain statins,[3] genes encoding organic anion–transporting polypeptides,[12] some of which are associated with statin elimination, and genes involved in ubiquinone (coenzyme Q_{10}) deficiency.[13] Genetic associations with statin-induced myopathy, myalgia, or intolerance have been reported,[14,15] but none were statistically convincing, owing to the large numbers of candidate genes and single-nucleotide polymorphisms (SNPs) assessed.[13-17] Moreover, the apparent differences in the risk of myopathy in those studies may have been confounded by differences in statin regimens and concomitant use of other drugs.[4] The comparatively large number of cases of myopathy among patients who were taking a high dose of simvastatin in SEARCH and the inclusion of well-matched controls from the same population allowed us to conduct a genomewide association study with good power to detect genetic variants that have plausibly large effects.

METHODS

PARTICIPANTS AND SAMPLES IN SEARCH

Between September 1998 and October 2001, investigators in the SEARCH trial randomly assigned 12,064 participants from the United Kingdom who had had a myocardial infarction to receive either 80 mg or 20 mg of simvastatin daily.[10] Approval was obtained from the ethics committees of the participating institutions, and all participants gave written informed consent. At each follow-up assessment (at 2, 4, 8, and 12 months and then every 6 months), participants were questioned about new, unexplained muscle pain or weakness, and blood was drawn for measurements of creatine kinase and alanine aminotransferase levels at a central laboratory. By September 2006, "definite" myopathy (i.e., muscle symptoms, with creatine kinase levels that were more than 10 times the upper limit of the normal range) had developed in 49 of the 6031 participants who had been assigned to receive 80 mg of simvastatin. An additional 49 participants were considered to have "incipient" myopathy on the basis of their safety blood profile (a creatine kinase level that

was more than both 3 times the upper limit of the normal range and 5 times the baseline level, plus an alanine aminotransferase level that was more than 1.7 times the baseline value without an elevated alanine aminotransferase level alone at any other visit), irrespective of whether there were muscle symptoms. In contrast, only 2 definite and 6 incipient cases were identified among the 6033 participants who had been assigned to receive 20 mg of simvastatin.

The myopathy study was designed, analyzed, and interpreted jointly by members of the writing committee. The data were gathered as part of randomized trials designed and conducted by the Clinical Trial Service Unit, Oxford, United Kingdom, and genotyping was performed by the Centre National de Génotypage, Paris. The members of the writing committee drafted this report on behalf of the SEARCH Collaborative Group (see the Supplementary Appendix, available with the full text of this article at www.nejm.org) and take responsibility for the completeness, accuracy, and integrity of the data.

GENOTYPING AND SEQUENCING IN SEARCH

The genomewide association study was restricted to the 96 participants in whom myopathy (definite in 48 and incipient in 48) developed while they were taking 80 mg of simvastatin daily as part of SEARCH and for whom buffy-coat samples were available. Among the remaining participants who were assigned to receive 80 mg of simvastatin, 96 controls were selected who were matched with the case subjects with respect to sex, age, estimated glomerular filtration rate, and use or nonuse of amiodarone at baseline. Case subjects and controls were not known to be related; one case subject who had classified himself as having non-European ancestry was excluded. Multidimensional scaling of the matrix of genomewide identity-by-state distances was used to identify persons with potentially different ancestry or other outliers[18]; four participants appeared to cluster away from the remaining case subjects and controls (and a sensitivity analysis was performed with these participants excluded). The DNA concentration was measured by means of fluorescence staining (PicoGreen method, Invitrogen), and its quality examined by means of gel electrophoresis and polymerase-chain-reaction (PCR) amplification of two microsatellite markers; we obtained adequate DNA from 85 case subjects and 90 controls (see Table 1 in the Supplementary Appendix). The Sentrix HumanHap300-Duo BeadChip (Illumina), which contains 318,237 SNPs, was used,[19] and 316,184 SNPs (99.4%) passed data-quality checks (after exclusion of 1098 SNPs that were not successfully genotyped in any participant, 813 that were missing in more than 10% of the participants, 139 that were monomorphic in this population, and 3 that deviated from Hardy–Weinberg equilibrium among controls [$P<1.6\times10^{-7}$; $P<0.05$ with the Bonferroni correction]).

After the genomewide analysis, we resequenced exons within *SLCO1B1* in 83 case patients and 89 controls who had adequate DNA available and included an additional 38 genotyped and 141 imputed[20] variants (using HapMap CEU[21] as a reference population) with a nonzero minor-allele frequency in the case–control analysis. The Illumina panel does not cover variation in *CYP3A4*, which is a plausible candidate for statin-induced myopathy,[3] so we also resequenced this gene in 54 case subjects and 62 controls with adequate DNA remaining and included 20 genotyped and 11 imputed variants in the analysis. (Further details are available in Table 2 and the Methods section in the Supplementary Appendix.)

REPLICATION IN THE HEART PROTECTION STUDY

Between July 1994 and May 1997, a total of 20,536 patients in the United Kingdom with preexisting occlusive vascular disease or diabetes were randomly assigned to receive either 40 mg of simvastatin daily or placebo as part of the Heart Protection Study.[22] At each follow-up assessment (at 4, 8, and 12 months and then every 6 months), participants were questioned about any new, unexplained muscle pain or weakness, and blood was drawn for measurements of creatine kinase and alanine aminotransferase levels at a central laboratory. During an average follow-up of 5 years, 24 cases of myopathy (10 definite plus 14 incipient) were identified among the 10,269 participants who were assigned to receive 40 mg of simvastatin (with 23 of the cases identified while the participants were taking the study statin) versus 12 cases (4 definite plus 8 incipient) among the 10,267 who were assigned to receive placebo (with 3 of the cases identified while the participants were taking nonstudy statin). DNA was extracted from 19,856 participants (97%), and the rs4149056 and

rs2306283 SNPs in *SLCO1B1* were successfully genotyped in 16,664 participants who had classified themselves as having European ancestry. Among those participants, we tested the associations of the two SNPs with the risk of myopathy and with the reduction in LDL cholesterol.

STATISTICAL ANALYSIS

Standard 1-degree-of-freedom (df) (trend) and 2-df (genotypic) tests of association with genotype, and case–control odds ratios for myopathy from logistic regressions, were calculated with the use of SAS (SAS Institute, version 8) and PLINK (version 1.00).[23] For genomewide associations, uncorrected P values smaller than 5×10^{-7} were considered to provide strong evidence of an association, whereas those between 5×10^{-5} and 5×10^{-7} were considered to provide only moderate evidence.[24] Haploview was used to estimate linkage disequilibrium and plot association results.[25] Haplotype frequencies and associated risks were estimated with the use of the haplo.stats package[26] in R.[27] Physical positions and alleles are expressed in terms of the forward strand of the reference human genome (National Center for Biotechnology Information [NCBI] build 36) unless otherwise stated. Ensembl version 46, NCBI dbSNP build 127, and published reports[12,28,29] were used to classify SNP locations and coding status. The attributable risk of myopathy with 80 mg of simvastatin daily was estimated by means of a life-table analysis; participants who were taking amiodarone at baseline were excluded because their simvastatin dose was subsequently reduced, owing to a high risk of myopathy. (Further details are available in the Methods section in the Supplementary Appendix.)

RESULTS

CHARACTERISTICS OF THE PARTICIPANTS

The use of amiodarone at baseline was found to be associated with an increased risk of definite or incipient myopathy among participants who were assigned to receive 80 mg of simvastatin daily in SEARCH,[11] with a relative risk of 8.8 (95% confidence interval [CI], 4.2 to 18.4) during the first year of follow-up (Table 1). After detection of this association early in the trial, participants taking amiodarone who had been assigned to receive 80 mg of simvastatin were switched to treatment with 20 mg of simvastatin, which may explain the less extreme relative risk observed subsequently with concomitant amiodarone use. We also observed small increases in the risk of myopathy among older participants and women, as well as among those with evidence of impaired renal function and those who were taking calcium antagonists at baseline.

GENOMEWIDE ASSOCIATION STUDY

The genomewide association study involved 85 participants with suspected myopathy and 90 controls, all of whom were taking 80 mg of simvastatin daily (Table 1 in the Supplementary Appendix). Single-SNP analysis yielded one strong association of myopathy with the noncoding rs4363657 SNP located within intron 11 of *SLCO1B1* on chromosome 12 ($P=4\times10^{-9}$; $P=0.001$ with the Bonferroni correction). No associations between myopathy and SNPs in any other region yielded an uncorrected P value of less than 10^{-5} (Table 2 and Fig. 1). The prevalence of the rs4363657 C allele was 0.13 among the controls. We calculated that the odds ratio for myopathy was 4.3 (95% CI, 2.5 to 7.2) per copy of the C allele and 17.4 (95% CI, 4.8 to 62.9) among CC homozygotes as compared with TT homozygotes. There was little evidence of deviation from Hardy–Weinberg equilibrium. In addition, the results did not seem to be affected by population substructure or other potential sources of systematic deviation: the chi-square value for rs4363657 was well outside the 95% confidence interval for the quantile–quantile plot, whereas values for all of the other genotyped SNPs were within this confidence interval (see the figure and the Methods section in the Supplementary Appendix). The summary statistics for the genomewide screen are in the repository of the National Institutes of Health Genotype and Phenotype database (dbGaP) (accession number, phs000141.v1.p1).

CANDIDATE GENOTYPING AND HAPLOTYPE ANALYSIS

In light of the strong association between myopathy and rs4363657, we identified additional SNPs within *SLCO1B1* and 20 kb of flanking sequence (10 kb proximal and 10 kb distal to the gene) by resequencing or by imputation from genotyped SNPs. Table 2a in the Supplementary Appendix shows associations with myopathy for a total of 56 genotyped and 141 imputed SNPs in this region. Of these, two genotyped (and nine imputed)

Table 1. Relative Risk of Myopathy Associated with Selected Baseline Characteristics among 6031 Participants Assigned to 80 mg of Simvastatin Daily in SEARCH.

Baseline Characteristic	No. of Participants	Definite or Incipient Myopathy in First Year (N = 56)		Definite or Incipient Myopathy in Later Years (N = 42)		Definite or Incipient Myopathy at Any Time (N = 98)	
		No.	Relative Risk (95% CI)	No.	Relative Risk (95% CI)	No.	Relative Risk (95% CI)
Age							
<65 yr	3019	17	1.0	14	1.0	31	1.0
≥65 yr	3012	39	2.3 (1.3–4.1)	28	2.0 (1.1–3.9)	67	2.2 (1.4–3.4)
Sex							
Male	5005	42	1.0	30	1.0	72	1.0
Female	1026	14	1.6 (0.9–3.0)	12	2.0 (1.0–3.9)	26	1.8 (1.1–2.8)
Estimated glomerular filtration rate							
≥60 ml/min/1.73 m²	5209	41	1.0	30	1.0	71	1.0
<60 ml/min/1.73 m²	822	15	2.4 (1.3–4.3)	12	2.6 (1.3–5.1)	27	2.5 (1.6–3.9)
Creatinine							
<85 μmol/liter (1.0 mg/dl)	2731	14	1.0	15	1.0	29	1.0
≥85 μmol/liter (1.0 mg/dl)	3300	42	2.5 (1.4–4.6)	27	1.5 (0.8–2.8)	69	2.0 (1.3–3.1)
Use of amiodarone							
No	5893	47	1.0	39	1.0	86	1.0
Yes	138	9	8.8 (4.2–18.4)	3	3.5 (1.1–11.6)	12	6.4 (3.4–12.1)
Use of calcium antagonists							
No	4459	29	1.0	32	1.0	61	1.0
Yes	1572	27	2.7 (1.6–4.5)	10	0.9 (0.4–1.8)	37	1.7 (1.2–2.6)
Diabetes mellitus							
No	5398	49	1.0	33	1.0	82	1.0
Yes	633	7	1.2 (0.6–2.7)	9	2.3 (1.1–4.9)	16	1.7 (1.0–2.9)

Table 2. Genomic Regions Associated with Myopathy in the Genomewide Association Study.*

Chromosome	SNP	Position	P Value for Trend (1 df)	P Value for Genotypic Test (2 df)	Risk Allele	Other Allele	Risk-Allele Frequency		P Value for Hardy–Weinberg Equilibrium among Controls	Odds Ratio (95% CI) per Risk Allele	Odds Ratio for Heterozygotes	Odds Ratio for Homozygotes	Gene
							Cases	*Controls*					
Strong evidence of association													
12p12	rs4363657	21259989	4.1×10^{-9}	2.5×10^{-8}	C	T	0.46	0.13	1.8×10^{-1}	4.3 (2.5–7.2)	4.4	17.4	*SLCO1B1*
Moderate evidence of association													
1p12	rs2490197	118656353	2.6×10^{-5}	1.4×10^{-4}	A	G	0.39	0.18	7.3×10^{-1}	3.0 (1.7–5.0)	2.7	11.4	
	rs6665507	118661127	2.7×10^{-5}	1.5×10^{-4}	T	C	0.39	0.19	7.3×10^{-1}	3.0 (1.7–5.0)	2.7	11.2	
	rs10494209	118664994	3.4×10^{-5}	1.8×10^{-4}	A	C	0.39	0.19	7.3×10^{-1}	2.9 (1.7–4.9)	2.6	11.2	
	rs6428744	118726966	3.6×10^{-5}	1.1×10^{-4}	T	C	0.48	0.26	4.1×10^{-1}	2.6 (1.6–4.2)	1.9	9.9	
2p11	rs404892	76157421	1.9×10^{-1}	4.0×10^{-5}	T	C	0.54	0.47	6.0×10^{-5}	1.3 (0.9–2.0)	5.4	1.9	
	rs7563058	76196590	1.2×10^{-1}	2.6×10^{-5}	T	C	0.55	0.46	1.1×10^{-4}	1.4 (0.9–2.1)	5.8	2.1	
2q21	rs901225	134444137	1.2×10^{-4}	4.9×10^{-5}	A	G	0.18	0.06	2.3×10^{-1}	4.3 (2.0–9.5)	5.8	0.0	
2q33	rs1975583	200934147	5.2×10^{-5}	3.2×10^{-5}	C	A	0.46	0.25	8.7×10^{-2}	2.5 (1.6–4.1)	4.3	4.4	*DNAPTP6*
	rs295129	200937718	6.7×10^{-5}	1.3×10^{-5}	G	A	0.52	0.31	2.5×10^{-2}	2.4 (1.5–3.8)	5.1	4.6	*DNAPTP6*
6p24	rs1348	10557244	3.9×10^{-5}	1.8×10^{-4}	C	T	0.32	0.13	$1.0\times10^{+0}$	3.0 (1.7–5.1)	2.4	19.4	
7p15	rs17160152	31449058	2.6×10^{-5}	1.5×10^{-4}	T	C	0.25	0.08	$1.0\times10^{+0}$	3.9 (2.0–7.7)	3.6	†	
9p23	rs610789	9578336	1.6×10^{-2}	3.1×10^{-5}	A	G	0.71	0.59	3.1×10^{-2}	1.7 (1.1–2.7)	0.3	1.4	*PTPRD*
	rs598356	9578824	2.6×10^{-2}	2.7×10^{-5}	T	G	0.71	0.59	3.1×10^{-2}	1.6 (1.1–2.6)	0.3	1.3	*PTPRD*
	rs656074	9589879	1.5×10^{-2}	2.3×10^{-5}	T	C	0.70	0.57	3.0×10^{-2}	1.7 (1.1–2.7)	0.3	1.6	*PTPRD*
9p23	rs986974	13805721	4.6×10^{-5}	1.7×10^{-4}	C	T	0.26	0.09	5.7×10^{-1}	3.6 (1.9–6.8)	3.9	6.6	
9q31	rs1516895	107923972	3.7×10^{-5}	1.3×10^{-4}	T	C	0.84	0.65	1.0×10^{-1}	3.2 (1.8–5.5)	1.7	6.1	
10q21	rs10826143	59467137	2.0×10^{-2}	3.6×10^{-5}	C	T	0.55	0.43	5.4×10^{-2}	1.7 (1.1–2.7)	6.3	3.1	
12q24	rs1859836	124791023	1.2×10^{-3}	3.3×10^{-5}	T	C	0.72	0.57	1.2×10^{-3}	2.2 (1.4–3.7)	0.5	2.2	
	rs1558062	124819460	1.1×10^{-3}	2.9×10^{-5}	A	G	0.68	0.51	2.9×10^{-3}	2.2 (1.4–3.5)	0.6	3.1	
15q23	rs6494940	69592172	1.2×10^{-4}	4.5×10^{-5}	A	G	0.88	0.71	1.2×10^{-1}	3.1 (1.7–5.6)	0.5	2.1	*THSD4*
17p12	rs2323178	14242338	2.2×10^{-5}	7.0×10^{-5}	C	T	0.60	0.36	6.5×10^{-1}	2.6 (1.6–4.1)	1.8	7.0	
17q12	rs11870629	28870661	3.9×10^{-5}	2.0×10^{-4}	G	A	0.27	0.10	5.9×10^{-1}	3.5 (1.9–6.6)	3.0	†	*ACCN1*
19q13	rs1618536	50563446	1.7×10^{-5}	5.5×10^{-5}	T	C	0.58	0.35	1.7×10^{-1}	2.7 (1.7–4.4)	2.0	8.2	*ERCC2*
20q11	rs6059106	31188763	4.5×10^{-5}	2.0×10^{-4}	A	G	0.34	0.16	1.1×10^{-1}	3.2 (1.8–5.8)	2.8	†	
20q13	rs1355598	51358916	9.0×10^{-1}	3.3×10^{-5}	A	C	0.30	0.29	4.1×10^{-2}	1.0 (0.6–1.7)	2.5	0.0	*TSHZ2*

* P values lower than 5×10^{-7} were considered to provide strong evidence of an association, whereas those between 5×10^{-5} and 5×10^{-7} were considered to provide moderate evidence of an association.

† Odds ratios for homozygotes were not calculated when there were no rare-allele homozygotes among the controls.

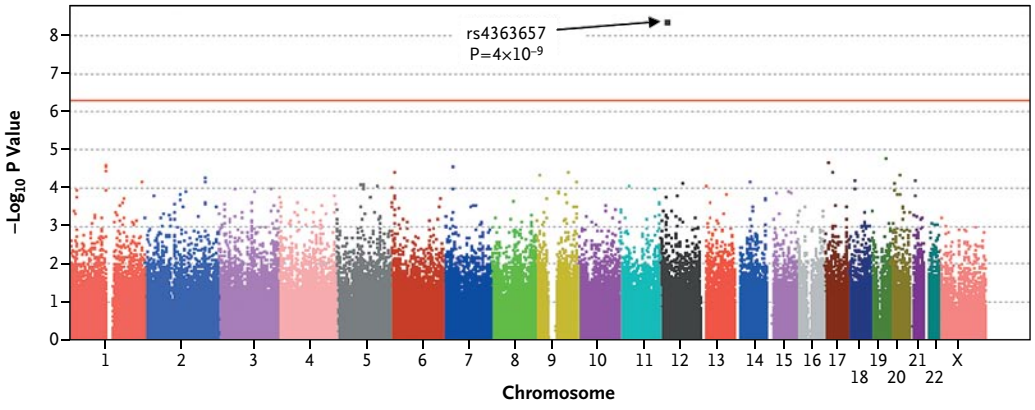


Figure 1. Results of Tests for a Trend in the Association between Myopathy and Each SNP Measured in the Genome-wide Association Study.

P values are shown for each SNP measured among 85 participants with myopathy and 90 matched controls who were taking 80 mg of simvastatin daily. Analyses are based on 316,184 of the 318,237 SNPs (99.4%) on the Sentrix HumanHap300-Duo BeadChip (Illumina). A result above the horizontal red line indicates strong evidence of an association ($P<5\times10^{-7}$).

SNPs were in nearly complete linkage disequilibrium with rs4363657 ($r^2>0.95$ for each). But among them, only rs4149056 (Val174Ala) in exon 6 was "nonsynonymous" (i.e., altering the encoded protein): the prevalence of its C allele was 0.13 among controls, with odds ratios for myopathy of 4.5 (95% CI, 2.6 to 7.7) per copy of the C allele and 16.9 (95% CI, 4.7 to 61.1) among CC homozygotes as compared with TT homozygotes ($P=2\times10^{-9}$, with four missing results imputed).

We identified five other nonsynonymous variants in *SLCO1B1*, including three that were relatively common: rs2306283 (44% frequency of the G allele in controls), rs11045819 (18% frequency of the A allele), and rs34671512 (8% frequency of the C allele) (Table 2a in the Supplementary Appendix). There was only moderate linkage disequilibrium between rs4149056 and these three variants ($r^2<0.20$ for each pairwise comparison). In haplotypes with rs4149056, both the rs2306283 G allele and the rs3471512 C allele were associated with lower risks of myopathy that were of borderline significance ($P=0.03$ and $P=0.06$, respectively), whereas rs11045819 did not appear to influence the risk.

SUBGROUP FINDINGS FOR rs4149056

The odds ratios for myopathy associated with rs4149056 did not differ significantly according to whether the myopathy was definite or incipient or according to baseline age, sex, estimated glomerular function, or use or nonuse of amiodarone, although the study had limited power to detect modest differences (Fig. 2). When we excluded the four participants who were identified as having potentially different ancestry, the P value for rs4149056 changed only slightly, from 2.4×10^{-9} to 2.0×10^{-9} (and there was no alteration in the genomic regions classified as strongly significant).

ATTRIBUTABLE RISK OF MYOPATHY

We selected controls on the basis of the fact that myopathy had not developed in them, and hence, there was a lower probability that they had the rs4149056 C allele, which is associated with an increased risk of myopathy. After allowing for this selection bias, the population prevalence of the C allele was estimated to be 0.15 (which is consistent with the range of 0.14 to 0.22 reported previously among people of European ancestry[27]). On the basis of this prevalence, a life-table analysis was used to estimate the cumulative risk of myopathy among participants taking 80 mg of simvastatin daily, according to their status with respect to the rs4149056 genotype (Fig. 3). CC homozygotes had an 18% cumulative risk, with myopathy occurring primarily during the first year, whereas the CT genotype was associated with a cumulative risk of about 3%. In contrast, the cumulative risk of myopathy was only 0.6% among TT homozygotes who were taking 80 mg of simvastatin. Overall, more than 60% of these myo-

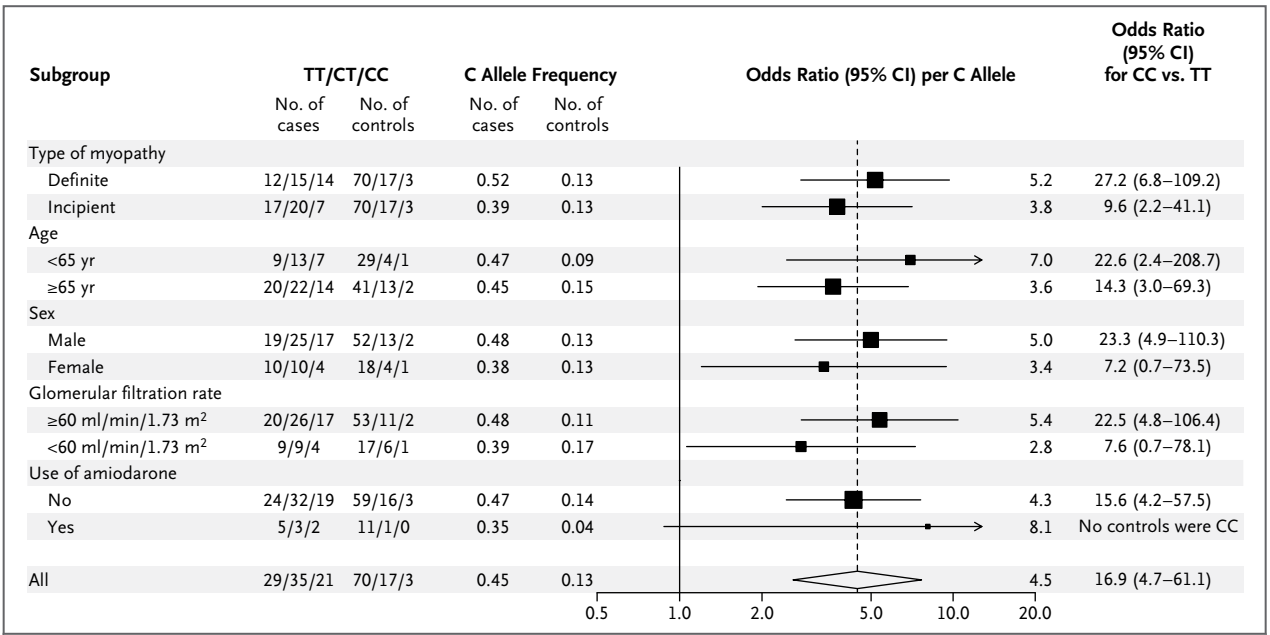


Figure 2. Odds Ratios for Myopathy Associated with the *SLCO1B1* rs4149056 Genotype among Subgroups of Patients Taking 80 mg of Simvastatin Daily.

Black squares indicate odds ratios (with area proportional to the amount of statistical information in each subdivision), and horizontal lines indicate 95% CIs (ending with an arrowhead when the CI extends beyond the scale). The overall odds ratio and its 95% CI are indicated by an unshaded diamond.

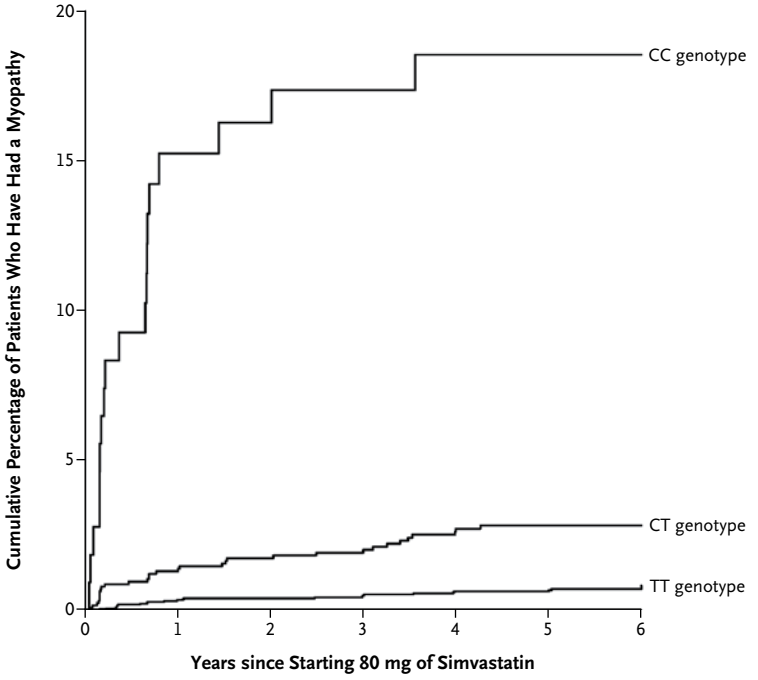
pathy cases could be attributed to the rs4149056 C variant in *SLCO1B1*.

REPLICATION IN THE HEART PROTECTION STUDY

Among 16,664 genotyped participants in the Heart Protection Study,[29] the rs4149056 and rs2306283 variants were not associated with significant differences in pretreatment LDL cholesterol levels (Table 3 in the Supplementary Appendix). Before randomization, all of these participants took 40 mg of simvastatin daily for 4 to 6 weeks, and the mean (±SE) reduction in the LDL cholesterol level was 40.57±0.12%. When both variants were considered together, the reductions were 1.28±0.25% smaller per copy of the rs4149056 C allele (P<0.001), and 0.62±0.18% larger per copy of the rs2306283 G allele (P<0.001). Overall in the Heart Protection Study, there were 23 definite or incipient cases of myopathy among participants who were taking their assigned 40 mg of simvastatin, as compared with 9 cases among participants who were assigned to take a placebo and were not taking nonstudy statin. Consequently, in contrast with SEARCH, only about half of the myopathy cases among participants taking simvastatin in the Heart Protection Study are likely to have been the result of treatment with statins. Even so, the comparison within the Heart Protection Study between the 21 genotyped participants with myopathy who were taking 40 mg of simvastatin and the 16,643 genotyped controls without myopathy confirmed that the rs4149056 SNP is associated with myopathy (P=0.004), albeit with a less extreme relative risk of 2.6 (95% CI, 1.3 to 5.0) per copy of the C allele. This large number of genotyped people without myopathy provides an alternative control population for the SEARCH cases, yielding an odds ratio for myopathy of 4.7 (95% CI, 3.5 to 6.4) per copy of the rs4149056 C allele, with the even smaller P value of 3×10^{-28}.

COMPARISONS WITH PREVIOUS STUDIES

To our knowledge, no previously published study has provided statistically conclusive evidence of associations of genetic variants with statin-induced myopathy (Table 4 in the Supplementary Appendix). In a study of eight candidate genes in 10 subjects with myopathy and 26 controls, an association with *SLCO1B1* SNPs was reported,[17] but those results were not statistically robust after



Genotype	Population Frequency	Cumulative No. and Percentages with Myopathy							
		Year 1				Year 5			
				Attributable to gentoype				Attributable to gentoype	
		no.	%	*no.*	*% of total*	*no.*	%	*no.*	*% of total*
TT	0.730	12	0.34	0	0	21	0.63	0	0
CT	0.249	17	1.38	12.8	75	32	2.83	24.9	78
CC	0.021	16	15.25	15.6	98	19	18.55	18.4	97
All genotypes	1.000	45	0.91	28.4	63	72	1.56	43.3	60

Figure 3. Estimated Cumulative Risk of Myopathy Associated with Taking 80 mg of Simvastatin Daily, According to *SLCO1B1* rs4149056 Genotype.

adjustment for multiple comparisons. In the present study, we observed no significant associations between myopathy and SNPs in any of the other genes previously reported to be associated with myopathy (Table 4 in the Supplementary Appendix) or with statin pharmacokinetics (Table 5 in the Supplementary Appendix). In particular, there was no significant association of myopathy with the 20 genotyped and 11 imputed SNPs in the *CYP3A4* gene, which is involved in simvastatin clearance[30] (Table 2b in the Supplementary Appendix). It has been reported that 10% of people with statin-induced myopathy who were referred for genetic testing had, or were carriers for, one of three inherited metabolic myopathies (McArdle's disease, carnitine palmitoyltransferase II deficiency, or myoadenylate deaminase deficiency),[31] but we observed no significant associations between myopathy and SNPs in those genes (Table 5 in the Supplementary Appendix).

DISCUSSION

We provide compelling evidence that at least one common variant in the *SLCO1B1* gene substantially alters the risk of simvastatin-induced myopathy. Among patients taking 20 to 40 mg of simvastatin daily (or standard doses of other statins), the incidence of myopathy is typically only about 1 per 10,000 patients per year,[4] and the effect of these gene variants on the absolute risk of myopathy is likely to be small (as indicated by our results among participants in the Heart Protection Study). In contrast, the risk of myopathy may be substantially

increased in patients who take 80 mg of simvastatin daily (and some other high-dose statin regimens), as well as in those who are also receiving certain other drugs[2-4] (e.g., cyclosporine and, as we found in SEARCH, amiodarone[11]). Hence, the use of those drugs in subjects who are taking such high doses of statins and who have the C allele of the rs4149056 polymorphism may produce particularly high risks of myopathy (Fig. 2).

SLCO1B1 encodes the organic anion–transporting polypeptide OATP1B1, which mediates the hepatic uptake of various drugs, including most statins and statin acids.[12] Several clinical studies have investigated associations between rs4149056 *SLCO1B1* genotypes and statin pharmacokinetics.[12] Although not all those studies yielded significant results, the collective evidence indicates that statin blood concentrations are higher in people with the C allele (Table 6 in the Supplementary Appendix). Five of those studies also examined haplotypes of rs4149056 and rs2306283 and, in aggregate, suggest that the G variant of rs2306283 is associated with lower statin concentrations (data not shown), which is consistent with the lower risk of myopathy observed in SEARCH. Genetic variants that slow the hepatic uptake of a statin might also be expected to reduce its effect on lowering cholesterol. Our data from the Heart Protection Study[22] confirm that these variants do cause small differences in the amount of reduction in LDL cholesterol produced by simvastatin.

The Illumina HumanHap300-Duo panel is estimated from HapMap CEU samples to provide about 75% genomic coverage at $r^2 \geq 0.8$ for common SNPs in people of European descent (see the Methods section in the Supplementary Appendix). Given the numbers of case subjects and controls, the present genomewide association study had only about 50% power to detect odds ratios of about 4 for common variants at an uncorrected P value of less than 5×10^{-7}. Hence, the existence of other genetic variants that carry a relative risk of myopathy of 2 to 4 cannot be ruled out by this analysis. Genes with prior evidence of links with myopathy might instead be regarded as candidates that require less extreme P values to provide good evidence of an association. Tables 4 and 5 in the Supplementary Appendix list approximately 100 such SNPs, which represent about 1/3000 of the genome screen; therefore, a P value of less than 1.5×10^{-3} (i.e., $3000\times5\times10^{-7}$) might be considered significant for these candidates. No such P values were obtained, however, for any of the SNPs studied in those regions.

In conclusion, this genomewide study has identified common genetic variants in *SLCO1B1* that are associated with substantial alterations in the risk of simvastatin-induced myopathy. These findings are likely to apply to other statins because myopathy is a class effect, and *SLCO1B1* polymorphisms affect the blood levels of several statins. Moreover, these variants may be relevant to the effects of other classes of drugs transported by OATP1B1 (e.g., the oral hypoglycemic agent repaglinide[32]). Consequently, the genotyping of *SLCO1B1* polymorphisms may be useful in the future for tailoring both the statin dose and safety monitoring (especially when statins are used in combination with certain other drugs and during the first year of treatment, when the absolute risk of myopathy is greatest) in order to obtain the benefits of statin therapy more safely and effectively.

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APPENDIX

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