ORIGINAL ARTICLE

Pharmacogenomic Study of Statin-Associated Muscle Symptoms in the ODYSSEY OUTCOMES Trial

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BACKGROUND: Statin-associated muscle symptoms (SAMS) are the most frequently reported adverse events for statin therapies. Previous studies have reported an association between the p.Val174Ala missense variant in *SLCO1B1* and SAMS in simvastatin-treated subjects; however, evidence for genetic predictors of SAMS in atorvastatin- or rosuvastatin-treated subjects is currently lacking.

METHODS: ODYSSEY OUTCOMES (Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab; n=18 924) was a double-blind, randomized, placebo-controlled study evaluating the efficacy and safety of alirocumab (a PCSK9 [proprotein convertase subtilisin/kexin type 9] inhibitor) in acute coronary syndrome patients receiving high-intensity statin therapy. The goal of this pharmacogenomic analysis was to identify genetic variants associated with atorvastatin- and rosuvastatin-mediated SAMS among ODYSSEY OUTCOMES subjects who consented to participate in the genetic study (n=11 880). We performed multi-ancestry exome-wide and genome-wide association studies and gene burden analysis across 2 phenotypes (clinical SAMS [n=10 617] and creatine kinase levels [n=9630]).

RESULTS: A novel genome-wide significant association for an intronic variant (rs6667912) located within *TMEM9* (odds ratio [95% CI], 1.39 [1.24–1.55]; $P=3.71\times10^{-8}$) for patients with clinical SAMS (cases=894, controls=9723) was identified. This variant is located ≈30 kb upstream of *CACNA1S*, a locus associated with severe SAMS. We replicated 2 loci, at *LINC0093* and *LILRB5*, previously associated with creatine kinase levels during statin treatment. No association was observed between p.Val174Ala (rs4149056) in *SLCO1B1* and SAMS (odds ratio [95% CI], 1.03 [0.90–1.18]; $P=0.69$).

CONCLUSIONS: This study comprises the largest discovery exome-wide and genome-wide association study for atorvastatin- or rosuvastatin-mediated SAMS to date. These novel genetic findings may provide biological/mechanistic insight into this drug-induced toxicity, and help identify at-risk patients before selection of lipid-lowering therapies.

Key Words: acute coronary syndrome ■ atorvastatin ■ creatine kinase ■ drug-related side effects and adverse reactions ■ genome-wide association study ■ pharmacogenetics ■ rosuvastatin calcium

The HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase inhibitors (also known as statins) are the most prescribed lipid-modifying medications globally and are the first-line treatment for primary and secondary prevention of atherosclerotic cardiovascular disease.[1] Muscle symptoms (eg, myalgia and myopathy) are among the most common adverse events associated with statin use and are collectively referred to as statin-associated muscle symptoms (SAMS). SAMS comprise a clinical spectrum ranging from benign muscle cramping/weakness to severe, life-threatening rhabdomyolysis.[2,3] This adverse drug reaction can often lead to discontinuation or dosage reduction of statins.[4] Since statins are effective lipid-lowering treatments in high-risk patients, SAMS-dependent statin discontinuation increases the risk of developing major adverse cardiovascular events.[5]

The estimated incidence of SAMS varies depending on study design and clinical definition, but occurs in 5% to

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Nonstandard Abbreviations and Acronyms

CK	creatine kinase
ExWAS	exome-wide association study
GWAS	genome-wide association study
IGFN1	Immunoglobulin like and fibronectin type III domain containing 1)
Klf5	kruppel like factor 5
LD	linkage disequilibrium
HMG-CoA	3-hydroxy-3-methyl-glutaryl-coenzyme A
MAF	minor allele frequency
OATP1B1	Solute carrier organic anion transporter family member 1B1
ODYSSEY OUTCOMES	Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab
OR	odds ratio
PCSK9	proprotein convertase subtilisin/kexin type 9
Q1	first quartile
Q3	third quartile
SAMS	statin-associated muscle symptoms
SOAT1	sterol O-acyltransferase 1
ULN	upper limit of normal

30% of first-time statin users.[3,6] Several clinical characteristics (eg, age, excess body weight, diabetes, fibromyalgia, and hypothyroidism), external factors (eg, concurrent drug therapies), and genetic factors have been implicated as risk factors for SAMS.[3,6,7] A commonly used nonspecific biomarker to assess SAMS severity is the level of serum creatine kinase (CK), an enzyme released from injured muscle cells.[6] CK elevations, however, do not always correlate well with SAMS, as this drug-related toxicity can occur in the absence of CK elevations, and CK elevations can occur due to many conditions other than SAMS.[8] Thus, there are no reliable clinical predictors or biomarkers of SAMS risk for patients in whom statin therapy is indicated.

To date, several genetic variants have been reported to be associated with increased susceptibility to SAMS.[9] However, the only variant consistently associated with this phenotype has been rs4149056 (c.521T>C, p.Val174Ala) in the *SLCO1B1* gene, which encodes a basolateral transporter (Solute carrier organic anion transporter family member 1B1, [OATP1B1]) responsible for the hepatic uptake of several statins.[10,11] This association is strong with simvastatin but has failed to show consistent association with atorvastatin- or rosuvastatin-mediated SAMS.[12–14] While studies have shown clear association of *SLCO1B1* variation with atorvastatin and rosuvastatin pharmacokinetics,[15] these findings do not always correlate with SAMS occurrence,[10] suggesting potential involvement of additional factors and/or pharmacodynamic pathways.[3]

Atorvastatin and rosuvastatin are the most potent and among the most prescribed statins in higher-income countries.[16,17] However, in contrast to simvastatin-mediated SAMS,[11,18] genetic studies investigating atorvastatin- and rosuvastatin-mediated SAMS have been potentially underpowered statistically.[10] The largest atorvastatin-mediated SAMS discovery genome-wide association study (GWAS; n=2529; cases = 28, controls = 2501) to date found no genome-wide significant associations.[19] Candidate gene approaches have been investigated for rosuvastatin-mediated SAMS,[13,20,21] but no published rosuvastatin-mediated SAMS discovery GWAS currently exist. To gain a better understanding of these drug-related adverse effects, larger studies are needed to identify additional genetic predictors of atorvastatin- and rosuvastatin-mediated SAMS.

The ODYSSEY OUTCOMES trial (Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab; n=18924) was a double-blind, randomized, placebo-controlled clinical trial to evaluate the safety and efficacy of the PCSK9 (proprotein convertase subtilisin/kexin type 9) inhibitor alirocumab in patients with acute coronary syndrome and elevated atherogenic lipoproteins, despite optimized statin therapy, in 57 countries across 5 continents.[22] At the time of randomization, 88.8% of trial subjects were taking high-dose atorvastatin (40 or 80 mg) or rosuvastatin (20 or 40 mg). The remainder of participants were treated with a lower-intensity statin regimen or no statin for reasons that included SAMS or elevated CK with prior statin treatment.

The aim of this study was to identify genetic variants associated with atorvastatin- and rosuvastatin-mediated SAMS by using clinical trial data for 11880 ODYSSEY OUTCOMES subjects who consented to genetic studies and who had genome-wide genotyping and exome sequencing data available for analysis (see Table 1 for additional demographic information). Candidate gene and exome-wide association study (ExWAS) and GWAS analyses were performed for a clinical and a biochemical phenotype. This study comprises the largest multi-ancestry discovery ExWAS/GWAS cohort for atorvastatin- or rosuvastatin-mediated SAMS to date.

METHODS

This multicenter study was approved by the appropriate institutional review boards and all trial participants provided written informed consent.[22] Detailed description of methodology may be found in the Supplemental Material, including inclusion and exclusion criteria and selection of phenotypes in Table S1 and Figure S1. Individual clinical and genomic data presented in this study are not publicly available due to informed consent and patient privacy restrictions.

Table 1. Demographics and Baseline Characteristics of ODYSSEY OUTCOMES Subjects and Those Included in Pharmacogenomic Analysis: Distribution Among Phenotypes

Clinical characteristic	ODYSSEY OUTCOMES trial population (n=18924)	ODYSSEY OUTCOMES PGx analysis subgroup (n=11880)	CK maximum value during the treatment period ExWAS/GWAS subgroup (n=9630)	Baseline statin intolerance or investigator-documented SAMS ExWAS/GWAS subgroup (n=10617)	
				Cases (n=894)	Controls (n=9723)
Age, y, median (IQR)*	58.0 (52.0–65.0)	58.0 (52.0–65.0)	58.0 (52.0–64.0)	61.0 (54.0–67.0)	58.0 (52.0–65.0)
Female sex, n (%)*	4762 (25.2)	3021 (25.4)	2333 (24.2)	252 (28.2)†	2391 (24.6)
Body mass index, median (IQR)*	27.9 (25.2–31.1)	28.2 (25.5–31.5)	28.3 (25.5–31.4)	28.4 (25.7–31.2)	28.3 (25.6–31.6)
Self-reported race, n (%)*					
White	15024 (79.4)	10157 (85.5)‡	8320 (86.4)‡	819 (91.6)†	8365 (86.0)
Asian	2498 (13.2)	818 (6.9)‡	549 (5.7)‡	49 (5.5)	546 (5.6)
Black	473 (2.5)	323 (2.7)	256 (2.7)	14 (1.6)†	282 (2.9)
Other/unknown	929 (4.9)	582 (4.9)	505 (5.2)	12 (1.3)†	530 (5.5)
Biomarkers at randomization, median (IQR)					
Creatine kinase, IU/L	102.0 (73–148)§	103.0 (73.0–151.0)‖	104.0 (75.0–151.0)	105.0 (74.0–166.0)¶	103.0 (74.0–150.0)#
HbA$_{1c}$, %	5.8 (5.5–6.3)	5.8 (5.5–6.3)	5.8 (5.5–6.2)	5.8 (5.5–6.1)	5.8 (5.5–6.3)
Medical history before index acute coronary syndrome, n (%)					
Current smoker	4560 (24.1)	2901 (24.4)	2409 (25.0)‡	169 (18.9)†	2469 (25.4)
Diabetes*	5444 (28.8)	3264 (27.5)‡	2500 (26.0)‡	214 (23.9)†	2695 (27.7)
Fibromyalgia	129 (0.7)	80 (0.7)	56 (0.6)	18 (2.0)†	53 (0.5)
Hepatic/hepatobiliary disorder	530 (2.8)	316 (2.7)	233 (2.4)	15 (1.7)	245 (2.5)
Hypertension	12,249 (64.7)	7757 (65.3)	6075 (63.1)‡	585 (65.4)	6308 (64.9)
Hypothyroidism (controlled)*	645 (3.4)	474 (4.0)‡	331 (3.4)	69 (7.7)†	348 (3.6)
Renal impairment	623 (3.3)	436 (3.7)	291 (3.0)	34 (3.8)	355 (3.7)
Statin medication at randomization, n (%)					
Atorvastatin (40/80 mg)	13,428 (71.0)	8821 (74.3)	7965 (82.7)	194 (21.7)	8034 (82.6)
Rosuvastatin (20/40 mg)	3384 (17.9)	1832 (15.4)	1665 (17.3)	43 (4.8)	1689 (17.4)
Other**	2112 (11.2)	1227 (10.3)	0 (0)	657 (73.5)††	0 (0)‡‡
Interacting concomitant medications at randomization, n (% of patients taking victim drug)§§					
Atorvastatin DDI‖‖	1331 (9.9)	886 (10.0)	715 (9.0)	21 (10.8)	792 (9.9)
Rosuvastatin DDI¶¶	164 (4.8)	93 (5.1)	80 (4.8)	4 (9.3)	82 (4.9)

CK indicates creatine kinase; DDI, drug-drug interaction; ExWAS, exome-wide association study; GWAS, genome-wide association study; HbA$_{1c}$, hemoglobin A$_{1c}$; IQR, interquartile range; IU, international units; ODYSSEY OUTCOMES, Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab; PGx, pharmacogenomic; and SAMS, statin-related muscle symptoms.

*Included as a covariate in the ExWAS/GWAS regression model for both phenotypes.

†Statistically significant difference (P<0.05) from control group.

‡Statistically significant difference (P<0.05) from ODYSSEY OUTCOMES trial population.

§A total of 18897 subjects from the ODYSSEY OUTCOMES trial population with CK level drawn at randomization.

‖A total of 11865 subjects from the PGx subgroup with CK level drawn at randomization.

¶A total of 892 subjects from the baseline statin intolerance or investigator-documented SAMS ExWAS/GWAS case subgroup with CK level drawn at randomization.

#A total of 9713 from the baseline statin intolerance or investigator-documented SAMS ExWAS/GWAS control subgroup with CK level drawn at randomization.

**Includes lower doses of atorvastatin/rosuvastatin, other statins, or no statin.

††Subjects with baseline statin intolerance due to muscle symptoms with or without elevated CK during the run-in (n=657) were not taking high-dose atorvastatin or rosuvastatin at randomization. During the run-in, 555 subjects were deemed intolerant to both atorvastatin and rosuvastatin, while 102 subjects had medical history of statin intolerance (specific statin medications unknown) before enrollment.

‡‡Subjects not taking high-dose atorvastatin or rosuvastatin at randomization because of a reason other than statin intolerance due to muscle symptoms with or without elevated CK were not included in the analysis/control group.

§§Data on atorvastatin or rosuvastatin DDIs presented from treatment period only; victim drug = atorvastatin or rosuvastatin.

‖‖Medications that interact with atorvastatin included but were not limited to: azithromycin, cyclosporine, clarithromycin, diltiazem, fenofibrate, fluconazole, gemfibrozil, and ritonavir.

¶¶Medications that interact with rosuvastatin included but were not limited to: cobicistat, cyclosporine, gemfibrozil, and ritonavir.

RESULTS

ExWAS/GWAS Results for Baseline Statin Intolerance or Treatment Period SAMS

This case-control ExWAS/GWAS included trial subjects with either baseline statin intolerance (documented muscle symptoms with or without elevated CK before randomization) or SAMS while taking high-dose atorvastatin or rosuvastatin during the treatment period (cases=894; controls=9723). The results for this SAMS ExWAS/GWAS are shown in the Manhattan plot in Figure 1A, while the top associated locus is visualized in Figure 1B. Table 2 contains a detailed list of genetic associations that reached genome-wide (P<5×10^{-8}) or suggestive (P<1×10^{-6}) significance. Table 3 provides
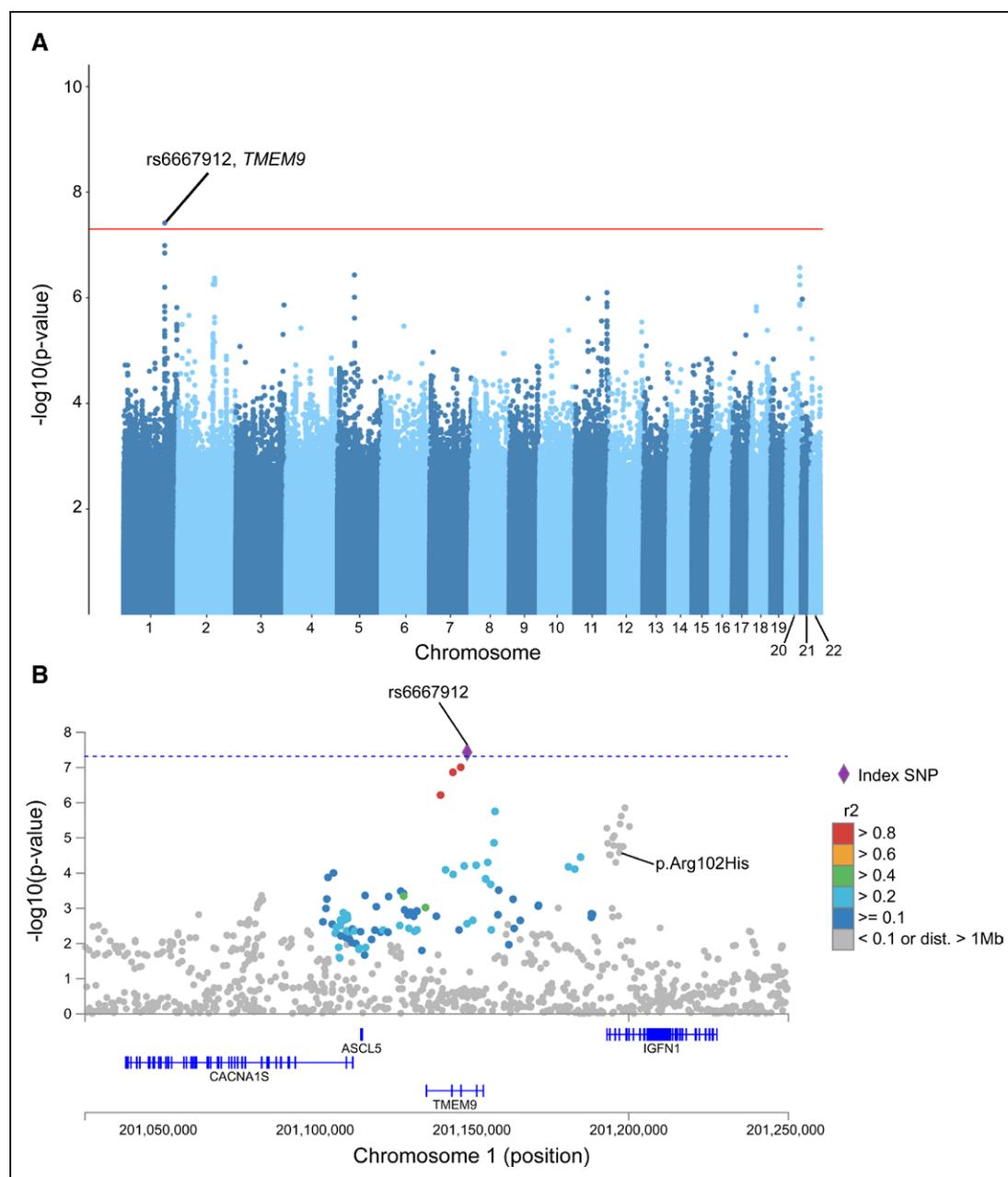
Figure 1. Manhattan and locus plot of baseline statin intolerance or investigator-documented statin-associated muscle symptoms (SAMS) during treatment period exome-wide association study (ExWAS)/genome-wide association study (GWAS; cases=894; controls=9723).
A, Manhattan plot with the y axis representing—log10 P values derived from logistic regression analysis of 8 921 030 genetic variants. The x axis represents chromosome and position (build GRCh38). The top associated locus in *TMEM9* (rs6667912, P=3.71×10^{-8}) is labeled. The dotted line represents the genome-wide significance cutoff (P<5×10^{-8}). **B**, Locus plot of top ExWAS/GWAS association rs6667912 (*TMEM9*) with baseline statin intolerance or SAMS during the treatment period. A missense variant in *IGFN1* (p.Arg102His) with modest association (P=2.49×10^{-5}) was a finding independent from the top association (rs6667912) and is also labeled. Rs6667912 (*TMEM9*) is located ≈30 kb 5′ of *CACNA1S* (locus previously associated with severe SAMS). Several modest associations (P<1×10^{-3}) were also reported in the *CACNA1S* locus. The dotted line represents the genome-wide significance cutoff (P<5×10^{-8}). SNP indicates single-nucleotide polymorphism.

the top exonic region variant associations. A common (minor allele frequency [MAF]=0.34) intronic variant in *TMEM9* (rs6667912) achieved a genome-wide significant association with this phenotype (odds ratio [95% CI], 1.33 [1.20−1.48]; P=3.71×10^{-8}; Table 2). Sensitivity analyses indicate that this association was consistent in

both alirocumab and placebo treatment groups and irrespective of whether SAMS occurred before or following randomization. This variant in *TMEM9* (rs6667912) was also highly significant in the European subgroup analysis (odds ratio [95% CI], 1.39 [1.24−1.55]; P=6.01×10^{-9}; Figure S2) and was significantly associated with a

Table 2. Top Associated Loci for the ExWAS/GWAS of Baseline Statin Intolerance or Investigator-Documented SAMS During the Treatment Period

Baseline statin intolerance or investigator-documented SAMS during treatment period ExWAS/GWAS results (cases=894; controls=9723)						
Chr:Pos:Ref:Alt	rsID-risk allele	Region	Gene*	RAF	OR (95% CI)	P Value
1:201148730:C:G	rs6667912-G	Intronic	TMEM9	0.34	1.33 (1.20–1.48)	3.71×10⁻⁸†
20:58072670:C:T	rs76443348-T	Intergenic	C20orf85	0.01	2.55 (1.78–3.66)	3.22×10⁻⁷
2:154457782:G:A	rs17815112-A	Intergenic	GALNT13	0.43	0.77 (0.70–0.85)	4.10×10⁻⁷
5:68066791:AC:A	rs35136807-A	Intergenic	PIK3R1	0.26	1.54 (1.30–1.81)	4.36×10⁻⁷
2:147667570:C:G	rs7564037-G	Intergenic	ACVR2A	0.10	0.57 (0.46–0.71)	5.78×10⁻⁷
11:127318262:A:AC	rs11399393-AC	Intergenic	LINC02712/KIRREL3	0.45	1.30 (1.17–1.44)	7.19×10⁻⁷

Genome-wide ($P<5×10^{-8}$) and suggestive ($P<1×10^{-6}$) associations derived from logistic regression for ExWAS/GWAS of baseline statin intolerance or investigator-documented SAMS during the treatment period in the ODYSSEY OUTCOMES cohort. Only the top index variant (lowest P value) is shown per LD cluster ($r^2>0.2$). Alt indicates alternative allele; Chr, chromosome; ExWAS, exome-wide association study; GWAS, genome-wide association study; LD, linkage disequilibrium; ODYSSEY OUTCOMES, Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab; OR, odds ratio; Pos, position; RAF, risk allele frequency; Ref, reference allele; rsID, reference variant cluster ID; and SAMS, statin-associated muscle symptoms.

*Nearest gene is reported.

†Genome-wide significant P values.

skeletal muscle splicing quantitative trait loci in *TMEM9*[23] ($P=1.1×10^{-8}$; Table S2). No other variants reached genome-wide significance. Also at this locus, an analysis conditioned on the *TMEM9* (rs6667912) variant identified a common (MAF=0.40) missense variant in *IGFN1* (rs4915221, p.Arg102His) which showed a modest, but independent association (odds ratio [95% CI], 1.24 [1.12–1.37]; $P=2.49×10^{-5}$; Table 3) and was in weak linkage disequilibrium (LD; $r^2=0.36$) with an intronic *IGFN1* variant that had an association just below the suggestive significance threshold ($P=1.03×10^{-6}$).

CK Maximum Value During Treatment Period ExWAS/GWAS Results

The median (first quartile [Q1]–third quartile [Q3]) increase from baseline CK for subjects taking high-dose atorvastatin or rosuvastatin at randomization included for analysis (n=9630) was 87 (45–167) IU/L. In this cohort, median (Q1–Q3) CK considering all postrandomization measurements was 114 (80–167) IU/L, and the median peak postrandomization CK level was 188 (127–292) IU/L. Maximum CK levels >4× or

>10× the upper limit of normal (ULN) occurred in 317 and 58 patients, respectively.

An ExWAS/GWAS for maximum circulating CK level (as a continuous variable) during the trial treatment period was conducted in subjects taking high-dose atorvastatin or rosuvastatin at randomization (n=9630). Figure 2A shows a Manhattan plot of the findings. The top associated locus is shown in Figure 2B. Table 4 contains a detailed list of genetic associations that reached genome-wide or suggestive significance. Table 5 provides the top exonic region variant associations. A total of 8 variants, all within the *LINC00393* region on chromosome 13, reached genome-wide significance when tested for association. The top association, rs7993814 (β [95% CI], 0.08 [0.06–0.12]; $P=9.77×10^{-9}$; Table 4), is a common variant located at position 13:73612794 (build GRCh38) and falls within the *LINC00393* noncoding RNA in a region between the *KLF5* and *KLF12* genes. All top 8 variant associations are in strong LD ($r^2≥0.8$). No variants in other loci reached genome-wide significance for this phenotype. However, associations for several variants in *LILRB5* reached suggestive significance (Tables 4 and 5). This includes a common missense

Table 3. Top Associated Exonic Loci for ExWAS of Baseline Statin Intolerance or Investigator-Documented SAMS During the Treatment Period

Baseline statin intolerance or investigator-documented SAMS during treatment period ExWAS results (cases=894; controls=9723)						
Chr:Pos:Ref:Alt	rsID-risk allele	Protein change	Gene	RAF	OR (95% CI)	P value
15:52251418:G:T	rs72734946-T	Ser545Tyr	MYO5C	0.01	2.17 (1.52–3.08)	1.6×10⁻⁵
1:201197255:G:A	rs4915221-G	Arg102His	IGFN1	0.43	1.24 (1.12–1.37)	2.5×10⁻⁵
1:248488770:G:C	rs199723306-C	Ser61Asn	OR2T5	0.08	1.41 (1.17–1.69)	2.5×10⁻⁴
11:72232917:C:A	rs61749195-A	Leu632Ile	INPPL1	0.01	1.83 (1.33–2.52)	2.1×10⁻⁴
4:185458581:T:C	rs6827370-C	Gln669Arg	CCDC110	0.12	0.74 (0.63–0.88)	3.4×10⁻⁴

Top 5 exonic region associations were derived from logistic regression for ExWAS of baseline statin intolerance or investigator-documented SAMS during the treatment period in the ODYSSEY OUTCOMES cohort. Only the top index variant (lowest P value) is shown per LD cluster ($r^2>0.2$). Alt indicates alternative allele; Chr, chromosome; ExWAS, exome-wide association study; LD, linkage disequilibrium; ODYSSEY OUTCOMES, Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab; OR, odds ratio; Pos, position; RAF, risk allele frequency; Ref, reference allele; rsID, reference variant cluster ID; and SAMS, statin-associated muscle symptoms.
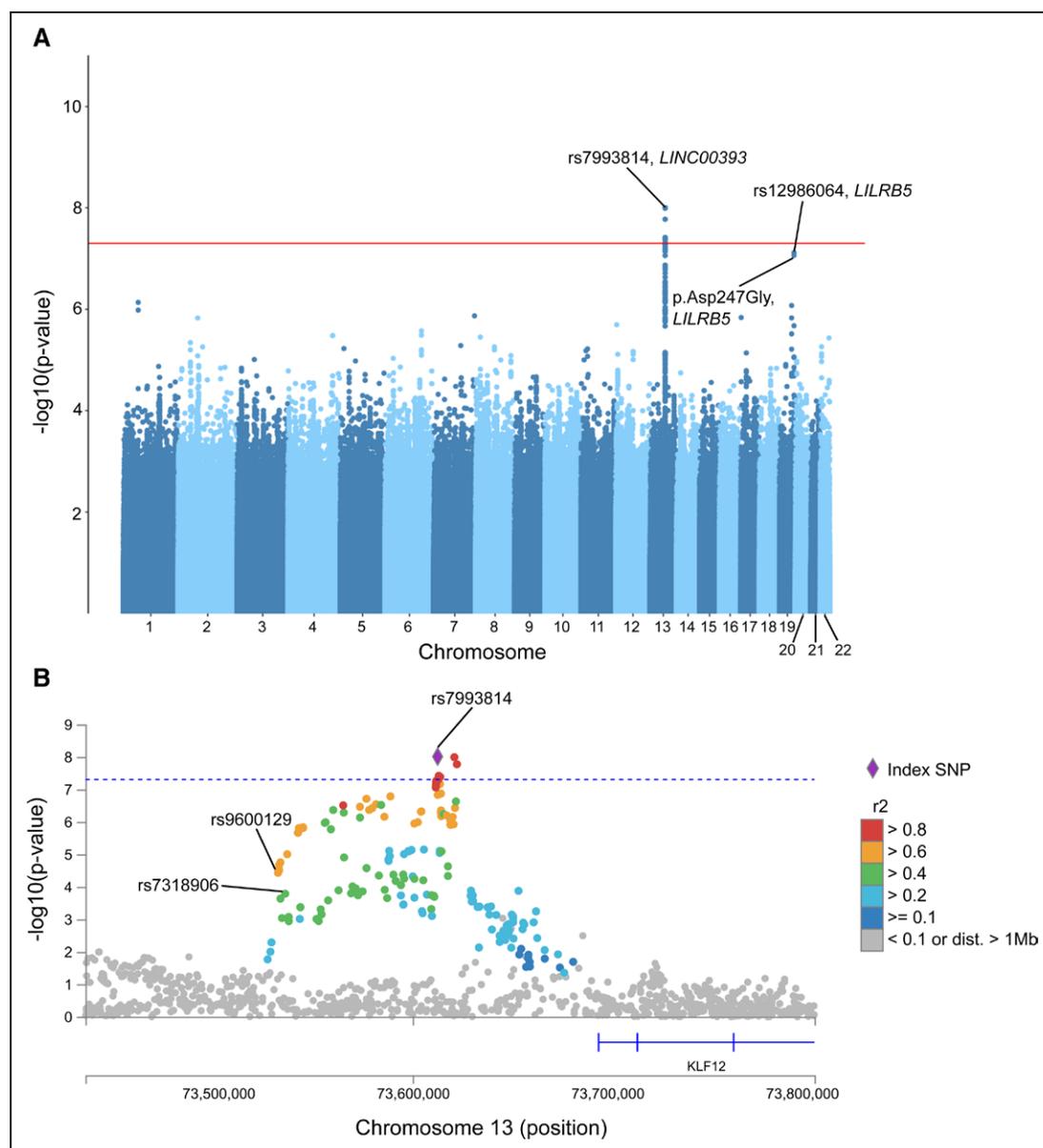
Figure 2. Manhattan plot and locus plot of maximum creatine kinase (CK) during treatment period exome-wide association study (ExWAS)/GWAS (n=9630).
A, Manhattan plot with the y axis representing—log10 P values derived from linear regression analysis of 8 921 030 genetic variants. The x axis represents chromosome and position (build GRCh38). The top associated loci in *LINC00393* (rs7993814, P=9.77×10^{-9}) and *LILRB5* (rs12986064, P=7.64×10^{-8}; p.Asp247Gly, P=8.68×10^{-8}) are labeled. The dotted line represents the genome-wide significance cutoff (P<5×10^{-8}). **B**, Locus plot of top association (rs7993814, *LINC00393*) and variants previously associated with CK levels in statin and nonstatin users (rs9600129, *LINC00393*; rs7318906, *LINC00393*). Both rs9600129 (r²=0.61) and rs7318906 (r²=0.48) are in modest linkage disequilibrium (LD) with the top association/reference variant (rs7993814). The top variant (rs7993814) is located ≈80 kb 3′ of *KLF12*. Both rs7993814 and rs9600129 have associated skeletal muscle expression quantitative trait loci (eQTLs) in *KLF5* (not pictured, located ≈500 kb from top association, rs7993814). The dotted line represents genome-wide significance cutoff (P<5×10^{-8}). SNP indicates single-nucleotide polymorphism.

variant (rs12975366, p.Asp247Gly; P=8.68×10^{-8}; Table 5) which had previously been associated with SAMS and CK levels.[24–26] A rare (MAF=~0.02) intronic variant in *KANK4* (rs149062268, P=7.30×10^{-7}; Table 4) also showed a suggestive association with this phenotype.

ExWAS/GWAS analysis was also performed for CK >4× ULN (cases=317, controls=9313) and CK >10× ULN (cases=58, controls=9572) using the same methodology as detailed above. No genome-wide or suggestive associations of note were identified for either phenotype (Figure S3).

Phenotype Intersection Analysis

Among patients who developed SAMS after randomization who were eligible for CK analysis (n=219), median

Table 4. Top Associated Loci for ExWAS/GWAS of CK Maximum Value During the Treatment Period

CK maximum value during treatment period ExWAS/GWAS results (n=9630)						
Chr:Pos:Ref:Alt	rsID-risk allele	Region	Gene*	RAF	Per allele β (95% CI)	P value
13:73612794:C:T	rs7993814-T	Intergenic	LINC00393/KLF12	0.38	0.08 (0.06 to 0.12)	9.77×10^{-9}†
19:54251270:T:C	rs12986064-C	Intronic	LILRB5	0.47	−0.10 (−0.06 to −0.14)	7.64×10^{-8}
1:62285404:G:A	rs149062268-A	Intronic	KANK4	0.02	−0.23 (−0.14 to −0.32)	7.30×10^{-7}

Genome-wide ($P<5 \times 10^{-8}$) and suggestive ($P<1 \times 10^{-6}$) associations derived from linear regression for ExWAS/GWAS of the maximum CK value during the treatment period in the ODYSSEY OUTCOMES cohort. Only the top index variant (lowest P value) is shown per LD cluster ($r^2 > 0.2$). Per-allele β-effect size was reported based on RINTed values. Alt indicates alternative allele; Chr, chromosome; CK, creatine kinase; ExWAS, exome-wide association study; GWAS, genome-wide association study; LD, linkage disequilibrium; ODYSSEY OUTCOMES, Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab; Pos, position; RAF, risk allele frequency; Ref, reference allele; RINT, rank-based inverse normalized transformation; rsID, reference SNP cluster ID; and SAMS, statin-associated muscle symptoms.

*Nearest gene is reported.

†Genome-wide significant P values.

(Q1–Q3) peak CK was 222 (146–356) IU/L. Additionally, for subjects with peak postrandomization CK in the first through the fourth quartiles, 1.5%, 2.3%, 2.5%, and 3.4% developed SAMS, respectively.

An intersection analysis did not identify any genetic associations stronger than $P<1 \times 10^{-4}$ across the clinical (binary SAMS) and biochemical (CK levels) phenotypes. Genome-wide and suggestive associations listed in Tables 2 and 3 were also investigated and did not show any association ($P<0.05$) with the other phenotype.

Candidate Gene/Variant Analysis

No variants in candidate gene regions previously associated with atorvastatin and/or rosuvastatin pharmacokinetics or pharmacodynamics showed genome-wide significant associations with either phenotype. No genome-wide or suggestive associations were seen in the SLCO1B1 locus for either phenotype. Of note, no association was observed between p.Val174Ala (rs4149056) in SLCO1B1 and baseline statin intolerance/investigator-documented SAMS (odds ratio [95% CI], 1.03 [0.90–1.18]; $P=0.69$) or maximum CK levels (β [95% CI], 0.003 [−0.04 to 0.04]; $P=0.87$) for this cohort. In addition to variants in LILRB5, some variants previously associated with CK levels in both statin users and nonusers showed genome-wide and suggestive significance (Table S3).

Gene Burden Analysis

One exome-wide significant association ($P<1 \times 10^{-6}$) was identified for a singleton mask (M3) in SOAT1 with the baseline statin intolerance or investigator-documented SAMS during treatment period phenotype (Table S4). Additionally, one candidate gene mask (ABCB1, M2) demonstrated a near exome-wide significant association ($P=3.23 \times 10^{-5}$) with the same phenotype. No exome-wide significant associations were observed with the CK maximum value during the treatment period phenotype.

DISCUSSION

This large multi-ancestry ExWAS/GWAS analysis of SAMS and CK levels in atorvastatin/rosuvastatin-treated patients from the ODYSSEY OUTCOMES trial identified a novel genome-wide association on chromosome 1 for an intronic variant in the TMEM9 gene (rs6667912, $P=3.71 \times 10^{-8}$; Table 2, Figure S4) in patients with either statin intolerance before randomization or who developed SAMS while taking high-dose atorvastatin or rosuvastatin during the treatment period. This finding was even more significant in the European-only subgroup analysis ($P=6.01 \times 10^{-9}$; Figure S2). TMEM9 is expressed ubiquitously across most human tissues[23] and encodes a transmembrane protein involved in vesicular transport through the Wnt/β-catenin pathway.[27,28] A recent

Table 5. Top Associated Exonic Loci for ExWAS of CK Maximum Value During the Treatment Period

CK maximum value during treatment period ExWAS results (n=9630)						
Chr:Pos:Ref:Alt	rsID-risk allele	Protein change	Gene	RAF	Per allele β (95% CI)	P value
19:54255498:T:C	rs12975366-C	Asp247Gly	LILRB5	0.42	−0.08 (−0.11 to −0.05)	8.7×10^{-8}
10:84196512:C:A	rs12781048-A	His53Gln	CDHR1	0.04	−0.16 (−0.23 to −0.08)	3.1×10^{-5}
15:40266281:A:G	rs3743135-G	His215Arg	PAK6	0.08	0.10 (0.05 to 0.17)	5.8×10^{-5}
17:66220697:C:T	rs8178847-T	Arg154His	APOH	0.06	0.12 (0.06 to 0.17)	6.8×10^{-5}
19:55391573:G:A	rs45448592-A	Gly115Glu	RPL28	0.06	0.12 (0.06 to 0.18)	1.2×10^{-4}

Top 5 exonic region associations were derived from linear regression for ExWAS of the maximum CK value during the treatment period in the ODYSSEY OUTCOMES cohort. Per-allele β-effect size was reported based on RINTed values. Only the top index variant (lowest P value) is shown per LD cluster ($r^2 > 0.2$). Alt indicates alternative allele; Chr, chromosome; CK, creatine kinase; ExWAS, exome-wide association study; LD, linkage disequilibrium; ODYSSEY OUTCOMES, Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab; Pos, position; RAF, risk allele frequency; Ref, reference allele; RINT, rank-based inverse normalized transformation; and rsID, reference variant cluster ID.

study demonstrated significant upregulation of *Tmem9* skeletal muscle mRNA in a muscular atrophy mouse model, suggesting its potential role in skeletal muscle energy conservation.[29] Additionally, the *TMEM9* locus is located near *CACNA1S* (≈30 kb upstream), a region previously associated with severe myopathy in patients taking atorvastatin, rosuvastatin, or simvastatin.[30] The significantly associated *CACNA1S* missense variants previously reported by Isackson et al[30] had MAF<0.01 and were, therefore, not included in the present ExWAS/GWAS analysis. Thirteen *CACNA1S* variants from the prior study were investigated separately and were not associated ($P<0.05$) with either phenotype. Several intronic *CACNA1S* variants and a single missense variant (rs12742169, p.Leu458His) showed modest associations ($P<1.0×10^{-3}$) in our data set (Figure 1B and Table S3). Interestingly, the p.Leu458His variant was not in LD ($r^2<0.2$) with the top *CACNA1S* intronic variants, and showed similar association when conditioned on these variants, suggesting an independent finding. Last, a conditional analysis with the top *TMEM9* variant (rs6667912) identified intronic and missense variants in the nearby *IGFN1* gene with associations of $P<1×10^{-5}$, which may suggest another independent SAMS association at this locus (Figure 1B). *IGFN1* is expressed almost exclusively in skeletal muscle[23] and encodes immunoglobulin- and fibronectin-like protein domains. IGFN1 (Immunoglobulin like and fibronectin type III domain containing 1) downregulates protein synthesis during muscle denervation,[31] suggesting a potential role in muscle wasting. Interestingly, *Igfn1* and *Tmem9* were both significantly downregulated in skeletal muscle following treatment with a promyogenic agent (ie, soluble activin type IIB receptor)[32] and upregulated following treatment with an atrophy-inducing agent (ie, activin A).[33]

CK is an enzyme released into systemic circulation following skeletal muscle or myocardial injury and is a commonly used nonspecific biomarker to assess SAMS severity. Our ExWAS/GWAS analysis of CK levels in atorvastatin/rosuvastatin-treated patients identified a significant genome-wide association with a variant in the *LINC00393* noncoding RNA on chromosome 13 (Table 4). Variants in *LINC00393* had previously been reported to be associated with CK levels in statin and nonstatin users in Icelandic (rs7318906)[25] and Japanese (rs9600129)[34] populations. These variants are located <10 kb away from the top association (rs7993814) identified in our study and are in partial LD (rs7318906, r^2=0.48; rs9600129, r^2=0.61) with this variant (Figure 2B). These previously associated variants showed modest association (rs7318906, β [95% CI], −0.06 [−0.03 to −0.09]; P=2.30×10^{-4}$; rs9600129, β [95% CI], 0.07 [0.03–0.10]; P=3.30×10^{-5}$) in our study (Table S3). Notably, rs7993814 (the top finding in our study) and rs9600129 (the variant previously associated with CK levels in a Japanese population[34]) both have

expression quantitative trait loci in *KLF5* associated with reduced gene expression in skeletal muscle (Table S2).[23] *KLF5* is located ≈500 kb from the top association in this study (rs7993814) and encodes a zinc-finger transcription factor involved in the promotion and suppression of cell proliferation; it is ubiquitously expressed in human tissues. While no previous association has been reported for *KLF5* with CK levels or SAMS, Klf5 (kruppel like factor 5) was shown to be an essential regulator of skeletal muscle regeneration and differentiation in mice.[35] However, its mechanistic role in human skeletal muscle and CK levels needs further investigation. Additionally, validated colocalization data is needed to confirm the link between *LINC00393* variation and *KLF5* expression. We also examined several candidate genes reported to be associated with CK levels. Variants in *LILRB5* have been consistently associated with CK levels in statin and nonstatin users.[36] A *LILRB5* missense variant (p.Asp247Gly), previously associated with CK in 2 large European ancestry studies[25,26] and SAMS independent of CK levels,[24] had an association (P=8.68×10^{-8}$) with CK levels in our analysis that nearly reached genome-wide significance (Table 5). However, the top association in *LILRB5* in our study was an intronic variant (rs12986064; P=7.64×10^{-8}$) that had not been previously associated with CK levels (Table 4). This variant (rs12986064) was in partial LD (r^2=0.61) with p.Asp247Gly.

Of note, some of the strongest genetic associations (Tables 2 through 5) were observed to have protective effects (ie, associated with decreased risk of SAMS or lower maximum CK levels). While the same protective association with CK levels has been previously observed with rs12975366[25] in *LILRB5*, the physiological mechanism has yet to be elucidated despite speculation that LILRB5 is involved in systemic CK clearance.[25,26] Variation in *GALNT13*, *KANK4*, *ACVR2A*, *CCDC110*, and *CDHR1* has not been previously associated with SAMS or CK levels, and it is unclear how these variants might exert their protective effects from a mechanistic standpoint.

Other phenotypes during the treatment period, such as CK elevation (>4× ULN) in the presence of muscle symptoms (ie, clinical myopathy) attributed to statin use by study investigator (19 cases) and statin-induced rhabdomyolysis (9 cases), were also investigated. Both traits were underpowered due to small case sizes and not included in this study. Additionally, atorvastatin/rosuvastatin pharmacokinetic and pharmacodynamic candidate genes and previous associations were investigated, none of which reached genome-wide or suggestive significance (Table S3).

While candidate gene/variant analysis failed to show any variants with genome-wide significance, some variants did show modest associations. An intronic variant in *CACNA1S* (rs12239772, P=8.08×10^{-5}$) had modest association with the baseline statin intolerance

or investigator-documented SAMS during treatment period phenotype (Table S3). The lack of any association between p.Val174Ala (rs4149056, *SLCO1B1*) with SAMS or CK levels in our study is consistent with results from other atorvastatin/rosuvastatin pharmacogenetic studies[12–14,37] and suggests that this association is specific for simvastatin-treated patients.

Genetic burden analysis identified one exome-wide association ($P<1\times10^{-6}$) between a singleton mask (*SOAT1*) and the baseline statin intolerance or investigator-documented SAMS during treatment period phenotype (Table S4). Interestingly, the protein product of *SOAT1* (sterol O-acyltransferase 1) acts downstream from HMG-CoA reductase (statin pharmacological target) and is responsible for esterification of intracellular cholesterol as a mechanism to store excess cholesterol before removal by efflux.[38] *SOAT1* variation has not been previously associated with SAMS, and the potential mechanistic role of this association is unclear. We also observed a modest association ($P=2.81\times10^{-5}$) between a candidate gene mask (*ABCB1*) and the binary SAMS phenotype. *ABCB1* encodes the drug efflux transporter P-glycoprotein, and a small study (n=98) showed an association with a common *ABCB1* missense variant and atorvastatin-mediated SAMS.[39] No exome-wide associations were observed with the CK maximum value during treatment period phenotype.

An inherent limitation of our study is that this clinical cohort was primarily assembled to investigate the efficacy and safety of alirocumab, rather than genetic predictors of SAMS. While ODYSSEY OUTCOMES did not exclude statin intolerant patients from study randomization, subjects with concomitant disease states (eg, uncontrolled hypothyroidism, significant renal or hepatic disease) that may increase SAMS risk were excluded, resulting in the potential for a healthy-user bias in our study population. The study protocol did not mandate statin withdrawal and/or rechallenge for muscle symptoms as per the standardized definition of SAMS for pharmacogenomic studies,[40] but rather specified statin dose reduction and/or substitution of a nonstatin lipid-lowering agent. Identification of SAMS cases with concomitant CK elevations <4× ULN during the treatment period was also restricted, preventing stratification of SAMS cases with and without CK elevation, and further limited the study's ability to adhere to phenotype standardization. This may explain the absence of association with *SLCO1B1*. Another limitation of our study is the absence of a control group not treated with a statin. Muscle symptoms and mild-to-moderate CK elevations are common in the absence of statin treatment, and frequently occur in placebo arms of randomized trials with statins.[41] Recent literature has highlighted uncertainties surrounding associations between statin use and muscle symptoms in the absence of extreme CK elevation.[42,43] Accordingly, some muscle symptoms or CK elevations attributed to statin treatment by investigators may have had another cause (eg, excessive physical activity). We are unable to explore whether the genetic associations identified in the current analysis also exist with muscle symptoms or elevated CK in the absence of statin use. Another limitation is that the reported genetic findings do not discriminate between statins, since many participants categorized as having SAMS before randomization were intolerant to both atorvastatin and rosuvastatin, and in some cases other statins. A strength of this study is that it was multiracial and international, including 818 Asian and 323 Black patients in the overall pharmacogenomic analysis subgroup. However, the proportion of patients of non-European ancestry is still too small to confidently generalize these results across different ancestry populations. It should also be noted that White patients were significantly over-represented in the genetic substudy compared with the parent trial (Table 1). The results identified here will need to be replicated in other external clinical trial datasets.

The findings from this study demonstrate the complex, multifactorial nature of SAMS and highlight the need for more large-scale studies investigating associations of rarer variants (MAF<0.01) with this drug-related adverse event. Results of this study implicate genes likely involved in different pharmacodynamic pathways in skeletal muscle (eg, calcium signaling [*CACNA1S*] and cell differentiation/proliferation [*IGFN1*, *KLF5*, *TMEM9*]). In summary, novel genome-wide associations in *TMEM9* (rs6667912) and *LINC00393* (rs7993814) are reported and warrant further investigation through validation in separate cohorts and in vitro studies to understand their potential role in SAMS.

Supplemental Material

Supplemental Methods
Tables S1–S5
Figures S1–S5
References [44–62]

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