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Genetic and clinical factors are associated with statin-related myotoxicity of moderate severity: a case-control study

Brief title: *genetics, sex and statin related myalgia*

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ABSTRACT

We evaluated the contribution of patient specific clinical and genetic factors to statin-related muscle toxicity (SRM) without significant creatine kinase elevation (125 cases related to simvastatin or atorvastatin and 481 controls). The association between 12 SNPs in nine candidate genes and clinical factors with SRM was evaluated. Of the 12 SNPs genotyped, only rs4149056 in *SLCO1B1* was associated with SRM in univariate analysis (with any statin, OR=1.73, 95 % CI= 1.14-2.62, P = 0.010) and this association was influenced by sex (P= 0.006) and BMI (P=0.02). In multivariate and binary logistic regression analyses, *SLCO1B1* rs4149056 genotype (OR=1.66, 95% CI: 1.08-2.54, P=0.014) and sex (OR=1.72, 95% CI =1.15 - 2.59, P=0.006) were independently associated with muscle toxicity related to statin treatment. Patient-specific genetic and clinical factors associated with increased systemic exposure to statins are implicated in the full spectrum of SRM, including myalgia in addition to severe myopathy.

INTRODUCTION

Although statins are generally well tolerated, statin-associated side-effects are a common problem in routine clinical practice, leading to dose reduction, non-adherence or discontinuation of therapy, with consequent poor control of LDL-cholesterol and increased risk of preventable cardiovascular morbidity and mortality (1). Statin-associated musculoskeletal symptoms (SAMS) without significant elevation of creatine kinase is the most common adverse event leading to discontinuation of therapy (2). However, therapy can be restored in the majority of statin-intolerant patients when re-challenged (3). Indeed, in large clinical trials, minor muscle symptoms occur at similar frequency, regardless of allocation to statin or placebo suggesting that in most patients, SAMS are not of pharmacological origin (4, 5). Conversely, true statin-related muscle toxicity (SRM), with characteristic symptoms which resolve quickly on discontinuation of therapy and recur with re-challenge, is demonstrated in only a minority of SAMS cases under double-blind conditions (6, 7). The phenotype of SRM ranges from tolerable myalgia to intolerable muscle pain and weakness with elevation of creatine kinase (CK) due to breakdown of muscle tissue mandating immediate withdrawal of statin therapy (8). The graded classification proposed by Alfirevic et. al. (8) defines this spectrum on a scale of 0-6 according to the severity of symptoms, CK elevation and end organ damage. Although more severe forms of muscle toxicity, such as myopathy and rhabdomyolysis (SRM4-5), are infrequent even at higher statin doses (8, 9), clinicians considering re-challenge in patients intolerant of the high intensity statin regimes recommended for effective cardiovascular prevention face a difficult decision - how to distinguish patients with minor muscle symptoms of non-pharmacological origin from those with "true SRM" of moderate severity (SRM 2-3) who are at risk of progression to more severe myopathy, and in whom the high cost, non-statin alternative therapies such as PCSK9 inhibitors may be justified.

There is consistent evidence that patient-specific clinical factors, such as advanced age (10), female sex (11, 12), low body mass (13), hepatic and renal dysfunction (14) and drug- drug interactions (15), contribute to SRM (16). There is also compelling evidence that genetic factors, including those that influence statin disposition and localization in skeletal muscle increase the risk of SRM in susceptible individuals (16). The best genetic predictor for severe forms of statin-related myopathy is the rs4149056 polymorphism in the *SLCO1B1* gene, which codes for the organic anion-transporting polypeptide OATP1B1 (9). The existence of an association between *SLCO1B1* rs4149056 and simvastatin-induced myopathy has a well-established pharmacokinetic basis (17) and has been confirmed in clinical studies by several investigators (18-21) but is clearly demonstrated only at higher doses and when CK elevation is included in the definition of SRM(18, 22) but not at lower doses of statins or milder SRM(23). It is less clear whether this genetic variant is a useful predictor of SRM with atorvastatin, the most widely prescribed high potency statin (24). However, the association between statin use and myopathy is a class effect and if less severe SRM is pharmacological in origin, an association might be expected with common variants in *SLCO1B1* or other genes related to statin disposition and muscle exposure, including those involved in drug metabolism and muscle membrane transport (25, 26). Thus, the possible contributions of common SNPs in a selection of these genes, together with patient specific clinical factors, were evaluated in patients treated with the highest recommended doses of simvastatin and atorvastatin in a lipid clinic based case-control study, designed to include the less severe end of the SRM spectrum (SRM2) which most commonly accounts for discontinuation of therapy in routine practice (8).

RESULTS

Characteristics of study subjects

A total of 606 patients [125 cases and 481 controls], all of whom were of self-reported European ancestry were recruited into the study. The demographic and clinical characteristics of the study cohorts are summarized in **Table 1**. All cases reported myalgia without significant CK elevation (>4 times ULN) and were classified as SRM2. One case deteriorated to SRM4 (severe myopathy) but was later diagnosed as polymyositis, not statin-related and was therefore excluded from the data analysis. In the case group there was a significantly greater proportion of females than the control group (58.4% vs 44.1 %; $P=0.004$). A greater proportion of patients in both the case and the control groups were treated with simvastatin than atorvastatin (107 of 125, 85.6 % and 297 of 481, 61.7 % respectively; $P < 0.001$). In both groups, simvastatin 40 mg/day was the most frequently prescribed while the most common dosage regimen for atorvastatin was 80 mg/day (**Table 1**). The mean age of the controls (60.93 years; 95 % CI: 59.82 –62.05) and the cases (60.29 years; 95 % CI: 58.10 -62.48) were similar. There was no significant difference in BMI between the cases (median 28.5 kg/m², interquartile range 26.4 - 31.7) and the controls group (median 28.5 kg/m², interquartile range 25.8 -32.0). There was a marked difference ($P<0.001$) in total duration of statin treatment between the cases (median 8 months, interquartile range 3 -18) and the controls (median 24 months, interquartile range 12 - 65). There was no significant association between SRM and the presence of comorbidities reported to enhance the risk of SRM, such as type 2 diabetes, impaired renal function, hypothyroidism and hypertension. Approximately 17-30 % of the cases and of controls reported concurrent exposure to drugs which have the potential to interact with the pharmacokinetics of statins, including fibrates, calcium antagonists and CYP3A4 inhibitors. However with the exception of warfarin (fewer patients in the case group; $p =0.008$) and dihydropyridine calcium antagonists (fewer patients in the control group; $p= 0.031$) the patient-reported use of such agents was similar in cases compared to the controls. A list of all concomitant medications deemed to interact with statins which were taken by the study

patients is provided in the **supplementary Table S1**. Compared to the control subjects, cases had higher concentrations of serum lipids at the time of recruitment, on maximum tolerated lipid lowering therapy, which were well above recommended treatment goals for high risk primary and secondary prevention (**Table 1**).

Environment-gene association with SRM

The genotype frequencies of all tested SNPs were checked for their consistency with Hardy-Weinberg Equilibrium (HWE) (**Table 2**). Frequencies for one SNP, rs1050450 in *GPX1* were not in Hardy-Weinberg equilibrium and this SNP was not studied further. Of the remaining 12 SNPs studied, none were significantly different to the frequencies reported in the GBR (British in England and Scotland) derived from 1000 genomes browser (<http://browser.1000genomes.org/index.html>) and met the analysis criteria of having a minor allele frequency >0.01. Subsequently these 12 SNPs were included in further analyses. All SNPs were successfully genotyped in 99.2 % of the patients (blood samples were missing for 5 individuals belonging to the control group). When these 12 SNPs were tested in dominant models (**Table 2**) in a univariate analysis, only the SNP in *SLCO1B1* [rs4149056, (c.521T>C; V174A)] was associated with SRM (P=0.010). There was a significantly higher proportion of carriers of the minor C allele (heterozygotes and homozygotes) in the case group, irrespective of the type of statin used, compared to the controls; 37.6 % vs 25.8 % (**Figure 1-A**) with odds ratio (OR) of 1.73 (95% CI: 1.14- 2.62). There was no significant difference in the frequency of C allele carriers between the simvastatin and atorvastatin intolerant cases (**Figure 1-B**). Polymorphisms in other genes, including SNPs in previously proposed gene candidates for SRM i.e. *GATM* (rs9806699) and *COQ2* (rs4693075), showed no significant difference between cases and controls.

In multivariate logistic regression analyses adjusted for patient age, sex, BMI and the 11 remaining genotypes, *SLCO1B1* rs4149056 (OR=1.72, 95% CI: 1.15- 2.59; P=0.014) and

female sex [OR=1.72 (95% CI =1.15 - 2.59; P=0.006)] were the only variables which were independently associated with SRM (**Table 3**). In the statin-intolerant group the relative risk of SRM was higher in females and it increased further in patients with C variant allele irrespective of sex (**Table 4**). In the C allele carriers, the BMI was significantly lower in the cases (N=47, Mean BMI 27.9 kg/m²) than the controls (N=123, Mean BMI 29.8 kg/m²) a mean difference in BMI of 1.9 kg/m² (P=0.02).

According to binary logistic regression analysis only sex (P=0.004), *SLCO1B1* genotype (P=0.011) and logged BMI in C allele carriers (P=0.014,) were found to be significantly associated with SRM.

DISCUSSION

A modern dilemma faced by clinicians who prescribe statins is how to decide which, if any, patients with muscle symptoms in the absence of significant CK elevation have “true” statin-related muscle toxicity (SRM), related to the pharmacology of statins, and which have non-specific musculoskeletal symptoms reflecting a high background prevalence in patients eligible for statin treatment. In order to determine whether pharmacological factors contribute to less severe statin-related muscle toxicity in patients referred to a specialist lipid clinic, we examined the association between SRM2-3 defined by de-challenge and re-challenge, and polymorphisms in candidate genes implicated in statin disposition and safety alongside patient demographics and clinical features. Our findings of a clear association between SRM2 and both the *SLCO1B1* rs4149056 polymorphism and sex is consistent with findings reported previously in both moderate and severe cases (9, 15, 18) which provides further evidence indicating that SRM of moderate severity is, at least in part, of pharmacological origin and appears related to altered systemic and muscular drug exposure. Therefore, the symptoms reported by patients with moderate SRM, as defined in our cohort,

cannot be attributed solely to a “nocebo effect” or misattribution of symptoms, and imply that tailored strategies for lipid lowering therapy may reduce the risk of SRM.

Our findings of the association between female sex and SRM is in concordance with previous findings from clinical trials (9, 18) and epidemiological studies reporting that, female sex (linked to small body frame), along with patient age and frailty, increase susceptibility to SRM(27). In the entire study cohort, we found no association between patient age and BMI and SRM; however, the *SLCO1B1* C allele carriers in the case group, irrespective of age, had a significantly lower BMI. Our data supports the view that low BMI is an endogenous risk factor for SRM (2, 28), perhaps because low BMI, which is linked to smaller vascular volumes and reduced muscle mass, could lead to greater statin systemic exposure, thus exacerbating the risk of SRM, particularly in the C allele carriers.

Systemic exposure to statins appears to be attributable, to an extent, to the *SLCO1B1* rs41490565 polymorphism resulting in altered hepatic drug uptake. It has been demonstrated that, relative to the wild-type *TT* genotype, homozygosity for the C allele increases the area under the plasma concentration versus time (AUC) by 221% for active simvastatin acid (29), significantly more than for other statins, including pitavastatin (162%-191%), atorvastatin (144%), pravastatin (57 %-130%), rosuvastatin (62%-117%) and least for fluvastatin (19%) (15). Although previous studies have shown conflicting results on the susceptibility to SRM with other statins (11, 18, 20, 21, 30), there may be an increased risk for SRM among *SLCO1B1* rs4149056 carriers using atorvastatin, as suggested by Pasanen and colleagues (31). Whether *SLCO1B1* rs4149056 truly confers susceptibility among atorvastatin-intolerant cases remains inconclusive but the effects of the variant on the pharmacokinetics of atorvastatin (31) are certainly smaller than seen with simvastatin (32). It is also known that hepatic uptake of atorvastatin is mediated not only by OATP1B1 but also by OATP1B3 (33) and atorvastatin and its lactone appear to have lesser inhibitory effect

on mitochondrial complex III activity (34). In the present study, we found no evidence of contributions from any of the newly proposed candidate genes toward SRM. Surprisingly, we found no association between *CYP3A4**22 and SRM, even though the SNP rs35599367 has been associated with reduced CYP3A4 activity, which is involved in the metabolism of both simvastatin and atorvastatin and has been previously demonstrated to contribute to variability in simvastatin efficacy in the Dutch population (35).

In our study, patients were identified as SRM cases on the basis of having received either simvastatin or atorvastatin and discontinued treatment because of intolerable muscle symptoms which later resolved on drug withdrawal and recurred on re-challenge, without significant increase in CK. As the statin rechallenge was unblinded and often undertaken prior to referral we expect that a proportion of cases who failed rechallenge without CK elevation did not have true SRM as has been demonstrated by double-blind rechallenge with statins(7). While measurement of CK is recommended as a biomarker of muscle damage in symptomatic patients (9, 14, 36) and is important for classifying SRM according to severity, it is non-specific and cannot be used alone for establishing SRM. Since CK levels during initial exposure were frequently unavailable (at the time of recruitment the majority of cases had already stopped statin therapy), we cannot exclude the possibility that the severity grading in some of our patients may have been underestimated, potentially misclassifying SRM3 or even SRM4 cases as SRM2. However, this is also likely to reflect the situation in clinic practice and although challenging, prompt measurement of CK in symptomatic patients remains of central importance for the clinical assessment of SRM.

Our study suggests that, among people of European ancestry, *SLCO1B1* rs4149056 is a predisposing factor for SRM across the full spectrum of severity (SRM2 or greater) with simvastatin and atorvastatin at the highest recommended dose (40 mg and 80 mg respectively). It has been proposed that the *SLCO1B1* rs4149056 polymorphism may help identify patients at risk of progression to more severe statin-related myopathy. For

simvastatin users, possession of the C allele had a diagnostic sensitivity and specificity of 70.4% and 73.7%, respectively, giving positive and negative likelihood ratios of 2.7 and 0.4 for the prediction of definite or incipient myopathy during 5 years of 80 mg/day simvastatin use (37). However, in view of the low positive predictive value for progression to severe myopathy and high frequency of the C allele in statin tolerant patients, in our opinion the possession of the C allele is not a clinically useful diagnostic test to identify patients at risk of SRM.

In conclusion, our results demonstrate that symptoms reported by patients with SRM of moderate severity are at least in part, of pharmacological origin and cannot be attributed solely to a “nocebo effect”. *SLCO1B1* rs4149056 genotype is an important susceptibility factor which together with female gender, is associated with the full spectrum of SRM seen in patients treated with high doses of simvastatin and atorvastatin. However, we found no association between SRM and polymorphisms in any of the newly proposed gene candidates studied, and an accurate, predictive diagnostic test for susceptibility to SRM remains elusive. In view of their proven safety and low cost, statins will remain in clinical use for the foreseeable future. Therefore, in order to improve the already overwhelmingly positive benefit-risk ratio of statins clinicians must adopt a pragmatic approach to reduce the risk of SRM when prescribing statins, where possible avoiding the use of simvastatin which has the greatest potential for toxicity, by choosing lower initiation doses in women and other patients with lower body mass index and by avoiding co-administration of potentially interacting drugs.

METHODS

Patient recruitment, blood collection and case definitions

Ethical approval for the study was provided by NRES Committee North East - County Durham & Tees Valley. Patients requiring statin therapy for primary or secondary prevention of cardiovascular disease were recruited between May 2011 and October 2014 from outpatients attending the Lipid and Cardiology Clinics at the Royal Victoria Infirmary, Newcastle upon Tyne. Patients who had received a daily dose of simvastatin 40 mg or atorvastatin 80 mg (the two most commonly prescribed statins in our locality) for at least 6 months without new onset of muscle symptoms were defined as the statin tolerant control group. Patients who had previously received either simvastatin 40 mg or atorvastatin 80 mg but had discontinued the drug because of new muscle symptoms (intolerable muscle pain or weakness, with or without elevation of creatine kinase, onset within 6 months of starting therapy) which resolved on withdrawal and recurred with re-challenge at the same or a reduced dose were considered to have SRM and to be eligible for recruitment as cases. In each case the severity of muscle toxicity was graded according to the classification system based on the severity of muscle related symptoms (tolerable or intolerable) and the CK elevation (less than 4 times the upper normal limit in SRM2) proposed by Alfirevic and co-workers (8). The local laboratory reference ranges for creatine kinase were as follows: Male 10-190 U/L; Female 10-160 U/L.

After obtaining informed written consent, the medical records of those previously intolerant of simvastatin and/or atorvastatin were reviewed and a structured face-to-face interview was conducted by a trained research nurse. Patient demographics (including age, sex, height and weight), co-morbidities and medications were recorded. The indication for statin therapy, the daily doses and type of statins used and the regional distribution and temporal pattern of symptoms of muscle pain or weakness were recorded. In addition to routine serum biochemical measurements, each patient provided a venous blood sample (4 ml) which was transferred into EDTA tubes and stored at -80 °C for later genotyping.

Single nucleotide polymorphism (SNP) selection, DNA extraction and genotyping

SNPs of known functional significance were selected based on previous findings(9, 11, 38-42). Additionally, rs1050450 and rs713041 in glutathione peroxidases 1 and 4 (GPX1 and GPX4), respectively, were selected on the basis of decreased GPX activity associated with these SNPs reported by several previous studies (43-47) (**Table 5**). While there is evidence for the involvement of OATP2B1 and various multidrug resistance-associated proteins (MRPs) in transport of statins in striated muscle (48), there is no information available on the involvement of monocarboxylate transporters (MCTs), which are abundantly expressed in the skeletal muscle, and their polymorphisms in relation to SRM. We hypothesized that SRM could be promoted by the increased uptake of statins and/or accumulation of endogenous monocarboxylate metabolites (e.g. lactate) in skeletal muscle which mainly involve MCTs, possibly MCT1 and MCT4 (25, 26). Since there was no prior information available on the influence of either MCT1 (*SLC16A1*) or MCT4 (*SLC16A3*) gene on statin-related myopathy, tag-SNP selection was employed for these two genes (**Table 6**) with tag-SNPs in both MCT1 and MCT4 selected from the HapMap website (<http://www.hapmap.org>). The data from tagging approach from the HapMap website captured 100 % of alleles with mean r^2 0.994 and 1.0, for the *SLC16A1* and *SLC16A3* genes, respectively.

DNA extraction and genotyping

DNA extraction was carried out using perchloric acid-chloroform extraction method as described by Daly and co-workers (49). Most SNPs were genotyped by TaqMan assays designed and supplied by Applied Biosystems in a ABI StepOne Real Time PCR system (Paisley, UK) under the following thermocycling conditions: 10 min at 95 °C followed by 40 cycles of denaturation at 95 °C for 15s and annealing/extension at 60 °C for 1 min.

Genotypes for polymorphisms in ABCC2 (rs717620) and ABCG2 (rs2231142) together with

one SNP in SLC16A1 (rs1049434) were determined by PCR-RFLP (50) using the conditions summarized in **Table 6**.

Sample size calculation

Assuming a rs4149056 *SLCO1B1* minor allele frequency of 0.14 in our statin-tolerant patients, we calculated that a sample size of 600 patients (500 controls and 100 cases) with 85% power would detect an OR of 2 at a significance value of 0.05. We hypothesized that a multitude of other factors contribute to intolerance to simvastatin and atorvastatin, including clinical and environmental factors and common variants in the genes that mediate statin systemic exposure. However, as there is no *priori* information available on the variance in drug exposure (plasma simvastatin/atorvastatin concentration), and the quantitative effect of each genetic variant and other confounders including patient characteristics on the risk of statin muscle toxicity, we considered this sample size to be sufficient to detect any association of comparable magnitude.

Statistical analysis

Statistical analyses for all clinical parameters (expressed as mean, standard deviation, 95 % confidence interval, CI) were performed using Minitab statistical software (version 16, Coventry, UK). For univariate analysis, chi-square statistics were used for categorical variables and t-tests for continuous variables. Where necessary data were log transformed to achieve normality. For multivariate analysis, by taking a dichotomous variable (cases vs controls) as dependent variable, binary logistic regression was performed to assess independent patient, clinical and genetic risk factor/s associated with the cases. Comparison of genotype frequencies for one or two variant alleles between cases vs controls was made using binary logistic regression; Hosmer and Lemeshow goodness-of-fit test was used as an indicator of the validity at the last step of iterations. A dominant model for genotypes [i.e. wild-type vs (heterozygous + homozygous genotype)] was used for all SNPs studied due to

homozygous mutants being rare. For all analyses, a p-value of <0.05 was taken as being statistically significant.

STUDY HIGHLIGHTS

- What is the current knowledge on the topic?

The existence of an association between *SLCO1B1* rs4149056 and simvastatin-induced myopathy has been demonstrated only at higher drug doses and when CK elevation is included in the definition of statin-associated musculoskeletal symptoms.

- What question did this study address?

Are statin-associated muscle symptoms without significantly raised CK levels caused by statins or are they a placebo effect?

- What this study adds to our knowledge?

Patient-specific genetic and clinical factors associated with increased systemic exposure to statins are implicated in the full spectrum of SRM, from myalgia to severe myopathy.

- How this might change clinical pharmacology or translational science?

Pharmacological mechanisms contribute to the full spectrum of SRM including myalgia without CK elevation. A wider understanding of the predisposing factors to SRM will enable clinicians to optimize management while minimizing untoward effects in susceptible patients.

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CONFLICT OF INTEREST/ DISCLOSURES

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

NSB was involved in genetic and data analyses. DN was involved with study design and responsible for patient identification. PA was involved in study design and statistical analysis of data. CB was involved with preliminary cellular work which led to the identification and involvement of MCTs in statin transport. AKD supervised and designed the genetic analyses. FK was involved in study design and data analysis. NSB and FK drafted the manuscript. All authors were involved in redrafting of the manuscript.

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FIGURE LEGEND

Figure 1: Distribution of SLCO1B1 rs4149056 genotype between cases and controls (A) and between simvastatin and atorvastatin users in the case group (B)

SUPPLEMENTARY FILE LEGEND

Supplementary Table S1: Proportion of patients taking drugs known to interact with statins

Table S1: Proportion of patients taking drugs known to interact with statins

Drug	Control; n (%)	Cases; n (%)
Amiodarone	1(0.2)	0 (0.0)
Ciclosporin	0 (0.0)	0 (0.0)
Colchicine	0 (0.0)	2 (1.6)
Azithromycin	2 (0.4)	0 (0.0)
Clarithromycin	0 (0.0)	0 (0.0)
Erythromycin	1 (0.2)	0 (0.0)
Digoxin	9 (1.9)	1 (0.8)
Amlodipine	29 (6.0)	14 (11.2)
Lercanidipine	4 (0.8)	1 (0.8)
Diltiazem	4 (0.8)	2 (1.6)
Nifedipine	3 (0.6)	2 (1.6)
Verapamil	2 (0.4)	1 (0.8)
Ezetimibe	29 (6.0)	8 (6.4)
Bezafibrate	0 (0.0)	1 (0.8)
Fenofibrate	23 (4.8)	2 (1.6)
Itraconazole	0 (0.0)	0 (0.0)
Ketoconazole	0 (0.0)	0 (0.0)
Terbinafine	0 (0.0)	0 (0.0)
Sildenafil	1 (0.2)	1 (0.8)
Warfarin	51 (10.6)	3 (2.4)
PPI (-prazole)	138 (28.7)	19 (15.2)
Total	159 (33.1)	37 (29.6)

Table 1: Demographic and clinical characteristics of statin-tolerant (controls) and statin-intolerant (cases) subjects

Variables	Controls (n=481)	Cases (n=125)	P-value
Sex			0.004
Female, N (%)	212 (44.1%)	73 (58.4%)	
Male, N (%)	269 (55.9%)	52 (41.6%)	
Age at enrolment (years), mean (95 % CI)	60.9 (59.8-62.1)	60.3 (58.1 -62.5)	0.610
Body mass index (kg/m ²), median (LQ, UQ)	28.5 (25.8, 32.0)	28.5 (26.4, 31.7)	0.933
Duration on statin (months), median (LQ, UQ)	24 (12, 65)	8 (3, 18)	<0.001
Statin, N (%)			<0.001
Simvastatin,	297 (61.9)	107 [‡] (85.6)	
10 mg/day	-	8 (7.5)	
20 mg/day	4 (1.3)	15 (14.0)	
40 mg/day	286 (96.3)	78 (72.9)	
80 mg/day	7 (2.4)	4 (3.7)	
Atorvastatin,	183 (38.2)	18 [#] (14.4)	
10 mg/day	1 (0.5)	1(5.6)	
20 mg/day	2 (1.1)	-	
30 mg/day	1 (0.5)	-	
40 mg/day	12 (6.6)	1 (5.6)	
80 mg/day	167 (91.3)	15 (83.3)	
Plasma lipids, mean (± SD)			
Cholesterol, mmol/L	4.62 (1.72)	6.45 (1.71)	<0.001
Triglyceride, mmol/L	1.85 (1.46)	2.28 (1.49)	<0.001
HDL-cholesterol, mmol/L	1.45 (0.45)	1.48 (0.44)	0.852
Other medical conditions, N (%)			
Type 2 Diabetes	53 (11.0)	8 (6.4)	0.196
Hypertension	123 (25.6)	35 (28.0)	0.543
Hypothyroidism	28 (5.8)	11 (8.8)	0.216
Chronic Kidney Disease 3A	35 (7.3)	5(4.0)	0.249
Chronic Kidney Disease 3B-5	32 (6.7)	3 (2.4)	0.097
None	70 (14.6)	20 (16)	0.617
Statin interacting drugs, N (%)			
Digoxin	9 (1.9)	2 (1.6)	0.839
Fibrates	24 (5.0)	4 (3.2)	0.396
CYP3A4 inhibitor	3 (0.6)	0 (0)	-
Calcium antagonist (dihydropyridine)	36 (7.4)	17 (13.4)	0.031
Calcium antagonist (non-dihydropyridine)	6 (1.2)	3 (2.4)	0.342
Aspirin	148 (30.7)	27 (21.6)	0.129
Warfarin	51 (10.6)	3 (2.4)	0.008
Ezetimibe	29 (6.0)	8 (6.4)	0.877
Sildenafil	1 (0.2)	1 (0.8)	0.303

[‡] Initiation dose could not be confirmed for two individuals

[#]Initiation dose could not be confirmed for one individual

Table 2: Association between individual SNPs and SRM by univariate analysis

Gene and SNP	Geno- type	Frequency, n		P-value ^a	P-value	OR	95% CI for OR
		Controls ^b	Cases				
<i>SLCO1B1</i> rs4149056	WT	353	78	0.547	0.010	1.73	1.14- 2.62
	Het	112	45				
	Mut	11	2				
<i>ABCC2</i> rs717620	WT	300	80	0.626	0.917	0.96	0.64-1.44
	Het	158	40				
	Mut	18	5				
<i>ABCG2</i> rs2231142	WT	374	104	0.690	0.318	0.75	0.45 -1.26
	Het	94	19				
	Mut	7	2				
<i>CYP3A4</i> rs35599367 (*22)	WT	422	111	0. 729	1.000	1.00	0.54-1 .88
	Het	51	14				
	Mut	2	0				
<i>COQ2</i> rs4693075	WT	190	51	0.856	0.918	0.97	0.65- 1.44
	Het	219	57				
	Mut	66	17				
<i>GATM</i> rs9806699	WT	252	64	0.052	0.763	1.08	0.73-1.61
	Het	175	48				
	Mut	47	13				
* <i>GPX1</i> rs1050450	WT	245	63	<0.001	ND	ND	ND
	Het	160	52				
	Mut	54	10				
<i>GPX4</i> rs713041	WT	137	34	0.106	0.739	1.09	0.70-1.70
	Het	251	68				
	Mut	84	23				
<i>SLC16A1</i> rs1049434	WT	130	39	0.059	0.435	0.84	0.55-1.29
	Het	254	59				
	Mut	88	27				
rs3849174	WT	308	70	0.639	0.077	1.45	0.97-2.16
	Het	151	51				
	Mut	16	4				
rs4301628	WT	206	63	0.437	0.189	0.75	0.51-1.12
	Het	219	52				
	Mut	50	10				
<i>SLC16A3</i> rs7503429	WT	130	36	0.797	0.823	0.95	0.61-1.47
	Het	236	82				
	Mut	103	7				
rs12453290	WT	217	64	0.128	0.270	0.80	0.54-1.18
	Het	219	47				
	Mut	40	14				

*The rs1050450 SNP was excluded from subsequent analyses.

^a P-value for HWE calculated in control group and compared with CEU population.

^b Genotype results for all the SNPs investigated were unavailable for 5 individuals from control group.

WT; wild type, Het; Heterozygous genotype, Mut; Homogenous mutants, OR; Odds ratio, ND; not determined.

Table 3: Association between patient sex, rs4149056 SNP and SRM (adjusted for patient age, sex, BMI and other 11 genotypes) according to binary logistic regression analysis

Dependent Variables	Controls	Cases	Explanatory variables	P-value [#]	OR	95% CI for OR [¶]
Statin-related muscle toxicity	481*	125*	Patient sex	0.006	1.72	1.15-2.59
			<i>SLCO1B1</i> (rs4149056)	0.014	1.66	1.08-2.54
			<i>ABCC2</i> (rs717620)	0.800	0.96	0.64-1.49
			<i>ABCG2</i> (rs2231142)	0.228	0.74	0.44-1.25
			<i>CYP3A4</i> *22 (rs35599367)	0.884	0.98	0.52-1.86
			<i>COQ2</i> (rs4693075)	0.837	0.98	0.65-1.48
			<i>GATM</i> (rs9806699)	0.747	1.03	0.68-1.54
			<i>GPX4</i> (rs703041)	0.733	1.09	0.69-1.70
			<i>SLC16A1</i> (rs1049434)	0.456	0.83	0.49-1.44
			<i>SLC16A1</i> (rs3849174)	0.121	1.29	0.78-2.04
			<i>SLC16A1</i> (rs4301628)	0.406	0.80	0.49-1.33
			<i>SLC16A4</i> (rs7503429)	0.990	0.95	0.61-1.51
			<i>SLC16A4</i> (rs12453290)	0.310	0.81	0.54-1.22

* Genotypes from 6 individuals (5 in controls and 1 case) were not included in the analysis.

[#] P-value for comparison of genotypes frequencies possession of one or two variant alleles between cases vs controls

[¶] Odds ratio describes the odds to be a case relative to a control when having at least one variant allele (or being female).

Table 4: The relative risk of SRM in male and female *SLCO1B1* rs4149056 carriers (TC+CC genotypes) compared to wild-type (TT genotype) and wild-type female compared to male (TT genotype)

Category	OR	95 % CI	P-value
Male (<i>TC +CC</i>) vs male <i>TT</i> genotypes	2.43	1.3140, 4.4843	0.0046
Female (<i>TC +CC</i>) vs female <i>TT</i> genotypes	2.93	1.5847, 5.4279	0.0006
Female <i>TT</i> vs Male <i>TT</i> genotype	2.25	1.3550, 3.7212	0.0017

Table 5: SNPs selected for genotyping on the basis of previous genotyping studies on SRM

Gene	SNP	Evidence of the association with SRM/ reason for the selection	Reference(s)
<i>SLCO1B1</i>	rs4149056	Encodes a liver-specific member of the organic anion transporter family. The protein is involved in the removal of drug compounds such as statins. OR for SRM = 4.5.	(9, 18)
<i>ABCC2</i>	rs717620	Protein encoded by this gene is included in the superfamily of ATP-binding cassette (ABC) transporters. ABC proteins transport various molecules across extra- and intra-cellular membranes. Associated with switches to other cholesterol-lowering drugs during simvastatin therapy.	(38)
<i>ABCG2</i>	rs2231142	Protein encoded by this gene is included in the superfamily of ATP-binding cassette (ABC) transporters. ABC proteins transport various molecules across extra- and intra-cellular membranes. Polymorphism markedly affects PKs of simvastatin acid.	(40, 42)
<i>CYP3A4</i>	rs35599367 (*22 allele)	Encodes a member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins are monooxygenases that catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. Associated with statin metabolism and response.	(35, 39, 42)
<i>COQ2</i>	rs4693075	Encodes an enzyme that functions in the final steps in the biosynthesis of CoQ (ubiquinone), a redox carrier in the mitochondrial respiratory chain and a lipid-soluble antioxidant. Polymorphism associated with statin intolerance and myopathy.	(11)
<i>GATM</i>	rs9806699	Gene encodes a mitochondrial enzyme that belongs to the amidinotransferase family. The enzyme is involved in creatine biosynthesis. Meta-analysis odds ratio for SRM for this polymorphism = 0.60	(41)
<i>SLC16A1</i>	rs9429505	Encodes for the monocarboxylate transporter (MCT1) which is abundantly expressed in the membrane of striated muscle.	(26)
<i>SLC16A3</i>	rs7503429	Encodes for the monocarboxylate transporter (MCT4) which is abundantly expressed in the membrane of striated muscle.	(26)

Table 6 : Tag SNPs in SLC16A1 and SLC16A3 genes selected for genotyping and the primers, PCR conditions and restriction enzymes used for ABCC2 (rs717620), ABCG2 (rs2231142) and SLC16A1 (rs1049434) genotyping by PCR-RFLP

Gene	Test	Alleles captured	Assay	Primer set	PCR conditions¶	PCR product (bp)	Restriction enzyme (Recognition site)
SLC16A1 (MCT1)	rs9429505	rs7552903, rs4839272, rs2149036, rs4839270, rs3849174 , rs7536532, rs9429505, rs7518984	ABCC2 rs717620	Forward 5'-TGTCATCCACTGTTTCAATG - 3' Reverse 5'-CTGGACTGCGTCTGGATC- 3'	Annealing at 54°C for 1 min	193	TaqI (TCGA)
	rs7556664	rs3789592, rs7169, rs1049434 , rs6537765, rs7556664					
	rs12028967	rs4301628 , rs12028967	ABCG2 rs2231142	Forward 5'- GTCTCATTA AATGCTATTT - 3' Reverse 5'- CTCTTGAATG ACCCTGTTGA - 3'	Annealing at 50°C for 35 sec	151	MseI (TTAA)
SLC16A3 (MCT4)	rs7503429 rs12453290	rs7503429 rs12453290	SLC16A1 rs1049434	Forward 5'- TCAATGAACAACTGGTATGA- 3' Reverse 5'- ATGGAGACTACAAATACACA-3'	Annealing at 55°C for 1 min	387	Bccl (CCATC)

SNPs in bold text were chosen for genotyping based upon MAF >0.20 value obtained from 1958 British birth cohort website <http://www.b58cgene.sgul.ac.uk/>.

¶ Apart from the annealing temperatures described above, all PCR assays involved 35 cycles, with denaturation at 94°C for 1 min, extension at 72°C for 1 min and were held in the last cycle at 72°C for 7 min to ensure elongation except for rs2231142. For rs2231142, the period for each step in each cycle was 35 sec instead of 1 min. The ABCG2 and SLC16A1 assays were developed for this project. The ABCC2 assay was described previously (42).