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Association of Common Variants in the Human Eyes Shut Ortholog, *EYS*, with Statin-Induced Myopathy: Evidence for Additional Functions of *EYS*

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Competing Interests

The authors declare no financial conflicts of interest.

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Abstract

Introduction—Of nearly 38 million people in the U.S. receiving statin therapy, 0.1–0.5% experience severe or life-threatening myopathic side effects.

Methods—We performed a genome-wide association study (GWAS) in patients with severe statin myopathy versus a statin-tolerant group to identify genetic susceptibility loci.

Results—Replication studies in independent groups of severe statin myopathy (n=190) and statintolerant controls (n=130) resulted in the identification of three SNPs, rs9342288, rs1337512 and rs3857532, in the eyes shut homolog (*EYS*) on chromosome 6 suggestive of an association with risk for severe statin myopathy ($p=0.0003$ – 0.0008). Analysis of *EYS* cDNA demonstrated that *EYS* gene products are complex and expressed with relative abundance in the spinal cord as well as in the retina.

Discussion—Structural similarities of these *EYS* gene products to members of the Notch signaling pathway and to agrin suggest a possible functional role in the maintenance and regeneration of the structural integrity of skeletal muscle.

Keywords

Statin; myopathy; genetic association; notch; agrin; muscle; spinal cord

Introduction

Statin therapy has proven to be highly successful for treatment of hypercholesterolemia and prevention of cardiovascular disease ^{1,2}. Common side effects are muscle pain and weakness ^{3,4} which, in rare cases, progress to severe myopathic conditions and rhabdomyolysis ^{5,6}. The exact biological mechanism of statin-induced myopathy is unknown. Statins have been shown to cause both structural changes in skeletal muscle ⁷ and changes in gene expression in statin-tolerant patients ⁸. Higher drug doses produce myotoxic effects in animal studies ⁹. Proposed mechanisms for statin-induced myopathies primarily involve additional pathways influenced by HMG-CoA inhibition beyond cholesterol synthesis such as coenzyme Q synthesis ^{10,11}, isoprenylation and N-glycosylation ¹². A number of neuromuscular disorders have been reported coincident with statin treatment including metabolic myopathies ¹³, polymyositis ¹⁴, dermatomyositis ¹⁵, malignant hyperthermia ¹⁶, polyneuropathy ¹⁷ and amyotrophic lateral sclerosis-like syndrome ¹⁸. This association with a wide variety of neuromuscular diseases suggests that statin therapy compromises the neuromuscular system. Therefore, identifying individuals who are at risk based on underlying genetic susceptibility and/or because of undiagnosed neuromuscular disease may potentially allow for the development of pharmacogenetic approaches to disease prevention. A study reported in 2008 examined a group of patients who developed statin-induced myopathy associated with high-dose simvastatin therapy and identified a strong association with variants of the *SLCO1B1* gene ¹⁹. We have used the genome-wide association study (GWAS) approach in an attempt to identify unrecognized genes that may contribute to the pathology of severe statin myopathy. Here we report evidence for the association of three SNPs within a region of chromosome 6 which contains the recently identified gene, eyes shut homolog (*EYS*) ^{20,21} and encodes for protein products related to Notch and agrin.

Materials and Methods

Subjects

Clinical and histopathologic information was used as summarized in Table 1 and described in detail below to classify individuals according to phenotype. Test and control subjects were derived from more than 30 medical centers in 20 states and provinces in the USA, Canada and Australia representative of no single geographic location. Test subjects were specifically identified as having severe statin-induced myopathy as defined below. Control subjects were recruited at collaborating centers following at least 6 months of statin therapy without myopathic symptoms. Individuals who were not Caucasian were excluded due to small sample size. The study was approved by the Health Sciences Institutional Review Board of the University at Buffalo, and participants provided written informed consent.

Severe Statin Myopathy

Patients with severe statin myopathy were defined as those who had symptoms of severe and incapacitating muscle pain and/or weakness with onset following initiation of statin therapy. The time of onset of symptoms may have varied, however the association with statin therapy must have been clear to the referring physician. Within this definition there are those who recover when therapy is terminated and those who continue to have severe symptoms post-therapy that may become progressively worse with time. Individuals with severe statin myopathy generally have serum creatine kinase (CK) levels >4 times the upper limit of normal (ULN) suggestive of various degrees of rhabdomyolysis⁶, however some have no elevation in CK yet still may have serious symptoms of myalgia and/or weakness²².

Cases with Severe Statin Myopathy for the Pooled GWAS—This group consisted of 39 individuals (67% male) with severe statin myopathy. Subjects ranged in age from 21 to 83 years (mean age 61 ± 14 yrs). Prominent features for inclusion in this group included the requirement of either progressive or persistent myopathic symptoms for weeks to indefinite periods post-therapy, muscle weakness, muscle pain, elevated serum CK and fatigue. Muscle biopsy data was available for 22 of these patients. The findings were relatively nonspecific and included fiber atrophy (45%), the presence of storage material (lipid or glycogen, 60%), and either histochemical or electron microscopic evidence for increased mitochondrial content (33%). Patients were not biopsied during or just after an attack of rhabdomyolysis.

Cases with Severe Statin Myopathy for the Replication Study—A total of 190 Caucasian subjects (67% male) with characteristics of severe statin myopathy similar to those chosen for the pooling experiment were included in this group. Subjects ranged in age from 31 to 85 years (mean age 57 ± 11 yrs). Prominent symptoms included persistent and/or progressive symptoms post-therapy, muscle weakness, muscle pain or cramps and elevated serum CK ≥ 4 times ULN. Muscle biopsy data were available for 45 patients. Prominent histopathologic findings included fiber atrophy (67%); presence of storage material (24%); and evidence for increased mitochondrial content (18%).

Statin-tolerant Controls—The pooled GWAS control group was comprised of an age- and gender-adjusted group of 40 statin-tolerant Caucasian subjects (65% male) ranging in age from 42 to 81 years (mean age 57 ± 11 yrs) who had remained asymptomatic for at least 6 months during therapy, and most had experience extended periods with ongoing therapy. On followup, they were excluded from this control group if they developed myalgias on statins. A group of 130 Caucasian statin-tolerant subjects were used as controls for the fine mapping study (70% female). Subjects ranged in age from 29 to 83 years (mean age 59 ± 12 yrs). These individuals had no muscle symptoms during ≥ 6 months of statin therapy.

Statin Therapy

Information was available on the type of statin used for therapy in 90% of cases and controls who were taking statins. Atorvastatin was the most common statin used in all groups, including 66% of severe statin myopathy subjects and 84% of statin-tolerant controls. Dosage information was difficult to obtain, because most patients were prescribed several different statins over time at various dosages in an attempt to eliminate myopathic side effects. Our goal was to record the statin that first produced myopathic symptoms and, when possible, the dosage. Of those taking atorvastatin for which dosages were available, greater than 50% had taken or were taking the lowest dose (10 mgs). Among the severe cases, a wider variety of statins were represented. Simvastatin was used in 20% of cases versus only 6% of statin-tolerant controls. Other statins represented among severe cases included cerivastatin (5%) and pravastatin (9%). Among statin-tolerant controls, 10% took lovastatin.

DNA Isolation & Pooled GWAS

Genomic DNA was prepared from whole blood collected in EDTA or from skeletal muscle with Puregene DNA isolation kits (Gentra Systems). All genomic DNAs used for the pooled experiment were prepared from whole blood. Thirty-nine individuals with severe statin myopathy and 40 statin-tolerant controls were used for this experiment. For the pooled DNA analysis, the Affymetrix 100K SNP microarray (https://www.affymetrix.com/support/downloads/manuals/100K_manual.pdf) was used, with two pools of 19 and 20 cases and two pools of 20 controls each, run in parallel; according to standard protocols²³. Data from the GWAS has been submitted to the NIH GEO database, <http://www.ncbi.nlm.nih.gov/geo/>, accession number GSE17574.

Validation of the Pooled GWAS

The validation included available DNA from 31 severe statin myopathy cases and 40 statin-tolerant controls from the same individuals included in the pooled GWAS. Insufficient amounts of DNA were available for 8 of the original 39 SM pool samples. An iPLEX Gold assay system (Sequenom, San Diego, CA), which involves multiplex PCR amplification followed by single nucleotide primer extension and analysis by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), was used through the Roswell Park Cancer Institute MassArray Facility (Buffalo, NY). Three multiplex assays were designed using Sequenom software to genotype a total of 107 SNPs selected for further testing based on results of the pooled GWAS. Six of the SNP assays failed and results for the remaining 101 SNPs are shown in Supplementary Tables S1 and S2.

Replication in Independent (Non-Pooled) Samples

MALDI-TOF MS was used to type 101 SNPs from 67 cases and 42 controls who were independent of the individuals used in the pooled GWAS.

Fine Mapping Study

Twenty seven SNPs in a 400 kb region surrounding rs1337512 were genotyped on an Illumina BeadStation 500GX instrument using the Infinum II genotyping assays following manufacturer's recommendations. These 27 SNPs were predicted by Tagger software (Haploview v.4.0^{24,25}) to capture the allelic variation in the 335 SNPs present in the region surrounding rs1337512 (chr6:64,950,000–65,350,000). Fine mapping was performed with 190 cases and 130 controls, and the results for 25 SNPs are shown in Table 2. Results for two of the SNPs, rs9351261 and rs13217762, deviated significantly from Hardy-Weinberg equilibrium ($p < 0.01$) and are not included.

Statistical Analysis for genetic association tests

SNP tests for the pooled GWAS were performed using the methods of Sham et al.²⁶ and Macgregor et al.²⁷. For the remaining SNP association tests (non-pooled), the quality of the genotyping data was assessed by predetermined quality control inclusion criteria (MAF>5%, SNP call rate>90%, and HWE p value>0.01 among the controls). Allelic association tests and genotype-based association tests were conducted using standard chi-square analysis²⁸ and logistic regression, respectively, with and without adjustment for age and sex. We estimated both asymptotic p -values and Bonferroni adjusted p -values.

cDNA Analysis

First strand cDNA was prepared from total RNA from human spinal cord, skeletal muscle, brain, and retina (Clontech, Mountain View, CA) with AffinityScript reverse transcriptase (Stratagene, La Jolla, CA). PCR amplification primers spanning the *EYS* gene (Figure S1, Table S5) were designed based on the computer predicted sequences in this region beginning with XM_371829.2 and continuing with *EGFL11* (NM_198283), XM_371829.4 and XM_001126083. Regions of the cDNA spanning the entire *EYS*/spasm transcript were PCR amplified from first strand cDNA with Easy-A high-fidelity PCR cloning enzyme (Stratagene), purified with QIAquick columns (Qiagen, Valencia, CA) and directly sequenced. PCR products were cloned into TOPO-pCR2.1 (Invitrogen, Carlsbad, CA), and individual clones containing different sized inserts were also sequenced. Sequencing was performed with the Big Dye Terminator v3.1 Cycle Sequencing kit (ABI) on an ABI 3100 Genetic Analyzer by the Roswell Park Cancer Institute Biopolymer Facility (Buffalo, NY).

Bioinformatics

DNA sequence obtained from the cDNA cloning experiments was aligned with the human genomic sequence with human BLAT search software (<http://genome.ucsc.edu/cgi-bin/hgBlat>). Comparisons with other species and EST databases were performed with BLAT and BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) software. Protein domain homologies were identified with SMART protein domain (<http://smart.embl-heidelberg.de/>) and pFAM database (<http://pfam.sanger.ac.uk/>) software.

Results

A genome-wide association study was conducted in genomic DNA from patients with severe myopathic symptoms initiated with statin therapy. We required that persistent and/or progressive symptoms be present post-therapy for inclusion. A pooled sample approach was used²⁶, with validation in individual samples, followed by replication and fine mapping studies according to Figure 1. The pooled groups included a test group of individuals with severe statin-induced myopathy and a control group of statin-tolerant individuals on statin therapy for at least 6 months with no myopathic symptoms (see Methods). The pooled analysis identified 3,995 significantly associated SNPs with $p<0.05$. From these SNPs, 101 were selected for further testing based on three criteria: 1) the significance within the pooling experiment (based on z^2), 2) the location within putative candidate genes, and 3) the presence of multiple apparent associations located within one gene or within 2000 bp of each other (see supplementary material, Tables S1,S3,S4).

The group of 101 selected SNPs was genotyped in 31 of the 39 severely affected individuals and each of the 40 control individuals from the pooling experiment. Of the 101 selected SNPs, 26 were significantly associated with statin-induced myopathy in allelic association tests ($p<0.05$).

A replication study was designed to examine the 101 selected SNPs in an independent group of cases and controls. Of the 26 SNPs that remained significant in the individual analysis of pooled samples, two (rs1337512 on chromosome 6 and rs1476647 on chromosome 7) achieved statistical significance in the replication study ($p < 0.05$). The strongest of these apparent associations, rs1337512, which was also located within a possible candidate gene, was pursued by a fine mapping study. The chromosome 7 SNP, rs1476647, was not further pursued based on the non-significance of age and sex-adjusted estimates (Table S1).

Fine mapping of the surrounding region of rs1337512 in *EYS* allowed for further characterization of the identified association. The rs1337512 SNP and 26 additional SNPs spanning this region on chromosome 6 were genotyped and tested for genetic association in larger groups of severe statin myopathy and statin-tolerant individuals (Table 2). The composition and characteristics of these groups are described in Materials and Methods and Table 1. Allelic association tests identified two additional SNPs, rs9342288 ($p = 0.0008$) and rs3857532 ($p = 0.0003$), with equivalent or lower p values than rs1337512 ($p = 0.0008$). These associations remained significant following Bonferroni corrections for testing 27 SNPs ($p = 0.02$ – 0.008) but did not survive correction for the GWAS. With respect to genotypic risk, estimates for individuals carrying one and two minor alleles were similar, suggesting dominance, with an odds ratio (OR) of 2.65 (95% CI: 1.51–4.67).

Post hoc power calculations reveal the fine mapping study was powered to detect ORs of approximately 1.60 for the more common variants ($MAF > 0.3$). For larger effect sizes, such as that identified for rs1337512 (crude OR, 95% CI: 1.82, 1.21–2.74), we were fully powered to detect this genetic association, given our sample size²⁹.

The rs1337512, rs9342288 and rs3857532 SNPs are located within the *EYS* gene on chromosome 6^{20,21} which spans approximately 2 Mb from position 64,430,039 to 66,417,118. During the initial discovery of this association, rs1337512 was located in a region of chromosome 6 annotated as LOC389405. We used PCR amplification of cDNA from human skeletal muscle, spinal cord and brain with primers spanning different combinations of putative exons from predicted coding sequences for genes surrounding the region of association on chromosome 6. This led to the identification of a larger transcript that was only expressed in spinal cord and essentially identical to *EYS*/spacemaker (spam) which was identified during the course of this study^{20,21} (Figures 2,S2). The results of these experiments show that the *EYS* gene products are complex, resulting from many differentially spliced variants, and they are expressed with relative abundance in the spinal cord and retina (Figure 3). The predominant form of the spam transcript as shown in Figure 2 is the result of direct sequencing of PCR products from human spinal cord cDNA. The spinal cord spam transcript contains 45 exons, which encodes 3196 amino acid residues containing a signal peptide, 28 EGF-like domains and 5 laminin G domains (Figures 2,S2). In our analysis, we identified numerous alternatively spliced *EYS* transcripts and multiple promoters. Regions of the 3' end of the *EYS* gene beginning with exon 37 are more widely expressed in other tissues, but the full length transcript was only identified in retina and spinal cord (Figure 3). This extensive alternative splicing is also found in the homologous agrin gene in which numerous transcript variants are responsible for the multiple distinct biological activities of their gene products³⁰.

Discussion

While the *EYS* gene was originally reported as an eye-specific gene^{20,21}, our finding of expression in the spinal cord is consistent with findings in *Drosophila* where *EYS*/spam expression occurs in the photoreceptor cells of the eye, the central nervous system and peripheral sensory ganglia³¹. Two different functions of *Drosophila EYS*/spam have been

identified by genetic screens. Spam is essential for the formation of a matrix-filled intra-rhabdomeral space within ommatidia of the *Drosophila* retina^{31,32}. This function is consistent with the finding that mutations in human *EYS* result in retinitis pigmentosa^{20,21}. Second, *Drosophila* spam has been identified in a screen for a heat-sensitive loss of locomotor function³³. Extracellular spam was shown to protect mechanoreceptor neurons and to be capable of protecting other cell types from hyper-osmotic shock. Cell damage of motor neurons and/or skeletal muscle cells by hyper-osmotic shock is a possible mechanism in the pathology of exercise intolerance and statin-induced myopathy, i.e. human spam could protect motor neurons and/or skeletal muscle cells from exercise-induced damage.

The sequence similarity of the EGF-like repeats in the N-terminal region of spam to the EGF-like repeats of Notch1 suggests a role in the Notch signaling pathway, since proteins containing similar clusters of EGF-like domains in the Notch pathway have been shown to bind to each other. Members of the Notch family and their ligands play fundamental roles in development,³⁴ and mutations within these genes are responsible for a number of human diseases including aortic valve disease and the most common form of hereditary stroke disorder (CADASIL)^{35–37}. The sequence similarity of spam to Notch1, along with the observed expression of spam in spinal cord, is intriguing in view of the involvement of the Notch signaling pathway in satellite cell differentiation to myoblasts and regeneration of muscle tissue after exercise³⁸. A reduction in the ability of satellite cells to regenerate due to reduced Notch signaling has been specifically proposed as the reason for the decline of regenerative potential in aging muscle^{38,39}.

Laminin G domains bind to a wide variety of ligands including proteins, carbohydrates and steroids^{40–42}. The three agrin laminin G domains, which in human sequence comparisons are most similar to spam LG1–5, are known to specifically bind at least four different proteins⁴³. Structure/function studies with laminin G domains of agrin suggest that binding to α -dystroglycan concentrates agrin at the muscle surface facilitating the activation of muscle-specific tyrosine-protein kinase receptor³⁰. It is therefore possible that motor neuron-derived spam could bind α -dystroglycan and allow its specific interaction with an as yet unidentified specific receptor to be expressed by muscle cells. Similarly, α -dystroglycan binding by spam could serve to localize the N-terminal Notch-like ligand to the extracellular matrix in proximity to satellite cells where it could act as a positive regulator of the Notch pathway.

The significant SNP-myopathy associations we identified within intron 26 of the *EYS* gene near the first laminin G domain (LG1) are located 38–85 kb from exon 27, and it is therefore difficult to predict a functional effect. It is possible that one of these or a more strongly associating variant within this region has long range regulatory effects on *EYS* expression or splicing that result in a loss or alteration of function in neuromuscular tissue. Splicing variations could potentially prevent the laminin G multidomain region from binding to its specific receptor or interfere with α -dystroglycan binding which would impair extracellular localization of spam and indirectly prevent specific receptor binding by either the laminin G or Notch-like domains. Loss-of-function sequence variations in *EYS* could thus result in a diminished capacity for muscle regeneration, and this would be consistent with the symptomatic manifestations of statin-induced myopathy.

A strong association of polymorphic variants in the *SLCO1B1* gene only with high dose (80 mg) simvastatin has recently been found with a genome-wide scan of a different group of statin myopathy patients¹⁹. We did not genotype the reported SNPs rs4149056 or rs4363657 in our pooled GWAS, but we did interrogate rs2900478 which is in strong LD with the two aforementioned SNPs. The resulting statistic was not significant in our group of severe statin myopathy patients. The *SLCO1B1* product, a transporter protein (OATP1B1), is predicted to

be involved in simvastatin clearance. When tested for an association with atorvastatin, the recent STRENGTH study showed that there was an increased risk in *SLCO1B1* carriers taking atorvastatin, however, the finding was not statistically significant⁴⁴. Pravastatin was the only other cholesterol-lowering drug tested, and there was no association found in carriers of the *SLCO1B1* variant. No other agents (rosuvastatin, lovastatin, or fluvastatin) have been evaluated as yet for an association with the *SLCO1B1* variant⁴⁵. Therefore, the association we describe with *EYS* and severe statin myopathy across a variety of statins and dosages is the first that provides possible insight into a mechanism for statin myopathy.

The possibility of population stratification cannot be ruled out, since we did not explicitly control for it in the study design. In this study, where cases are rare in the population and obtained from multiple centers, it was particularly difficult to obtain a hospital-based control group that perfectly represents the source population. We considered the use of statin-tolerant controls to at least be superior to using unexposed subjects from the general population.

The results of this GWAS, replication and fine mapping study provide the first reported suggestive evidence that genetic variants (rs1337512, 3857532 and 9342288), within the *EYS* gene, are associated with severe statin myopathy and suggest that only one copy of the variant is necessary to confer this risk. Our study also demonstrates that *EYS* gene products are complex, resulting from numerous alternative splicing patterns, and relatively abundant in the spinal cord in addition to the retina. A proposed mechanism for *EYS* gene involvement in severe statin myopathy is that loss of function variants may result in diminished capacity for the regeneration of damaged muscle. This biologic plausibility supports the *EYS* gene as a candidate gene for susceptibility to statin-induced myopathy that warrants further replication and functional study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

<i>AMPD1</i>	adenosine monophosphate deaminase 1
<i>CADASIL</i>	cerebral autosomal dominant arteriopathy with sub-cortical infarcts and leukoencephalopathy
<i>CEU</i>	Utah residents with Northern and Western European ancestry
<i>CI</i>	confidence interval
<i>CK</i>	creatine kinase
<i>CPT2</i>	carnitine palmitoyltransferase 2
<i>EDTA</i>	ethylenediaminetetraacetic acid
<i>EGF</i>	epidermal growth factor-like

EYS	eyes shut homolog
FDR	false discovery rate
GP	general population
GWAS	genome-wide association study
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
LD	linkage disequilibrium
LG	laminin G
MAF	minor allele frequency
MALDI-TOF MS	matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
OR	odds ratio
PCR	polymerase chain reaction
PYGM	glycogen phosphorylase
RT-PCR	reverse transcriptase-polymerase chain reaction
SE	standard error
SLC01B1	Solute carrier organic anion transporter family member 1B1
SM	severe statin myopathy
SNP	single nucleotide polymorphism
spam	spacemaker
ULN	upper limit of normal

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Figure 1. Multi-stage study design

***EYS/spam*, spinal cord**

Figure 2. Exon and protein domain structure of *EYS/spam* identified in human spinal cord cDNA

EGF-like repeats are indicated in the protein representation in gold and laminin G modules are blue. The green arrow indicates the location of the SNPs associated with statin myopathy, rs9342288, rs1337512 and rs3857532, in intron 26.

Figure 3. PCR amplification of the 3' end of the *EYS* gene

EYS cDNA was amplified from retina, spinal cord, skeletal muscle and brain cDNA. Lane 1) PCR amplification with primers spanning *EYS* exons 27–43, lane 2) exons 37–43, lane 3) exons 39–43, lane 4) exons 27–45, lane 5) exons 37–45, lane 6) exons 39–45. Only transcripts from the 3' region of the gene, beginning with exons 37 or 39 but not 27, are amplifiable from skeletal muscle and brain.

Table 1

Characteristics of Study Subjects

Patients	Age (y)	Gender	Prominent Symptoms	Muscle Pathology
Severe Statin Myopathy				
Pooled GWAS (n=39)	61±14 [21–83]	67% male	Progr/persistent symptoms post-therapy (100%); muscle weakness (59%); muscle pain (44%); fatigue (28%); elev CK ≥4XULN (51%; 40% of these had elev CK ≥10XULN)	Fiber atrophy (45%); lipid/ glyc storage (60%); elev mitochond content (33%)
Replication (n=190)	57±11 [31–85]	67% male	Progr/persistent symptoms post-therapy (25%); muscle weakness (28%); muscle pain (34%); fatigue (24%); elev CK ≥4XULN (70%; 43% of these had elev CK ≥10XULN)	Fiber atrophy (67%); lipid/ glyc storage (24%); elev mitochond content (18%)
Statin-Tolerant Controls				
Pooled GWAS (n=40)	57±11 [42–81]	65% male	No myopathic symptoms for ≥ 6mos of statin therapy	NA
Replication (n=130)	59±12 [29–83]	70% female	No myopathic symptoms for ≥ 6mos of statin therapy	NA

y, years; progr, progressive; elev CK ≥4XULN, elevated plasma creatine kinase ≥4 times the upper limit of normal; glyc, glycogen; mitoch, mitochondria; exerc intol, exercise intolerance

Table 2

Allelic Association Results for 25 SNPs in the *EYS* Gene

SNP	chr6 position	MAF cases n=190	MAF controls n=130	p	OR (95% CI)
rs4509103	64900230	0.03	0.03	0.88	1.08 (0.41–2.82)
rs9353681	64984651	0.19	0.26	0.03, 0.82*	0.66 (0.45–0.96)
rs12208631	65051529	0.05	0.05	0.71	0.87 (0.43–1.79)
rs11752403	65061880	0.06	0.06	0.78	1.10 (0.57–2.14)
rs10944484	65067404	0.15	0.21	0.04, 0.99*	0.66 (0.44–0.99)
rs626316	65067582	0.31	0.23	0.02, 0.45*	1.55 (1.08–2.23)
rs17479806	65142654	0.02	0.04	0.41	0.68 (0.26–1.73)
rs644929	65168776	0.39	0.28	0.003, 0.09*	1.67 (1.18–2.34)
rs9342288	65187046	0.38	0.25	0.001, 0.02*	1.81 (1.28–2.57)
rs7754782	65189913	0.03	0.03	0.96	0.98 (0.37–2.60)
rs9451737	65193357	0.01	0.02	0.59	0.68 (0.17–2.75)
rs1337512	65193820	0.38	0.25	0.001, 0.02*	1.81 (1.28–2.57)
rs9451846	65219452	0.01	0.02	0.81	0.85 (0.23–3.21)
rs16895413	65224009	0.03	0.01	0.19	2.32 (0.63–8.50)
rs6934305	65225433	0.02	0.01	0.13	3.13 (0.67–14.6)
rs3857532	65233665	0.42	0.28	0.0003, 0.008*	1.87 (1.33–2.63)
rs2993117	65243245	0.08	0.05	0.18	1.56 (0.81–3.00)
rs2993118	65243525	0.42	0.34	0.04, 0.95*	1.42 (1.02–1.97)
rs12189763	65244987	0.29	0.28	0.81	1.04 (0.74–1.48)
rs2050621	65245338	0.27	0.24	0.44	1.16 (0.80–1.66)
rs2782295	65266980	0.28	0.30	0.50	0.89 (0.63–1.25)
rs16880108	65273495	0.28	0.25	0.48	1.14 (0.79–1.63)
rs587592	65276741	0.28	0.30	0.52	0.89 (0.63–1.26)
rs17403480	65278124	0.10	0.13	0.15	0.70 (0.42–1.14)
rs10494881	65286066	0.11	0.13	0.50	0.85 (0.52–1.37)

MAF: Minor allele frequency, SM: severe statin myopathy, SC: statin-tolerant control group

* Bonferroni-adjusted p value for 27 SNPs.

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