Title

Laboratory Medicine in the Clinical Decision Support for Treatment of Hypercholesterolemia Pharmacogenetics of Statins

Journal

Clinics in Laboratory Medicine, 36(3)

ISSN

0272-2712

Authors

Ruaño, Gualberto
Seip, Richard
Windemuth, Andreas
et al.

Publication Date

2016-09-01

DOI

10.1016/j.cll.2016.05.010

Peer reviewed

HHS Public Access

Author manuscript

Clin Lab Med. Author manuscript; available in PMC 2017 September 01.

Published in final edited form as:

Clin Lab Med. 2016 September ; 36(3): 473–491. doi:10.1016/j.cll.2016.05.010.

Laboratory Medicine in the Clinical Decision Support for Treatment of Hypercholesterolemias: Pharmacogenetics of Statins

Gualberto Ruaño, M.D., Ph.D.[1], **Richard Seip, Ph.D.**[2], **Andreas Windemuth, Ph.D.**[3], **Alan H. B. Wu, Ph.D.**[4], and **Paul D. Thompson, M.D.**[5]

[1]Genomas Inc., Hartford, CT

[2]Sanofi Genzyme, Cambridge, MA

[3]Cyclica Inc., Cambridge, MA

[4]San Francisco General Hospital, San Francisco, CA

[5]Division of Cardiology, Hartford Hospital, Hartford, CT

Keywords

Statins; Myopathy; Coenzyme Q_{10}; Pharmacogenetics; Physiogenomics statins; pharmacogenetics; myalgia; myopathy; lipid metabolism; personalized medicine; PCSK9 inhibitors

Personalized Medicine of Statins

One of the promises of the Human Genome Project is individualization of patient care based on highly heterogeneous innate metabolic factors determined by DNA typing of gene polymorphisms. Translation of such gene polymorphisms into clinical decision support for personalized healthcare is the basis for DNA guided medicine. Statin responsiveness is an area of high research interest given the success of the drug class in the treatment of hypercholesterolemia and in primary and secondary prevention of cardiovascular disease. Interrogation of the patient's genetic status for variants will eventually guide hyperlipidemic intervention.

Statins selectively and competitively inhibit the intracellular enzyme hydroxymethylglutaryl Coenzyme A reductase (HMGCoA reductase) that is expressed to different degrees in various tissues. HMG CoA reductase is the rate-limiting enzyme in cholesterol biosynthesis.

Correspondence address: Gualberto Ruaño, M.D., Ph.D., Genomas Inc., 67 Jefferson Street, Hartford, CT 06106 USA, g.ruano@genomas.net, TEL: (860) 972-4574, FAX: (860) 545-4575.

The views presented in this article are strictly those of the authors, and do not represent the opinions of the companies Genomas, Sanofi or Cyclica which employ, respectively, Dr. Ruaño, Dr. Seip, and Dr. Windemuth. Dr. Wu reports no conflicts.

In addition to the inhibition of cholesterol synthesis, the inhibition of HMGCoA activity reduces synthesis of geranyl and farnesyl products, leading to decreased isoprenylation of proteins and possible impairrment of many varied cellular functions. Statin entry into cells can be gated, and metabolic pathways for the drugs of this class are varied and drug dependent.

Statins are the most prescribed drugs in the United States [1] and the world [2]. Atorvastatin, simvastatin, and rosuvastatin comprise 85% of the prescriptions written in the U.S. [1] The success of the drug class in primary and secondary prevention of cardiovascular disease [3] has fostered increasingly aggressive usage and dosing.

Statin Efficacy

Administered at maximum dosages, the most common statins—atorvastatin, simvastatin, rosuvastatin, and pravastatin—lower low-density lipoprotein cholesterol (LDLC) by 37–57% in patients with primary hypercholesterolemia [4–7]. The magnitude of the LDLC response differs according to phenotypic, demographic, and as yet unexplained characteristics [8].

Although ~50% of the variability in plasma LDLC is estimated to be due to inheritance[9], only a small number of common and multiple rare gene variants that contribute to the phenotype are known [9–11]. Pharmacogenetic studies of LDLC lowering associated with statin therapy have focused mainly on genes in cholesterol synthetic, lipoprotein lipid transport, and pharmacokinetic pathways showing that single nucleotide polymorphisms in genes of cholesterol metabolism such as *HMGCR* [12–15] and lipoprotein transport such as *APOE* [15–24], and *LIPC* [25] can influence the statins' ability to lower LDLC levels. Variants in pharmacokinetic genes such as *SLCO1B1* which encodes the organic ion transporter protein 1B1, and *CYP7A1* which encodes the cytochrome p450 7A1 protein, can also affect LDLC lowering with statins [24, 26]. Recent findings have begun to extend the repertoire of gene variants associated with statin efficacy to new mechanisms of drug action. The *KIF6* gene codes for a cytoskeletal protein involved in intracellular transport of protein complexes, membrane organelles, and mRNA [27]. The Trp719Arg substitution in the protein enhances the efficacy of statin therapy apparently through pleiotropic effects [28]. In the absence of statin therapy, variants in genes such as *APOE* [29–31], *APOB* [30, 31], *NPC1L1* [32], *PSCK9* [31, 33], *CELSR2* [30], *PSRC1* [30], *SORT1* [30, 31], and *LDLR* [9, 30, 31] affect LDLC. Because baseline LDLC to some extent predicts the magnitude of LDLC lowering with statins, there may be overlap in the genes that regulate LDLC metabolism and statin-mediated LDLC lowering.

Our previous physiogenomic studies have generated hypothetical mechanisms related to statin-induced myositis [34], and myalgia [35]. We have also employed physiogenomic analysis to further investigate gene associations to LDLC in patients receiving statin therapy [36]. We found new evidence of opposing effects associated with an intronic variant near the ACACB mitochondrial binding domain, rs34274, and a SNP near the cAMP-dependent phosphorylation site, rs2241220, to LDLC-lowering in patients receiving statin therapy (Figure 1). In this study we had employed a cohort of 202 subjects receiving statin therapy

and genotyped for an array consisting of 384 SNPs distributed across physiological pathways represented by 222 genes.

Genotype:phenotype associations in large cohorts have confirmed loci at *APOB*, *APOE-APOC1-APOC4-APOC2*, *LDLR*, *HMGCR* and *PCSK9* [10] and discovered intergenic SNPs in the chromosomal regions 1p13 (near *CELSR2*, *PSRC1* and *SORT1)* and 19p13 (near *CILP2* and *PBX4)* [10] that are associated to LDL cholesterol in patients with elevated cholesterol. Genome-wide studies have shown that common, non-coding SNPs in HMG-CoA reductase are significantly associated with LDL cholesterol levels but that the effect sizes are relatively small, a 5% difference in LDL level [10]. The addition of genotype scoring consisting of summing the number of risk markers among the eleven SNPs just mentioned, has significantly improved risk classification in cardiovascular disease prone subjects in large cohorts.

In addition, variants in the gene which encodes *cholesteryl ester transport protein (CETP)*, though not associated with total LDLC, have been linked to LDL subfractions [37]. LDL subfractions vary in atherogenicity, and variability in activity of the cholesteryl ester transport protein is hypothesized to play a role the modification of their size and density. In this regard, the investigation of CETP haploptypes in relation to LDL subfractions warrants further investigation [38].

Statin Safety

The main clinically relevant safety risk is statin-induced neuromyopathy evidenced as a constellation of neuromuscular side effects. Neuromyopathies are disabling to 3–20% of patients on statins, require alteration of therapy, and reduce compliance [35, 39–41]. Neuromyopathies include myalgias (pain, weakness, aches, cramps) and myositis (typically monitored by elevation of serum creatine kinase [CK] activity) [39]. Neuromyopathies vary in extent among drugs and from patient to patient. Were there a system to predict the safety and efficacy of the pre-eminent statin drugs according to the genome of each patient, a clinician could optimize the selection from among atorvastatin, simvastatin, and rosuvastatin. Alternatively a patient's genomic profile may prove incompatible with statins, and the clinician could decide to avoid the drug class.

We have considered myalgias and myositis independently under the broader diagnosis of myopathy. Myalgias occur in patients often with no or little CK elevation and CK is not necessarily elevated in the presence of histopathological evidence of statin associated muscle damage [36, 42]. Only in the clinically rare condition of rhabdomyolysis is the relationship between myopathy, extremely elevated CK, and clinical severity, incontrovertible [43]. There is a need to identify novel surrogate markers that can better predict high risk of myalgia in patients taking statins [12].

Despite the limitations of CK measurement with respect to its specificity to statin neuromyopathy [44], in clinical studies increased activity of serum creatine kinase (CK) has provided the predominant means for assessing the degree of myopathic severity. The elevation of CK activity to 10-fold ULN (upper limit of normal) indicates severe statin-

induced neuromyopathy [45]. Pharmacokinetic gene-focused hypotheses have their basis on the increased plasma statin concentrations resulting from decreased first pass hepatic clearance (variation in drug transporters) and metabolism (variation in cytochrome p450 and glucuronidation pathways) [46].

Previous research linked polymorphisms in the *SLCO1B1* gene to elevated serum creatine kinase activity (myalgia) in patients receiving simvastatin therapy. *SLCO1B1* encodes for the OATB1 protein (organic anion transporter B1) which is a regulator of hepatic statin uptake. In patients selected for a high degree of CK elevation, the pharmacokinetic gene *SLCO1B1* *5 variant rs4363657 SNP is associated with CK elevation [42], likely through linkage disequilibrium with the non-synonymous SNP rs4149056 (Val174Ala) or the rs4149080 SNP in the 13th intron [47]. The relationship between *SLCO1B1* and CK elevation has been independently confirmed [47]. This association may be simvastatin-specific and not an effect seen with all statins.

A subsequent report assessed *SLCO1B1* polymorphisms in relation to clinically reported myalgia during rosuvastatin therapy [48]. Patients enrolled in the JUPITER trial without prior cardiovascular disease (CVD) or diabetes who had LDLC < 130 mg/dL and C-reactive protein ≥ 2 mg/L were randomly allocated to rosuvastatin 20 mg or placebo and followed for first CVD events and adverse effects. The SNPs rs4363657 and rs4149056 in *SLCO1B1* were assessed for association to clinically reported myalgia, which was determined as self-reported symptoms. Clinical myalgia frequency over 1.9 years of follow-up was not different between rosuvastatin and placebo, and the authors conclude that myalgia is not associated with the SNPs of interest in patients taking rosuvastatin.

Phenotypic expression of myalgia is quite variable [40, 49] and mechanistic explanations of the pharmacodynamic bases of neuromuscular side effects have incorporated diverse and complex pathways [39, 41]. These have been recently summarized [50]. Statin interactions with HMG-CoA reductase homologue proteins may interfere with energy transduction processes [51, 52]. Some mechanisms find their basis in the possibility that statin inhibition of nonsteroidal molecules such as ubiquinone and isoprenoids triggers disruption in normal mitochondrial function, cell signaling, cell proliferation, and cell repair [39, 51, 53–62]. Specific proposed myalgia etiologies include decreased sarcolemmal [39] or sarcoplasmic reticular cholesterol [63], reduced production of ubiquinone or coenzyme Q_{10} [64], decreased prenylated proteins [39, 65], changes in fat metabolism [66], increased uptake of cholesterol [67] or phytosterols [68], failure to replace damaged muscle protein via the ubiquitin pathway [69], disruption of calcium metabolism in the skeletal muscle [70, 71] and inhibition of selenoprotein synthesis [56]. Pharmacodynamic genetic markers are generally unknown, though progress to identify candidate markers has been made [50]. Vladutiu et al. [72] have reported increased prevalence of heterozygosity among known markers for a number of inherited muscle metabolic diseases.

We illustrate 2 approaches: hypothesis-free genome wide association studies and hypothesis-led candidate gene studies.

Hypothesis-free approach: Genome-wide SNP associations

We pursued a hypothesis-free genome-wide association study (GWAS) probing the association of 865,483 SNPs across all chromosomes in a group of 812 outpatients undergoing statin therapy for hyperlipidemia [73]. The study sample was enriched with patients diagnosed as having statin-induced neuromyopathy, accomplished through recruitment of patients treated at specialized lipid clinics. The results confirmed the association of 3 out of 31 previously identified candidate genes including *COQ2* and *CPT2* involved in myocellular energy transfer and provide a novel association through the neuronally expressed *CLN8* gene. The *Manhattan plot* in Figure 2 shows the association log-scores for all 865,483 SNPs to myalgia as a function of their genomic location. In GWAS, a *Manhattan plot* is a scatter diagram, where genomic location coordinates are displayed along the X-axis, while the negative logarithm of the association *p*-value for each SNP is displayed on the Y-axis. Thus each dot on the *Manhattan plot* signifies a SNP, and the strongest associations have the greatest "height" because the negative logarithms of the smallest *p*-values will be the greatest values. For example, a *p*-value of 10^{-10} will have a value of 10 in the plot.

At a threshold log-score of 7.2 (calculated from the negative logarithm_{10} of *0.05 ÷ 865,483*), no associations reached genome-wide significance after correction for the 865,483 multiple comparisons, but there are a number of interesting associations that are suggestive. The full set of suggestive associations is listed in Table 1. The highest log-score in the study, 6.3, was achieved by SNP rs4693570 on chromosome 4, located 100 kb downstream of the gene for para-hydroxybenzoate--polyprenyltransferase *(COQ2),* whose enzyme product catalyzes one of the final reactions in the biosynthesis of Coenzyme Q_{10}. *COQ2* was also one of our top candidate genes. SNP rs11980747, with a log-score of 6.1 ($p < 2{\cdot}10^{-6}$, $R^2 = 3.1\%$), is located within the gene for the heavy chain of axonemal dynein (*DNAH11*). SNPs rs2738466, rs14158, rs2116897, and rs2569537 on chromosome 19 were associated with log-scores of 5.6, 5.5, 5.4, and 5.3 respectively ($p < 5{\cdot}10^{-6}$, $R^2 = 2.9\%$), and located in linkage within the gene for low density lipoprotein receptor *(LDLR).* At a log-score of 5.1 ($p < 10^{-5}$, $R^2 = 2.6\%$), there is SNP rs7014327, located within the gene ceroid-lipofuscinosis, neuronal 8 *(CLN8).*

Figure 3 shows the genomic locus and the SNP effect on myalgia for the four genes identified above. The figures show log-scores of association for all SNPs within 200 kb of the index SNP along the chromosome, and on the right show the effect of the variant allele on the probability of myalgia in subgroups of patients taking one of the three major statins. In all cases the loci show linkage disequilibrium, indicated by the fact that there are other associated SNPs in the direct vicinity of the index SNP. Interestingly, the *CLN8* SNP shows a very strong drug-dependent effect. There is no effect at all in the group of patients taking either rosuvastatin or simvastatin, but a strong effect ($p < 2{\cdot}10^{-7}$, $R^2 = 5.2\%$) in the group of patients taking atorvastatin. The difference in effect between the subpopulations is significant ($p < 0.05$) under the t-statistic.

Not shown, but also interesting at $p < 10^{-5}$, corresponding to a log-score of 5.0 or better, are 2 additional SNPs not associated with any gene and a SNP in the gene *MAGI2*, membrane associated guanylate kinase, WW and PDZ domain. The protein encoded by *MAGI2*

interacts with atrophin-1. Atrophin-1 contains a polyglutamine repeat, expansion of which is responsible for dentatorubral and pallidoluysian atrophy. This encoded protein is characterized by two WW domains, a guanylate kinase-like domain, and multiple PDZ domains. It has structural similarity to the membrane-associated guanylate kinase homologue (MAGUK) family. The $p < 10^{-5}$ threshold represents a posterior likelihood of true association of >10%. SNP rs7267722, on chromosome 20 with a log-score of 6.1 ($p < 7{\cdot}10^{-7}$, $R^2 = 3.3\%$), is not associated with any gene and located in the middle of a ~400 kb intergenic region.

Hypothesis-led approach: Validation of candidate genes

We also assembled a list of 31 candidate genes with known or hypothetical pharmacodynamic or pathological roles in myalgia as assessed from several previous publications. Table 2 lists the candidates with literature references and our validation results. There are 9 genes with an adjusted log-score above 1.3 ($p < 0.05$) and 3 that are statistically significant in the face of multiple comparisons among loci: *COQ2* ($p < 3{\cdot}10^{-5}$), *ATP2B1* ($p < 0.001$), and *DMPK* ($p < 0.002$).

COQ2—The *COQ2* gene encodes mitochondrial para-hydroxybenzoate-polyprenyltransferase, an enzyme that catalyzes one of the final reactions in the biosynthesis of Coenzyme $Q10_{10}$ (CoQ_{10}), the prenylation of parahydroxybenzoate with an all-trans polyprenyl group [74]. CoQ_{10} (ubiquinone) serves as a redox carrier in the mitochondrial respiratory chain and is a lipid-soluble antioxidant. CoQ_{10} blood concentrations are lowered by statins, and CoQ_{10} deficiency has been linked to mitochondrial myopathy.

DMPK—The *DMPK* gene encodes Myotonin-protein kinase (*MT-PK*), a serine-threonine kinase that has been implicated in myotonic dystrophy type I, a inherited multisystemic disease characterized by wasting of the muscles (muscular dystrophy), cataracts, heart conduction defects, endocrine changes, and myotonia. Newly diagnosed patients with inherited myopathies have been reported to have a higher exposure rate to statins [75], suggesting that statins may have contributed to the onset of symptoms.

CLN8—We identified a new candidate, *CLN8*, which has a strong effect only in patients taking atorvastatin and not taking either rosuvastatin or simvastatin. *CLN8* has not been implicated directly with myopathy, but it is associated with Pompe's disease, which in turn has been associated with statin induced myopathy. Its drug dependent association supports the hypothesis that the myopathy side effect is mediated by different pathways for different statins. Further research to elucidate the biological association of *CLN8* with myopathy and statins, and to confirm its association in a follow-up study of patients treated with statins is needed.

Compared to other recent GWAS studies, this study is limited in statistical power, but it is the largest examination of statin induced myalgia to date. The associations determined in our study confirm connections between common myalgia and genes involved in biochemical pathways that have been previously implicated in statin mylagia. Other candidate genes tested here were not validated. One of these, the *SLCO1B1* gene demonstrates variants that

are strongly associated to elevated CK during simvastatin therapy [42, 47]. Our study [73] observed a different phenotype and tested only a limited number of patients receiving simvastatin. The present study detected no association of *SLCO1B1* to myalgia probably as a consequence of its broader set of statins and phenotype.

We successfully validated *COQ2*, *ATP2B1*, and *DMPK* as a candidate gene for statin induced myalgia. The candidate genes *COQ2*, *ATP2B1*, and *DMPK*, representing pathways involved in myocellular energy transfer, calcium homeostasis, and myotonic dystonia, respectively, were validated as markers for the common myalgia observed in patients receiving statin therapy.

Multi-Gene Models

These three genes integrated into a physiogenomic predictive model could be relevant to myalgia diagnosis and prognosis in clinical therapeutics. We had identified a clear tendency for the risk alleles (*COQ2*-G, *DMPK*-G, and *ATP2B1*-T) to be dominant, and there was a substantial effect of each allele on the probability of developing myalgia [73]. The genotypic effect of the markers on the probability of myalgia is detailed in Figure 4. The combined effect of the three markers is quantified, showing that of the 2 subjects with no risk alleles, none have myalgia, and of the 40 subjects with the full complement of 6 risk alleles, 70% have myalgia. The study confirmed 3 previously identified markers. Among the 377 patients diagnosed clinically as having statin myalgia, all have at least one risk allele from the 3 validated genes.

The SINM study sought to identify genetic markers for statin myopathy that can be used to predict which patients are most likely to develop myopathic complaints during statin treatment and to aid in objectifying the diagnosis of statin myopathy. Combining the *COQ2*, *ATP2B1*, and *DMPK* markers into a physiogenomic panel to represent candidate pathways and deriving a risk score, establishes a prototype system to predict the onset of myalgia and to aid in diagnosis [73]. Despite subjectivity in diagnosis of statin myalgia, the study identified previously suspected candidate genes with logical relationships to muscle metabolism as contributing to statin myalgia.

Biotechnology drugs for treatment of hypercholesterolemias

Over the last 5 years, the pharmaceutical industry has developed new therapies such as nucleic acid anti-sense compounds and monoclonal antibodies for the treatment of severe genetic hypercholesterolemias. These molecules may be extended to treatment of patients who are refractory or intolerant of statin treatment.

One such mRNA inhibitor is mipomersen, an antisense oligonucleotide that specifically binds to *APOB* mRNA, rendering it susceptible to degradation. Mipomersen inhibits hepatic formation and release of nascent apolipoprotein B100-containing lipoproteins and can lower LDLC in patients whose LDL particle recognition and hepatic uptake mechanism is defective. Another drug, lomitapide, a small molecule inhibitor of microsomal triglyceride transfer protein, inhibits transfer of lipid into developing chylomicrons in enterocytes and very low density lipoprotein particles in hepatocytes, resulting in fewer apolipoprotein

B100- and B48-containing particles. At present these agents remain specifically targeted to patients with confirmed genetic homozygous hypercholesterolemia.

Monoclonal antibodies have been synthesized to inhibit proprotein convertase subtilisin / kexin 9 (PCSK9) activity. Through binding to PCSK9, the inhibitors disrupt the intracellular traffic that normally degrades the LDL receptor. PCSK9 antibodies appear effective and safe in Phase III trials. In the class, 2 monoclonal antibodies produced by different pharmaceutical companies were FDA approved as of late 2015. The agents are indicated for use in patients with heterozygous familial cholesterolemia (HeFH) and/or patients with atherosclerotic vascular disease on maximally tolerated statins, who require additional LDL reduction. The US target population for these drugs, when patients who are unable to tolerate maximal statin therapy are included, is sizable. In the USAGE study, a survey of approximately 10,000 current and former statin users, 12% of patients on statins discontinued therapy and 62% of these patients cited side effects as the reason for discontinuation [76]. More than 86% of patients who discontinued therapy because of side effects cited muscle pain or weakness as the reason. Based upon these data, it has been estimated that approximately 6% of statin users, representing more than 2 million adults in the United States, ceased therapy because of muscle pain or weakness and are therefore statin intolerant [77]. We expect statin pharmacogenetics to play a significant role in the selection and confirmation of patients unlikely to be well managed with the more conventional and far less expensive statins.

Clinical Scenarios

Statins will remain the first-line therapy in the foreseeable future for patients with high cardiovascular disease risk that is traditionally founded on elevated LDL cholesterol. We have created a prototype decision support matrix which incorporates statin-specific SNP markers of both efficacy and safety with the intent to provide decision making guidance. The quantitative comparison of the merits of each drug offers more clinical resolution than generalized single-drug outcomes [78]. With such pharmacogenetic decision support tools, the value to the physician is the capacity to address the complexity and variety of clinical scenarios and appropriately evaluate the need for non-statin drugs. In Figure 5, we describe three clinical scenarios from actual records of patients in our clinical registry.

Scenario 1—The patient was a Caucasian male with history of statin therapy for elevated cholesterol and a successful reduction in LDL cholesterol to a level of 85 mg/dL with statin therapy, well below the target level of 100 mg/dL. Statin associated myopathy was diagnosed by the treating physician. Analysis of the patient’s genome, focused on genotypes of the decision support matrix, did not reveal a statin-wide adversity, but clear and dramatic differences in predicted safety and efficacy among the three statins.

According to the decision support matrix, simvastatin is preferred based on predicted responses in the lowest quartiles for predicted log CK (low CK activity) and predicted LDL cholesterol (42 mg/dL) (green cells), and predicted responses for myalgia that were in the middle two quartiles of patient distributions. In contrast, the SINM PhyzioType predicts undesirable responses for all four parameters (myalgia, plasma CK activity, LDL

cholesterol, HDL cholesterol) for rosuvastatin (red cells) and safety and efficacy responses to atorvastatin that are not highly favorable or unfavorable (yellow cells). This patient would not be a candidate for PCSK9 inhibitor

Scenario 2—This patient had been priorly treated with simvastatin 5 mg, atorvastatin 10 mg, and rosuvastatin 10 mg. The patient complained of mild discomfort on each of the drugs but the physician was unconvinced and diagnosed “possible statin-associated myopathy”. The LDL cholesterol level was 189 mg/dL. In this case, the decision support matrix provided a clinical guidance toward atorvastatin as the best choice of statin to treat the patient’s hypercholesterolemia based on predicted LDL cholesterol level of 115.9 mg/dL which is an efficacy prediction that is favorable compared to rosuvastatin and simvastatin, but still sub par). The support matrix is already pointing to limitations of statin treatment, which may render this patient as one to treat with statin as a last trial in anticipation of a switch to PCSK9 inhibitor.

Scenario 3—The patient had been treated with lovastatin 20 mg, which lowered LDL cholesterol to 88 mg/dL (satisfying target goal achievement) but caused mild discomfort which was interpreted by the treating clinician as possible statin associated myalgia. Rosuvastatin 5 mg was subsequently tried. In this case, the decision support matrix revealed no clear superior statin for treatment. Rosuvastatin had the best profile based on highly favorable efficacy with respect to predicted LDL cholesterol level of 78.7 mg/dL and the prediction of non-myalgia, but the prediction of an elevated CK (ln[CK]=5.76, or 317 CK activity units) presents a major risk. This patient would be a good candidate for PCSK9 inhibitors.

Clinical Pharmacogenetic Testing

Testing for cardiovascular risk factors is available from direct-to-consumer companies. Selected clinical laboratories specializing in high-resolution lipid profiling have begun offering heart disease markers as an adjunct service.

A number of academic centers and commercial laboratories have embraced *SLCO1B1* testing and have begun offering it clinically. However, this marker is limited to extreme myopathy, and appears to be simvastatin specific. Commercial insurers in USA do not cover the test.

Testing for myopathies is available from the Robert Guthrie Biochemical and Molecular Genetics Laboratory at SUNY Buffalo [72]. It is implemented for genetic myopathies, but not for statin-related effects.

In June 2011, Genomas announced a product development grant from the National Institute of General Medical Sciences to develop genetic tests and DNA-guided diagnostic systems for optimal selection of statins and for improved delivery of statin therapy for the treatment of cardiovascular disease, obesity and diabetes. This pioneering project is in progress and harbors the potential to provide clinicians and physicians with newly developed genetic tests and a decision support system which will allow them to manage statins, prescribe and dose

these drugs on a DNA-guided, personalized basis to more effectively guide the therapy for each patient. This decision support may be relevant to the prescription of biotechnology drugs to statin intolerant and recalcitrant patients.

Genetic testing for statin efficacy and safety is thus currently available, but faces questions as to its clinical utility and validity by the healthcare payers. We predict however, that such testing will become standard once its economic value and return on the investment is demonstrated in selected instances.

Acknowledgments

Supported in part by the NIGMS NIH Small Business Innovation Research Grant R44 GM-085201 *System for DNA-Guided Optimization and Personalization of Statin Therapy.* The ClinicalTrials.gov identifier for the SINM study is *NCT00767130*. Dr. Ruaño is Principal Investigator of the study and President and Founder of Genomas Inc.

References

1. Findlay, S. Consumer Reports. Consumers Union; Yonkers, NY: 2007. Prescription and price trends October 2005 to December 2006 and potential cost savings to Medicare from increased use of lower cost statins. Consumer Reports. 2-19-2007. Yonkers, NY, Consumers Union.
2. Visiongain, L. Statins: The World Market, 2009–2024. London, UK: 2009.
3. Waters DD. What the statin trials have taught us. Am J Cardiol. 2006; 98(1):129–134. [PubMed: 16784935]
4. Jones P, et al. Comparative dose efficacy study of atorvastatin versus simvastatin, pravastatin, lovastatin, and fluvastatin in patients with hypercholesterolemia (the CURVES study). Am J Cardiol. 1998; 81(5):582–7. [PubMed: 9514454]
5. Stein EA, et al. Efficacy and safety of simvastatin 80 mg/day in hypercholesterolemic patients. The Expanded Dose Simvastatin U.S. Study Group. Am J Cardiol. 1998; 82(3):311–6. [PubMed: 9708659]
6. Ballantyne CM, et al. Efficacy and safety of rosuvastatin 40 mg alone or in combination with ezetimibe in patients at high risk of cardiovascular disease (results from the EXPLORER study). Am J Cardiol. 2007; 99(5):673–80. [PubMed: 17317370]
7. Sacks FM, et al. Relationship between plasma LDL concentrations during treatment with pravastatin and recurrent coronary events in the Cholesterol and Recurrent Events trial. Circulation. 1998; 97(15):1446–52. [PubMed: 9576424]
8. Simon JA, et al. Phenotypic predictors of response to simvastatin therapy among African-Americans and Caucasians: the Cholesterol and Pharmacogenetics (CAP) Study. Am J Cardiol. 2006; 97(6): 843–50. [PubMed: 16516587]
9. Burnett JR, Hooper AJ. Common and rare gene variants affecting plasma LDL cholesterol. Clin Biochem Rev. 2008; 29(1):11–26. [PubMed: 18566665]
10. Kathiresan S, et al. Six new loci associated with blood low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides in humans. Nat Genet. 2008; 40(2):189–97. [PubMed: 18193044]
11. Kathiresan S, et al. Polymorphisms associated with cholesterol and risk of cardiovascular events. N Engl J Med. 2008; 358(12):1240–9. [PubMed: 18354102]
12. Chasman DI, et al. Pharmacogenetic study of statin therapy and cholesterol reduction. JAMA. 2004; 291(23):2821–7. [PubMed: 15199031]
13. Krauss RM, et al. Variation in the 3-hydroxyl-3-methylglutaryl coenzyme a reductase gene is associated with racial differences in low-density lipoprotein cholesterol response to simvastatin treatment. Circulation. 2008; 117(12):1537–44. [PubMed: 18332269]
14. Polisecki E, et al. Genetic variation at the LDL receptor and HMG-CoA reductase gene loci, lipid levels, statin response, and cardiovascular disease incidence in PROSPER. Atherosclerosis. 2008; 200(1):109–14. [PubMed: 18261733]

15. Thompson JF, et al. Comprehensive whole-genome and candidate gene analysis for response to statin therapy in the Treating to New Targets (TNT) cohort. Circ Cardiovasc Genet. 2009; 2(2): 173–81. [PubMed: 20031582]
16. Donnelly LA, et al. Apolipoprotein E genotypes are associated with lipid-lowering responses to statin treatment in diabetes: a Go-DARTS study. Pharmacogenet Genomics. 2008; 18(4):279–87. [PubMed: 18334912]
17. Maitland-van der Zee AH, et al. Apolipoprotein-E polymorphism and response to pravastatin in men with coronary artery disease (REGRESS). Acta Cardiol. 2006; 61(3):327–31. [PubMed: 16869455]
18. Tavintharan S, et al. Apolipoprotein E genotype affects the response to lipid-lowering therapy in Chinese patients with type 2 diabetes mellitus. Diabetes Obes Metab. 2007; 9(1):81–6. [PubMed: 17199722]
19. Pedro-Botet J, et al. Apolipoprotein E genotype affects plasma lipid response to atorvastatin in a gender specific manner. Atherosclerosis. 2001; 158(1):183–93. [PubMed: 11500190]
20. Sousa MO, et al. Lack of association between the APOE genotype and the response to statin treatment in patients with acute ischemic episodes. Med Clin (Barc). 2008; 130(11):401–4. [PubMed: 18394363]
21. Pena R, et al. Effect of apoE genotype on the hypolipidaemic response to pravastatin in an outpatient setting. J Intern Med. 2002; 251(6):518–25. [PubMed: 12028507]
22. Chiodini BD, et al. Apolipoprotein E polymorphisms influence effect of pravastatin on survival after myocardial infarction in a Mediterranean population: the GISSI-Prevenzione study. Eur Heart J. 2007; 28(16):1977–83. [PubMed: 17567623]
23. Thompson JF, et al. An association study of 43 SNPs in 16 candidate genes with atorvastatin response. Pharmacogenomics J. 2005; 5(6):352–8. [PubMed: 16103896]
24. Zhang W, et al. SLCO1B1 521T-->C functional genetic polymorphism and lipid-lowering efficacy of multiple-dose pravastatin in Chinese coronary heart disease patients. Br J Clin Pharmacol. 2007; 64(3):346–52. [PubMed: 17439540]
25. Zambon A, et al. Common hepatic lipase gene promoter variant determines clinical response to intensive lipid-lowering treatment. Circulation. 2001; 103(6):792–8. [PubMed: 11171785]
26. Takane H, et al. Pharmacogenetic determinants of variability in lipid-lowering response to pravastatin therapy. J Hum Genet. 2006; 51(9):822–6. [PubMed: 16917677]
27. Hirokawa N, Noda Y. Intracellular transport and kinesin superfamily proteins, KIFs: structure, function, and dynamics. Physiol Rev. 2008; 88(3):1089–118. [PubMed: 18626067]
28. Iakoubova OA, et al. Polymorphism in KIF6 gene and benefit from statins after acute coronary syndromes: results from the PROVE IT-TIMI 22 study. J Am Coll Cardiol. 2008; 51(4):449–55. [PubMed: 18222355]
29. Bennet AM, et al. Association of apolipoprotein E genotypes with lipid levels and coronary risk. JAMA. 2007; 298(11):1300–11. [PubMed: 17878422]
30. Chasman DI, et al. Genetic loci associated with plasma concentration of low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, apolipoprotein A1, and Apolipoprotein B among 6382 white women in genome-wide analysis with replication. Circ Cardiovasc Genet. 2008; 1(1):21–30. [PubMed: 19802338]
31. Willer CJ, et al. Newly identified loci that influence lipid concentrations and risk of coronary artery disease. Nat Genet. 2008; 40(2):161–9. [PubMed: 18193043]
32. Cohen JC, et al. Multiple rare variants in NPC1L1 associated with reduced sterol absorption and plasma low-density lipoprotein levels. Proc Natl Acad Sci U S A. 2006; 103(6):1810–5. [PubMed: 16449388]
33. Cohen JC, et al. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. N Engl J Med. 2006; 354(12):1264–72. [PubMed: 16554528]
34. Ruaño G, et al. Physiogenomic analysis links serum creatine kinase activities during statin therapy to vascular smooth muscle homeostasis. Pharmacogenomics. 2005; 6(8):865–72. [PubMed: 16296949]
35. Ruaño G, et al. Physiogenomic association of statin-related myalgia to serotonin receptors. Muscle Nerve. 2007; 36(3):329–35. [PubMed: 17600820]

36. Ruaño G, et al. Physiogenomic analysis of statin-treated patients: domain-specific counter effects within the ACACB gene on low-density lipoprotein cholesterol? Pharmacogenomics. 2010; 11(7): 959–71. [PubMed: 20602615]
37. Shim H, et al. A multivariate genome-wide association analysis of 10 LDL subfractions, and their response to statin treatment, in 1868 Caucasians. PLoS One. 2015; 10(4):e0120758. [PubMed: 25898129]
38. Winkelmann BR, et al. Haplotypes of the cholesteryl ester transfer protein gene predict lipid-modifying response to statin therapy. Pharmacogenomics J. 2003; 3(5):284–96. [PubMed: 14583798]
39. Thompson PD, Clarkson P, Karas RH. Statin-associated myopathy. JAMA. 2003; 289(13):1681–1690. [PubMed: 12672737]
40. Bruckert E, et al. Mild to moderate muscular symptoms with high-dosage statin therapy in hyperlipidemic patients--the PRIMO study. Cardiovasc Drugs Ther. 2005; 19(6):403–14. [PubMed: 16453090]
41. Ghatak A, Faheem O, Thompson PD. The genetics of statin-induced myopathy. Atherosclerosis. 2010; 210(2):337–43. [PubMed: 20042189]
42. Link E, et al. SLCO1B1 variants and statin-induced myopathy--a genomewide study. N Engl J Med. 2008; 359(8):789–99. [PubMed: 18650507]
43. Ruaño, G.; Windemuth, A.; Holford, TR. Physiogenomics: Integrating Systems Engineering and Nanotechnology for Personalized Medicine. In: Bronzino, JD., editor. The Biomedical Engineering Handbook. CRC Press; Cleveland, OH: 2005. p. 1-9.
44. Seip, RL., et al. Statin-Induced Neuromyopathy. In: Murray, MF.; Babyatsky, MW.; Giovanni, MA., editors. Clinical Genomics. Practical Applications in Adult Care. McGraw-Hill Education; New York: 2014. p. 38-44.
45. McKenney JM, et al. Final conclusions and recommendations of the National Lipid Association Statin Safety Assessment Task Force. Am J Cardiol. 2006; 97(8A):89C–94C.
46. Seip, RL.; Duconge, J.; Ruaño, G. Genotype-Guided Statin Therapy. In: Wu, AHB.; Yeo, J., editors. Pharmacogenomic Testing in Current Clinical Practice: Implementation in the Clinical Laboratory. Humana Press, Springer Science+Business Media; New York: 2011. p. 155-174.
47. Voora D, et al. The SLCO1B1*5 genetic variant is associated with statin-induced side effects. J Am Coll Cardiol. 2009; 54(17):1609–16. [PubMed: 19833260]
48. Danik JS, et al. Lack of association between SLCO1B1 polymorphisms and clinical myalgia following rosuvastatin therapy. Am Heart J. 2013; 165(6):1008–14. [PubMed: 23708174]
49. Stroes ES, et al. Statin-associated muscle symptoms: impact on statin therapy-European Atherosclerosis Society Consensus Panel Statement on Assessment, Aetiology and Management. Eur Heart J. 2015; 36(17):1012–22. [PubMed: 25694464]
50. Needham M, Mastaglia FL. Statin myotoxicity: a review of genetic susceptibility factors. Neuromuscul Disord. 2014; 24(1):4–15. [PubMed: 24176465]
51. Rosenson RS. Current overview of statin-induced myopathy. Am J Med. 2004; 116(6):408–16. [PubMed: 15006590]
52. Wilke RA, Mareedu RK, Moore JH. The Pathway Less Traveled: Moving from Candidate Genes to Candidate Pathways in the Analysis of Genome-Wide Data from Large Scale Pharmacogenetic Association Studies. Curr Pharmacogenomics Person Med. 2008; 6(3):150–159. [PubMed: 19421424]
53. Arora R, Liebo M, Maldonado F. Statin-induced myopathy: the two faces of Janus. J Cardiovasc Pharmacol Ther. 2006; 11(2):105–12. [PubMed: 16891287]
54. Law M, Rudnicka AR. Statin safety: a systematic review. Am J Cardiol. 2006; 97(8A):52C–60C.
55. Phillips PS, et al. Statin-associated myopathy with normal creatine kinase levels. Ann Intern Med. 2002; 137(7):581–5. [PubMed: 12353945]
56. Moosmann B, Behl C. Selenoprotein synthesis and side-effects of statins. Lancet. 2004; 363(9412): 892–4. [PubMed: 15031036]
57. Sinzinger H, Schmid P, O'Grady J. Two different types of exercise-induced muscle pain without myopathy and CK-elevation during HMG-Co-enzyme-A-reductase inhibitor treatment. Atherosclerosis. 1999; 143(2):459–60. [PubMed: 10217378]

58. Franc S, et al. A comprehensive description of muscle symptoms associated with lipid-lowering drugs. Cardiovasc Drugs Ther. 2003; 17(5–6):459–65. [PubMed: 15107601]
59. Sewright KA, Clarkson PM, Thompson PD. Statin myopathy: incidence, risk factors, and pathophysiology. Curr Atheroscler Rep. 2007; 9(5):389–96. [PubMed: 18001622]
60. Dirks AJ, Jones KM. Statin-induced apoptosis and skeletal myopathy. Am J Physiol Cell Physiol. 2006; 291(6):C1208–12. [PubMed: 16885396]
61. Hanai J, et al. The muscle-specific ubiquitin ligase atrogin-1/MAFbx mediates statin-induced muscle toxicity. J Clin Invest. 2007; 117(12):3940–51. [PubMed: 17992259]
62. Hansen KE, et al. Outcomes in 45 patients with statin-associated myopathy. Arch Intern Med. 2005; 165(22):2671–6. [PubMed: 16344427]
63. Draeger A, et al. Statin therapy induces ultrastructural damage in skeletal muscle in patients without myalgia. J Pathol. 2006; 210(1):94–102. [PubMed: 16799920]
64. Marcoff L, Thompson PD. The role of coenzyme Q10 in statin-associated myopathy: a systematic review. J Am Coll Cardiol. 2007; 49(23):2231–7. [PubMed: 17560286]
65. Santos RD. What are we able to achieve today for our patients with homozygous familial hypercholesterolaemia, and what are the unmet needs? Atherosclerosis Supplements. 2014; 15(2): 19–25. [PubMed: 25257073]
66. Phillips PS, Haas RH. Statin myopathy as a metabolic muscle disease. Expert Rev Cardiovasc Ther. 2008; 6(7):971–8. [PubMed: 18666847]
67. Yokoyama M, et al. Effects of lipoprotein lipase and statins on cholesterol uptake into heart and skeletal muscle. J Lipid Res. 2007; 48(3):646–55. [PubMed: 17189607]
68. Paiva H, et al. High-dose statins and skeletal muscle metabolism in humans: a randomized, controlled trial. Clin Pharmacol Ther. 2005; 78(1):60–8. [PubMed: 16003294]
69. Urso ML, et al. Changes in ubiquitin proteasome pathway gene expression in skeletal muscle with exercise and statins. Arterioscler Thromb Vasc Biol. 2005; 25(12):2560–6. [PubMed: 16224050]
70. Mohaupt MG, et al. Association between statin-associated myopathy and skeletal muscle damage. CMAJ. 2009; 181(1–2):E11–8. [PubMed: 19581603]
71. Guis S, et al. In vivo and in vitro characterization of skeletal muscle metabolism in patients with statin-induced adverse effects. Arthritis Rheum. 2006; 55(4):551–7. [PubMed: 16874775]
72. Vladutiu GD. Genetic predisposition to statin myopathy. Curr Opin Rheumatol. 2008; 20(6):648–55. [PubMed: 18946323]
73. Ruaño G, et al. Mechanisms of statin-induced myalgia assessed by physiogenomic associations. Atherosclerosis. 2011; 218(2):451–6. [PubMed: 21868014]
74. Forsgren M, et al. Isolation and functional expression of human COQ2, a gene encoding a polyprenyl transferase involved in the synthesis of CoQ. Biochem J. 2004; 382(Pt 2):519–26. [PubMed: 15153069]
75. Sailler L, et al. Increased exposure to statins in patients developing chronic muscle diseases: a 2-year retrospective study. Ann Rheum Dis. 2008; 67(5):614–9. [PubMed: 17768174]
76. Cohen JD, et al. Understanding Statin Use in America and Gaps in Patient Education (USAGE): an internet-based survey of 10,138 current and former statin users. J Clin Lipidol. 2012; 6(3):208–15. [PubMed: 22658145]
77. Esperion Therapeutics Inc. Targeting Unmet Patient Needs: Finding new therapies for lowering LDLC for patients with hypercholesterolemia and a history of statin intolerance and for patients with residual risk. Plymouth, MI: 2013. p. 1-5.http://www.esperion.com/wp-content/uploads/2013/06/Esperion-Target-Needs-Whitepaper.pdf
78. Ridker PM, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. The New England journal of medicine. 2008; 359(21):2195–207. [PubMed: 18997196]

KEY POINTS

- Genotype:phenotype associations in large cohorts have confirmed loci at APOB, APOE-APOC1-APOC4-APOC2, LDLR, HMGCR and PCSK9 that are associated with LDL cholesterol in patients with elevated cholesterol.
- Variants in the gene which encodes cholesteryl ester transport protein (CETP), though not associated with total LDLc, have been linked to LDL subfractions.
- Research has linked polymorphisms in the SLCO1B1 gene to elevated serum creatine kinase activity (myalgia) in patients receiving simvastatin therapy.
- COQ2, ATP2B1, and DMPK, representing pathways involved in myocellular energy transfer, calcium homeostasis, and myotonic dystonia, respectively, have been validated as markers for the common myalgia observed in patients receiving statin therapy, and integrated into a physiogenomic predictive model for myalgia diagnosis and prognosis in clinical therapeutics.
- We expect statin pharmacogenetics to play a significant role in the selection and confirmation of patients for PCSK9 inhibitors.
- Genetic testing for statin efficacy and safety is currently available, but faces questions as to its clinical utility and validity by the healthcare payers; however, we predict that such testing will become standard once predictive multi-gene models and clinical decision support are brought to market.

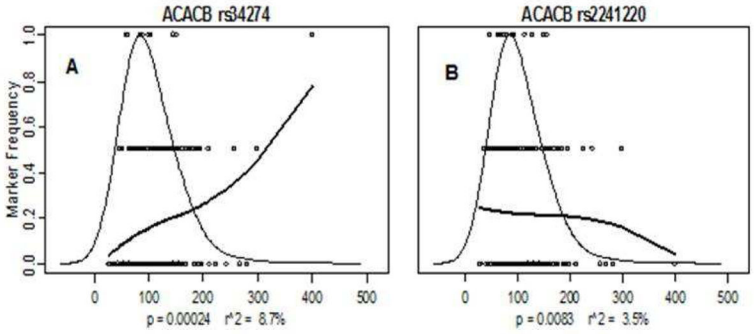

Figure 1.

Associations to LDL cholesterol lowering through for *ACACB* rs34274 (left panel) and *ACACB* rs2241220 (right). Each circle represents a subject (genotype), with the horizontal axis specifying the low density lipoprotein cholesterol, and the vertical axis the genotype: bottom circles – homozygous for major allele, middle circles – heterozygous, top circles – homozygous for minor allele. A LOESS fit of the allele frequency as a function of LDLC (thick line) is shown. LOESS: LOcally-wEighted Scatter plot Smooth.

(*From Ruaño G, Thompson PD, Kane JP, et al. Physiogenomic analysis of statin-treated patients: domain-specific counter effects within the ACACB gene on low-density lipoprotein cholesterol? Pharmacogenomics. 2010 Jul;11(7):959–71; with permission.)*

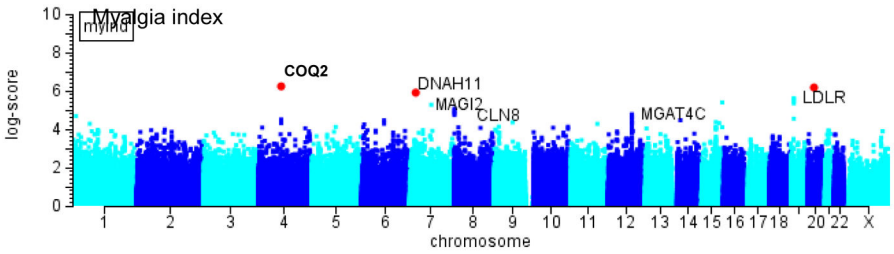

Figure 2.

Manhattan plot for Myalgia index. The scale on the ordinate represents statistical significance as the log-score given by $s = -\log_{10} p$, and the abscissa represents the chromosomal location, with the chromosome boundaries indicated by the coloring of the data points. The strongest SNP associations (log-score > 5) are indicated by a filled circle (●), and the top six markers are labeled with the name of the associated gene for 865,483 SNPs.

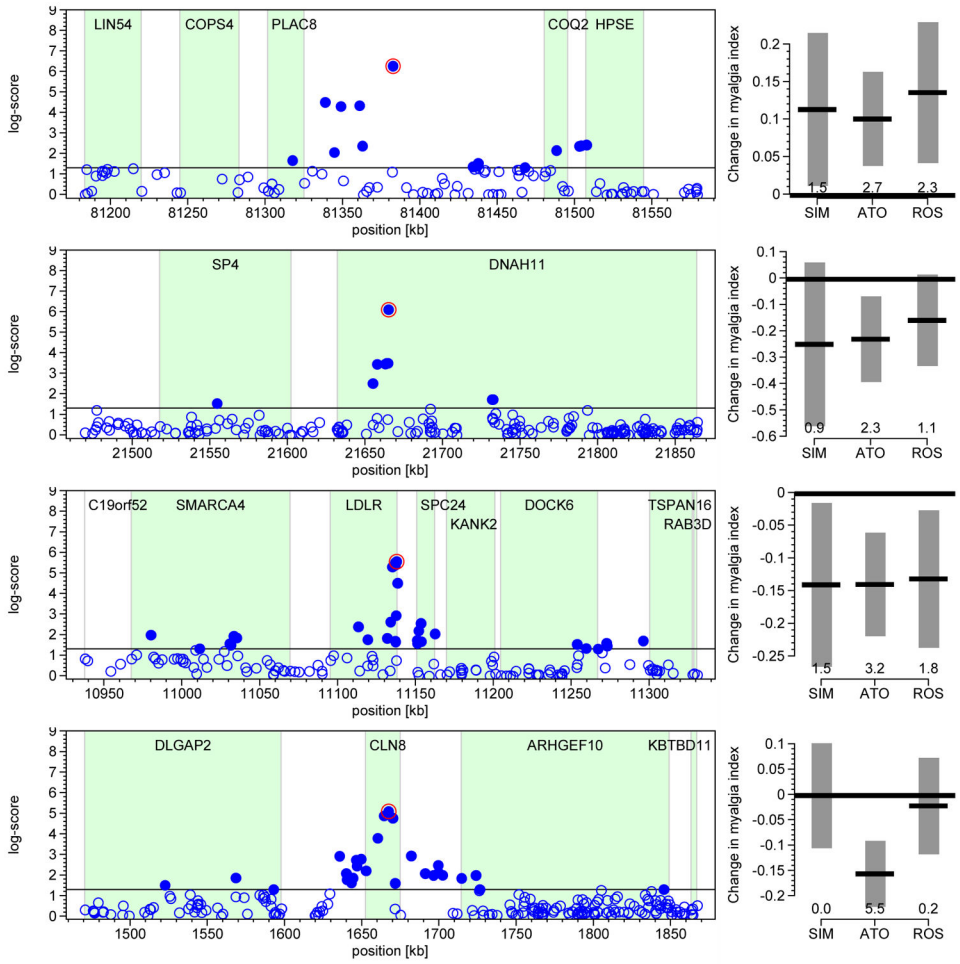

Figure 3.

Genomic locus and effect on myalgia for four of the genes identified in the whole-genome screen. The panels show log-scores of association for all SNPs within 200 kb of the index SNP along the chromosome, and on the right show the effect of the variant allele on the probability of myalgia in subgroups of patients taking one of the three major statins. (Top Panel from Ruaño G, Windemuth A, Wu AH, et al. Mechanisms of statin-induced myalgia assessed by physiogenomic associations. Atherosclerosis, 2011. 218(2): p. 451–6; with permission)

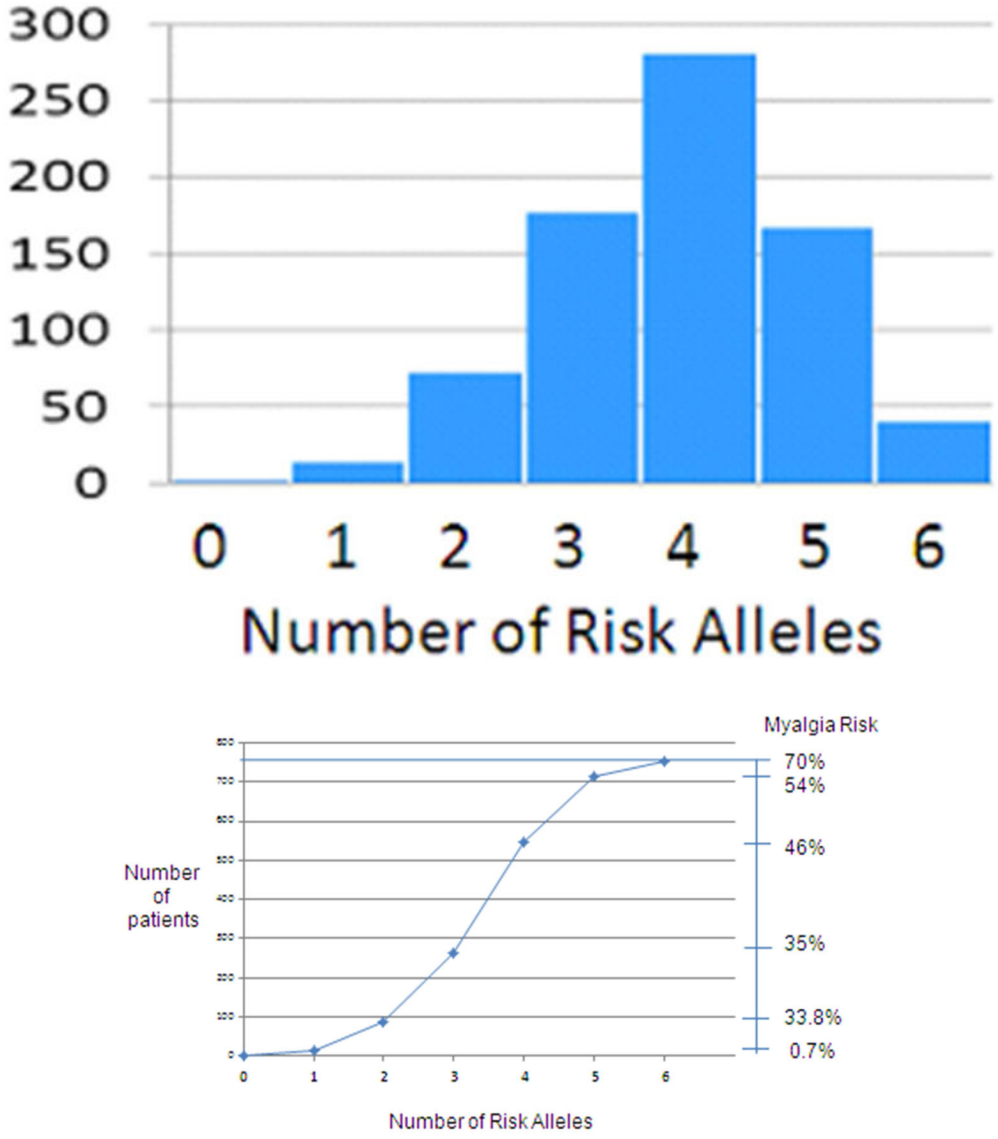

Figure 4.

Left panel: Frequency distribution showing the numbers of patients carrying from 0 to 6 risk alleles, respectively, at the 3 polymorphic sites rs4693570 (*COQ2*, para-hydroxybenzoate-polyprenyltransferase), rs6732348 (*DMPK*, myotonin, protein kinase), and rs17381194 (Plasma membrane calcium-transporting ATPase 1). All patients were treated with statins and ~40% were diagnosed with statin myalgia. ***Right panel:*** Statin myalgia risk index curve based on the same SNP markers. According to the function, a patient with 0 or 1 risk allele has less than 1% chance of experiencing myalgia on statin. A patient with 5 risk alleles has a 54% chance and a patient with 6 risk alleles, a 70% chance. As the number of predictive SNP markers in the model is increased, it may be possible to refine further the prediction of statin myalgia beyond 70%.

#1 Drug / Phenotype	Myalgia Index	logCK	LDL
Rosuvastatin	0.51 (77%)	5.38 (81%)	256.75 (97%)
Atorvastatin	0.22 (47%)	4.48 (40%)	137.15 (65%)
Simvastatin	0.28 (54%)	-2.24 (0%)	42.57 (2%)

#2 Drug / Phenotype	Myalgia Index	logCK	LDL
Rosuvastatin	0.02 (26%)	5.00 (67%)	162.89 (81%)
Atorvastatin	-0.70 (0%)	4.24 (27%)	115.87 (51%)
Simvastatin	0.04 (28%)	0.93 (0%)	207.37 (93%)

#3 Drug / Phenotype	Myalgia Index	logCK	LDL
Rosuvastatin	-0.13 (14%)	5.76 (90%)	78.66 (23%)
Atorvastatin	-0.16 (13%)	5.46 (84%)	175.25 (86%)
Simvastatin	-0.04 (21%)	-1.64 (0%)	209.41 (93%)

Figure 5.

SINM PhyzioType results and drug recommendations for 3 patients (Patients #1, #2, #3). The PhyzioType Result is a matrix defined by predictions for 3 phenotypes (Myalgia Index, locCK, LDL) in response to 3 drugs (rosuvastatin, atorvastatin, simvastatin). The values on the left of the cells are predicted responses, with percentile ranks in parentheses on the right. The color coding of the cells with the predictions for each patient represents their quartile rank in a population distribution: RED, worst quartile with unfavorable response; GREEN, best quartile with favorable response; YELLOW, second and third quartiles with intermediate response.

Table 1

Hypothesis-free associations for myalgia index at a suggestive significance level *($p < 10^{-5}$).* The log-score is the negative logarithm of the *p*-value. A log-score of 5 corresponds to *p*-value of 10^{-5}.

rs id	Chr.	log-score	Coeff.	Freq.	r^2	*Symbol*	Gene Name
rs4693570	4	6.3	0.11	58%	3.3%	*COQ2*	Coenzyme Q10 (100 kb downstream)
rs11980747	7	6.1	−0.25	95%	3.2%	*DNAH11*	Dynein, axonemal, heavy chain 11
rs7267722	20	6.1	0.09	36%	3.3%	rs7267722	no gene link
rs2738466	19	5.6	−0.12	26%	2.9%	*LDLR*	Low density lipoprotein receptor
rs14158	19	5.5	0.12	73%	2.9%	*LDLR*	Low density lipoprotein receptor
rs1023781	15	5.5	−0.10	36%	2.9%	rs1023781	no gene link
rs2116897	19	5.4	0.12	73%	2.8%	*LDLR*	Low density lipoprotein receptor
rs12668317	7	5.3	−0.12	76%	2.8%	*MAGI2*	Membrane associated guanylate kinase, WW and PDZ domain containing 2
rs2569537	19	5.3	0.12	73%	2.8%	*LDLR*	Low density lipoprotein receptor
rs7014327	8	5.1	−0.10	31%	2.6%	*CLN8*	Ceroid-lipofuscinosis, neuronal 8 (epilepsy, progressive with mental retardation)

Table 2

Candidate genes and their associations to myalgia index. The log-score is the negative algorithm of the association *p*-value for each gene. Log-scores greater than 1.3 *(p < 0.05)* are shown. Log-scores 2.8 or greater *(p < 0.015)* indicate genes statistically significant (bolded) after adjusting for testing of 31 multiple genes in the selected set of candidates (Bonferroni correction). Log-scores below 1.3 are not shown and are reported as ns (not significant).

Pathway or pathology	Chr	*Symbol*	log-score	Gene
Serotonin receptors [36]	11	*HTR3B*	ns	5-Hydroxytryptamine (serotonin) receptor 3B
	10	*HTR7*	ns	5-Hydroxytryptamine (serotonin) receptor 7
Organic anion transporter [42]	12	*SLCO1B1*	ns	Solute carrier organic anion transporter family, member 1B1
Vascular genes [35]	3	*AGTR1*	ns	Angiotensin II receptor, type 1
	7	*NOS3*	1.4	Nitric oxide synthase 3 (endothelial cell)
	17	*ACE*	ns	Angiotensin I converting enzyme (peptidyl-dipeptidase A) 1
Muscle phosphorylase [41]	11	*PYGM*	2.0	Phosphorylase, glycogen, muscle
Glucosidase	17	*GAA*	ns	Glucosidase, alpha; acid
Carnitine related [41]	11	*CPT1A*	ns	Carnitine palmitoyltransferase 1A (liver)
	22	*CPT1B*	ns	Carnitine palmitoyltransferase 1B (muscle)
	1	*CPT2*	ns	Carnitine palmitoyltransferase II
Myoadenylate deaminase [41]	1	*AMPD1*	ns	Adenosine monophosphate deaminase 1 (isoform M)
Mitochondrial energy [41]	**4**	***COQ2***	**4.4**	**Coenzyme Q2 homolog, prenyltransferase (yeast)**
	9	*APTX*	ns	Aprataxin
Muscular dystrophy [40]	**19**	***DMPK***	**2.8**	**Dystrophia myotonica-protein kinase**
	X	*DMD*	ns	Dystrophin
	18	*AQP4*	ns	Aquaporin 4
Calcium transport [41]	**12**	***ATP2B1***	**3.1**	**ATPase, Ca++ transporting, plasma membrane 1**
	16	*ATP2A1*	ns	ATPase, Ca++ transporting, cardiac muscle, fast twitch 1
	17	*ATP2A3*	ns	ATPase, Ca++ transporting, ubiquitous
	19	*RYR1*	ns	Ryanodine receptor 1 (skeletal)
	1	*RYR2*	ns	Ryanodine receptor 2 (cardiac)
	3	*ATP2B2*	1.5	ATPase, Ca++ transporting, plasma membrane 2
	3	*ATP2C1*	1.5	ATPase, Ca++ transporting, type 2C, member 1
	7	*RYR3*	1.7	Ryanodine receptor 3
	X	*ATP2B3*	ns	ATPase, Ca++ transporting, plasma membrane 3
	16	*ATP2C2*	ns	ATPase, Ca++ transporting, type 2C, member 2
	12	*ATP2A2*	ns	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2
	1	*ATP2B4*	1.8	ATPase, Ca++ transporting, plasma membrane 4

Pathway or pathology	Chr	*Symbol*	log-score	Gene
Regulator microtubule dynamics [41]	8	*FAM82B*	ns	Family with sequence similarity 82, member B
Rippling muscle disease [41]	3	*CAV3*	ns	Caveolin 3