ABSTRACT: We employed physiogenomic analyses to investigate the relationship between myalgia and selected polymorphisms in serotonergic genes, based on their involvement with pain perception and transduction of nociceptive stimuli. We screened 195 hypercholesterolemic, statin-treated patients, all of whom received either atorvastatin, simvastatin, or pravastatin. Patients were classified as having no myalgia, probable myalgia, or definite myalgia, and assigned a myalgia score of 0, 0.5, or 1, respectively. Fourteen single nucleotide polymorphisms (SNPs) were selected from candidates within the 5-HT receptor gene families ([5a-hydroxytryptamine receptor genes (HTR) 1D, 2A, 2C, 3A, 3B, 5A, 6, 7] and the serotonin transporter gene (SLC6A4). SNPs in the HTR3B and HTR7 genes, rs2276307 and rs1935349, respectively, were significantly associated with the myalgia score. Individual differences in pain perception and nociception related to specific serotonergic gene variants may affect the development of myalgia in statin-treated patients.


PHYSIOGENOMIC ASSOCIATION OF STATIN-RELATED MYALGIA TO SEROTONIN RECEPTORS

GUALBERTO RUANO, MD, PhD,1 PAUL D. THOMPSON, MD,2 ANDREAS WINDEMUTH, PhD,1 RICHARD L. SEIP, PhD,1,2 AMIT DANDE, MD,2 ALEXEY SOROKIN, MD,2 MOHAN KOCHERLA, MS,1 ANDREW SMITH, BS,3 THEODORE R. HOLFORD, PhD,4 and ALAN H. B. WU, PhD3

1 Genomas, Inc., 67 Jefferson Street, Hartford, Connecticut 06102, USA
2 Department of Cardiology, Hartford Hospital, Hartford, Connecticut, USA
3 Department of Laboratory Medicine, University of California, San Francisco and the San Francisco General Hospital, San Francisco, California, USA
4 Department of Biostatistics, Yale University School of Medicine, New Haven, Connecticut, USA

Accepted 14 March 2007

The 3-hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) reductase inhibitors or statins are the most commonly prescribed drugs worldwide because of their ability to reduce atherosclerotic vascular events.41 Statins are generally well tolerated but can produce a variety of adverse drug reactions (ADRs). Most of these ADRs are myopathic, ranging from myalgia with or without elevations in serum creatine kinase (CK) to clinically important rhabdomyolysis.6,8,17–49 However, neuropathic ADRs, including changes in cognition, mood, or behavior,16 and autoimmune ADRs, including myositis,13 are increasingly being recognized. The prevalence of statin-related myopathic complaints has increased with the frequency of statin use, and their incidence has increased with the use of higher doses.

Various mechanisms have been proposed for statin-induced myopathy including altered pharmacokinetics due to drug metabolism or drug–drug interactions,9,54 physiochemical properties of the drugs, effects on metabolic end products such as coenzyme Q10,5 and interference with metabolic pathways regulating muscle repair.51 Statin use may also unmask asymptomatic metabolic myopathies.18,52,53 Effects on cellular regulatory proteins causing activation of molecular pathways, ultimately leading to apoptosis, have also been proposed.47 However, none satisfactorily explains the heterogeneity of the adverse effects and the range of clinical symptomatology.

It is also not clear why some patients experience asymptomatic serum CK elevations during statin therapy, whereas other patients experience myopathic symptoms such as myalgia without serologic

Abbreviations: ADR, adverse drug reaction; AGTR1, angiotensin II type 1 receptor; CEPH, the HapMap reference population; CK, creatine kinase; EDTA, ethylene-diamine tetraacetic acid; FDR, false discovery rate; ERK, extracellular signal–regulated kinases; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; HTR1D, 2A, 2C, 3A, 3B, 5A, 6, and 7, 5-hydroxytryptamine (serotonin) receptor 1D, 2A, 2C, 3A, 3B, 5A, 6, and 7, respectively; NOS3, nitric oxide synthase 3; SLC6A4, solute carrier family 6, member 4; SNP, single nucleotide polymorphism

Key words: myalgia; nociception; physiogenomics; serotonin receptors; statins

Correspondence to: G. Ruano; e-mail: g.ruano@genomas.net

© 2007 Wiley Periodicals, Inc.
Published online 28 June 2007 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/mus.20871
Physiogenomics of Statin Myalgia  
Physiology of muscle pain. Statins can produce a peripheral neuropathy, raising the possibility that individual differences in neuronal perception of pain affect the frequency of myopathic complaints. The neurotransmitter serotonin influences pain perception and has been implicated in the pathogenesis of pain syndromes. Serotonin receptors have also been associated with rheumatic conditions clinically characterized by muscle weakness and pain.

We used physiogenomics to determine whether polymorphisms in serotonergic genes affected the report of statin-related myalgia. Physiogenomics is a medical application of sensitivity analysis, an engineering discipline. The physiogenomic approach has been successfully applied to cardiovascular and neuropsychiatric studies of drugs and diet. Sensitivity analysis is the study of the relationship between the input and the output of a model and, more specifically, utilizing systems theory, it analyzes how variation of the input leads to changes in output quantities. Physiogenomics utilizes the variability in genes as input, measured by single nucleotide polymorphisms (SNPs), and determines how the SNP frequency among individuals relates to the variability in physiologic characteristics, the output. Using this approach, we have previously shown that serum CK levels during statin therapy are associated with genes affecting vascular smooth muscle and raised the novel hypothesis that statins may affect vascular function. The present report suggests that statin-related myalgia may be related to certain serotonin receptors.

MATERIALS AND METHODS

Patient Enrollment. Patients treated with statins for at least 1 month were recruited from Hartford Hospital clinics. They provided written informed consent to participate in the study, which was approved by the Hartford Hospital institutional review board. All patients were recruited and entered into the study by one investigator (A.W.). Subjects were recruited if they were on atorvastatin, simvastatin, or pravastatin, and excluded if they were on other statins or multiple lipid-lowering medications. Valid genotype data were obtained for 195 patients (78 women, 117 men), of whom 82% were Caucasian by self-report. The mean age was 68 ± 13 years. Statin name and dose were obtained by self-report. There were a total of 107 patients on atorvastatin (45 on 10 mg, 29 on 20 mg, 18 on 40 mg, 2 on 80 mg, and 13 on an unspecified dose), 69 patients on simvastatin (1 on 5 mg, 7 on 10 mg, 21 on 20 mg, 28 on 40 mg, 1 on 60 mg, 9 on 80 mg, and 2 on an unspecified dose), and 19 patients on pravastatin (5 on 20 mg, 7 on 40 mg, and 7 on 80 mg).

The patients were interviewed and assessed by a qualified investigator as to whether they suffered from myalgia or “muscle pain” (definite myalgia, 39 patients) or not (no myalgia, 144 patients). For some patients, myalgia symptoms were likely but assessed as ambiguous (probable myalgia, 12 patients). A myalgia score of 1 was assigned to patients with definite myalgia when any of the following criteria could be established from the patient interview: (1) muscle pain began concurrently with the initiation of statin therapy; (2) muscle pains coincided with an increase in statin dosing; (3) muscle pains resolved when the inducing statin was switched to another statin; or (4) muscle pains resolved when statin therapy was discontinued altogether. A myalgia score of 0.5 was assigned to patients with probable myalgia if they complained of muscle pain, but the underlying etiology was not clear or possibly resulted from a comorbidity unrelated to statin therapy. A myalgia score of 0 was assigned to patients with no myalgia, which occurred when there was complete denial of any muscle pain by the patient.

Laboratory Analysis. Blood was either collected prospectively or retrieved from routine clinical analysis. Samples were collected into tubes containing either ethylene-diamine tetraacetic acid (EDTA) or citrate for DNA extraction. The blood was centrifuged, and the plasma was assayed within 2 days for total CK activity using an analyzer (Cobas Integra; Roche Diagnostics, Indianapolis, Indiana). The normal reference range was <200 U/L for men and <140 U/L for women. The DNA was extracted from leukocytes in 1 ml of whole blood using a DNA isolation kit (Puregene Gentra; Qiagen, Valencia, California).

Gene Selection and Genotyping Technology. Nine candidate genes were selected for their role in serotonergic neurotransmission, which has been widely implicated in pain detection and processing in the brain, spinal cord, and peripheral tissues. The genes are listed in Table 1 with brief descriptions of their function in the footnote. Genotyping was performed using the Illumina BeadArray platform and the GoldenGate assay (Table 1). Covariates between the myalgia score and SNPs were analyzed using stepwise, multiple linear regression. Age, gender, race, statin, and dose were not significantly associated with the myalgia score. To test for association, a linear regression model was constructed including the SNP genotype.
SNP genotype was coded quantitatively as a numerical variable indicating the number of minor alleles: 0 for major homozygotes; 1 for heterozygotes; and 2 for minor homozygotes. The \( F \)-statistic \( P \)-value for the SNP variable was used to evaluate the significance of association (Table 2).

**Statistical Analysis.** The endpoint in this study has a discrete, non-normal distribution, so a linear regression test based on the \( F \)-distribution is not automatically valid. To establish validity, we performed an independent calculation of the \( P \)-values using permutation testing.\(^{12,22}\) Permutation testing requires extensive computation, but the resulting \( P \)-values are non-parametric, that is, they are valid regardless of the endpoint distribution. The agreement between permutation \( P \)-values and those from the \( F \)-distribution was very good (\( R^2 = 98\% \), RMSD = 0.055), and

### Table 1. Assay details for SNPs analyzed.

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Chromosome</th>
<th>Major</th>
<th>Minor</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTR1D</td>
<td>rs676643</td>
<td>1</td>
<td>G</td>
<td>A</td>
<td>AGTTTCATCTTGAGCGCTATCT(A/G)AGCTAGCTAACTCGTCC</td>
</tr>
<tr>
<td>HTR2A</td>
<td>rs659734</td>
<td>13</td>
<td>T</td>
<td>C</td>
<td>CTGTAGAGAATTTAAGCTGAAGAA(A/G)TCATAAAAGGAAAGCAGTCA</td>
</tr>
<tr>
<td>HTR2C</td>
<td>rs6312</td>
<td>13</td>
<td>A</td>
<td>G</td>
<td>ACCATAGTACATGGAC(A/G)ACCTGTAGCTAACTCGTCC</td>
</tr>
<tr>
<td>HTR2C</td>
<td>rs539748</td>
<td>X</td>
<td>A</td>
<td>G</td>
<td>GGGAAATATATCTTGAGC(A/G)ATATAAGGAGGATATAAT</td>
</tr>
<tr>
<td>HTR3A</td>
<td>rs6318</td>
<td>X</td>
<td>G</td>
<td>C</td>
<td>TTGCGCTATTATGCGTATCGAATCTGGAAGCCAGG</td>
</tr>
<tr>
<td>HTR3B</td>
<td>rs1150226</td>
<td>11</td>
<td>C</td>
<td>T</td>
<td>TTATGTCACCTCGGGAAGTAA(A/G)AGATGTGTCATCCCTGTTCTC</td>
</tr>
<tr>
<td>HTR5A</td>
<td>rs276807</td>
<td>11</td>
<td>A</td>
<td>G</td>
<td>AAGCCTTTCTCTGCGTATCCGTGAGAAGAC</td>
</tr>
<tr>
<td>HTR5B</td>
<td>rs2276307</td>
<td>11</td>
<td>A</td>
<td>G</td>
<td>GGGAGCGGAGTCGTGAGAAGAC</td>
</tr>
<tr>
<td>HTR6</td>
<td>rs1440451</td>
<td>7</td>
<td>G</td>
<td>C</td>
<td>GGGCGCCAGAAGCGAGGGCGC(A/G)TGACCGGAGTTCAGGAG</td>
</tr>
<tr>
<td>HTR6</td>
<td>rs1805054</td>
<td>1</td>
<td>C</td>
<td>T</td>
<td>GCGCGCCAGAAGCGAGGGCGC(A/G)TGACCGGAGTTCAGGAG</td>
</tr>
<tr>
<td>HTR7</td>
<td>rs1935349</td>
<td>10</td>
<td>G</td>
<td>A</td>
<td>TTATGATGTGTCATCGGGAAGTAA(A/G)AGATGTGTCATCCCTGTTCTC</td>
</tr>
<tr>
<td>HTR7</td>
<td>rs1891311</td>
<td>10</td>
<td>A</td>
<td>G</td>
<td>AAATGAGGAGTTCATCTGGAAGGAGTTCATCCCTGTTCTC</td>
</tr>
<tr>
<td>SLC6A4</td>
<td>rs140700</td>
<td>17</td>
<td>G</td>
<td>A</td>
<td>ATCCTTTCTCGGACACCGCGCCT(A/G)CCCCGCTCTTCTCAAAGGCTTCT</td>
</tr>
<tr>
<td>SLC6A4</td>
<td>rs2020933</td>
<td>17</td>
<td>T</td>
<td>A</td>
<td>TTTGGTCGCCAGAAAAGTGAACC(A/T)GGTCAATGGATTATTTATGA</td>
</tr>
</tbody>
</table>

Shown are chromosome location of the gene (chromosome), sequence of the most common allele (major), and sequence of the least common allele (minor).

HTR1D encodes a serotonin receptor that is active in migraine.\(^{34}\) HTR5A encodes one implicated in several neuropsychiatric disorders,\(^{35}\) and HTR2C encodes one that causes decreased food intake when stimulated by an agonist.\(^{19}\) Pain related to serotonin release from platelets is mediated by the HTR2 receptors.\(^{19}\) HTR3A and 3B encode homologous ligand-gated ion channels that may be involved in psychiatric disorders such as schizophrenia and bipolar disorder.\(^{46}\) HTR5A encodes a serotonin receptor expressed in certain brain areas but with unclear physiological roles.\(^{45}\) HTR6 encodes one that shares high affinity for several therapeutically important antidepressant and antipsychotic drugs,\(^{33}\) and HTR7 encodes one that is a possible schizophrenia susceptibility factor\(^{22}\) with additional roles in pain.\(^{33}\) SLC6A4 is a high-affinity, Na\(^+\)/K\(^+\) -dependent serotonin transporter localized in presynaptic neuronal membranes; it participates in active clearing of serotonin from synaptic spaces and is the target of serotonin selective reuptake inhibitor class of drugs.\(^{31}\)

### Table 2. Genes and SNPs analyzed for associations with myalgia.

<table>
<thead>
<tr>
<th>Function</th>
<th>Gene</th>
<th>SNP</th>
<th>MAF CEPH</th>
<th>MScore</th>
<th>log CK</th>
<th>Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotonin receptors</td>
<td>HTR1D</td>
<td>rs676643</td>
<td>17.1%</td>
<td>0.125</td>
<td>0.79</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>HTR2A</td>
<td>rs659734</td>
<td>6.6%</td>
<td>0.425</td>
<td>0.54</td>
<td>-0.06</td>
</tr>
<tr>
<td></td>
<td>HTR2C</td>
<td>rs6312</td>
<td>7.2%</td>
<td>0.392</td>
<td>0.43</td>
<td>-0.06</td>
</tr>
<tr>
<td></td>
<td>HTR2C</td>
<td>rs539748</td>
<td>15.3%</td>
<td>0.673</td>
<td>0.51</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>HTR3A</td>
<td>rs1150226</td>
<td>15.4%</td>
<td>0.270</td>
<td>0.41</td>
<td>-0.05</td>
</tr>
<tr>
<td></td>
<td>HTR3B</td>
<td>rs3758987</td>
<td>28.0%</td>
<td>0.740</td>
<td>0.82</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>HTR3B</td>
<td>rs2276307</td>
<td>23.8%</td>
<td>0.211</td>
<td>0.68</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>HTR5A</td>
<td>rs1440451</td>
<td>1.5%</td>
<td>0.524</td>
<td>1.00</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>HTR6</td>
<td>rs1805054</td>
<td>20.8%</td>
<td>0.853</td>
<td>0.57</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>HTR7</td>
<td>rs1935349</td>
<td>14.8%</td>
<td>0.026</td>
<td>0.96</td>
<td>0.13</td>
</tr>
<tr>
<td>Serotonin transporter</td>
<td>SLC6A4</td>
<td>rs140700</td>
<td>8.5%</td>
<td>0.435</td>
<td>0.38</td>
<td>-0.06</td>
</tr>
<tr>
<td></td>
<td>SLC6A4</td>
<td>rs2020933</td>
<td>9.4%</td>
<td>0.391</td>
<td>0.67</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Shown are gene function, HUGO gene symbol, and dbSNP identifier for each locus. Also shown is the minor allele frequency (MAF) as determined in our study population, the allele frequency in the CEPH population (Utah residents of central European ancestry) of the HapMap project, the \( P \)-value of association to the myalgia score (MScore), and the \( P \)-value of association to the serum creatine kinase activity for comparison. The coefficient indicates the average increase (or decrease) in the myalgia score for each copy of the minor allele. The SNPs in boldface type were significantly associated with myalgia score.
the ranking of all SNPs is identical under both, with the exception of rs659734, which is not significantly associated and changes in \( P \)-value from 0.43 to 0.5, and in rank from 8 to 10. Because they are based on a Monte Carlo simulation, the permutation \( P \)-values have a stochastic component and vary slightly from simulation to simulation. For this reason, we report here the more reproducible values from the \( F \)-distribution.

As an alternative test for significance, we performed a chi-square test on the contingency table linking genotype with myalgia outcome. We found the same two SNPs to be significant, although less so (\( P = 0.017 \) and 0.03). This is likely due to the fact that a contingency table does not assume a dose-response relationship between allele and phenotype, which makes it the less sensitive test if such a relationship is present. We also performed a logistic regression on a binary endpoint of no myalgia vs. probable or definite myalgia. Again, the \( P \)-values are significant (\( P = 0.01 \) and 0.02), but not as strong as those from the linear regression. This is explained by the loss of power inherent in the loss of distinction between probable and definite myalgia. To account for the multiple testing of 14 SNPs, we calculated adjusted \( P \)-values using Benjamini and Hochberg’s false discovery rate (FDR) procedure.\(^{10,11,32}\)

**RESULTS**

The distribution of the myalgia phenotypes in the study population is shown in Figure 1. It features a trough between the definite phenotypes to account for probable myalgia. There was no significant difference in the proportion of myalgia patients among the patients treated with atorvastatin, simvastatin, and pravastatin.

Table 2 shows the results of the SNP association tests. Only HTR3B:rs2276307 and HTR7:rs1935349 were significantly associated. The \( R^2 \) values, which indicate the proportion of the observed variation explained by each SNP, were 4% and 3%, respectively. The coefficients were 0.14 and 0.13, meaning that each allele leads to an increase of the myalgia score of 14%. The false discovery rates under multiple testing were 8% and 17%, respectively. Table 2 also indicates the allele frequency for all SNPs as observed in our study population. These agree remarkably well with the ones reported by the HapMap project for the CEPH population of Caucasians, except that HTR6:rs1805054 is not available in the HapMap.

No SNP was significantly associated with serum CK activity (Table 2). We previously reported SNPs to be significantly associated with statin-related CK activity for the angiotensin II type 1 receptor (AGTR1) and nitric oxide synthase 3 (NOS3) genes.\(^{37}\) Neither gene was associated with the myalgia score.

Figure 2 shows physiogenomic representations of myalgia severity by depicting for each gene the SNP frequency in the subpopulation with no myalgia compared to those in the subpopulations with probable and definite myalgia. For SNPs with a strong association, the marker frequency will be significantly different between myalgia scores of 0 and 1. Conversely, if a marker is neutral, the frequency will be independent of myalgia score and the plot will be essentially flat. For example, the first panel in Figure 2 shows the plot for SNP rs2276307 of the HTR3B gene. The frequency of the minor allele is \(< 20\%\) in subjects with myalgia scores of 0, whereas it approaches 35% in subjects with myalgia scores of 1 and has an intermediate frequency in subjects with myalgia scores of 0.5. This finding indicates a strong association between the HTR3B marker and myalgia. As the frequency of the minor allele is higher in patients with definite myalgia, HTR3B SNP rs2276307 is considered a risk marker for statin-related myalgia.

**DISCUSSION**

Statin-related muscle toxicity may consist of statin myopathy, myalgia (muscle complaint without serum CK elevation), myositis, or rhabdomyolysis.\(^{39}\) Of these, myalgia is the most common and adversely
affects quality of life and compliance with these medications.

The myalgia rate of 20% in the present study is higher than the myopathy rates of 10% demonstrated in most published studies.4,20,50 Myalgia per se has rarely been examined in statin clinical trials.48 Industry-sponsored trials have relied on serum CK elevations to document statin myopathy. In contrast, we inquired directly about muscle pain. We believe that this approach elicits muscle discomfort–related complaints from patients more frequently than other approaches and is also a strategy that uncovers subtle symptoms. Other strategies might also have reduced the rate of myopathy in industry trials. High-risk patients were often not recruited. Some large trials, such as the Heart Protection Study,21 used a “run-in” period before the study to exclude patients intolerant of or non-compliant with the medications.4,20,50

We examined the relationship between serotonin metabolism and statin myalgia because the serotonergic system has been implicated in clinical syndromes with muscle pain and tenderness, such as certain rheumatic diseases.24,28 Serotonin has also been implicated in other neurological disorders including migraine2 and epilepsy3 in addition to its well-known role in psychiatric conditions.35,44 Various lines of evidence implicate the serotonin receptors in nociception.1,14,33,43 We found a statistically significant relationship between myalgia and two SNPs (rs2276307 and rs1935349) in genes HTR3B and HTR7, which encode serotonin receptors. These results suggest that gene polymorphisms producing individual differences in pain perception may have an important role in patients’ reports of muscle pain.

Previous pharmacogenetic studies of statins have concerned mostly cholesterol- and muscle-related genes.27,55 We have examined the possibility that SNPs in genes expressed in neurological pathways affect the incidence of statin myalgia. We found associations between genes involved in serotonergic function and statin myalgia. Consequently, it is possible that “statin myopathy” may be a constellation of independent syndromes with varying innate predispositions in the population and diverse physiological mechanisms encompassing various gene pathways.

The present study has various limitations. We focused only on potential class-wide effects. The subjects studied were a diverse group of patients who were treated with different statins for differing periods of time. Only 40 patients reported definite statin myalgia. Myalgia is necessarily subjective as there are no objective measurement instruments to quantify this condition. We examined only statistically significant associations that may not reflect cause and effect. It is also possible that the significant SNPs are in linkage disequilibrium with other genes responsible for statin myopathy. Further research is ongoing to substantiate these findings in additional patient cohorts and to discover new drug-specific associations using genome-wide arrays.
As more physiological pathways and eventually the entire genome are screened, a multi-gene model can be developed where an individual’s configuration of various significant SNPs can reliably predict the probability of statin-related adverse events for each patient. Generalized clinical use of such diagnostics for DNA-guided medical management may help to improve statin tolerability and safety as these drugs are deployed ever more widely in treatment and prevention of many cardiovascular disorders.

This research was funded by grants from the Hartford Hospital Research Administration and by Genomas internal research and development funds.

REFERENCES


