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Increased Frequency of DRB1*11:01 in Anti-HMG-CoA Reductase-Associated Autoimmune Myopathy

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Abstract

Objective—Here, we investigated the association of anti-HMG-CoA reductase (HMGCR) myopathy with human leukocyte antigen (HLA) class I and II antigens.

Methods—HLA antigens were determined in (a) 20 white and 8 black anti-HMGCR patients, (b) 487 white and 167 black controls, and (c) 51 white subjects with mild self-limited statin intolerance.

Results—White anti-HMGCR patients had a higher frequency of the combination DR11; DQA5; DQB7 than controls or statin intolerant subjects (70 vs 17%, $p = 4.1 \times 10^{-7}$, OR 11.7, 95% CI 4.0-35.3 and 70 vs 21%, $p = 5.4 \times 10^{-4}$, OR = 8.3, 95% CI = 2.2-33.9, respectively). This combination was not increased in black anti-HMGCR subjects compared to controls (13 vs 3%, p = 0.2, OR = 4.6, 95% CI = 0.2-53.3). However, DR11 was increased in black anti-HMGCR patients compared to controls (88 vs 21%, p = 0.0002, OR = 26.4, 95% CI = 3.1-590.3). High resolution mapping showed that 95% with DR11 had DRB1*11:01. DQA1 and DQB6 were less frequent in white anti-HMGCR positive patients compared to controls (25 vs 64%, $p = 5.5 \times 10^{-4}$, OR = 0.2, 95% CI = 0.1-0.5 and 0 vs 45%, $p = 2.1 \times 10^{-5}$, OR = 0.0, 95% CI = 0.0-0.3, respectively). DRB11 was not associated with particular disease features.

Conclusion—DRB1*11:01 is associated with increased risk of anti-HMGCR myopathy in whites and blacks. These findings suggest a mechanistic link between statin exposure, increased HMGCR expression, and the possible presentation of HMGCR-derived peptide(s) by DRB1*11:01.

INTRODUCTION

Several groups have described patients with a statin-associated myopathy which continues to progress even after the statin is discontinued (1–3). We recently discovered that these patients, as well as a minority without statin exposure, have antibodies recognizing HMGCR, the pharmacologic target of statins; in those anti-HMGCR positive patients who were 50 years-old or over at the time weakness developed, greater than 90% were exposed to statins (4). A favorable response to immunosuppression, the increased expression of MHC I on biopsied myofibers, and the presence of autoantibodies suggest that anti-HMGCR

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myopathy is an immune-mediated process (3, 4). Furthermore, anti-HMGCR antibodies are specific for those with immune-mediated myopathy and were not found in large populations of patients with statin exposure, including those with self-limited musculoskeletal side-effects associated with statin use (5).

Antigen presentation of peptides to T cells depends upon the HLA system and an increased risk of developing many autoimmune diseases, including autoimmune myopathy, is frequently associated with specific HLA alleles (6). For example, in Caucasians, DRB1*0301 is a risk factor for developing an inflammatory myopathy and especially for developing anti-Jo-1 (7), the most prevalent autoantibody associated with autoimmune myopathy.

Here, we investigated whether HLA class I and II antigens are associated with increased risk of developing anti-HMGCR myopathy or influence other aspects of this disease.

PATIENTS AND METHODS

Study Populations

Study population 1: Between May 2002 and March 2011, 937 patients seen by a faculty member at the Johns Hopkins Myositis Center with suspected myopathy as defined by proximal muscle weakness, elevated CK levels, myopathic EMG findings, muscle edema on magnetic resonance imaging (MRI), and/or myopathic features on muscle biopsy were enrolled in a longitudinal study. Sera from all subjects were screened for the presence of anti-HMGCR autoantibodies by ELISA as previously described (4). Positive sera were confirmed by immunoprecipitating *in vitro* transcribed and translated HMGCR protein as described (4). Sex, maximum creatine kinase (CK) levels, age at onset of weakness, and the presence of statin exposure at the onset of weakness was documented for all anti-HMGCR positive patients.

Study population 2: 51 patients affected by familial hypercholesterolemia (FH) due to LDLR gene mutations evaluated at the Chicoutimi Hospital Lipid Clinic and ECOGENE-21 Clinical Research Center (Chicoutimi, Quebec, Canada) who presented with signs and symptoms of muscular intolerance to statins were described in a previous report (5) and were also included in this study. The degree of myalgias and muscular weakness was self-reported by subjects as part of a detailed questionnaire. The clinical evaluation also included plasma CK and myoglobinuria assessment. These patients were screened for anti-HMGCR antibodies as described above.

Control population: 487 European American (white) and 167 African American (black) potential organ donors were recruited at the Johns Hopkins Hospital and were included as normal controls. Further demographic information regarding these controls was not available.

HLA typing

All anti-HMGCR positive subjects and normal controls and the statin-intolerant FH subjects were typed at an intermediate resolution antigen level for HLA-DRB1, -DQA, and DQB. All anti-HMGCR positive subjects with a DRB1*11 antigen underwent high resolution typing to define the specific DRB1 alleles. Initial intermediate level typing of HLA DR alleles and typing for DQA and DQB alleles was performed by reverse sequence-specific oligonucleotide probe hybridization (LABType SSO, One Lambda, Inc., Canoga Park, CA). High resolution definition of DR11 alleles was performed by sequencing based typing (SBT). AlleleSEQR HLA-DRB1 SBT reagents (Abbott Molecular Diagnostics, Des Plaines, IL) were used for SBT of Exon 2 of HLA-DRB1. Polymerase chain (PCR) and SBT

reactions were performed on the GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA). Sequencing data was collected using a 3500XL Genetic Analyzer (Applied Biosystems, Foster City, CA) and HLA-DRB1 allele assignments were interpreted using ATF software (Conexio Genomics, Western Australia).

Statistical analysis

Eighteen HLA types (2 HLA-A, 3 HLA-B, 4 HLA-DR, 4 HLA-DQA, 5 HLA-DQB) had frequencies > 20% in either white anti-HMGCR patients or white normal controls. Only these antigens were analyzed for association with anti-HMGCR susceptibility using the Fisher exact test. Because 18 HLA types were considered in the analysis, a Bonferroni correction factor of 18 was applied, and an uncorrected p value < 0.00277 was considered significant. Possible associations with prevalence of statin exposure, titers of anti-HMGCR antibodies, gender, mean age at onset of weakness, and maximal CK levels were analyzed by using the Fisher exact test or *t* test.

Standard protocol approvals, registrations, and patient consents

IRB and/or ethics review board approval and participants' written informed consent was obtained from each participant.

RESULTS

Screening by ELISA followed by confirmation with immunoprecipitation revealed that 52 of 937 patients (5.5%) enrolled at the Myositis Center were positive for anti-HMGCR antibodies. Among the anti-HMGCR positive subjects, DNA samples were available from 20 white and 8 black subjects. Each of these subjects presented with proximal weakness and elevated serum CK levels (> 2000 IU/L in each case). Muscle biopsies were available for review from 24 of 28 subjects and, in each case, revealed a necrotizing myopathy as is typical of patients with anti-HMGCR-associated myopathy (3); one of these cases also had prominent endomysial and perivascular inflammation.

Intermediate level resolution antigen typing for HLA-DRB1, -DQA, and DQB were performed on all subjects and the complete results for anti-HMGCR subjects of both races are included as Supplementary Table 1. Table 1 shows the HLA antigens found with frequency of > 20% in either white patients or normal controls. Antigens with significant increased frequencies in anti-HMGCR positive subjects compared to normal controls included: DR11 (70 vs 18%, $p = 1.2 \times 10^{-6}$), DQA5(75 vs 40%, p = 0.0022), and DQB7(75 vs 35%, $p = 5.5 \times 10^{-4}$). The antigen combination HLA-DR11; -DQA5; -DQB7 was found in 70% of white anti-HMGCR positive subjects compared to 17% of normal controls ($p = 4.1 \times 10^{-7}$).

Given that DNA samples from only 8 black anti-HMGCR myopathy patients were available for analysis, comprehensive comparisons with black controls were not performed. However, we noted that the antigen combination HLA-DR11; -DQA5; -DQB7 was not increased in the 8 black anti-HMGCR positive subjects compared to 167 black controls (13 vs 3%, p = 0.3). Interestingly, however, the frequency of DR11 was markedly increased in black anti-HMGCR positive patients compared to black controls (88 vs 21%, p = 0.0002).

To determine whether the antigen combination HLA-DR11; -DQA5; -DQB7 is uniquely associated with anti-HMGCR myopathy or is also associated with relatively mild forms of self-limited statin intolerance, we HLA typed 51 white FH patients with statin-intolerance requiring the cessation of treatment. The clinical characteristics of these patients have been detailed elsewhere (5) and are included here in Supplementary Table 2. In brief, 41 patients experienced myalgias, 17 patients became weak, and 9 patients had mild CK elevations or

myoglobinuria that resolved once statin exposure was discontinued. None of these 51 patients had positive anti-HMGCR titers (5). Anti-HMGCR myopathy subjects had a higher frequency of the antigen combination HLA-DR11; -DQA5; -DQB7 compared to those with self-limited statin-related musculoskeletal side-effects (70 vs 21%, $p = 5.4 \times 10^{-4}$).

We sequenced the DR locus alleles in all DR11 positive black and white patients with anti-HMGCR myopathy. This showed that DRB1*11:01 was present in 13 of 14 (93%) white patients and 7 of 7 (100%) black subjects with the DR11 antigen. .

The clinical characteristics of anti-HMGCR patients of both races with and without DR11 are shown in Table 2. Neither the gender, race, age at onset of weakness, prevalence of statin exposure, mean CK levels, nor mean anti-HMGCR titers were significantly associated with this antigen.

We also looked for negative correlations between HLA alleles and anti-HMGCR myopathy. Indeed, Table 1 shows that DQA1 and DQB6 were decreased in frequency among white anti-HMGCR positive patients compared to controls (25 vs 64%, $p = 5.5 \times 10^{-4}$ and 0 vs 45%, $p = 2.1 \times 10^{-5}$, respectively).

DISCUSSION

In this study, we found that the antigen combination HLA-DR11; -DQA5; -DQB7 was more prevalent among whites with anti-HMGCR myopathy than among normal controls. This antigen combination was not increased in a cohort of subjects with relatively mild self-limited statin-intolerance, indicating that it is not an immunogenetic risk factor for all forms of statin sensitivity.

In blacks, the DR11 antigen but not the antigen combination HLA-DR11; -DQA5; -DQB7 was strongly associated with anti-HMGCR myopathy. Since DR11 is strongly associated with anti-HMGCR myopathy in both races, DRB11, rather than another component of the antigen combination HLA-DR11; -DQA5; -DQB7 most likely confers the risk of anti-HMGCR myopathy. However, we cannot exclude the possibility that the observed association with HLA DR11 reflects linkage disequilibrium with other genes that influence susceptibility to anti-HMGCR myopathy. Indeed, the HLA gene contains numerous genes that participate in the immune response, including complement factors, cytokines, and heat shock proteins.

Sequencing showed that the DR11:01 allelewas present in 13 of 20 (65%) whites and 7 of 8 (88%) blacks with anti-HMGCR myopathy. We did not sequence the DR11 in our control subjects. However, a recent study showed that DR1*11:01 was found in only 38 of 539 (7%) white controls and 29 of 263 (11%) black controls (8). Using these published values, the odds ratios for the presence of DRB1*11:01 in anti-HMGCR myopathy patients vs. controls is 24.5 ($p = 3.2 \times 10^{-10}$) in whites and 56.5 ($p = 3.1 \times 10^{-6}$) in blacks. This represents one of the strongest associations between an immunogenetic risk factor and a myositis autoantibody (6).

In a previous study. O'Hanlon and colleagues reported the HLA types of 571 European American subjects with inflammatory myopathies and various myositis autoantibodies (7). They found that 4 out 5 (80%) anti-Ku positive myositis subjects had DR11 but that only 56 of 343 (16.3%) other myositis patients had this antigen. The association between DR11 and the development of anti-Ku was statistically significant ($p = 0.049$). However, no other myositis autoantibodies or myositis subgroups (e.g., dermatomyositis or polymyositis) were associated with DR11. High resolution phenotyping was not performed to determine

whether the anti-Ku positive subjects had DRB1*1101 or one of the many other DRB1*11 alleles.

DRB1*1101 has been associated with development of anti-Ro with anti-La in neonatal lupus (9) and resistance to chronic Lyme disease arthritis (10). However, to the best of our knowledge, this is the first time this HLA allele has been implicated as a risk factor for developing a form of autoimmune myopathy. Interestingly, although numerous distinct HLA alleles confer increased risk of developing autoimmune myopathy in either whites or blacks, only DR11 (in anti-HMGCR myopathy) and DRB1*0301 (in dermatomyositis (11, 12)) are found at increased frequencies in both racial groups.

In diseases such as Guillain-Barre' syndrome (13) and inclusion body myositis (14), specific HLA alleles are associated with disease severity and other clinical features. However, in this study of patients with anti-HMGCR myopathy, we did not find an association between DRB1*11 and mean CK levels, age at onset of disease, autoantibody titers, or the prevalence of statin exposure.

In addition to the positive association with DR11, this study demonstrated that DQA1 and DQB16 were negatively associated with developing anti-HMGCR myopathy in white patients. Although this suggests these HLA alleles are protective against developing anti-HMGCR myopathy in patients exposed to statins, the mechanism(s) underlying this association is unknown.

This study has several limitations. First, the number of anti-HMGCR positive subjects studied was relatively small. Second, all of the studied subjects were from one cohort of patients recruited at a single center; confirming the association of DR11 with anti-HMGCR myopathy in another large cohort of anti-HMGCR subjects will be important. Finally, it may be that DR11 confers susceptibility to statin-induced myositis, rather than anti-HMGCR antibodies per se. However, since statin exposure is common in controls as well as in polymyositis and dermatomyositis patients without anti-HMGCR antibodies (3), it's not currently clear how to define "statin-induced" myositis patients in the absence of anti-HMGCR antibodies.

As with other autoimmune diseases, the autoimmune myopathies are thought to result when genetically susceptible individuals are exposed to specific environmental triggers (15). We previously demonstrated that statins, an environmental exposure causing increased expression of HMGCR protein, are strongly associated with the development of anti-HMGCR myopathy (4). In this study, we show that those with DRB1*1101 are also at a particularly increased risk of developing anti-HMGCR myopathy. This association suggests the possibility that DRB1*11:01 preferentially presents a strongly immunogenic HMGCR-derived peptide that is generated when HMGCR is over-expressed in the presence of statins.

Interestingly, statins are known to decrease the expression of class II HLA molecules by down-regulating the expression of the class II transactivator (16). Thus, an additional explanation for the association between DRB11, statin exposure, and the development of anti-HMGCR myopathy includes the possibility that, unlike other studied HLA genes, DRB11 gene expression is not down-regulated by statin exposure. Future studies will be needed to clarify the mechanisms underlying the interaction between statin use, the DRB1*11:01 allele, and the development of anti-HMGCR myopathy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Significance and Innovations

1. We have identified the class II allele DRB1*11:01 as a strong genetic risk factor for developing anti-HMGCR-associated myopathy.
2. The class II alleles DQA1 and DQB6 appear to be protective against developing anti-HMGCR-associated myopathy.

Table 1

HLA class I and II antigens in white anti-HMGCR myopathy patients and white controls[a]

HLA	anti-HMGCR (%) n=20	controls (%) n=487	OR (95% CI)	p value
Class I				
A2	10 (50)	243 (50)	1.0 (0.4–2.7)	1.0
A3	7 (35)	139 (29)	1.3 (0.5–3.7)	0.6
B35	6 (30)	83 (17)	2.1 (0.7–6.0)	0.1
B44	9 (45)	129 (26)	2.3 (0.8–6.1)	0.08
B60	5 (25)	51 (10)	2.9 (0.9–8.8)	0.06
Class II				
DR4	8 (40)	164 (34)	1.3 (0.5–3.5)	0.6
DR7	7 (35)	138 (28)	1.4 (0.5–3.8)	0.6
DR11	14 (70)	89 (18)	10.4 (3.6–31.4)	1.2×10^{-6} [b]
DR15	0 (0)	138 (28)	0 (0.0–0.6)	0.003
DQA1	5 (25)	315 (64)	0.2 (0.1–0.5)	5.5×10^{-4} [b]
DQA2	7 (35)	137 (28)	1.4 (0.5–3.8)	0.6
DQA3	9 (45)	171 (35)	1.5 (0.6–4.0)	0.5
DQA5	15 (75)	194 (40)	4.5 (1.5–14.5)	0.0022 [b]
DQB2	7 (35)	174 (36)	1.0 (0.3–2.7)	1.0
DQB5	5 (25)	133 (27)	0.9 (0.3–2.7)	1.0
DQB6	0 (0)	217 (45)	0.0 (0.0–0.3)	2.1×10^{-5} [b]
DQB7	15 (75)	172 (35)	5.5 (1.8–17.6)	5.5×10^{-4} [b]
DQB8	5 (25)	108 (22)	1.2 (0.4–3.5)	0.8
DR11;DQA5;DQB7	14 (70)	81 (17)	11.7 (4.0–35.3)	4.1×10^{-7}

Abbreviations: CI = confidence interval; HLA = human leukocyte antigen; OR = odds ratio.

[a] Set of alleles with frequency >20% in either patients or controls was analyzed by Fisher exact test.

[b] $p < 0.00277$ was considered significant using the Bonferroni correction for n = 18 comparisons.

Table 2

Clinical characteristics of all anti-HMGCR subjects with and without DRB1*11

	with DRB1*11, n = 21	without DRB1*11, n = 7	p value
Male, n (%)	10 (48)	2 (29)	0.7
Black race (%)	7 (33)	1 (14)	0.6
Age in years at onset (SD)	53.0 (15.2)	57.9 (9.8)	0.4
Statin-exposed (%)	17 (81)	6 (86)	1.0
Median CK (SD)	11558 (8960)	10454 (4865)	0.8
Anti-HMGCR ELISA titer (SD)	1.24 (0.48)	1.05 (0.27)	0.3