Uncoupling the Roles of HLA-DRB1 and HLA-DRB5 Genes in Multiple Sclerosis


*J Immunol* 2008; 181:5473-5480; doi: 10.4049/jimmunol.181.8.5473

http://www.jimmunol.org/content/181/8/5473

---

**References**  This article cites 53 articles, 12 of which you can access for free at: http://www.jimmunol.org/content/181/8/5473.full#ref-list-1

**Subscription**  Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**  Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**  Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts

---

*The Journal of Immunology* is published twice each month by The American Association of Immunologists, Inc., 1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 2008 by The American Association of Immunologists All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.
Uncoupling the Roles of HLA-DRB1 and HLA-DRB5 Genes in Multiple Sclerosis

Stacy J. Caillier,* Farren Briggs,† Bruce A. C. Cree,* Sergio E. Baranzini,* Marcelo Fernandez-Viña,‡ Patricia P. Ramsay,† Omar Khan,§ Walter Royal III,¶ Stephen L. Hauser,* Lisa F. Barcellos,*,†† and Jorge R. Oksenberg2* 

Genetic susceptibility to multiple sclerosis (MS)3 is associated with the MHC located on chromosome 6p21. This signal maps primarily to a 1-Mb region encompassing the HLA class II loci, and it segregates often with the HLA-DQB1*0602, -DQA1*0102, -DRB1*1501, -DRB5*0101 haplotype. However, the identification of the true predisposing gene or genes within the susceptibility haplotype has been handicapped by the strong linkage disequilibrium across the locus. African Americans have greater MHC haplotype diversity and distinct patterns of linkage disequilibrium, which make this population particularly informative for fine mapping efforts. The purpose of this study was to establish the telomeric boundary of the HLA class II region affecting susceptibility to MS by assessing genetic association with the neighboring HLA-DRB5 gene as well as seven telomeric single nucleotide polymorphisms in a large, well-characterized African American dataset. Rare DRB5*null individuals were previously described in African populations. Although significant associations with both HLA-DRB1 and HLA-DRB5 loci were present, HLA-DRB1*1503 was associated with MS in the absence of HLA-DRB5, providing evidence for HLA-DRB1 as the primary susceptibility gene. Interestingly, the HLA-DRB5*null subjects appear to be at increased risk for developing secondary progressive MS. Thus, HLA-DRB5 attenuates MS severity, a finding consistent with HLA-DRB5’s proposed role as a modifier in experimental autoimmune encephalomyelitis. Additionally, conditional haplotype analysis revealed a susceptibility signal at the class III AGER locus independent of DRB1. The data underscore the power of the African American MS dataset to identify disease genes by association in a region of high linkage disequilibrium. The Journal of Immunology, 2008, 181: 5473–5480.

*Department of Neurology, University of California, San Francisco, CA 94143; †Division of Epidemiology, School of Public Health, University of California, Berkeley, CA 94720; ‡Division of Laboratory Medicine, University of Texas, Cancer Center, Houston, TX 77030; ††Department of Neurology, Wayne State Medical School, Detroit, MI 48201; §Maryland Center for Multiple Sclerosis Treatment and Research, University of Maryland School of Medicine, Baltimore, MD 21201

Received for publication July 2, 2008. Accepted for publication August 16, 2008.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was funded by grants from the National Institutes of Health (R01 NS046297, U19AI067152, K23 NS048869-01, and R01NS049510) and the National Multiple Sclerosis Society (RG3060C8).

2 Address correspondence and reprint requests to Dr. Jorge R. Oksenberg, Department of Neurology, University of California, San Francisco, 513 Parnassus Avenue, Medical Science Building, Room S-256, San Francisco, CA 94143-0435. E-mail address: jorge.oksenberg@ucsf.edu

3 Abbreviations used in this paper: MS, multiple sclerosis; AGE, advanced glycation end product; CHM, conditional haplotype method; EAE, experimental autoimmune encephalomyelitis; LD, linkage disequilibrium; MBP, myelin basic protein; OR, odds ratio; SNP, single nucleotide polymorphisms.

Copyright © 2008 by The American Association of Immunologists, Inc. 0002-1767/08/52.00
end-products, a member of the Ig superfamily and mediator of chronic inflammatory reactions (16).

Materials and Methods

Subjects

The primary dataset studied consisted of 1635 African American individuals, including 769 MS cases, 124 parents, and 742 unrelated control individuals. All MS subjects met established diagnostic criteria (17). MS phenotypes were characterized by systematic chart review as previously described (19). Global estimation of European ancestry was documented based on genotyping of 186 informative SNPs in 713 (92.7%) MS cases and 500 (67.4%) controls (19). Mean European ancestry proportions in African American MS cases and controls were not statistically different (p > 0.10).

SNP genotyping. DRB5. All study participants were screened for the presence of DRB5 using a validated gene-specific TaqMan assay. An internal positive control (β-globin) was included in each well to confirm that the reaction amplified successfully. PCR was conducted in a total volume of 10 µl containing 20 ng DNA, 0.5 µM DRB5-specific primers (forward 5'-ACGTTTCTCTGTCGACGCTTAA-3', reverse 5'-TGGACCCATCTCCTCCAAA-3'), 0.45 µM control primers (forward 5'-ACGGGAAGCCAGGACAGAAGA-3', reverse 5'-AGGGACTGCCGCCCACACTAAA-3'), 0.125 µM VIC-labeled DRB5-specific probe (5'-ACCAAGCCAGAAGAGATCTCCGACG-3'), and 0.125 µM FAM-labeled control probe (5'-TCTACCCTGACGACGGTTGTCTGAT-3'). Amplification was performed in an ABI Prism 7900HT Sequence Detection System with an initial 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Samples are considered to contain at least one copy of the DRB5 gene if the respective Ct exceeds a preestablished threshold. The second exon for DRB5 was then sequenced for allele determination.

SNP genotyping. AGER (rs1635). AGER (rs2076530), BTN2L2 (rs2076530), AGER (rs2076600, rs1035798, rs184003), and MICA (rs1051796, rs1063653) SNP genotyping (Fig. 1) was completed in the African American dataset (n = 1635 individuals) using ABI custom TaqMan assays designed on File Builder 2.0 software. TaqMan SNP genotyping assays are conducted in 384-well plates using TaqMan Universal PCR Master Mix on an ABI 7900HT Sequence Detection System using SDS 2.0 software. Similarly, two AGER SNPs (rs2076600, rs1035798) were genotyped in white MS cases and controls for confirmatory analyses. The entire AGER gene was sequenced in 10 African American MS patients and 10 African American controls in an effort to locate any causative SNPs in the gene, but no novel alleles were found.

Table I. Clinical and demographic features of the dataset

<table>
<thead>
<tr>
<th></th>
<th>African American</th>
<th>African American</th>
<th>White MS Cases</th>
<th>White Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total N</td>
<td>769</td>
<td>866</td>
<td>487</td>
<td>434</td>
</tr>
<tr>
<td>Female/male ratio</td>
<td>3.8:1</td>
<td>1.27:1</td>
<td>2.2:1</td>
<td>2.0:1</td>
</tr>
<tr>
<td>% European ancestry</td>
<td>22 ± 11.5</td>
<td>23 ± 15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean age of onset in years</td>
<td>32.6 ± 9.5</td>
<td>33.6 ± 9.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean disease duration in years</td>
<td>9.82 ± 7.9</td>
<td>8.82 ± 9.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relapsing remitting cases (%)</td>
<td>431, 58.5%</td>
<td>340, 69.8%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a For all markers, including HLA, allele frequencies were similar between female and males study participants (data not shown).
b European ancestry in African Americans was documented based on genotyping of 186 informative SNPs in 713 (92.7%) MS cases and 500 (67.4%) controls (19). Mean European ancestry proportions in African American MS cases and controls were not statistically different (p > 0.10).
c Family controls consist of 124 parents (62 nontransmitted chromosomes); 742 unrelated controls were also used.

d Family controls consist of 124 parents (62 nontransmitted chromosomes); 742 unrelated controls were also used.

FIGURE 1. Genomic organization of the MHC region of human chromosome 6p21.3. Location of DRB1, DRB5, and the seven SNPs in four genes covering a 1.2-Mb segment telomeric to the DRB1 gene. Each filled box represents the relative size and location of each gene in relation to each other. dbSNP rs numbers are listed below each gene.
SNPs were found. Additional genotype data for CEPH (CEU) and Yoruban (YRI) International HapMap project samples (60 unrelated individuals from each group or 240 total chromosomes) was available for 13,787 (CEU) and 13,820 (YRI) extended MHC region SNPs (827,220 and 829,200 genotypes, respectively) spanning 7.8 Mb (www.hapmap.org, and also, additional SNP data provided kindly by Illumina) for comprehensive LD analyses between the AGER locus SNPs and other surrounding MHC loci.

Ager RNA expression

Transcriptional activity of Ager in lymph nodes and spinal cord of experimental allergic encephalomyelitis (EAE) mice was determined as part of a genome-wide longitudinal expression study previously reported (21, 22). Normalized expression values for these genes were subjected to hierarchical clustering using Euclidean distance and average linkage as the distance metrics.

Statistical analysis

All genotypes were tested for deviation from Hardy-Weinberg expectations in African American and white MS cases and controls using PyPop (version 0.6) (23) or Haplovie (version 4.0) (24). Affected family-based controls (nontransmitted parental alleles or "AFBAC") were derived for MHC region SNPs and class II HLA loci in the African American dataset as previously described (25) and combined with data from unrelated controls, when possible, to increase statistical power for association tests. p-values, odds ratios, and confidence intervals for allele or genotype heterogeneity tests were derived using the Fisher’s exact test. Gene x phenotype correlations used Kaplan-Meier survival estimates and a Cox proportional hazard model.

Results

Table I lists the clinical and demographic features of the study participants. An increased disease risk associated with the DRB1*15 (both DRB1*1501 and *1503) and DRB1*03 alleles was observed (Table II), as previously reported for a subset of this dataset (15). After accounting for DRB1*15 and *03 effects, no other DRB1 alleles demonstrated evidence for association (data not shown). DRB1*15 haplotypes carry two functional DRβ-chain genes, DRB1 and DRB5, and two different DR dimers can thus be formed by pairing with the nonpolymorphic DRα-chain (31).
Since the DRB5 locus is carried exclusively on DRB1*15 and *16 haplotypes, as expected, a strong association with MS was observed with this locus as well (odds ratio (OR) = 1.40, p = 0.0002, Table II). However, DRB5*null strong individuals were previously described in populations with African ancestry (32) and offer the opportunity to distinguish between independent effects of DRB1 and DRB5. To address this hypothesis, DRB1-DRB5 two-locus haplotypes were assigned in MS cases and controls (Table II). While 100% of observed DRB1*1501 haplotypes in this dataset included the DRB5*0101 gene (overall frequency = 6.4% and 2.9% in cases and controls, respectively), heterogeneity was observed for DRB5 on DRB1*1503 haplotypes: 18 (1.2%) MS cases and 7 (0.4%) controls carried the DRB1*1503-DRB5*null haplotypes. Similar to DRB1*1501, the most common DRB5 allele on DRB1*1503 haplotypes was *0101 (>90% of haplotypes). Interestingly, DRB1*1503 was associated with MS in the absence of DRB5 (OR = 2.89, 95% CI = 1.15–2.95, p = 0.008). The independent association with AGER was confirmed in a white MS dataset (Tables V and VI). Analyses for rs1035798 in African Americans and rs2070600 in whites conditioned on DRB1 using the independent effect test implemented in WHAP yielded very similar results. Both AGER SNPs showed evidence for association when conditioned on the DRB1 genotype using WHAP (p < 0.01 for rs1035798 in African Americans and p < 0.0001 for rs2070600 in whites, data not shown), which were similar to results shown for the conditional haplotype analysis (Tables IV and VI). This is compatible with independent contributions from both AGER and DRB1 to MS susceptibility. The entire AGER gene was sequenced in 10 African American MS patients and 10 African American controls in an effort to locate suggestive causative SNPs in the gene, but no novel SNPs were found.

To further assess the involvement of AGER in neuroinflammation, we interrogated a large longitudinal gene expression study of relapsing EAE (21, 22). In that study, microarrays were used to monitor the expression of 22,000 genes in spinal cord and lymph nodes of NOD mice at several stages after immunization with the encephalitogenic peptide myelin oligodendrocyte glycoprotein (MOG)35–55. We thus mined the transcriptional dataset to examine

### Table III. Results for MHC region SNPs in African American MS cases and controls*

<table>
<thead>
<tr>
<th>Locus/Allele</th>
<th>Case</th>
<th></th>
<th>Control</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Allele 1 (Frequency)</td>
<td>Allele 2 (Frequency)</td>
<td>Allele 1 (Frequency)</td>
<td>Allele 2 (Frequency)</td>
</tr>
<tr>
<td>rs1051796, MICAex4</td>
<td>1 = C, 2 = T</td>
<td>890 (0.579)</td>
<td>648 (0.421)</td>
<td>914 (0.569)</td>
</tr>
<tr>
<td>rs1063653, MICAex4</td>
<td>1 = A, 2 = G</td>
<td>1020 (0.663)</td>
<td>518 (0.337)</td>
<td>1094 (0.680)</td>
</tr>
<tr>
<td>rs184003, AGERint7/8</td>
<td>1 = G, 2 = T</td>
<td>1209 (0.786)</td>
<td>329 (0.214)</td>
<td>1289 (0.803)</td>
</tr>
<tr>
<td>rs1035798, AGERint3/4</td>
<td>1 = C, 2 = T</td>
<td>1484 (0.965)</td>
<td>54 (0.035)</td>
<td>1498 (0.932)</td>
</tr>
<tr>
<td>rs2070600, AGERex3</td>
<td>1 = G, 2 = A</td>
<td>1526 (0.992)</td>
<td>12 (0.008)</td>
<td>1582 (0.984)</td>
</tr>
<tr>
<td>rs2076530, BTNL2</td>
<td>1 = A, 2 = G</td>
<td>1093 (0.711)</td>
<td>445 (0.289)</td>
<td>1082 (0.673)</td>
</tr>
<tr>
<td>rs2395182, HLA-DRA</td>
<td>1 = T, 2 = G</td>
<td>1090 (0.709)</td>
<td>448 (0.291)</td>
<td>1181 (0.735)</td>
</tr>
</tbody>
</table>

*p-values, odds ratios, and 95% CI derived using Fisher’s exact test, two-sided. All analyses were performed in STATA (version 9.2).

---

### Table IV. AGER/BTLN2 SNP allele associations with MS in African Americans in the absence of DRB1*15 and DRB1*15/*03*

<table>
<thead>
<tr>
<th>Locus/Allele</th>
<th>DRB1*15 Negative Case and Control Haplotypes (Total 2N = 2587)</th>
<th>DRB1*15 and *03 Negative Case and Control Haplotypes (Total 2N = 1345)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p Value</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>rs1035798, AGERint3/4</td>
<td>1 = C, 2 = T</td>
<td>0.0003</td>
</tr>
<tr>
<td>rs2070600, AGERex3</td>
<td>1 = G, 2 = A</td>
<td>0.13</td>
</tr>
<tr>
<td>rs2076530, BTNL2</td>
<td>1 = A, 2 = G</td>
<td>0.32</td>
</tr>
</tbody>
</table>

*p = 0.004.

---

Since the DRB5 locus is carried exclusively on DRB1*15 and *16 haplotypes, as expected, a strong association with MS was observed with this locus as well (odds ratio (OR) = 1.40, p = 0.0002, Table II). However, DRB5*null strong individuals were previously described in populations with African ancestry (32) and offer the opportunity to distinguish between independent effects of DRB1 and DRB5. To address this hypothesis, DRB1-DRB5 two-locus haplotypes were assigned in MS cases and controls (Table II). While 100% of observed DRB1*1501 haplotypes in this dataset included the DRB5*0101 gene (overall frequency = 6.4% and 2.9% in cases and controls, respectively), heterogeneity was observed for DRB5 on DRB1*1503 haplotypes: 18 (1.2%) MS cases and 7 (0.4%) controls carried the DRB1*1503-DRB5*null haplotypes. Similar to DRB1*1501, the most common DRB5 allele on DRB1*1503 haplotypes was *0101 (>90% of haplotypes). Interestingly, DRB1*1503 was associated with MS in the absence of DRB5 (OR = 2.89, 95% CI = 1.15–2.95, p = 0.008). The independent association with AGER was confirmed in a white MS dataset (Tables V and VI). Analyses for rs1035798 in African Americans and rs2070600 in whites conditioned on DRB1 using the independent effect test implemented in WHAP yielded very similar results. Both AGER SNPs showed evidence for association when conditioned on the DRB1 genotype using WHAP (p < 0.01 for rs1035798 in African Americans and p < 0.0001 for rs2070600 in whites, data not shown), which were similar to results shown for the conditional haplotype analysis (Tables IV and VI). This is compatible with independent contributions from both AGER and DRB1 to MS susceptibility. The entire AGER gene was sequenced in 10 African American MS patients and 10 African American controls in an effort to locate suggestive causative SNPs in the gene, but no novel SNPs were found.

To further assess the involvement of AGER in neuroinflammation, we interrogated a large longitudinal gene expression study of relapsing EAE (21, 22). In that study, microarrays were used to monitor the expression of 22,000 genes in spinal cord and lymph nodes of NOD mice at several stages after immunization with the encephalitogenic peptide myelin oligodendrocyte glycoprotein (MOG)35–55. We thus mined the transcriptional dataset to examine...
the expression of Ager and its ligands, the S100-calgranulins. Prog-ressive increase in gene expression that correlated with disease symptoms was observed for Ager as well as for all the tested calgranulins except S100b. Notably, S100a8 and S100a9 showed marked up-regulation even before symptoms of EAE were evident (Fig. 3), followed by decreased expression during the recovery phase. Interestingly, a reverse pattern of expression (with concomitant down-regulation as disease progressed) of Ager and S100-calgranulins was observed in the lymph nodes of the same animals. This mirror-like pattern also applies to S100b, whose expression in lymph nodes peaks at the time of maximal disability.

**Discussion**

The HLA locus on chromosome 6p21 is the strongest genetic fac-tor identified as influencing MS susceptibility. However, previous attempts to isolate the susceptibility gene in this region did not provide consensus. The discovery of the causal variant was im- peded by the high degree of LD that characterizes the DRB1*1501 haplotypes in the high-susceptibility northern European populations (13). The rigidity of this haplotype is the result of recent population history and may indicate selection events (34). Because LD patterns differ between populations, the analysis of African Americans, who have substantially smaller blocks of disequilib-rium, is an attractive strategy to identify recombination events that will assist in the identification of disease genes. In a previous study of DRB1 and DQB1 alleles and haplotypes in an African American MS cohort, a selective association with DRB1*15 was revealed, establishing the centromeric boundary of the HLA class II DR-DQ association in MS and suggesting a primary role for the DRB1 gene in MS independent of DQB1*0602 (15). Conversely, the intro-duction of DQB1*0601 into DRB1*1502 transgenic mice re-duced EAE severity, suggesting modulatory effects on disease pro-gression (35). African American patients also exhibited a high degree of DRB1 allelic heterogeneity as disease association was found for DRB1*1501, DRB1*1503, and DRB1*0301 alleles. The HLA-DRB1*0301 association with MS confirmed here in African-Americans has been previously demonstrated in Sardinian patients (36), whereas HLA-DRB1*0301 transgenic mice are susceptible to proteolipid protein-induced EAE (37).

Altogether, the haplotypic features of the DRB1*1501-DQB1*X (X = non-0602) and DRB1*1503-positive chromosomes indicated an older African origin for the HLA-associated MS susceptibility genes, predating the divergence of human ethnic groups (15). The present analysis further narrows the susceptibility locus within the class II region to DRB1. A primary role for DRB1 in susceptibility to MS is consistent with a pathogenesis model that involves a T cell-mediated autoimmune response. Susceptibility may be then related to the known function of the encoded molecules in the normal immune response, Ag binding and presentation and T cell repertoire determination.

The crystal structure resolution of a DRea/DRB5*0101-EBV peptide complex revealed a marked structural equivalence to the DRB1*1501-myelin basic protein (MBP) peptide complex at the surface presented for TCR recognition (38), suggesting that EBV peptides with limited sequence identity with a myelin peptide could activate autoreactive T cells and initiate an autoimmune re-

**Table V. MHC region AGER SNPs and HLA-DRB1 in white MS cases and controls**

<table>
<thead>
<tr>
<th>Locus/Allele</th>
<th>Allele 1 (Frequency)</th>
<th>Allele 2 (Frequency)</th>
<th>p Value</th>
<th>Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRB1*15 1</td>
<td>1 = *1501, 2 = other</td>
<td>259 (0.266)</td>
<td>715 (0.734)</td>
<td>&lt;0.0001</td>
<td>3.09</td>
</tr>
<tr>
<td>DRB1*03 1</td>
<td>1 = *02, 2 = other</td>
<td>126 (0.130)</td>
<td>844 (0.870)</td>
<td>0.0330</td>
<td>1.39</td>
</tr>
<tr>
<td>rs1035798 AGERint3/4</td>
<td>1 = C, 2 = T</td>
<td>752 (0.772)</td>
<td>222 (0.228)</td>
<td>0.0020</td>
<td>1.29</td>
</tr>
<tr>
<td>rs2070600 AGERex3</td>
<td>1 = G, 2 = A</td>
<td>959 (0.985)</td>
<td>15 (0.015)</td>
<td>&lt;0.0001</td>
<td>3.41</td>
</tr>
</tbody>
</table>

* Total number of MS cases (N = 487); MS controls (N = 434).
* Fisher’s exact test, two sided.
* Fisher’s exact test p-values.

**Table VI. AGER SNP allele associations with MS in whites in the absence of DRB1*15 and DRB1*1503**

<table>
<thead>
<tr>
<th>Locus/Allele</th>
<th>DRB1*15 Negative Case and Control Haplotypes (Total 2N = 1492)</th>
<th>DRB1*15 and *03 Negative Case and Control Haplotypes (Total 2N = 1274)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p Value</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>rs1035798 AGERint3/4</td>
<td>1 = C, 2 = T</td>
<td>0.4649</td>
</tr>
<tr>
<td>rs2070600 AGERex3</td>
<td>1 = G, 2 = A</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*Total number of MS cases (N = 487); MS controls (N = 434); see Materials and Methods for DRB1 allele designations. UNPHASED (version 3.0.7) was used to assign haplotypes for cases and controls; see Materials and Methods. Evaluation of positive MHC SNP allele associations conditional on DRB1 was performed using the conditional haplotype method (CHM). p-values, odds ratios, and 95% CI derived using Fisher’s exact test, two-sided. Overall (omnibus) three-locus haplotype test (rs1035798–rs2070600–DRB1) was performed using WHAP, p < 0.0001.
a modulatory role of \textit{DRB5} gene products on the progression of human demyelinating disease.

The absence of \textit{DRB5} was observed only in the \textit{DRB1*1503} haplotypes (~74\% of the \textit{DRB1*15} samples in the African American cohort contain the *1503 allele vs 0\% of the white population). Although structural features of \textit{DRB1*1503} have not been described, the two \textit{DRB1*15} alleles differ only at position 30 (Tyr in *1501, His in *1503), away from the critical pockets anchoring the peptides. Immunological studies showed that both alleles are equally efficient in presenting the immunodominant epitope MBP peptide, whose interaction with microglia within the CNS is linked to sustained inflammation and neuronal toxicity and cell death (47); 3) amphoterin (high mobility group box chromosomal protein 1 or HMGB-1), a molecule with implications for neurite outgrowth (48); and 4) other uncharacterized cell surface molecules on bacteria and prions (49, 50). While a role for AGER in MS has not yet been established, there is strong evidence for its involvement in the activation of MBP-reactive CD4 T cells in EAE models (51). Furthermore, blocking AGER ameliorates the model disease by preventing the infiltration of encephalitogenic T cells into the CNS (51). Finally, a correlation between serum AGER levels and disease progression in MS was recently reported (52). Using transcriptional information from the CNS and lymph nodes of mice with EAE and controls, we show differential expression of Ager and its ligands, thus providing additional evidence for a potential role of Ager in EAE/MS. Previous results based on a small study sample also suggest that variation in \textit{AGER} may influence inflammatory responses (53); therefore, it is a plausible disease candidate for autoimmune conditions such as MS.

The intronic variant of \textit{AGER} (rs1035798) that was found associated with MS in African Americans is unlikely to be functional by itself. While two other polymorphisms in \textit{AGER} were also examined in this study (rs2070600, a rare missense variant located in exon 3, and rs184003, another intronic polymorphism), neither demonstrated evidence of association with MS in African Americans. On the other hand, the \textit{AGER} rs2070600 variant was strongly associated in the white MS case-control dataset. Neither of the associated AGER SNPs are in strong LD with each other, in either dataset ($r^2 < 0.02$ for pairwise correlation in African Americans and whites), which suggests a role for other rare variants within \textit{AGER} or at nearby loci. Importantly, the class III region within the MHC is the most gene-dense region of the human genome (54), and a comprehensive evaluation of all available MHC SNP data in CEU and YRI populations (see \textit{Materials and Methods}) shows that the associated AGER SNPs from this study (rs1035798 and rs2070600) are linked with several other class III region genes. These include \textit{AGPAT1}, \textit{PBX2}, and \textit{NOTCH4} ($r^2 > 0.6$ in CEU, \textit{EGFL8}, and \textit{CREBL1} loci ($r^2 > 0.6$) in both CEU and YRI and, finally, \textit{TNXB}, \textit{CYP21A2}, \textit{RDBP}, \textit{HS3A1}, and \textit{MSH5} loci ($r^2 > 0.9$) in YRI only, providing a long list of strong candidates for comprehensive mapping efforts.

The current data underscore the power of ethnically defined cohorts to identify disease genes by association for complex diseases. The data demonstrate that, in contrast to the prevailing single locus model, the MHC associations with MS result from complex, multilocus effects that span the entire region. The full characterization of the association range in informative datasets is important to understand MS susceptibility, as well as the role of genetics in progression and response to therapeutics.

\textbf{Acknowledgments}

We are grateful to the MS patients and their families for participating in this study. We thank Robin Lincoln, Wendy Chin, Hourieh Mousavi, and Rosa Guerrero for expert specimen management and Refugia Gomez for database management. We also acknowledge the contribution on non-MS African American samples from John Kane (University of
References


