HLA B*44

Protective effects in MS susceptibility and MRI outcome measures

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ABSTRACT

Objective: In addition to the main multiple sclerosis (MS) major histocompatibility complex (MHC) risk allele (*HLA DRB1*1501*), investigations of the MHC have implicated several class I MHC loci (*HLA A*, *HLA B*, and *HLA C*) as potential independent MS susceptibility loci. Here, we evaluate the role of 3 putative protective alleles in MS: *HLA A*02*, *HLA B*44*, and *HLA C*05*.

Methods: Subjects include a clinic-based patient sample with a diagnosis of either MS or a clinically isolated syndrome ($n = 532$), compared to subjects in a bone marrow donor registry ($n = 776$). All subjects have 2-digit HLA data. Logistic regression was used to determine the independence of each allele's effect. We used linear regression and an additive model to test for correlation between an allele and MRI and clinical measures of disease course.

Results: After accounting for the effect of *HLA DRB1*1501*, both *HLA A*02* and *HLA B*44* are validated as susceptibility alleles ($p_{A*02} = 0.00039$ and $p_{B*44} = 0.00092$) and remain significantly associated with MS susceptibility in the presence of the other allele. Although *A*02* is not associated with MS outcome measures, *HLA B*44* demonstrates association with a better radiologic outcome both in terms of brain parenchymal fraction and T2 hyperintense lesion volume ($p = 0.03$ for each outcome).

Conclusion: The MHC class I alleles *HLA A*02* and *HLA B*44* independently reduce susceptibility to MS, but only *HLA B*44* appears to influence disease course, preserving brain volume and reducing the burden of T2 hyperintense lesions in subjects with MS. **Neurology**® 2010;75:634–640

GLOSSARY

BPF = brain parenchymal fraction; **CIS** = clinically isolated demyelinating syndrome; **EDSS** = Expanded Disability Status Scale; **KIR** = killer immunoglobulin receptor; **MHC** = major histocompatibility complex; **MS** = multiple sclerosis; **MSSS** = Multiple Sclerosis Severity Score; **NK** = natural killer; **SNP** = single nucleotide polymorphism.

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the CNS that has a component of genetic susceptibility. The locus with the strongest association with susceptibility is the major histocompatibility complex (MHC), and this association has been well-validated in a multitude of studies.¹ The main risk haplotype for MS within the MHC is tagged by the *HLA DRB1*1501* allele.¹ However, there is mounting evidence that other haplotypes may also contribute to MS susceptibility in European populations.^{2–5} In particular, 3 class I MHC alleles have been proposed to be associated with susceptibility to MS after accounting for the effect of *HLA DRB1*1501*: *HLA A*02*, *HLA B*4402*, and *HLA C*05*.^{2,6–10} The most recent class I MHC allele, *HLA B*4402*, was discovered by imputing HLA alleles from single nucleotide polymorphism (SNP) genotype data and is consistent with a recent report that the *HLA Bw4*

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group of *HLA B* alleles (which includes *HLA B*4402*) is associated with reduced susceptibility to MS.^{8,11}

While imputation of SNP data provides good estimates of HLA type in many cases, the effectiveness of this method is allele-specific. SNP data can localize an effect, but there is, as yet, not a comprehensive set of surrogate SNPs for each MHC class I or II allele.¹² Thus, we turned to a novel, independent dataset of class I and II MHC typing data to validate the respective roles of these 3 MHC class I alleles. Our new data are of 2-digit resolution, meaning that, for example, *HLA B*4402* is one of several alleles captured under the *HLA B*44* rubric used in the HLA typing data that we have collected in the subjects from the Partners MS Center in Boston.

Here, we replicate the existence of *HLA DRB1*1501*-independent MHC class I susceptibility haplotypes and refine the relative roles of *HLA A*02*, *HLA B*44*, and *HLA C*05*. While the *HLA C*05* allele appears to be secondary to *HLA B*44*, *HLA A*02* is an independent risk allele. Secondly, we extend our analysis to explore the phenotypic associations of the protective *HLA A*02* and *HLA B*44* alleles in subjects with MS.

METHODS **Human subjects.** All subjects with MS met the revised McDonald diagnostic criteria¹³ or had a clinically isolated demyelinating syndrome (CIS); the latter subjects have a history of a single episode of inflammatory demyelination documented by a neurologist and had 2 or more periventricular or ovoid hyperintense T2 lesions of >3 mm on MRI.¹⁴ The control subjects consist of a population of 776 anonymous samples from unrelated subjects consecutively recruited as volunteer donors for a bone marrow registry by the C.W. Bill Young Department of Defense Marrow Donor Program. Because of the recruitment setting, individuals are unlikely to be related and are likely to

originate from different areas of the United States. The MS and control subjects are self-identified as non-Latino, non-Asian, non-African American subjects of European ancestry. The demographic profile of both sets of subjects is outlined in table 1.

Standard protocol approvals, registrations, and patient consents. The study was approved by the institutional review board of Partners Healthcare and written informed consent was obtained from all patients participating in the study.

Clinical and HLA typing data. Clinical data were obtained from the Partners MS Center Oracle relational database. At each clinic visit, the Expanded Disability Status Scale (EDSS) score is measured on each patient.¹⁵ Using the EDSS and disease duration recorded at the most recent clinic visit, the Multiple Sclerosis Severity Score (MSSS) of each subject was calculated using the Global MSSS table provided by Roxburgh and colleagues.¹⁶ A total of 374 patients had an EDSS and recorded disease duration of 30 years or less and contributed to the MSSS analysis. Details on the generation of the HLA typing data are available in appendix e-1 on the *Neurology*[®] Web site at www.neurology.org.^{17,18}

Radiographic data. The details of the Partners MS Center's collection of volumetric data have been previously described.¹⁹ In short, routine clinical MRI scans were obtained on a 1.5-Tesla magnetic resonance system (Signa, GE Medical Systems, Milwaukee, WI) using a standard birdcage quadrature coil as part of routine clinical practice at the Partners MS Center.²⁰ Each brain is segmented using an automated template-driven segmentation pipeline termed TDS+. ^{21,22} No healthy control subjects had radiographic data. A total of 378 patients (2,067 scans) with MHC class I information had radiographic data and recorded disease duration less than 40 years. A detailed description of our data extraction and quality control pipeline is available in appendix e-1. The final dataset consisted of 375 patients with 1,933 scans.

Statistical analysis. The allele frequencies for *HLA DRB1*1501*, *HLA A*02*, *HLA B*44*, and *HLA C*05* were calculated by tabulating the total number of alleles of each category. Genetic association was assessed using a standard χ^2 test completed on a 2 × 2 contingency table for each HLA allele tested. The association between other alleles at each loci was also considered using the same approach. In order to address the potential for association due solely to linkage disequilibrium, multiple logistic regression models were used to estimate the association between *HLA A*02*, *HLA B*44*, and *HLA C*05* and susceptibility controlling for *HLA DRB1*1501* and the other loci. In each case, the number of alleles was used as the predictor in the model. Interactions between alleles on the log odds scale were investigated using the logistic regression model. In addition, the association between the secondary loci and susceptibility was investigated in *HLA DRB1*1501*-/- patients. For comparison of clinical characteristics in the patients with MS, a Kruskal-Wallis test was used to compare the MSSS across the 3 genotype groups. For comparison of age at onset and radiographic characteristics (brain parenchymal fraction [BPF] and log-transformed T2 lesion volume), we compared the characteristics at last visit using linear regression with an additive effect of each allele. Multivariate models for the radiographic characteristics were also investigated to control for age, disease duration, and gender. For longitudinal MRI follow-up, the change over time in BPF and T2 lesion volume was investigated using a random intercept and slope model. For this approach, an additive effect of each allele was used as a predictor. To assess whether an allele had a significant effect on the change with time, the interaction between the allele and study time was investigated. The model also included

Table 1 Subject demographics		
	Brigham and Women's Hospital subjects with MS	Healthy organ donor
No. of subjects	532	776
Age at last visit, y	48.72 ± 11.15	—
Male:female	135:397	—
Mean age at symptom onset, y	33.06 ± 9.39	—
Mean EDSS at last visit	3.3 ± 2.6 (range 0.0–9.0)	—
CIS/RR/SP/PP/PP	17/351/123/4/37	—

Abbreviations: CIS = clinically isolated syndrome; EDSS = Expanded Disability Status Scale; PP = primary progressive; PR = progressive-relapsing; RR = relapsing-remitting; SP = secondary progressive.

Table 2 Associations with loci of interest

Allele	No. of cases/ controls	Allele frequency cases	Allele frequency controls	p Value	Odds ratio (95% CI ^a)
<i>HLA DRB1*1501</i>	532/776	0.26	0.15	2.44×10^{-12}	2.01 (1.65–2.46)
<i>HLA A*02</i>	526/776	0.21	0.28	2.71×10^{-5}	0.67 (0.55–0.81)
<i>HLA B*44</i>	531/776	0.094	0.14	2.39×10^{-4}	0.62 (0.48–0.80)
<i>HLA C*05</i>	526/776	0.054	0.083	6.22×10^{-3}	0.63 (0.45–0.88)

^a Exact 95% confidence interval for the odds ratio is provided.

baseline age, disease duration, and gender as potential confounders. To minimize heterogeneity due to disease course and biologic age, we also completed a restricted analysis among patients under age 65 years and within the first 10 years of disease (calculated from the onset of first symptom). All statistical analysis was completed in the statistical package R (<http://www.R-project.org>).

RESULTS This is the first assessment of the MHC in our collection of subjects with MS from the Partners MS Center. When the subjects with MS or CIS ($n = 532$) were compared to healthy organ donors ($n = 776$), the expected increased frequency of the *HLA DRB1*1501* allele among MS subjects was observed (table 2) ($p = 2.44 \times 10^{-12}$). Additional associations were observed at several MHC class I alleles including the 3 alleles of interest: *HLA A*02*, *HLA B*44*, and *HLA C*05*. Unlike our earlier study in subjects from the United Kingdom,² significant associations were not observed with *HLA DRB1*01* either before or after taking the effect of *HLA DRB1*1501* into account and only a marginal evidence of association was observed with *HLA DRB1*03* after accounting for *HLA DRB1*1501* in these subjects from the United States (tables e-1 and e-2). Given our data, these alleles were not included in further modeling, and we focused on the class I alleles since they displayed much more robust evidence of association. No other *DRB1* alleles were considered in the modeling since *HLA A*02* and *HLA B*44* were the first and second most associated allele among the tested loci after the effect of *HLA DRB1*1501* was taken into account.

To address whether the 3 HLA alleles of interest have independent effects, multiple logistic regression was used. After accounting for the effect of *HLA DRB1*1501*, all 3 alleles remained associated with

MS susceptibility (table 3). Given this residual association, a set of additional regression analyses examined the effect of each of the three alleles in the presence of the other alleles (table 3). This series of analyses demonstrated that the effect of the *HLA C*05* allele was secondary to that of the *HLA B*44* allele and *HLA A*02* allele since no significant residual association was observed with *HLA C*05* in the larger models. No significant gene/gene interactions were observed in these analyses. This confirms our earlier analysis that suggested the secondary role of *HLA C*05* relative to *HLA B*44* using SNP data in a different sample set.⁸ These analyses also examined the relative roles of the *HLA A*02* and *HLA B*44* alleles in the presence of the other allele. In our dataset, these 2 associations appeared to be distinct since a residual association with MS susceptibility remained for (1) *HLA A*02* in the presence of *HLA DRB1*1501* and *HLA B*44* and (2) *HLA B*44* in the presence of *HLA DRB1*1501* and *HLA A*02* (table 3). No significant gene/gene interaction between *HLA B*44* and *HLA A*02* was observed in this analysis. Thus, our study suggests the existence of 2 separate MHC class I associations, confirming both the recent report concerning *HLA B*44* and the published associations with *HLA A*02*.

To confirm these results using a different approach, we also analyzed our data after limiting the analysis to those subjects who were *HLA DRB1*1501*–/–. Table e-3 reports the results of these analyses, which mirror the results of the primary analysis. In particular, *HLA A*02* and *HLA B*44* had independent effects while *HLA C*05* appeared to have no residual effect in the presence of *HLA B*44*.

Given the existence of 2 MHC class I loci associated with MS susceptibility, the association between these loci and MS outcomes was examined. In our cross-sectional analyses, *HLA A*02* did not have a significant association with either our clinical measures—the age at symptom onset and MSSS—or measures derived from MRI—BPF and T2 hyperintense lesion volume at last visit (table 4). On the other hand, *HLA B*44* was associated with both MRI measures at last visit in both univariate ($p =$

Table 3 Associations of each loci in regression analyses of all subjects ($n = 1,308$)

Covariates	Association p values		
	<i>HLA A*02</i>	<i>HLA B*44</i>	<i>HLA C*05</i>
<i>HLA DRB1*1501</i>	0.00039	0.00092	0.014
<i>HLA DRB1*1501 HLA A*02</i>	—	0.0046	0.058
<i>HLA DRB1*1501 HLA B*44</i>	0.0020	—	0.50
<i>HLA DRB1*1501 HLA C*05</i>	0.0012	0.020	—

Table 4 Assessment of association between *HLA A*02* and *HLA B*44* genotypes and clinical/demographic/radiologic outcomes

	HLA B genotype				HLA A genotype			
	<i>B*44,B*44</i>	<i>B*44,X</i>	<i>X,X</i>	<i>p</i> Value	<i>A*02,A*02</i>	<i>A*02,X</i>	<i>X,X</i>	<i>p</i> Value
Clinical outcomes								
No. of subjects	9	57	359		25	123	272	
Mean age at symptom onset, <i>y</i> ($\pm$ SD)	31.32 $\pm$ 8.85	33.03 $\pm$ 8.53	33.25 $\pm$ 9.71	0.62 ^a	34.10 $\pm$ 8.47	32.08 $\pm$ 9.79	33.74 $\pm$ 9.51	0.37 ^a
Mean MSSS ($\pm$ SD) ^b	2.92 $\pm$ 3.09	3.74 $\pm$ 3.16	3.60 $\pm$ 2.87	0.82 ^c	4.44 $\pm$ 2.91	3.55 $\pm$ 2.96	3.55 $\pm$ 2.88	0.30 ^c
Radiographic outcomes								
No. of subjects	9	46	320		19	110	242	
Mean BPF ($\pm$ SD)	0.89 $\pm$ 0.03	0.86 $\pm$ 0.04	0.85 $\pm$ 0.05	0.032 ^a	0.84 $\pm$ 0.05	0.85 $\pm$ 0.05	0.85 $\pm$ 0.05	0.35 ^a
Mean T2 lesion volume ($\pm$ SD)	3.32 $\pm$ 2.14	4.65 $\pm$ 5.34	5.65 $\pm$ 5.10	0.032 ^a	6.26 $\pm$ 9.47	5.33 $\pm$ 5.18	5.44 $\pm$ 4.54	0.21 ^a

Abbreviations: BPF = brain parenchymal fraction; MSSS = Multiple Sclerosis Severity Score; X = *HLA B* allele other than *B*44* and *HLA A* allele other than *A*02*.

^a One degree of freedom linear regression model for additive allelic effect.

^b A total of 374 patients contributed to *B*44* analysis and 370 patients contributed to *A*02* analysis.

^c Two degrees of freedom Kruskal-Wallis test comparing the 3 groups.

0.03 for each measure, table 4) and multivariate analyses ($p = 0.02$ for BPF and $p = 0.03$ for T2 lesion volume). *HLA B*44* was associated with higher BPF and lower T2 lesion volume, which is consistent with the protective effect of this allele in terms of susceptibility. No significant association between *HLA B*44* and age at onset or MSSS was observed (table 4). In longitudinal analyses, neither allele was significantly associated with the rate of change in BPF or T2 lesion volume, but the direction of the effect was toward a protective effect for each allele (table 5). This suggests that subjects with an *HLA B*44* allele have a slower rate of brain atrophy and T2 hyperintense lesion accumulation than other subjects with MS.

A comprehensive assessment of evidence of association between *HLA A*, *B*, *C*, and *DRB1* alleles and MS susceptibility is provided in tables e-1 and e-2. Consistent with previous reports, the relatively uncommon *HLA DRB1*14* allele displayed modest evidence of a protective effect in terms of MS susceptibility.^{3,23} In addition, there is evidence of further heterogeneity in the MHC locus, with 3 sugges-

tive associations appearing in the analysis where the effects of *HLA A*02*, *HLA B*44*, and *HLA DRB1*1501* are accounted for (full model p) (tables e-1 and e-2): *HLA A*03*, univariate (no covariates) $p = 0.8$, full model $p = 0.001$; *HLA C*03*, univariate $p = 0.0002$, full model $p = 0.002$; and *HLA DRB1*11*, univariate $p = 0.06$, full model $p = 0.003$. Given the 50 alleles tested in this secondary analysis, study-wide significance is established as a full model $p < 0.001$. Of note, we saw a nonsignificant trend for a higher frequency of an *HLA DRB1*1501/*08* genotype in subjects with MS (data not shown); this genotype combination has previously been proposed as a high-risk genotype in relation to MS susceptibility.³

DISCUSSION In our focused analysis of the MHC class I region, we confirm that this chromosomal segment is associated with MS independently of the *HLA DRB1*1501* association. Specifically, we validate for the first time the earlier observation concerning the role of the *HLA B*44* allele and confirm the

Table 5 Assessment for evidence of interaction between *HLA A*02* and *HLA B*44* and change in MRI endophenotypes^a

	HLA B genotype				HLA A genotype			
	<i>B*44,B*44</i>	<i>B*44,X</i>	<i>X,X</i>	<i>p</i> Value	<i>A*02,A*02</i>	<i>A*02,X</i>	<i>X,X</i>	<i>p</i> Value
No. of subjects	9	46	320		19	110	242	
Estimated rate of BPF decline	−0.0025	−0.0027	−0.0029	0.59	−0.0023	−0.0026	−0.0030	0.24
Estimated change in log-transformed T2 lesion volume	−0.0031	0.011	0.025	0.15	0.0053	0.016	0.027	0.14

Abbreviations: BPF = brain parenchymal fraction; X = *HLA B* allele other than *B*44* and *HLA A* allele other than *A*02*.

^a The results presented are those using an additive model for the effect of *HLA A*02* and *HLA B*44*. A genotypic model similarly fails to yield evidence of interaction between genotype and the rate of change in BPF or T2LV.

role of the *HLA A*02* allele first identified in Scandinavian populations by demonstrating their association with MS susceptibility in an independent cohort of subjects with MS from the Partners MS Center. The results are consistent with other reports validating the role of the *HLA A*02* allele and implicating the *HLA Bw4* group of alleles.^{7,9,11,24} Further, we enhance the state of the field by demonstrating that these 2 effects are independent of one another and that the previously described association of the *HLA Cw*05* allele with susceptibility to MS² is likely to be due to linkage disequilibrium with *HLA B*44*.

In our dataset, both *HLA A*02* and *HLA B*44* have substantial effects on susceptibility ($OR_{A*02} = 0.67$ and $OR_{B*44} = 0.62$). These effects are larger than that of the non-MHC risk alleles discovered to date²⁵ and will play an important role as we assemble a comprehensive map of the genetic architecture of MS in coming years. Given the MHC's allelic heterogeneity, long-range linkage disequilibrium, and the large effect size of *HLA DRB1*1501*, it is not surprising that loci with effects of this magnitude are only being validated now. In fact, we suspect that additional susceptibility loci will be found within the MHC as part of future investigations in larger sample sets. While the 2 MHC class I alleles discussed here are clearly associated with susceptibility to MS, we should remember that they exist on haplotypes that extend over long physical distances and encompass many different polymorphisms. Thus, we cannot say that *HLA A*02* and *HLA B*44* are causal variants at this time; they simply represent the best markers of independent protective haplotypes within the MHC. It is also possible that multiple alleles in strong linkage disequilibrium with either allele could be required to achieve the protective effects that we have validated.

The association of MHC class I alleles with susceptibility to MS highlights the role of certain cell types, such as CD8+ and natural killer (NK) cells, in the onset of MS. These cell types harbor receptors for MHC class I, and these findings fit well with the reported dysfunction of CD8+ and NK cells in early stages of demyelinating disease.²⁶ Recently, in a large-scale scan of peripheral blood cell populations, we identified and validated a reduction in the frequency of a CD56+ NK cell population in untreated subjects who either had relapsing-remitting MS or a clinically isolated demyelinating syndrome.²⁷ Many other investigators have assessed the role of NK cells in MS, and, while some early studies were negative, the preponderance of the evidence available to date suggests that NK cell frequency is reduced in MS and that they may be dysfunctional.²⁸ The convergence of these immunologic and genetic data strongly argue for a comprehensive assessment of the complex interactions between MHC class I molecules and

their various receptors in subjects with MS, both at the genetic and functional level. An early effort in this direction used *HLA Bw4* and explored the role of killer immunoglobulin receptors (KIR) in MS susceptibility, including *KIR3DL1*, which is a receptor for this group of *HLA B* alleles.¹¹ While *KIR* alleles did not appear to be associated in this study of moderate size, modest effects typical of inflammatory disease susceptibility alleles cannot yet be excluded and will require the careful assessment of allele combinations between KIR and MHC class I alleles.

Interestingly, a protective effect of *HLA B*44* has been previously reported in subjects with *CD95/TNFRSF6* mutations resulting in autoimmune lymphoproliferative syndrome type 1a. Subjects with such mutations who also bear an *HLA B*44* allele were less likely to develop the full spectrum of autoimmune lymphoproliferative syndrome clinical features such as lymphadenopathy, splenomegaly, autoimmunity, and lymphoma.²⁹ Furthermore, *HLA B*44* homozygotes have also been reported to have lower NK cell activity, offering one suggestion as to the mechanism by which *HLA B*44* may reduce MS susceptibility.³⁰

While *HLA A*02* and *HLA B*44* are clearly validated as protective haplotypes in this and other studies,^{9,11,24} we see evidence that only the *HLA B*44* haplotype affects volumetric MRI measures (BPF and T2 hyperintense lesion volume): on average, subjects with MS with *HLA B*44* alleles had a larger brain volume and a lower lesion burden than subjects with MS who lacked this allele. Our secondary analysis of longitudinal data suggests that these correlations may arise from a reduction in the rates of brain atrophy and T2 lesion volume accumulation. Thus, the difference in brain volume at a subject's last MRI is less likely to be due to differences in baseline brain volume. However, further work is needed to confirm our observations relating these MS outcome measures and *HLA B*44* as well as an independent report of associations between *HLA A*02* and measures of MS disease course that we did not validate in our sample set.³¹ Overall, MS susceptibility alleles appear to have a limited role in MS disease severity so far,³²⁻³⁵ and dedicated studies are needed to explore the genetic architecture of disease course in MS.

As suggested by our comprehensive assessment of MHC alleles in table e-1, additional risk-associated and protective alleles, particularly in the *HLA C* locus, may well exist, and dedicated efforts to further explore this critical region are therefore warranted. Such efforts that include-high resolution HLA typing of multiple loci are needed to supplement the ongoing large SNP-based efforts: so far, HLA types are, for the most part, imperfectly tagged by SNP

markers and will be needed to identify the causal allele or alleles in each locus.

AUTHOR CONTRIBUTIONS

Statistical analysis was conducted by Dr. Brian Healy.

DISCLOSURE

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REFERENCES

1. Hauser SL, Oksenberg JR. The neurobiology of multiple sclerosis: genes, inflammation, and neurodegeneration. *Neuron* 2006;52:61–76.
2. Yeo TW, De Jager PL, Gregory SG, et al. A second major histocompatibility complex susceptibility locus for multiple sclerosis. *Ann Neurol* 2007;61:228–236.
3. Barcellos LF, Sawcer S, Ramsay PP, et al. Heterogeneity at the HLA-DRB1 locus and risk for multiple sclerosis. *Hum Mol Genet* 2006;15:2813–2824.
4. Harbo HF, Lie BA, Sawcer S, et al. Genes in the HLA class I region may contribute to the HLA class II-associated genetic susceptibility to multiple sclerosis. *Tissue Antigens* 2004;63:237–247.
5. Chao MJ, Barnardo MC, Lincoln MR, et al. HLA class I alleles tag HLA-DRB1*1501 haplotypes for differential risk in multiple sclerosis susceptibility. *Proc Natl Acad Sci USA* 2008;105:13069–13074.
6. Fogdell-Hahn A, Ligers A, Gronning M, Hillert J, Olerup O. Multiple sclerosis: a modifying influence of HLA class I genes in an HLA class II associated autoimmune disease. *Tissue Antigens* 2000;55:140–148.
7. Brynedal B, Duvefelt K, Jonasdottir G, et al. HLA-A confers an HLA-DRB1 independent influence on the risk of multiple sclerosis. *PLoS One* 2007;2:e664.
8. Rioux JD, Goyette P, Vyse TJ, et al. Mapping of multiple susceptibility variants within the MHC region for 7 immune-mediated diseases. *Proc Natl Acad Sci USA* 2009; 106:18680–18685.
9. Bergamaschi L, Leone MA, Fasano ME, et al. HLA-class I markers and multiple sclerosis susceptibility in the Italian population. *Genes Immun* 2010;11:173–180.
10. Burfoot RK, Jensen CJ, Field J, et al. SNP mapping and candidate gene sequencing in the class I region of the HLA complex: searching for multiple sclerosis susceptibility genes in Tasmanians. *Tissue Antigens* 2008;71:42–50.
11. Lorentzen AR, Karlsen TH, Olsson M, et al. Killer immunoglobulin-like receptor ligand HLA-Bw4 protects against multiple sclerosis. *Ann Neurol* 2009;65:658–666.
12. de Bakker PI, McVean G, Sabeti PC, et al. A high-resolution HLA and SNP haplotype map for disease association studies in the extended human MHC. *Nat Genet* 2006;38:1166–1172.
13. McDonald WI, Compston A, Edan G, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann Neurol* 2001;50:121–127.
14. Miller D, Barkhof F, Montalban X, Thompson A, Filippi M. Clinically isolated syndromes suggestive of multiple sclerosis, part 2: non-conventional MRI, recovery processes, and management. *Lancet Neurol* 2005;4:341–348.
15. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology* 1983;33:1444–1452.
16. Roxburgh RH, Seaman SR, Masterman T, et al. Multiple Sclerosis Severity Score: using disability and disease duration to rate disease severity. *Neurology* 2005;64:1144–1151.

17. Tu B, Mack SJ, Lazaro A, et al. HLA-A, -B, -C, -DRB1 allele and haplotype frequencies in an African American population. *Tissue Antigens* 2007;69:73–85.
18. Cao K, Chopek M, Fernandez-Vina MA. High and intermediate resolution DNA typing systems for class I HLA-A, B, C genes by hybridization with sequence-specific oligonucleotide probes (SSOP). *Rev Immunogenet* 1999;1:177–208.
19. Gauthier SA, Mandel M, Guttman CR, et al. Predicting short-term disability in multiple sclerosis. *Neurology* 2007;68:2059–2065.
20. Meier DS, Guttman CR. MRI time series modeling of MS lesion development. *Neuroimage* 2006;32:531–537.
21. Wei X, Warfield SK, Zou KH, et al. Quantitative analysis of MRI signal abnormalities of brain white matter with high reproducibility and accuracy. *J Magn Reson Imaging* 2002;15:203–209.
22. Liu L, Meier D, Polgar-Turcsanyi M, Karkocha P, Bakshi R, Guttman CR. Multiple sclerosis medical image analysis and information management. *J Neuroimaging* 2005;15:103S–117S.
23. Ramagopalan SV, Morris AP, Dymant DA, et al. The inheritance of resistance alleles in multiple sclerosis. *PLoS Genet* 2007;3:1607–1613.
24. Silva AM, Bettencourt A, Pereira C, et al. Protective role of the HLA-A*02 allele in Portuguese patients with multiple sclerosis. *Mult Scler* 2009;15:771–774.
25. De Jager PL, Jia X, Wang J, et al. Meta-analysis of genome scans and replication identify CD6, IRF8 and TNFRSF1A as new multiple sclerosis susceptibility loci. *Nat Genet* 2009;41:776–782.
26. McFarland HF, Martin R. Multiple sclerosis: a complicated picture of autoimmunity. *Nat Immunol* 2007;8:913–919.
27. De Jager PL, Rossin E, Pyne S, et al. Cytometric profiling in multiple sclerosis uncovers patient population structure and a reduction of CD8low cells. *Brain* 2008;131:1701–1711.
28. Segal BM. The role of natural killer cells in curbing neuroinflammation. *J Neuroimmunol* 2007;191:2–7.
29. Vacek MM, Schaffer AA, Davis J, et al. HLA B44 is associated with decreased severity of autoimmune lymphoproliferative syndrome in patients with CD95 defects (ALPS type Ia). *Clin Immunol* 2006;118:59–65.
30. Dubey DP, Alper CA, Mirza NM, Awdeh Z, Yunis EJ. Polymorphic Hh genes in the HLA-B(C) region control natural killer cell frequency and activity. *J Exp Med* 1994;179:1193–1203.
31. Zivadinov R, Weinstock-Guttman B, Zorzon M, et al. Gene-environment interactions between HLA B7/A2, EBV antibodies are associated with MRI injury in multiple sclerosis. *J Neuroimmunol* 2009;209:123–130.
32. Okuda DT, Srinivasan R, Oksenberg JR, et al. Genotype-Phenotype correlations in multiple sclerosis: HLA genes influence disease severity inferred by 1HMR spectroscopy and MRI measures. *Brain* 2009;132:250–259.
33. Baranzini SE, Wang J, Gibson RA, et al. Genome-wide association analysis of susceptibility and clinical phenotype in multiple sclerosis. *Hum Mol Genet* 2009;18:767–778.
34. Smestad C, Brynedal B, Jonasdottir G, et al. The impact of HLA-A and -DRB1 on age at onset, disease course and severity in Scandinavian multiple sclerosis patients. *Eur J Neurol* 2007;14:835–840.
35. Sombekke MH, Lukas C, Crusius JB, et al. HLA-DRB1*1501 and spinal cord magnetic resonance imaging lesions in multiple sclerosis. *Arch Neurol* 2009;66:1531–1536.

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