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Citation

Published Version
doi://10.1371/journal.pone.0011296

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A Major Histocompatibility Class I Locus Contributes to Multiple Sclerosis Susceptibility Independently from HLA-DRB1*15:01

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Abstract

Background: In Northern European descended populations, genetic susceptibility for multiple sclerosis (MS) is associated with alleles of the human leukocyte antigen (HLA) Class II gene DRB1. Whether other major histocompatibility complex (MHC) genes contribute to MS susceptibility is controversial.

Methodology/Principal Findings: A case control analysis was performed using 958 single nucleotide polymorphisms (SNPs) spanning the MHC assayed in two independent datasets. The discovery dataset consisted of 1,018 cases and 1,795 controls and the replication dataset was composed of 1,343 cases and 1,379 controls. The most significantly MS-associated SNP in the discovery dataset was rs3135391, a Class II SNP known to tag the HLA-DRB1*15:01 allele, the primary MS susceptibility allele in the MHC (O.R. = 3.04, p<1x10^-8). To control for the effects of the HLA-DRB1*15:01 haplotype, case control analysis was performed adjusting for this HLA-DRB1*15:01 tagging SNP. After correction for multiple comparisons (false discovery rate = 0.05) 52 SNPs in the Class I, II and III regions were significantly associated with MS susceptibility in both datasets using the Cochran Armitage trend test. The discovery and replication datasets were merged and subjects carrying the HLA-DRB1*15:01 tagging SNP were excluded. Association tests showed that 48 of the 52 replicated SNPs retained significant associations with MS susceptibility independently of the HLA-DRB1*15:01 as defined by the tagging SNP. 20 Class I SNPs were associated with MS susceptibility with p-values ≤1x10^-6. The most significantly associated SNP was rs4959039, a SNP in the downstream untranslated region of the non-classical HLA-G gene (Odds ratio 1.59, 95% CI 1.40, 1.81, p=8.45x10^-13) and is in linkage disequilibrium with several nearby SNPs. Logistic regression modeling showed that this SNP’s contribution to MS susceptibility was independent of the Class II and Class III SNPs identified in this screen.

Conclusions: A MHC Class I locus contributes to MS susceptibility independently of the HLA-DRB1*15:01 haplotype.

Citation: Cree BAC, Rioux JD, McCauley JL, Gourraud P-AF, Goyette P, et al. (2010) A Major Histocompatibility Class I Locus Contributes to Multiple Sclerosis Susceptibility Independently from HLA-DRB1*15:01, PLOS ONE 5(6): e11296. doi:10.1371/journal.pone.0011296

Editor: Christoph Kleinschnitz, Julius-Maximilians-Universität Würzburg, Germany

Received March 12, 2010; Accepted June 4, 2010; Published June 25, 2010

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Funding: This work was supported primarily by a grant from the National Institutes of Allergy and Infectious Diseases (AI067152). Additional support was received from the National Institute of Neurological Disease and Stroke (NS21799) to SLH and (K23 NS048869) to BACC. PLD was supported by a Harry Weaver Neuroscience Scholar Award of the National Multiple Sclerosis Society (NMSS). The IMSGC is supported by RO1NS049477. The authors also thank the NMSS and Nancy Davis Foundation for support of DNA collections. They acknowledge use of DNA from the British 1958 Birth Cohort collection (D. Strachan, S. Ring, W. McArdle and M. Pembrey), funded by the Medical Research Council grant G0000934 and Wellcome Trust grant 068545/Z/02. The Broad Institute Center for Genotyping and Analysis is supported by grant U54 RR020278 from the National Center for Research Resources. The funders had no role in study design, data collection, and analysis, decision to publish, or preparation in the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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* Membership of the International MHC and Autoimmunity Genetics Network (IMAGEN) is provided in the Acknowledgments.

* Membership of the International Multiple Sclerosis Genetics Consortium (IMSGC) is provided in the Acknowledgments.
Introduction

The autoimmune disease multiple sclerosis (MS) is one of the leading causes of neurological disability in young adults. Pathologically, the disease is characterized by focal areas of inflammation and demyelination (plaques) within the central nervous system with ensuing axonal damage. Although the etiology is not fully understood, MS is a complex genetic disorder and whole genome studies indicate that the major histocompatibility complex (MHC) on chromosome 6p21 represents the strongest genome-wide MS susceptibility locus [1,2].

In both Northern European and African descended populations, MS susceptibility is associated with alleles of the HLA Class II gene DRB1 [2–5] whereas the contribution of other genes within the extended MHC has been controversial [6–8]. Extensive linkage disequilibrium (LD) operating in the region [9–11], as well as marked polymorphism and high gene density, have complicated efforts to fully resolve the roles of HLA and non-HLA genes in MS susceptibility. Due to these inherent challenges, a comprehensive approach is needed to refine the contributions of the MHC to genetic risk for MS that includes a large and well-characterized dataset, dense concentration of markers, and appropriate methods to control for the extensive LD across the region.

A panel of single nucleotide polymorphisms (SNPs) selected for moderate LD across the 29 to 34 Mb region of the MHC was employed to map both HLA and non-HLA disease susceptibility signals [12]. Here we present the results of an analysis of two independent case control MS datasets using 958 SNPs adjusting for the effect of HLA-DRB1*15:01 whose extended haplotype spans the MHC.

Results

Case control study

Following quality control, 958 markers were genotyped in both datasets. In the discovery dataset the average number (standard deviation) of missing genotypes for cases was .0040 (.0031) and for controls was .0027 (.0035). In the replication dataset, the average number (standard deviation) of missing genotypes for cases was .0020 (.0060) for controls was .0022 (.0080). There was not a statistically significant difference in missing genotypes between cases and controls in either dataset.

Case control analysis was performed in the discovery dataset composed of 1018 cases and 1795 controls (Table S1) using 958 MHC spanning SNPs (Table S2, see Figure S1 for study design). Population stratification effects were controlled for by including sex and location of subject recruitment (United States versus United Kingdom) in the regression analyses. The Cochran Armitage trend test was used to identify MS associated SNPs and the false discovery rate (FDR = .05) was used to correct for multiple comparisons [13]. The most highly associated SNP was rs3153591 (odds ratio = 3.04, p < 10^{-8}), a Class II SNP known to tag the primary MS susceptibility allele HLA-DRB1*15:01 with very high sensitivity and specificity [11].

Using the trend test in the discovery dataset, a total of 501 SNPs in Class I, II and III regions showed statistically significant association with MS susceptibility; most of these associations were likely due to LD within extended haplotypes, particularly the one anchored by the HLA-DRB1*15:01 allele (Figure 1A). To correct for the effect of this haplotype, the trend test was performed adjusting for rs3153591 using the 958 SNPs (FDR = .05) and the number of significantly associated SNPs was reduced to 87 (Figure 1B).

A second independent dataset consisting of 1343 cases and 1379 controls was then used to replicate these associations (Table S1). All 950 markers were assessed in the replication dataset with the same association strategy adjusting for the HLA-DRB1*15:01 tagging SNP rs3153591 (FDR = .05). Only markers that were significantly associated in both cohorts, and had the same direction of association, were studied further. 52 such SNPs were significantly associated with MS susceptibility in both datasets (Table S3).

The merged HLA-DRB1*15:01(–) dataset

A merged cohort was next created by combining the discovery and replication datasets. The MAF for each SNP is reported for cases and controls in the merged dataset as well as the strength of association using the trend test (Table S3). A SNP in the downstream non-coding region of HLA-G (rs4959039) was the most significantly associated marker (p < 10^{-12}) in the merged cohort analysis, after adjusting for the HLA-DRB1*15:01 tagging SNP rs3153591 and potential stratification effects caused by sex, location (US versus UK), and dataset (discovery versus replication).

To further demonstrate that these 52 replicated SNP associations were independent from effects of the extended HLA-DRB1*15:01 haplotype, all subjects carrying at least one copy of this allele, as defined by the tagging SNP rs3153591, were dropped from the merged dataset to create a “HLA-DRB1*15:01(–)” dataset. This excluded a total of 2088 subjects (1277 cases and 811 controls) leaving a HLA-DRB1*15:01(–) dataset that consisted of 1075 cases and 2363 controls. Association tests were performed in this merged HLA-DRB1*15:01(–) dataset and significant associations were found for 48 of the 52 SNPs identified in the case control screens including all previously identified Class I and Class III SNPs (Table S4).

Using the genotype test for association in the HLA-DRB1*15:01(–) dataset, 20 Class I SNPs had p-values ≤ 10^{-8} (Table 1). The HLA-G linked rs4959039:A>G allele (rs4959039) continued to have the strongest association in this HLA-DRB1*15:01(–) dataset (odds ratio 1.59, 95% confidence intervals 1.40, 1.81, p < 8.45 × 10^{-15}). Importantly, rs4959039 and the other Class I SNPs associated with MS susceptibility are poorly correlated with the SNPs in the Class III and Class II regions as illustrated by the LD map (Figure 2). For example, the average (range) r^2 for rs4959039 with the Class III SNPs was .081 (.014 to .149) and for the Class II SNPs was .024 (.002 to .085).

In contrast to the poor correlations with the Class III and Class II SNPs, the LD map (Figure 2) shows that some of the associated Class I SNPs are closely linked. SNPs in the Class I region with p-values ≤ 10^{-8} that are in moderate to strong LD with each other (as defined by LD-R^2≥0.5) include: r2532382, r2517701 (HLA-80), r4713270 (HCG26P), r4713274 (HCGD), r2523946 (MICD), r3823355 (MICD), rs4959039 (in between HLA-G and HLA-A), r4713281 (HLA-A), r93939899 (HFA9), and r93939899 (HFA9). Using an algorithm to define haplotype blocks by LD-R^2≥0.5 an apparently separate Class I SNP cluster (rs362126, r2523393, r2743951) emerges that includes a tagging SNP for the HLA-B*44:02 allele (rs2523393), a recently identified MS protective allele [12,14].

Tests for independent association using logistic regression models

To confirm that the contribution to MS susceptibility of the rs4959039 SNP was independent of any residual Class II associations, logistic regression models were constructed. Because many of the 48 SNPs associated with MS susceptibility in the HLA-
Figure 1. Association test results for 958 SNPs spanning the MHC in the discovery dataset are shown. The location of the SNPs is depicted on the X-axis and the statistical significance of the association is depicted on the Y-axis. A: Discovery dataset (1018 cases and 1795 controls), 958 common SNP subset, FDR = .05, adjusted for sex and center (US versus UK), trend test. B: Discovery dataset, 958 common SNP subset, FDR = .05, adjusted for the HLA-DRB1*15:01 tagging SNP rs3135391), sex and center (US versus UK), trend test.

doi:10.1371/journal.pone.0011296.g001
Table 1. SNPs associated with MS susceptibility with genome-wide statistical significance in the merged dataset excluding all subjects who carry the HLA-DRB1*15:01 allele listed in order of highest to lowest statistical significance using the Cochran-Armitage trend test for association.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position</th>
<th>Class</th>
<th>Gene</th>
<th>Allele</th>
<th>MS Associated</th>
<th>Trend</th>
<th>Odds</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4959039</td>
<td>30065047</td>
<td>Class I</td>
<td>HLA-G</td>
<td>A</td>
<td>8.45×10^-13</td>
<td>1.59</td>
<td>1.40</td>
<td>1.81</td>
</tr>
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<td>rs9393889</td>
<td>30148062</td>
<td>Class I</td>
<td>RNF39</td>
<td>A</td>
<td>9.84×10^-13</td>
<td>0.63</td>
<td>0.56</td>
<td>0.72</td>
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<td>rs9357092</td>
<td>30092230</td>
<td>Class I</td>
<td>HCG9</td>
<td>A</td>
<td>1.17×10^-12</td>
<td>0.63</td>
<td>0.56</td>
<td>0.72</td>
</tr>
<tr>
<td>rs4713281</td>
<td>30086330</td>
<td>Class I</td>
<td>HLA-I</td>
<td>A</td>
<td>3.19×10^-12</td>
<td>0.63</td>
<td>0.56</td>
<td>0.72</td>
</tr>
<tr>
<td>rs4713274</td>
<td>30045471</td>
<td>Class I</td>
<td>MICD</td>
<td>C</td>
<td>5.11×10^-12</td>
<td>1.56</td>
<td>1.38</td>
<td>1.77</td>
</tr>
<tr>
<td>rs1736936</td>
<td>29902295</td>
<td>Class I</td>
<td>HCG4P8</td>
<td>C</td>
<td>2.22×10^-11</td>
<td>0.70</td>
<td>0.63</td>
<td>0.78</td>
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<tr>
<td>rs2523822</td>
<td>29936638</td>
<td>Class I</td>
<td>A</td>
<td>2.99×10^-11</td>
<td>1.51</td>
<td>1.34</td>
<td>1.70</td>
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<tr>
<td>rs4713270</td>
<td>30042675</td>
<td>Class I</td>
<td>HCG2P6</td>
<td>A</td>
<td>4.26×10^-11</td>
<td>0.66</td>
<td>0.58</td>
<td>0.75</td>
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<tr>
<td>rs3823355</td>
<td>30050061</td>
<td>Class I</td>
<td>MICD</td>
<td>C</td>
<td>7.74×10^-11</td>
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<td>1.33</td>
<td>1.70</td>
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<td>rs2734971</td>
<td>29942427</td>
<td>Class I</td>
<td>3.8–1.4</td>
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<tr>
<td>rs2239530</td>
<td>30260093</td>
<td>Class I</td>
<td>TRIM26</td>
<td>C</td>
<td>2.96×10^-10</td>
<td>0.65</td>
<td>0.57</td>
<td>0.74</td>
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<tr>
<td>rs2523393</td>
<td>29813637</td>
<td>Class I</td>
<td>FLJ35429</td>
<td>C</td>
<td>6.04×10^-10</td>
<td>0.72</td>
<td>0.65</td>
<td>0.80</td>
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<td>rs1541268</td>
<td>30211372</td>
<td>Class I</td>
<td>TRIM40</td>
<td>C</td>
<td>1.40×10^-9</td>
<td>0.66</td>
<td>0.58</td>
<td>0.76</td>
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<tr>
<td>rs2256266</td>
<td>29740296</td>
<td>Ext Cls I</td>
<td>MOG</td>
<td>A</td>
<td>2.67×10^-9</td>
<td>0.66</td>
<td>0.58</td>
<td>0.76</td>
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<td>rs2734951</td>
<td>29817212</td>
<td>Class I</td>
<td>FLJ35429</td>
<td>C</td>
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<td>1.37</td>
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<td>rs1611710</td>
<td>29936894</td>
<td>Class I</td>
<td>C</td>
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<td>0.74</td>
<td>0.66</td>
<td>0.82</td>
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<tr>
<td>rs2517701</td>
<td>30033950</td>
<td>Class I</td>
<td>HLA-B8</td>
<td>A</td>
<td>5.62×10^-8</td>
<td>1.41</td>
<td>1.26</td>
<td>1.58</td>
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<tr>
<td>rs2523946</td>
<td>30049921</td>
<td>Class I</td>
<td>MICD</td>
<td>C</td>
<td>8.69×10^-9</td>
<td>1.36</td>
<td>1.23</td>
<td>1.51</td>
</tr>
<tr>
<td>rs2256543</td>
<td>30045811</td>
<td>Class I</td>
<td>MICD</td>
<td>C</td>
<td>9.15×10^-9</td>
<td>1.36</td>
<td>1.22</td>
<td>1.51</td>
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<td>rs1362126</td>
<td>29798997</td>
<td>Class I</td>
<td>HLA-F</td>
<td>A</td>
<td>6.99×10^-9</td>
<td>0.75</td>
<td>0.67</td>
<td>0.83</td>
</tr>
</tbody>
</table>

The area under the receiver operator curve modeling the rs4959039 SNP alone was .617 showing that the contribution of these Class II SNPs is modest. Importantly, during the backward stepwise selection process all other Class I SNP clusters were dropped from the model suggesting that the Class I contribution to MS susceptibility is driven by the SNP cluster tagged by rs4959039.

To further understand the contributions of these loci to MS susceptibility two-locus haplotypes were constructed for SNPs rs2523393 (the HLA-B*44:02 tagging SNP) and rs4959039 (Table S6). This analysis defined a MS risk haplotype as rs2523393:T and rs4959039:A due to LD with rs5095039:A and the converse MS protective haplotype as rs2523393:T>rs4959039:G. Due to LD this analysis could not definitively prove that the influence of these loci on MS risk was independent. However, the heterozygous haplotype appears to be protective for MS risk (odds ratio = .71, p<9.73×10^-7) indicating that the protective haplotype is dominant.
Transmission disequilibrium test in HLA-DRB1*15:01 (-) trio families

As an additional test of association, the rs4959039 was assessed using the transmission disequilibrium test in a subset of the discovery dataset for whom parental genotyping was available. 347 trio families (affected individual plus both parents) that did not carry the HLA-DRB1*15:01 allele were genotyped for the rs4959039 SNP. The chromosome carrying the allele of rs4959039:A>G was transmitted 112 times and not transmitted 81 times in heterozygous trio families. Despite the small size of this...
family based dataset, a borderline level of statistical significance was observed \( (p = .046) \) supporting the validity of this SNP as an MS susceptibility locus using a family-based association test.

To determine whether the rs4959039:A>G allele adds to the risk of MS in **HLA-DRB1**/*15:01** subjects, bi-allelic haplotypes for rs3153391:T>G (the SNP that tags **HLA-DRB1**/*15:01*) and rs4959039:A>G individuals were constructed in the merged dataset (Table 2). Each bi-allelic haplotype was treated as a dichotomous variable in this analysis. The presence of the rs4959039:A>G allele contributed to MS susceptibility both in subjects who carry the **HLA-DRB1**/*15:01* allele as well as those that do not. In addition, the rs4959039:A>G allele appears to be additive to the effect of **HLA-DRB1**/*15:01* increasing the odds ratio for MS from 5.89 to 6.46, although the confidence intervals for the odds ratios of these haplotypes overlap.

**HLA-G** SNP associations from a meta-analysis genome-wide association study

Depending on the reference sequence the SNP rs4959039 maps to non-coding regions centromeric to **HLA-G** or **HLA-A**. The chromosome 6 *cox* reference sequence places this SNP in the intergenic non-coding region centromeric to **HLA-G** whereas the chromosome 6 *qbl* reference sequence maps the SNP centromeric to **HLA-A**. It appears that this SNP tags a possible ancestral duplication near both genes [16]. This observation raises the question as to whether the MS susceptibility signal associated with this SNP arises from alleles of **HLA-G**, **HLA-A**, or other nearby genes. Indeed, as presented above, many of the Class I SNPs identified in this study are in moderate to strong LD with each other.

A panel of different SNPs in the **HLA-G** locus was assessed using a dataset described in a recent genome wide association scan (GWAS) meta-analysis [14]. Although the published GWAS meta-analysis included subjects from the discovery dataset, these subjects were excluded from the following analysis to create an independent dataset consisting of 1606 MS cases and 5425 controls. In the GWAS meta-analysis 167 SNPs mapped to the **HLA-G** locus. After adjusting for **HLA-DRB1**/*15:01* using a tagging SNP and sex 63 of the 167 SNPs were associated with MS susceptibility with \( p \)-values \( \leq .01 \) (Table S7). The majority of the SNPs mapped to the untranslated region centromeric to **HLA-G**, some with \( p \)-Values \( \leq 1 \times 10^{-6} \) (rs1611715, rs3115627, rs2734982, rs2975033). 6 SNPs map within the **HLA-G** gene itself with \( p \)-Values \( \leq 1 \times 10^{-4} \). SNPs rs1611627, rs915668, rs 1736920 and rs1632933 are intronic SNPs whereas SNP rs1063320 maps to the 3’ end of the last exon of **HLA-G** and is transcribed but not translated. These data are consistent with the proposition that a MHC Class I MS susceptibility locus that is transcribed and independent of the extended **HLA-DRB1**/*15:01** haplotype maps to the region of the **HLA-G** gene.

**Summary**

This comprehensive SNP based analysis spanning the 29 to 34 kb region of the MHC shows that 52 SNPs in Class I, II and III regions of the MHC were associated with MS susceptibility in two independent datasets. Moreover, 20 of these SNPs were associated with MS susceptibility with \( p \)-values \( <1 \times 10^{-6} \) in a dataset that does not carry the extended **HLA-DRB1**/*15:01* haplotype. The most significant association was with rs4959039, a class I SNP near **HLA-G**. The association of this SNP with MS susceptibility appears to be independent of the effects of the other identified Class II and Class III SNPs.

**Discussion**

Using two case control datasets and a panel of SNPs specifically selected to capture the genetic variation within the MHC region we found that the MHC locus contributes to MS susceptibility, not only through the well recognized effect of **HLA-DRB1**/*15:01*, but also through independent contributions from a Class I locus. This study proves that, after the **HLA-DRB1**/*15:01* extended haplotype, the Class I region is the most significant contributor to MS susceptibility within the MHC. Importantly, these observations contrast with an earlier publication of a Canadian cohort which concluded that all Class I associations with MS susceptibility were due to LD with **HLA-DRB1**/*15:01* [5]. Although genetic heterogeneity might account for these differences, it is more likely that the structure of the current study, specifically the large dataset and denser set of informative markers, made possible the detection of independent effects of Class I and Class III genes.

**Class I genes and MS susceptibility**

The strongest **HLA-DRB1**/*15:01* independent MS association was with rs4959039, a SNP near the non-classical **HLA-G** gene. Several other SNPs in neighboring pseudogenes **HLA-80**, **HCG2P6**, **MICD** and **HLA-J** were also associated with MS susceptibility and are in LD with the rs4959039 SNP. These SNPs are not strongly linked to the SNP that tags the recently identified Class I MS protective allele **HLA-B**/*14:02* [12,14] and are independent of the major MS susceptibility allele **HLA-DRB1**/*15:01*. Because of the prohibitive cost we were unable to genotype classical **HLA** alleles in these large datasets to control for the possible contributions of **HLA-DRB1**/*03:01* [17] or other **HLA-DRB1** alleles. Nevertheless, logistic regression models that controlled for the 10 most statistically significant Class II SNPs, as well as the 8 Class III SNPs identified in this study,

<table>
<thead>
<tr>
<th><strong>Table 2.</strong> Paired marker analysis for <strong>HLA-DRB1</strong>/<em>15:01</em> and rs4959039 haplotypes in the merged dataset.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HLA-DRB1</strong></td>
</tr>
<tr>
<td><strong>HLA-DRB1</strong>/<em>X</em></td>
</tr>
<tr>
<td><strong>HLA-DRB1</strong>/<em>15:01</em>*</td>
</tr>
<tr>
<td><strong>HLA-DRB1</strong>/<em>15:01</em>*</td>
</tr>
<tr>
<td><strong>HLA-DRB1</strong>/<em>X</em></td>
</tr>
</tbody>
</table>

Two locus haplotypes were constructed and the odds ratio for association with MS susceptibility for each haplotype was tested in a logistic regression model treating each haplotype as a categorical variable. The odds ratio for the **HLA-DRB1**/*15:01* allele in the merged dataset was 3.50 \( (p<1.46 \times 10^{-10}) \). All results are adjusted for stratification effects caused by sex, location (US versus UK) and dataset (discovery versus replication). **DRB1**/*X* refers to subjects who do not carry the **HLA-DRB1**/*15:01* allele.

doi:10.1371/journal.pone.0011296.t002
demonstrated an independent allelic contribution of rs4959039 to MS susceptibility.

Although this association study cannot exclude the possibility that another closely linked HMC Class I gene, or genes, gives rise to the MS susceptibility signal detected by the rs4959039 SNP, it is clearly of interest that this SNP is in the 3' un-translated region of HLA-G. We conclusively demonstrated that this SNP’s association with MS susceptibility is independent of HLA-DRB1*15:01 and provided evidence that this SNP is not tightly linked to any of the Class III or Class II associations identified in this screen.

However, the rs4959039 SNP also maps to a duplication that is near HLA-A. HLA-A alleles were previously associated with MS susceptibility; the HLA-A*03 allele is thought to increase MS risk in HLA-DRB1*15:01 subjects [18,19] whereas the HLA-A*02 allele is thought to reduce MS risk [20]. Several lines of evidence suggest that the rs4959039 SNP’s association with MS might be through HLA-G rather than HLA-A*03. First, the HLA-A*03 allele is part of the extended HLA-DRB1*15:01 haplotype that was effectively excluded in this study. Second, the HLA-A*03 allele that was imputed in the discovery dataset is not tightly correlated with the rs4959039 SNP (r² = .002). Third, SNPs in HLA-A were not identified as disease-associated in either the discovery or the replication datasets. Lastly, using a different panel of HLA-G imputed SNPs from a genome wide meta-analysis in an independent dataset, multiple SNPs in the HLA-G locus were significantly associated with MS susceptibility after adjusting for HLA-DRB1*15:01. Thus we interpret our results as suggesting that the rs4959039 SNP association with MS risk is not through HLA-A*03. However, because typing of class I genes was unavailable for nearly the entire dataset we were unable to further analyze the relationship between the rs4959039 SNP and HLA-A*02, or other HLA-A alleles. Given that SNP rs4959039 tags a large haplotype block that includes HLA-A, mapping the class I susceptibility gene, or genes, will not only require classical typing of HLA-A but also could require an even larger dataset that excludes HLA-A alleles and MS susceptibility by the rs4959039 SNP (r² = .002).

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Thus, it is possible that a HLA-G associated haplotype could contribute to MS risk by influencing signaling via IL18RAP, IL7R and IL2RA receptors (IL18R) and interleukin 7 receptor (IL7R) [2].

Other Class I loci

In addition to the rs4959039 (near HLA-G) association, several other Class I SNPs associated with MS susceptibility were identified, replicated and shown to have independent effects. One group of SNPs tags the HLA-B*44:02 allele. Tagging SNPs for the closely linked HLA-C*0501 allele [7] did not survive the stringent criteria for association used in this study. These SNPs narrowly missed the cutoff for inclusion as candidates in the discovery dataset screen but were associated with MS susceptibility in the replication dataset screen. When these SNPs were included in the merged HLA-DRB1*15:01 (-) dataset, tagging SNPs for HLA-C*0501 [7] were significantly associated with MS susceptibility (data not shown). Logistic regression modeling suggested that the primary signal in the Class I region
arises from the locus identified by SNP rs4595039 although it remains possible that there could be independent contributions from other Class I loci.

Both HLA-C*05:01 and HLA-B*44:02 are reportedly protective alleles for MS susceptibility [7,12,14]. These neighboring alleles are in tight LD making discrimination between the effects of each allele challenging. In addition, different alleles of HLA-A may influence MS susceptibility in opposite directions. HLA-A*0201 may increase MS risk; however, this allele is part of the expanded haplotype shared by HLA-DRB1*15:01 and its proposed influence on MS susceptibility may be confounded by linkage to HLA-DRB1*15:01 [6]. In contrast, HLA-A*02:01 appears to have a protective effect [20]. This allele is also linked to the SNP identified in the present study, rs4595039. Functional studies, or fine mapping studies in populations with different patterns of LD, will be needed to determine whether the protective effect proposed for HLA-A*02:01 is mediated by linkage to an allele of HLA-G or other neighboring genes.

In summary, we found MHC SNP associations with MS susceptibility, independent from the primary influence of HLA-DRB1*15:01, in the Class I, Class II and Class III regions. The most significant contribution arises from the Class I region in the vicinity of the HLA-G gene. HLA-G, or another closely linked gene such as HLA-A, contributes to MS risk independently from the recently identified Class I allele HLA-B*44:02, as well as other Class II and Class III SNPs identified in the present study. Thus a Class I locus near HLA-G/HLA-A is a replicated locus within the MHC that contributes to MS risk independently of HLA-DRB1*15:01. The possible HLA-G association is particularly interesting because HLA-G is thought to function in induction of immune tolerance and is highly expressed in MS brain tissue. Further studies of functional polymorphisms in HLA-G, classical HLA typing, as well as studies in populations with different patterns of LD within the MHC, will help further define this locus's contribution to MS risk.

Methods

All study subjects signed written informed consent forms approved by the following local institutional review boards in accordance with the Declaration of Helsinki: Committee on Human Research (UCSF), CERDNT (MH), Human Subjects Research Office (University of Miami), Partners Healthcare IB/ Human Research Office, North Thames MREC, The North Shore - LIJ Health System IRB, Vanderbilt HRPP and Berkshire Research Ethics Committee.

The MS discovery dataset consists of 1018 cases (520 from the US and 498 from the UK) and 1795 controls (1049 from the US and 746 from the UK). All MS subjects met International Panel criteria for multiple sclerosis [39]. The control population was composed of samples from the United Kingdom 1958 birth cohort as well as a cohort of healthy subjects form The New York Cancer Project. The family based trio analysis was conducted on a subset, the tagging SNP rs3135391 was 100% sensitive and 100% specific for correctly calling HLA-DRB1*15:01 allele. Following identification of SNPs that were significantly associated with MS susceptibility in both datasets the discovery and replication datasets were included.

In the discovery dataset previous 2- or 4-digit typing of HLA-DRB1 was available for 27.6% of the dataset (N = 777). [14] In this subset, the tagging SNP rs3135391:T>C SNP that tags the HLA-DRB1*15:01 allele were excluded. MS associated SNPs where (Hardy Weinberg Equilibrium) HWE <0.01 in the control population of the merged dataset were dropped. 52 SNPs were significantly associated with MS susceptibility in both datasets.

In summary, we found MHC SNP associations with MS susceptibility, independent from the primary influence of HLA-DRB1*15:01, in the Class I, Class II and Class III regions. The most significant contribution arises from the Class I region in the vicinity of the HLA-G gene. HLA-G, or another closely linked gene such as HLA-A, contributes to MS risk independently from the recently identified Class I allele HLA-B*44:02, as well as other Class II and Class III SNPs identified in the present study. Thus a Class I locus near HLA-G/HLA-A is a replicated locus within the MHC that contributes to MS risk independently of HLA-DRB1*15:01. The possible HLA-G association is particularly interesting because HLA-G is thought to function in induction of immune tolerance and is highly expressed in MS brain tissue. Further studies of functional polymorphisms in HLA-G, classical HLA typing, as well as studies in populations with different patterns of LD within the MHC, will help further define this locus's contribution to MS risk.

Supporting Information

Figure S1 Study design summary. The 958 SNPs spanning the MHC used in the initial selections are listed in Supplemental Table 2. The 48 SNPs associated with MS in both datasets are listed in
Supplemental Table 3 and the 48 SNPs with $p$-values $\leq 1 \times 10^{-8}$ in the merged HLA-DRB1*15:01(-) dataset are listed in Table 1.

Table S1: Table S1. Case control datasets: The proportion of women to men in the control populations was well matched at the two study centers. However, the proportion of women to men in the MS subjects was significantly increased in the UK dataset.

Table S2: Table S2: 958 SNPs genotyped in both discovery and replication datasets. Ext = extended.

Table S3: Table S3: 52 SNPs significantly associated with MS susceptibility in the discovery and replication datasets using Cochran Armitage trend test, FDR = .05, adjusted for sex, center (US versus UK) and HLA-DRB1*15:01. The SNPs are listed in order of chromosomal position from telomere to centromere. The $p$-values for the merged dataset are unadjusted. rs2523393 is a tagging SNP for HLA-B*44:02 [12,14].

Table S4: Table S4: 48 SNPs significantly associated with MS susceptibility in the merged HLA-DRB1*15:01(-) dataset, using the trend test and adjusting for sex, center (US versus UK) and dataset (discovery versus replication). SNPs are listed in order of most to least statistical significance. Four class II SNPs identified in the discovery and replication datasets were no longer significantly associated with MS susceptibility in the HLA-DRB1*15:01(-) dataset: rs3129961, rs3135352, rs3135391, and rs3135388.

Table S5: Table S5: 48 SNPs that are associated with MS susceptibility in the HLA-DRB1*15:01(-) dataset are grouped together using an algorithm to define SNP clusters based on LD-R2 = .05 (moderate to strong LD) [15]. The 48 SNPs can be grouped into 20 SNP clusters and tagging SNPs for each cluster are designated by an asterisk. The SNPs are listed in order of cluster size with the largest cluster including 10 SNPs and the smallest SNP clusters include only single SNPs.

Table S6: Table S6: Two locus haplotypes for the SNPs rs2523393 (tags HLA-B*44:02) and SNP rs459039 (near HLA-G). A MS risk haplotype is rs2523393:T>G with rs459039:A>G and a MS protective haplotype is rs2523393:C>T with rs459039:G>A. The heterozygous haplotype appears to be protective suggesting a dominant effect of the protective haplotype. The $p$-values and odds ratios are adjusted for the covariates sex (men versus women) and cohort (discovery versus replication) to control for stratification.

References


Author Contributions

Conceived and designed the experiments: BACC JDR JLM PG PLDJ TJV PG IMAGEN IMSGC DBM DAH. Performed the experiments: BACC JDR JLM PG IMAGEN IMSGC DBM JLM PG PLDJ AS TJV PG IMAGEN IMSSC DBM DAH JLM MAPV AC SS JRO SLH. Analyzed the data: BACC JDR JLM PG JPM AS SS JRO SLH. Contributed reagents/materials/analysis tools: BACC JDR JLM PG PLDJ AS TJV PG IMAGEN IMSSC DBM DAH JLM MAPV AC SS JRO SLH. Wrote the paper: BACC JDR JLM PG JPM PLDJ AS TJV PG IMAGEN IMSSC DBM DAH JLM MAPV AC SS JRO SLH.

Acknowledgments


The International Multiple Sclerosis Genetics Consortium: Clinical and Sample Collection Groups (in order of the number of samples collected): University of Cambridge School of Clinical Medicine, Cambridge, United Kingdom — S. Sawcer (project coleader), M. Ban, A. Compston; University of California at San Francisco, San Francisco — J.R. Okenben (project coleader); B. Cree, S.L. Hauser; Brigham and Women’s Hospital, Boston — P.L. De Jager (project coleader), H.L. Weiner, D.A. Hafler. Project Management and Genotyping Centers: Harvard Center for Neurodegeneration and Repair, Boston — A.J. Ivinson (project leader); Brigham and Women’s Hospital, Boston — D.A. Hafler; Broad Institute of Harvard University and Massachusetts Institute of Technology, Cambridge, MA — S.B. Gabriel, D.B. Mirel; J.R. Okenben (project coleader); B. Cree, S.L. Hauser; Brigham and Women’s Hospital, Boston— P.L. De Jager, L.F. Barcellos, J.R. Oksenberg; University of California at Berkeley, Berkeley — L.F. Barcellos, J.R. Oksenberg; University of Cambridge School of Clinical Medicine, Cambridge, United Kingdom — S. Sawcer; University of Miami School of Medicine, Miami, FL — A. Pericak-Vance. Analysis Group: Massachusetts General Hospital, Boston — M.J. Daly (project coleader), P.L.W. de Bakker, Brigham and Women’s Hospital, Boston — P.L. De Jager, L.M. Maier; University of California at Berkeley, Berkeley — L.F. Barcellos, J.R. Oksenberg; University of Cambridge School of Clinical Medicine, Cambridge, United Kingdom — S. Sawcer; University of Miami School of Medicine, Miami, FL — A. Pericak-Vance, J.L. McCauley; and Vanderbilt University Medical Center, Nashville — L.J. Haines (project leader). The authors would like to thank all study participants and acknowledge Jordan Hiller for his consultations regarding use of JMP Genomics.

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16. www.sanger.ac.uk/HGP/Chr6/MHC/.


