Complex relation of **HLA-DRB1*1501**, age at menarche, and age at multiple sclerosis onset

**ABSTRACT**

**Objective:** To examine the relationship between 2 markers of early multiple sclerosis (MS) onset, 1 genetic (HLA-DRB1*1501) and 1 experiential (early menarche), in 2 cohorts.

**Methods:** We included 540 white women with MS or clinically isolated syndrome (N = 156 with genetic data available) and 1,390 white women without MS but with a first-degree relative with MS (Genes and Environment in Multiple Sclerosis [GEMS]). Age at menarche, HLA-DRB1*1501 status, and age at MS onset were analyzed.

**Results:** In both cohorts, participants with at least 1 HLA-DRB1*1501 allele had a later age at menarche than did participants with no risk alleles (MS: mean difference = 0.49, 95% confidence interval [CI] = [0.03–0.95], p = 0.036; GEMS: mean difference = 0.159, 95% CI = [0.012–0.305], p = 0.034). This association remained after we adjusted for body mass index at age 18 (available in GEMS) and for other MS risk alleles, as well as a single nucleotide polymorphism near the HLA-A region previously associated with age of menarche (available in MS cohort). Confirming previously reported associations, in our MS cohort, every year decrease in age at menarche was associated with a 0.65-year earlier MS onset (95% CI = [0.07–1.22], p = 0.027, N = 540). Earlier MS onset was also found in individuals with at least 1 HLA-DRB1*1501 risk allele (mean difference = −3.40 years, 95% CI = [−6.42 to −0.37], p = 0.028, N = 156).

**Conclusions:** In 2 cohorts, a genetic marker for earlier MS onset (HLA-DRB1*1501) was inversely related to earlier menarche, an experiential marker for earlier symptom onset. This finding warrants broader investigations into the association between the HLA region and hormonal regulation in determining the onset of autoimmune disease. Neurology Genet 2016;2:e88; doi: 10.1212/NXG.0000000000000088

**GLOSSARY**

BMI = body mass index; CI = confidence interval; CIS = clinically isolated syndrome; EDSS = Expanded Disability Status Scale; GEMS = Genes and Environment in Multiple Sclerosis; GRS = genetic risk score; GWAS = genome-wide association study; HLA = human leukocyte antigen; LD = linkage disequilibrium; MS = multiple sclerosis; SNP = single nucleotide polymorphism.

Understanding the cumulative effect of to-date disparate genetic and experiential risk factors on multiple sclerosis (MS) onset and course may be key to our ability to eventually predict and prevent the onset of clinical symptoms. The HLA-DRB1*1501 MS susceptibility allele has been previously associated with earlier age at MS onset. Across an individual's life history, the adolescent period may represent a critical window during which environmental factors modulate MS risk or age at onset. Among these, earlier menarche, a risk factor MS, has been tied with earlier onset of MS symptoms, although not always. Previous studies have sought to integrate genetic and experiential risk factors, for example, uncovering a striking interaction between obesity and HLA-DRB1*1501 in determining MS risk.
In the current study, we tested the association between HLA-DRB1*1501 and early menarche in 2 cohorts of white women, one with and one without MS. Secondarily, we confirmed the association between each marker and age at first MS symptoms in the cohort of affected women.

METHODS Participants. Individuals with MS. These participants were patients of the Partners Multiple Sclerosis Center, aged 18 or above, enrolled in the Comprehensive Longitudinal Investigation of Multiple Sclerosis at the Brigham and Women’s Hospital (CLIMB, www.climbstudy.org). Female participants were included in this study if they met the diagnostic criteria of relapsing remitting MS by the 2005 McDonald criteria or of clinically isolated syndrome (CIS).

Reproductive variables. A reproductive questionnaire, which is deployed to all active female CLIMB participants with a diagnosis of MS or CIS, was analyzed after a 60% response rate was achieved (on June 20, 2014), as reported previously. Among these 724 respondents, 675 provided an age at menarche. Participants with menarche data were older at first MS symptom than the 486 nonrespondents and participants without menarche data (33.8 vs 32.6 years, p < 0.05), but the groups did not differ in race, ethnicity, disease category, or Expanded Disability Status Scale (EDSS) at the most recent visit (p > 0.05 for each). The questionnaire variables included in the current analysis were (1) reported age at menarche and (2) response to whether a participant had “been told you were obese” during childhood and/or adolescence (yes/no).

Clinical data. Reproductive questionnaire responses were linked to demographic and clinical data available through CLIMB. Of the 675 participants who provided age at menarche and response to childhood obesity question in the reproductive questionnaire, we identified 540 participants for whom information about (3) residency at age 15 (north/middle/south United States; allowing us to categorize the latitude of residency at age 15 as a proxy for vitamin D exposure) and (4) smoking history (ever smoker/never smoker) was available. Of the 540 participants identified, 156 had genetic data available (see below, and table 1).

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| Table 1 | Demographic and genetic characteristics of women with (CLIMB cohort) and without (GEMS cohort) multiple sclerosis |
|---|---|---|
| Cohort | Discovery: | Replication: |
| | Participants with MS | Participants without MS |
| **Cohort** | **CLIMB Study (N = 156)** | **GEMS Study (N = 1,390)** |
| **Age at menarche, y, SD (range)** | 12.6 (1.5–8.0) | 12.7 (1.4–8.0) |
| **Age at survey, y, SD (range)** | 50.6 (10.1–50.6) | 34.0 (8.4–33.0) |
| **HLA-DRB1*1501 (rs3129889), n (%)** | | |
| 0 | 75 (48.1) | 843 (60.6) |
| 1 | 72 (46.2) | 497 (35.8) |
| 2 | 9 (5.8) | 50 (3.8) |
| **GRS 64, mean (SD, range)** | | |
| 10.1 (0.9–4.6) | 9.9 (0.9–6.5) |
| **GRS 59, mean (SD, range)** | | |
| 7.8 (0.6–2.9) | 7.7 (0.6–4.1) |

Abbreviations: GEMS = Genes and Environment in Multiple Sclerosis; GRS = calculated genetic risk score; IQR = interquartile range; MS = multiple sclerosis.

* p < 0.05.
RESULTS Demographic and disease characteristics. In our 2 cohorts of women, age at menarche was similar (12.6 and 12.7). As expected, the 156 women with MS had a higher GRS64 and GRS59 and were more likely to carry an HLA-DRB1*1501 risk allele, than were the 1,390 women in the GEMS cohort. The MS women also seemed to be older than the women without MS (table 1). Among the participants with MS, the 156 participants with genetic data available did not differ from the 384 participants without genetic data in terms of age at menarche (2-tailed test = −0.60; 95% confidence interval [CI] = [−0.35 to 0.18]; p = 0.55), age at first symptom (2-tailed t = 1.19; 95% CI = [−0.71 to 2.88]; p = 0.23), or EDSS at last visit (Mann-Whitney U test = 31; p = 0.11). However, there were no women with CIS among the genotyped population, likely reflecting inclusion criteria for genotyping (table 2).

Age at first symptoms. First, we confirmed that both the HLA-DRB1*1501 risk allele and age at menarche were associated with earlier age at first MS symptoms in our cohort of affected women (CLIMB). The presence of at least 1 HLA-DRB1*1501 allele was associated with earlier age at first symptoms (mean difference = −3.40, 95% CI = [−6.42 to −0.37], p = 0.028, N = 156). No association was found between any of the other 4 HLA alleles, or the overall genetic risk score GRS 64, and age at first symptoms (p > 0.10 for each).

Earlier age at menarche was also associated with earlier age at first MS symptom (figure 1). Specifically, the mean age at first symptoms was increased by 0.65 years for every 1-year increase in age at menarche (mean = 0.65; 95% CI = [0.07–1.22], p = 0.027, N = 540). This association remained (mean = 0.63; 95% CI = [0.05–1.21], p = 0.033) after we adjusted for 3 potential confounders (latitude at age 15, smoking history at first symptom, and obesity status in childhood or adolescence), none of which was significant in the multivariate model (p > 0.05 for each). Then, we ensured that the associations observed in the entire cohort were similar in the subset of participants with genetic data. We still observed that age at MS onset increased with increasing age at menarche, adjusting for the potential confounders. Specifically, for every 1-year increase in the age at menarche, the mean age at first symptoms increased by 0.35; but in the reduced sample of participants, this association was no longer significant (mean = 0.35; 95% CI = [−0.69 to 1.39]; p = 0.51; n = 156).

Genetic risk and age at menarche in 2 cohorts. Because both earlier menarche and genetic risk have previously been associated with earlier age at first symptoms, we sought to understand the association between menarche and genetic risk. Surprisingly, in our discovery cohort of women with MS, participants with at least 1 HLA-DRB1*1501 allele had a later age at menarche than participants with no risk alleles (2-tailed t = 2.12, 95% CI = [0.03–0.95]; p = 0.036; table 3). In the replication cohort of individuals without MS but with at least 1 first-degree relative with MS (GEMS), this was also true (2-tailed t = 2.15, 95% CI = [0.01–0.31], p = 0.032; table 3). When we combined the discovery and replication cohorts, having at least 1 HLA-DRB1*1501 risk allele was associated with a later age at menarche (2-tailed t = 2.69, 95% CI = [0.05–0.33]; p = 0.007). When the cohort effect was accounted for as a categorical variable in the regression model, our results remain unchanged (mean difference = 0.17; 95% CI = [0.04–0.31]; p = 0.011).

When we examined other genetic markers, a higher GRS64 score was also associated with later age at

Table 2: Demographic and disease characteristics of 540 white female participants with multiple sclerosis (CLIMB Study)

<table>
<thead>
<tr>
<th>Participants</th>
<th>Participants</th>
<th>Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>with complete covariate data (N = 540)</td>
<td>without genetics data (n = 384)</td>
<td>with genetics information (n = 156)</td>
</tr>
<tr>
<td><strong>Age at menarche, mean (SD; range)</strong></td>
<td>12.6 (1.4; 9.0–17.0)</td>
<td>12.6 (1.4; 9.0–17.0)</td>
</tr>
<tr>
<td><strong>Age at first symptom, mean (SD; range)</strong></td>
<td>33.6 (9.6; 11.5–64.6)</td>
<td>33.9 (9.64; 11.5–64.6)</td>
</tr>
<tr>
<td><strong>Disease category at last visit, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinically isolated syndrome</td>
<td>27 (5.0)</td>
<td>27 (7.0)</td>
</tr>
<tr>
<td>Primary progressive MS</td>
<td>12 (2.2)</td>
<td>11 (2.9)</td>
</tr>
<tr>
<td>Progressive relapsing MS</td>
<td>4 (0.7)</td>
<td>2 (0.5)</td>
</tr>
<tr>
<td>Relapsing-remitting MS</td>
<td>419 (77.6)</td>
<td>289 (75.3)</td>
</tr>
<tr>
<td>Secondary progressive MS</td>
<td>78 (14.4)</td>
<td>55 (14.3)</td>
</tr>
<tr>
<td>EDSS at last visit, median (IQR; range)</td>
<td>1.5 (1.5; 0.0–9.5)</td>
<td>1.5 (3.0; 0.0–8.0)</td>
</tr>
</tbody>
</table>

Abbreviations: EDSS = Expanded Disability Status Scale; IQR = interquartile range; MS = multiple sclerosis. *p < 0.05.
menarche in the individual and combined cohorts ($p < 0.05$, table 3). However, this was likely driven by the HLA-DRB1*1501 allele because there was no association between any of the other HLA alleles, or of the GRS59 (GRS64 without HLA SNPs), and age at menarche in either cohort ($p > 0.10$ for each analysis). In sensitivity analyses, we addressed the possibility that the HLA-DRB1*1501 effect was driven, through linkage disequilibrium (LD), by another SNP, namely, an HLA-A allele previously found to be associated with menarcheal age in a large GWAS (through the proxy rs16896742 SNP18). When we further adjusted our analyses for rs16896742 in the MS cohort, the association between HLA-DRB1*1501 and later menarcheal age remained (mean difference = 0.49, 95% CI = [0.03–0.95], $p = 0.037$).

In addition, in the GEMS cohort, for whom BMI at age 18 was available, with higher BMI itself associated with earlier age at menarche (mean difference = −0.07 years; 95% CI = [−0.08 to −0.05]; $p < 0.0001$, N = 1,388), adjusting for BMI at age 18 did not change the significance of the association between HLA-DRB1*1501 and age at menarche (mean difference = 0.1538; 95% CI = [0.029–0.278]; $p = 0.015$; N = 1,388).

**Summary of associations.** Altogether, a summary of the directionality of the findings between HLA-DRB1*1501, age at menarche, and age at first symptoms is represented in figure 2.

**DISCUSSION** In this analysis of a well-phenotyped cohort of women with MS, both earlier age at menarche and an established genetic risk factor for
Given such previous reports of synergistic associations between genetic and experiential factors in determining MS risk, we asked whether established MS genetic risk alleles, previously found to be associated with age of MS onset (for both HLA-DRB1*1501 and broader genetic risk variants), might underlie or enhance the association between menarcheal age and age of MS onset. We found a statistical trend between genetic risk factors for MS and earlier age at MS first symptoms, consistent with one previous report. Because there was no association between menarcheal age and age at MS onset in another large study, further cohorts are required to validate our observations and to assess the role of regional or epochal differences in menarcheal age and delay in MS diagnosis. Although the association between menarcheal age and symptom onset was expected, it was unexpected that an MS risk allele would show an association with later (rather than earlier) menarcheal age. The presence of HLA-DRB1*1501 was associated with later age at menarche, which in turn is associated with later age at MS onset.

There are few previous reports linking HLA alleles and menarcheal age. Given the recognized extent of LD in the HLA region on chromosome 6, one possible explanation for our finding might be that an allele in LD with HLA-DRB1*1501 might drive the apparent association between HLA-DRB1*1501 (which is in this scenario neutral) and later menarcheal age, even as other environmental factors are driving a relationship between earlier menarche and MS risk. However, we found no evidence that the other MS HLA risk alleles included in our GRS, or that an SNP near the HLA-A region previously found to show a genome-wide association with age of menarche, mitigated the association between HLA-DRB1*1501 and menarcheal age. Our findings hint at previously unexplored associations between immune regulation and pubertal timing, warranting not only replication in other data sets but also clarification of whether HLA-DRB1*1501 or an allele in LD with HLA-DRB1*1501 is driving the association with menarcheal age, as well as mechanistic explorations of the genetic and epigenetic regulation of immune activity during the pubertal transition.

The largest limitation of this study was that adiposity during the adolescent years was only incompletely captured for the MS participants; however, in the GEMS cohort, we were able to verify that the association between HLA status and menarcheal age was not influenced by BMI at age 18. The second limitation was the statistically significant, but minor in absolute terms, respondent biases in the reproductive survey. If respondents were older at MS onset than nonrespondents, they may have been less likely to have early menarche and to carry HLA-DRB1*1501.
risk allele; it is possible that with a higher response rate, we would have observed even stronger associations between genetic risk and menarcheal age. Third, only a subset of our participants had genetic data available for analysis, limiting the interpretability of data in the wider cohort of women and for women who are not white.

The findings in this study lend complexity to the analysis of early risk factors for MS onset, suggesting a more complex interplay between genetic risk factors and hormonal exposures in the development of inflammatory disease.

**AUTHOR CONTRIBUTIONS**


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**DISCLOSURE**

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