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

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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. ***Neurol 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.[1] The *HLA-DRB1*1501* MS susceptibility allele has been previously associated with earlier age at MS onset.[2–4] 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.[5] Among these, earlier menarche, a risk factor MS,[6–8] has been tied with earlier onset of MS symptoms,[9] although not always.[6] 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.[10]

Supplemental data at Neurology.org/ng

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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).[11] Female participants were included in this study if they met the diagnostic criteria of relapsing remitting MS by the 2005 McDonald criteria[12] 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.[13] Among these 724 respondents, 675 provided an age at menarche. Participants with menarche data were older at first MS symptom than the 486 non-respondents 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)[14] 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).

Table 1 Demographic and genetic characteristics of women with (CLIMB cohort) and without (GEMS cohort) multiple sclerosis

Cohort	Discovery: Participants with MS CLIMB Study (N = 156)	Replication: Participants without MS 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,[a] y, SD (range)	50.6 (10.1–50.6)	34.0 (8.4–33.0)
*HLA-DRB1*1501* (rs3129889_G), n (%)[a]		
0	75 (48.1)	843 (60.6)
1	72 (46.2)	497 (35.8)
2	9 (5.8)	50 (3.6)
GRS 64, mean (SD, range)[a]	10.1 (0.9–4.6)	9.9 (0.9–6.5)
GRS 59, mean (SD, range)[a]	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.
[a] $p < 0.05$.

Individuals with family history of MS. The 1,390 participants without MS were adult female participants in the Genes and Environment in Multiple Sclerosis (GEMS) study. This prospective cohort study was designed to map the sequence of events in the transition from health to MS, by enrolling individuals who have a first-degree relative with a diagnosis of MS.[1] At study enrollment, participants complete a detailed demographic, health, and reproductive questionnaire and provide samples for genetic analyses. GEMS participants were excluded from the current study if they themselves carried a diagnosis of MS.

Genotyping and genetic risk score. Genotyping of the individuals with MS[15] and GEMS participants[1] has been described previously. A genetic risk score (GRS) was designed from a list of 64 validated and replicated single nucleotide polymorphisms (SNPs) that are associated with MS susceptibility[2,16] and was the most up-to-date list as of 2011 when targeted genotyping of GEMS participants first began.[1] This list of 64 SNPs included 5 within the major histocompatibility complex or human leukocyte antigen (HLA) region (table e-1 at Neurology.org/ng). Each SNP was coded additively by the established risk allele and weighted by the natural log of the published odds ratio for MS susceptibility. The GRS59 refers to the GRS64 without the 5 HLA SNPs.

Standard protocol approvals, registrations, and patient consents. Institutional Review Board approval was granted by the Partners Human Research Committee.

Statistical analysis. All analyses were conducted using the Statistical Analysis System (SAS) 9.3 (Cary, NC). Given the low number of non-white participants (<7%), to mitigate the possible role of ancestry in genetic associations,[4,17] only white participants (fewer than 5% of whom identified as Hispanic) were included in this study. We compared the GEMS and MS genetic cohorts, as well as the MS participants with and without genetic data, using a 2-sample *t* test for continuous variables (ages, GRS), Fisher exact test for disease category, and 2-tailed Mann-Whitney *U* test for EDSS.

In our MS cohort, we first confirmed that the previously reported association between both age at menarche and genetic risk markers, and age at first symptoms, was present. To do this, we performed univariate analysis using a simple linear regression between each predictor (age at menarche, HLA alleles, GRS64, and GRS59) and age at first symptoms. We selected the rs3129889 SNP as our primary SNP, because it refers to the *HLA-DRB1*1501* MS susceptibility allele, which has been previously associated with earlier age at disease onset.[2–4] We examined SNPs in 2 ways; we report analyses examining SNPs as a categorical variable (at least 1 susceptibility allele vs none) using a 2-sample *t* test; the results were similar when we analyzed alleles as continuous variables (i.e., 0, 1, and 2; table e-2) using a linear regression model. We further assessed the potential confounding effect of reported obesity, latitude of residency at age 15, and smoking history, by including these variables in a multivariate analysis (multiple linear regression) for each of the models.

Then, in our primary analysis, we assessed the association between genetic risk markers (HLA alleles, GRS64 and GRS59) and age at menarche in both cohorts using multiple linear regression models. Here again, the rs3129889 SNP was our primary SNP. Because 1 HLA-A allele has previously been found to be associated with menarcheal age in a large genome-wide association study (GWAS) through the rs16896742 SNP, which is near HLA-A,[18] we further adjusted our analyses for rs16896742 (available in the MS cohort). Finally, we also adjusted for reported body mass index (BMI) at age 18 (available in the GEMS cohort).

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 $t = -0.60$; 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]; $p = 0.011$).

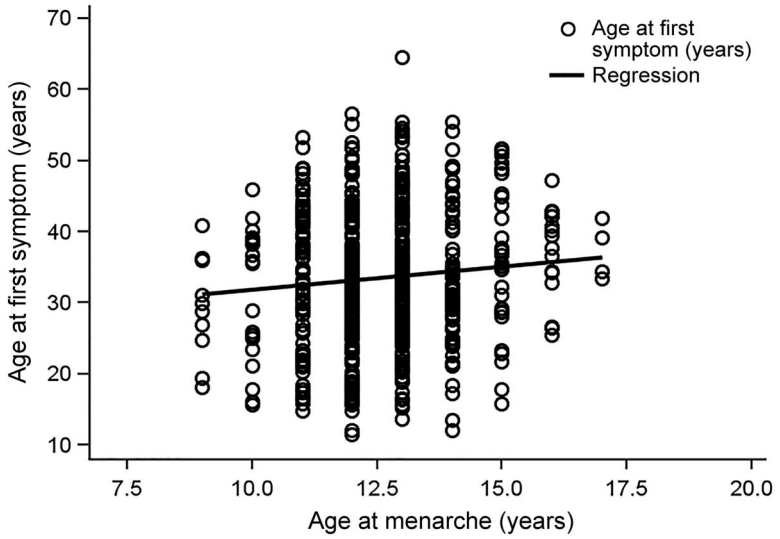
When we examined other genetic markers, a higher GRS64 score was also associated with later age at

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

	Participants with complete covariate data (N = 540)	Participants without genetics data (n = 384)	Participants with genetics information (n = 156)
Age at menarche, mean (SD; range)	12.6 (1.4; 9.0-17.0)	12.6 (1.4; 9.0-17.0)	12.6 (1.5; 9.0-17.0)
Age at first symptom, mean (SD; range)	33.6 (9.6; 11.5-64.6)	33.9 (9.64; 11.5-64.6)	32.83 (9.6; 13.5-56.5)
Disease category at last visit, n (%)[a]			
Clinically isolated syndrome	27 (5.0)	27 (7.0)	0 (0.0)
Primary progressive MS	12 (2.2)	11 (2.9)	1 (0.6)
Progressive relapsing MS	4 (0.7)	2 (0.5)	2 (1.3)
Relapsing-remitting MS	419 (77.6)	289 (75.3)	130 (83.3)
Secondary progressive MS	78 (14.4)	55 (14.3)	23 (14.7)
EDSS at last visit, median (IQR; range)	1.5 (1.5; 0.0-9.5)	1.5 (3.0; 0.0-8.0)	1.5 (1.5; 0.0-9.5)

Abbreviations: EDSS = Expanded Disability Status Scale; IQR = interquartile range; MS = multiple sclerosis.
[a] $p < 0.05$.

Figure 1 Earlier age at menarche is associated with earlier age at first symptoms



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 SNP[18]). When we further adjusted our analyses for rs16896742 in the MS cohort, the association between *HLA-DRB1*1501* and later menarcheal age remained (mean = 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

Table 3 Association between genetic risk markers and age at menarche in a discovery and replication cohort

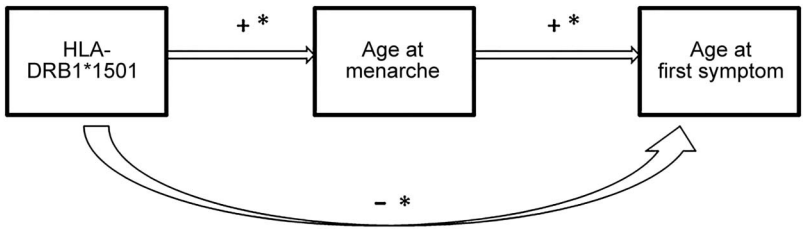
	Discovery: CLIMB Study (N = 156)				Replication: GEMS Study (N = 1,390)				Global (N = 1,546)			
	Adjusted mean difference	95% CI	2-tailed *t*	*p*	Adjusted mean difference	95% CI	2-tailed *t*	*p*	Adjusted mean difference	95% CI	2-tailed *t*	*p*
***HLA-DRB1*1501* (rs3129889_G)**												
0 vs 1 and 2	0.49	0.033 to 0.947	2.12	0.036[a]	0.159	0.014 to 0.307	2.15	0.032[a]	0.191	0.052 to 0.330	2.69	0.007[a]
GRS64	0.255	0.002 to 0.508		0.049[a]	0.087	0.009 to 0.165		0.029[a]	0.104	0.029 to 0.178		0.0064[a]
GRS59	0.225	−0.158 to 0.607		0.25	0.065	−0.055 to 0.185		0.29	0.082	−0.033 to 0.196		0.16

Abbreviations: CI = confidence interval; GEMS = Genes and Environment in Multiple Sclerosis; GRS = calculated genetic risk score.
[a] $p < 0.05$.

MS, *HLA-DRB1*1501* status, were associated with earlier onset of MS symptoms. However, when we examined the association between *HLA-DRB1*1501* status and age at menarche, in both a cohort of women at increased genetic risk for MS and our clinical cohort, this risk allele was associated with a later age at menarche.

The age at menarche has been decreasing over the past decades, and downstream events associated with earlier reproductive maturation have been hypothesized as one mechanism explaining an apparent increase in the female:male sex ratio in MS.[19] Our first observation, of an association between earlier menarche and earlier age at MS symptoms, adds to the growing literature about the role of changes at puberty in the development of MS risk.[5,6,9,20] Mechanistically, it is possible that this association is causal, through an effect of cycling gonadal hormones on immune regulation, inflammatory events, and ongoing neuronal development and susceptibility. This is supported by a recent report of increasing relapses as adolescent girls transition through puberty.[21] However, it is also possible that earlier menarche merely reflects an earlier adipose, proinflammatory childhood, and adolescent environment. There is an established link between adiposity and earlier menarche in healthy girls,[22,23] and many genes involved in the regulation of adiposity are also associated with timing of menarche.[24,25] An association between adolescent obesity and MS risk, first reported in the Nurses' Health Study,[26] has been replicated for both adult-onset[27,28] and childhood-onset MS[29]; and recently, higher adolescent BMI has also been associated with earlier age of MS onset.[30] The role for adiposity-related inflammatory mechanisms is supported by elevated adiposity markers such as leptin reported in MS,[31,32] as well as striking interactions between adolescent obesity and HLA risk genes in predicting adult-onset MS.[10]

Figure 2 **Flow diagram depicting the interaction between HLA risk allele, menarche, and age at first symptoms in female patients with multiple sclerosis**



Presence of *HLA-DRB1*1501* multiple sclerosis risk allele is associated with later age at menarche, which in turn is associated with later age at first symptoms. Presence of the *HLA-DRB1*1501* risk allele is associated with earlier age at first symptoms. A significant association/difference ($p < 0.05$) is represented by an asterisk (*). A positive association is represented by a plus sign (+) and a negative association by a negative sign (–).

Given such previous reports of synergistic associations between genetic and experiential factors in determining MS risk,[10] we asked whether established MS genetic risk alleles, previously found to be associated with age of MS onset (for both *HLA-DRB1*[2–4] and broader genetic risk variants[33,34]), 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.[9] Because there was no association between menarcheal age and age at MS onset in another large study,[6] 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.[35] Given the recognized extent of LD in the HLA region on chromosome 6,[36] 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,[18] 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-DRB*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

Study concept and design: R.B., T.C. Statistical analysis and interpretation of data: A.S.C., L.C. Acquisition of data and interpretation of results: R.B., Z.X. Manuscript drafting and revising: R.B., A.S.C., L.C., Z.X., P.L.D.J., T.C.

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DISCLOSURE

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