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SHORT COMMUNICATION

Parent-of-origin of *HLA-DRB1*1501* and age of onset of multiple sclerosis

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Multiple sclerosis (MS) is a complex neurological trait. Allelic variation in the MHC class II region exerts the single strongest effect on MS genetic risk. The clinical onset of the disease is extremely variable, and can range from the first to the ninth decade of life. Epidemiological studies have suggested a modest genetic component to the age of onset (AO) of MS. Previous studies have shown that *HLA-DRB1*1501* may be associated with a younger AO. Here, we sought to uncover any effect of *HLA-DRB1*1501* on the AO of MS in a large Canadian cohort. A total of 1816 MS patients were genotyped for *HLA-DRB1*. Patients carrying *HLA-DRB1*1501* were shown to have a small, but significantly lower, AO than patients without the allele (P=0.03). *HLA-DRB1*1501* was also shown to reduce the mean AO in both progressive and relapsing forms of the disease. An investigation of parent-of-origin effects indicated that the lower AO for *HLA-DRB1*1501* patients arises from maternally transmitted *HLA-DRB1*1501* haplotypes (maternal *HLA-DRB1*1501* mean AO=28.4 years, paternal=30.3 years; P=0.009). *HLA-DRB1*1501* exerts a modest, but significant effect on the AO of all forms of MS. Parent-of-origin effects at the MHC are further implicated in MS disease pathogenesis.
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INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system.[1] The cause of MS is unknown; however, epidemiological studies have shown that genetic factors are important in disease susceptibility.[2]

The HLA class II association exerts the strongest genetic effect in MS, with the *HLA-DRB5*0101–HLA-DRB1*150101–HLA-DQA1*0102–HLA-DQB1*0602*-extended haplotype conferring a relative risk of approximately 3, and homozygosity for this haplotype increasing the risk by over sixfold.[2] Other *HLA-DRB1* haplotypes also have a role in MS, especially by epistatic interaction.[2]

Most individuals have their clinical onset of MS between the ages of 20 and 40 years. The peak age of onset (AO) is 24 years in women and 25 years in men.[1] However, in large MS populations, the range of AO is very broad to an extent matched by few disease entities. Well-documented pathological cases are seen early in the first decade of life, as well as into the ninth decade.[1] Explanations for this wide AO distribution are largely unknown.

Studies have found that AO has a modest tendency at best to cluster in families, in affected parent–child pairs and in affected sibling pairs.[3–6] However, the evidence is conflicting,[7,8] perhaps implicating only a small role for genetic factors in MS AO. To this end, studies have highlighted a modest association of AO with the presence of *HLA-DRB1*1501*.[9–13] In this investigation, our aim was to determine whether there was any effect of *HLA-DRB1*1501* on AO in a large *HLA-DRB1*-genotyped Canadian MS population and to determine whether this was related to the parent-of-origin of *HLA-DRB1*1501* haplotypes.

MATERIALS AND METHODS

Participants

All individuals who participated in this study were ascertained through the ongoing Canadian Collaborative Project on the Genetic Susceptibility to MS (CCPGSMS), for which the methodology has been previously described.[14] A total of 1816 individuals with clinically definite MS were genotyped (1310 (72%) were female and 506 (28%) were male). These individuals were all Caucasian and of Northern European ancestry. The patients had a mean age of disease onset of 30.9 years and 82% of patients had a relapsing remitting/secondary progressive course, whereas the remainder had the primary progressive form of the disease.

Genotyping

HLA-DRB1 alleles were genotyped by an allele-specific PCR amplification method.[15] We obtained high-resolution *HLA-DRB1* genotypes with an additional 48 PCR reactions as described[16,17] (primer sequences available on request).

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Table 1 ANOVA table for linear model

	Df	*Sum Sqs*	*Mean Sq*	*F*	P-*value*
β_1	1	0.15	0.15	1.49	0.222
β_2	1	0.4	0.4	3.95	**0.047**
Residuals	1810	181.97	0.1		

Abbreviations: ANOVA, analysis of variance; Df, degrees of freedom; F, test statistic; Mean Sq, mean square, Sum Sqs, sum of squares.
P-value of association of HLA-DRB1*1501 and AO of MS is in bold.

Statistical analyses

Student's *t*-tests were used to compare age of onset between groups. Multiple linear regression was used to assess the relationships of sex and the presence or absence of *HLA-DRB1*1501* on AO. We fitted the additive model described by $(\mathrm{Log}(AO_i)=\beta_0+\beta_1 Sex_i+\beta_2 HLA\text{-}DRB1{*}1501_i+\varepsilon_i)$ by least squares. Both sex and *HLA-DRB1*1501* are categorical explanatory variables. It was necessary to log transform AO to satisfy the modeling assumptions and to justify using least squares.

RESULTS

A total of 1026 patients were positive for *HLA-DRB1*1501*. These individuals had an average age of MS onset of 30.4 years (standard deviation (s.d.)=9.77 years). The remaining 790 patients who were negative for *HLA-DRB1*1501* had an average AO of 31.4 years (s.d.=9.67 years). This difference was small (1 year), but significant; two-tailed Student's *t*-test $P=0.03$.

Table 1 presents the analysis of variance summary for the linear model. *HLA-DRB1*1501* did not account for a large amount of variance in AO (sum of squares=0.4), but it was a significant predictor of AO of MS ($P=0.047$), independent of sex. Individuals possessing *HLA-DRB1*15* have a mean clinical onset of MS 3% earlier than those not carrying the allele.

It was possible to assess the parental origin of *HLA-DRB1*1501* for 557 *HLA-DRB1*1501* heterozygous patients. There were 351 patients with a maternally transmitted *HLA-DRB1*1501* and 206 patients with a paternally transmitted *HLA-DRB1*1501*. Patients carrying a maternal *HLA-DRB1*1501* had an average AO of 28.4 years (s.d.=7.97 years) compared with a mean MS onset age of 30.3 years (s.d.=8.73 years) for MS patients carrying a paternal copy of this allele (two-tailed Student's *t*-test $P=0.009$).

We have previously characterized a benign and malignant MS cohort[16] (benign MS being patients with the relapsing–remitting clinical subtype of MS, wherein EDSS$\leqslant$3 was attained over a period greater than 20 years from disease onset ($n=112$) and malignant MS being cases who rapidly attained EDSS >6 within 5 years of disease onset with primary or relapsing progressive forms of the disease ($n=51$)). *HLA-DRB1*1501* was associated with a lower AO in both categories of patients (for benign *HLA-DRB1*1501*-positive patients, mean AO=24.5 years; for benign *HLA-DRB1*1501*-negative patients, mean AO=25.6 years (two-tailed Student's *t*-test $P=0.09$); for malignant *HLA-DRB1*1501*-positive patients, mean AO=36.3 years, for malignant *HLA-DRB1*1501*-negative patients, mean AO=38.1 years (two-tailed Student's *t*-test $P=0.13$)).

DISCUSSION

Previous studies have observed a modest effect of *HLA-DRB1*1501* on the AO of MS,[9,11] and we were able to confirm this in the Canadian population, as Canadian MS patients carrying this allele were likely to have a mean AO 1-year less than patients without this allele ($P=0.03$).

An earlier report also observed an association of *HLA-DRB1*1501* with lower ages of onset in both progressive and relapsing forms of the disease.[18] We were able to find a similar effect in benign and malignant MS patients, with *HLA-DRB1*1501*-carrying patients in each group having a lower mean AO, although this was not statistically significant because of the small numbers involved. The fact that relapsing–remitting and primary progressive MS have the same allelic frequency of *HLA-DRB1*1501* in conjunction with a similar AO, reducing the effect of this allele in both disease forms adds more genetic support to the notion that relapsing–remitting and primary progressive MS are the same disease. Indeed, natural history data have shown similar rates of disability accumulation in both disease forms once they enter the progressive phase;[19] it is merely that progression in relapsing–remitting MS is preceded by transient episodes of disease activity.

A recent epidemiological study has shown that affected pairs of relatives tend to be more alike for AO based on how closely related they are, with the greatest degree of similarity seen for concordant monozygotic pairs ($r=0.60$) and the least for first-cousin pairs ($r=0.10$).[20]

Dizygotic twins share the same number of genes as do siblings, but show a much stronger AO correlation ($r=0.54$ and $r=0.20$, respectively). Maternal half-siblings ($r=0.37$) are more strongly correlated than paternal half-siblings ($r=0.26$) for AO, indicating that maternal effects may have a more significant role in determining AO,[20] as has been described for disease susceptibility.[2] We have previously shown that maternally transmitted *HLA-DRB1*1501* confers a greater risk for MS than when paternally transmitted.[21] In this study, it seems that *HLA-DRB1*1501* from mothers may also influence AO to a greater extent than when transmitted from fathers. This analysis is based on a subset of the initial cohort, but is coherent with other data.[2] Gene–environment interactions or epigenetic factors are now implicated in the clinical features of MS, in addition to disease susceptibility.

In summary, in accordance with previous studies, we have shown a modest effect of *HLA-DRB1*1501* on the clinical onset of MS. It is probable that the presence of a major susceptibility allele is more likely to interact with disease-relevant epigenetic and environmental factors, increasing the likelihood of MS occurring earlier. It has been established for over 30 years that certain MHC class II haplotypes are more common in patients, although how these class II genes alter the risk of developing MS is not yet fully understood. Further work is needed to understand the complex role of HLA in MS.

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