The Inheritance of Resistance Alleles in Multiple Sclerosis

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Multiple sclerosis (MS) is a complex trait in which alleles at or near the class II loci HLA-DRB1 and HLA-DQB1 contribute significantly to genetic risk. HLA-DRB1*15 and HLA-DRB1*17-bearing haplotypes and interactions at the HLA-DRB1 locus increase risk of MS but it has taken large samples to identify resistance HLA-DRB1 alleles. In this investigation of 7,093 individuals from 1,432 MS families, we have assessed the validity, mode of inheritance, associated genotypes, and the interactions of HLA-DRB1 resistance alleles. HLA-DRB1*14, HLA-DRB1*11, HLA-DRB1*01, and HLA-DRB1*10-bearing haplotypes are protective overall but they appear to operate by different mechanisms. The first type of resistance allele is characterised by HLA-DRB1*14 and HLA-DRB1*11. Each shows a multiplicative mode of inheritance indicating a broadly acting suppression of risk, but a different degree of protection. In contrast, a second type is exemplified by HLA-DRB1*10 and HLA-DRB1*01. These alleles are significantly protective when they interact specifically in trans with HLA-DRB1*15-bearing haplotypes. HLA-DRB1*01 and HLA-DRB1*10 do not interact with HLA-DRB1*17, implying that several mechanisms may be operative in major histocompatibility complex-associated MS susceptibility, perhaps analogous to the resistance alleles. There are major practical implications for risk and for the exploration of mechanisms in animal models. Restriction of antigen presentation by HLA-DRB1*15 seems an improbably simple mechanism of major histocompatibility complex–associated susceptibility.

Introduction

Multiple sclerosis (MS) is a complex neurological disease with a strong genetic predisposition, as demonstrated by genetic-epidemiological studies [1–3]. However, the mapping of putative susceptibility genes has proven difficult. The imputation of many non–major histocompatibility complex (MHC) genes by analogies with experimental models and the drop in concordance between monozygotic and dizygotic twins, has not been validated by genome scans. In genome-wide studies with enough power to detect relatively small effects, the MHC has consistently been the only locus linked to MS [4]. This indicates that the MHC is the key susceptibility locus in MS and other susceptibility genes contribute relatively little to overall risk [31].

Accordingly, polymorphisms at the major histocompatibility locus (MHC) have undergone renewed study. Genes in the HLA class I region were originally shown to be associated with MS [5,6] but stronger associations were subsequently observed in the class II region of the MHC [7]. This class II association has been fine mapped to the extended haplotype HLA-DQA1*0102-DQB1*0602-DRB1*1501-DRB5*0101 [8,9].

The human leukocyte antigen (HLA) association in a large Canadian MS population was recently revisited [10] and the situation was found to be much more complex than originally conceived. The study of interactions was only productive when a large sample was analysed (n = 4,347 individuals). The HLA-DRB1*17 allele has long been known to be associated with susceptibility in other groups, in particular the Sardinian and the Swedish MS populations [11,12] and this was confirmed in the Canadian population.

There were clear indications of resistance alleles, in particular, HLA-DRB1*14, and the same approach was recently repeated in a study of American and European families [13] with similar results. Additionally, HLA-DRB1*08 and DRB1*01 showed effects on MS risk in the presence of HLA-DRB1*15 [10]. We present here an investigation of an expanded series of Canadian MS families aimed at elucidating the inheritance pattern of MS susceptibility and resistance alleles.

Results

A total of 7,093 individuals from 1,432 families have been typed as part of the Canadian Collaborative Project on the Genetic Susceptibility to MS (CCPGSMS). This includes 2,454 individuals with definite MS and 4,639 of their unaffected first-degree relatives.

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Abbreviations: CCPGSMS, Canadian Collaborative Project on the Genetic Susceptibility to MS; HLA, human leukocyte antigen; MHC, major histocompatibility complex; MS, multiple sclerosis; NT, not transmitted; OR, odds ratio; TDT, transmission disequilibrium test; TR, transmitted;
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Transmission Disequilibrium Test Analysis of All HLA-DRB1 Alleles

In the total sample, HLA-DRB1*15 was transmitted 953 times and not transmitted 392 times; \( x^2 = 233.99 (p = 8.0 \times 10^{-55} \). To avoid confounding results due to the over transmission of HLA-DRB1*15, transmissions from non-HLA-DRB1*15-bearing parents were examined. HLA-DRB1*17 was transmitted (TR) 306 times and not transmitted (NT) 222 times; odds ratio (OR) = 1.4, \( x^2 = 13.36 (p = 0.00026 \). HLA-DRB1*14 continues to be protective; TR = 23, NT = 66, OR = 0.35, \( x^2 = 20.78 (p = 5.2 \times 10^{-6} \) and HLA-DRB1*11 was also found to be significantly under transmitted (TR = 157, NT = 213) even after applying a Bonferroni correction for multiple testing \( p = 0.0036, p_c = 0.047 \). HLA-DRB1*08 \( (p = 0.0058 \) and HLA-DRB1*12 \( (p = 0.041 \) were over and under transmitted, respectively, but would not be significant after applying a Bonferroni correction.

Transmission of HLA-DRB1 alleles to unaffected offspring was also analysed. In the total sample, HLA-DRB1*15 was transmitted 518 times and not transmitted 616 times; \( x^2 = 8.47 (p = 0.0036, p_c = 0.047 \). Transmissions of HLA-DRB1*14 and HLA-DRB1*11 from non-HLA-DRB1*15-bearing parents were TR = 42, NT = 42 \( (p = 1 \) and TR = 142, NT = 161 \( (p = 0.28 \), respectively.

Mode of Inheritance of HLA-DRB1*15

The mode of inheritance of HLA-DRB1*15 was assessed by examining the segregation of the allele in various mating types (Table 1) where the genotypes of both parents were available. The genotype HLA-DRB1*15/X had a 3.1-fold increased risk of MS over HLA-DRB1*/X (where “X” refers to all alleles other than HLA-DRB1*15). An individual homozygous for HLA-DRB1*15 had a 1.8-fold increased risk over a heterozygous HLA-DRB1*15 individual; \( x^2 = 20.24 (p = 6.8 \times 10^{-6} \).

Table 1. The Inheritance of HLA-DRB1*15

| Mating Genotypes       | Offspring Genotype |
|------------------------|-------------------|
| 15/15 versus 15/X      |                  |
| 15/X by X/X            | NA                |
| 15/X by 15/X           | 107 by 56 (OR = 1.9) |
| 15/15 by 15/X          | 53 by 33 (OR = 1.6) |
| Total                  | 160 by 89 (OR = 1.8) |

\( \chi^2 \) value (p-value) for differences between categories:

- \( \chi^2 = 20.24 (p = 6.8 \times 10^{-6}; p_c = 1.6 \times 10^{-6} \)
- \( \chi^2 = 9.6 \times 10^{-3}; p_c = 2.1 \times 10^{-4} \)

In all tables, NA means that no matings of this type are available or offspring genotypes are not possible with parental genotypes.

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Mode of Inheritance of HLA-DRB1*17

The mode of inheritance for the HLA-DRB1*17 susceptibility allele was assessed in a similar manner. In the total sample, HLA-DRB1*17/17 or HLA-DRB1*17/X showed no increased frequency in the genotypes of the affected offspring (unpublished data). However, controlling for the effects of HLA-DRB1*15 by examining only those parents lacking HLA-DRB1*15 (n = 179 nuclear families), HLA-DRB1*17 heterozygotes increased MS risk by 1.6-fold over HLA-DRB1*X/X individuals (where “X” is neither HLA-DRB1*15 nor DRB1*17) (Table 2). Additionally, HLA-DRB1*17 homozygosity further increased risk by 2.2-fold compared to a HLA-DRB1*17 heterozygote.

To test the relative and interactive effects of HLA-DRB1*17 and HLA-DRB1*15, the offspring genotypes of the mating type HLA-DRB1*15/17 by HLA-DRB1*XX (where “X” is neither HLA-DRB1*15 nor DRB1*17) were examined. If the two alleles are codominant in their mode of inheritance, there should be an equal proportion of HLA-DRB1*17/X and HLA-DRB1*15/X offspring. In this mating type, there were 77 HLA-DRB1*15/X offspring compared to 26 HLA-DRB1*17/X offspring; \( \chi^2 = 25.25 (p = 5.0 \times 10^{-7}; p_c = 1.4 \times 10^{-5} \). A confirmation of this can be given by the mating HLA-DRB1*15/X by HLA-DRB1*17/X (where “X” is neither HLA-DRB1*15 nor DRB1*17). If HLA-DRB1*15 were dominant to HLA-DRB1*17, the numbers of HLA-DRB1*15/17 offspring and HLA-DRB1*15/X offspring should be equal. In this mating type, there were 74 HLA-DRB1*15/17 and 70 HLA-DRB1*15/X; \( \chi^2 = 0.11 (p = 0.74 \).

Mode of Inheritance of HLA-DRB1*14

The inheritance of the resistance allele, HLA-DRB1*14, was also examined. As with HLA-DRB1*17, the inheritance of HLA-DRB1*14 was examined with and without HLA-DRB1*15-bearing parents. In the presence of HLA-DRB1*15 and DRB1*17, the transmission of HLA-DRB1*14 was, expectedly, significantly under transmitted, OR = 0.31; \( x^2 = 29.8 (p = 4.8 \times 10^{-8}; p_c = 6.2 \times 10^{-7} \). When the over transmission of HLA-DRB1*15 and DRB1*17 was controlled for, the OR of HLA-DRB1*14 was significantly different from 1.0 (Table 3); OR = 0.27; \( p = 0.0096, p_c = 0.27 \). There were no HLA-DRB1*14 homozygous parents, unaffected siblings, or MS cases observed in the entire sample.

To further assess the relative effects of HLA DRB1*14 and DRB1*15, the offspring genotypes from HLA-DRB1*15/X by HLA-DRB1*14/X matings were examined (where “X” is...
neither HLA-DRB1*15 nor DRB1*14). Possible offspring genotypes are HLA-DRB1*15/14, DRB1*15/X, DRB1*14/X, and DRB1*X/X. If HLA-DRB1*15 acts dominantly, numbers of HLA-DRB1*15/14 offspring should equal numbers of HLA-DRB1*15/X offspring. Alternatively, if HLA-DRB1*14 acts dominantly over HLA-DRB1*15, the numbers of HLA-DRB1*15/14 offspring should be less than HLA-DRB1*15/X offspring. A total of 24 HLA-DRB1*15/X offspring and seven HLA-DRB1*15/14 offspring were observed ($p = 0.0017$, $p_c = 0.048$). The OR of HLA-DRB1*15/14 over HLA-DRB1*X/X (OR = 0.5) was not significantly different from expected, $\chi^2 = 1.6$, ($p = 0.21$). However, when the frequency of HLA-DRB1*15/14 was compared between affected ($n = 102/182$) and unaffected ($n = 30/2126$) siblings, the OR = 0.50, $\chi^2 = 10.63$ ($p = 0.0010$, $p_c = 0.028$). Only one affected and one unaffected individual per family were included and the individuals were chosen randomly.

**Mode of Inheritance of HLA-DRB1*11**

HLA-DRB1*11 was demonstrated to be a resistance allele in this cohort. To further evaluate the mode of inheritance of this resistance allele we repeated the previous inheritance analyses with HLA-DRB1*11 (Table 4). In HLA-DRB1*15-negative families, there were 69 HLA-DRB1*11/X offspring and 107 HLA-DRB1*X/X children (where “X” is neither HLA-DRB1*15 nor DRB1*11); OR = 0.64 ($p = 0.0042$, $p_c = 0.12$). HLA-DRB1*11/11 showed increased protection (OR = 0.44); however, this was not statistically significant. When the frequency of HLA-DRB1*11/11 was compared between affected ($n = 42/182$) and unaffected ($n = 13/2126$) siblings, the OR = 0.38, $\chi^2 = 5.02$ ($p = 0.025$, $p_c = 0.7$).

To examine the relative effects of HLA-DRB1*11 and HLA-DRB1*15, matings of HLA-DRB1*11/X by HLA-DRB1*15/X and HLA-DRB1*11/X and HLA-DRB1*15/15 (where “X” is neither HLA-DRB1*15 nor DRB1*11) were assessed. There were 57 HLA-DRB1*11/15 and 98 HLA-DRB1*15/X observed genotypes in the offspring. The number of HLA-DRB1*11/15 children was significantly decreased from the number of HLA-DRB1*15/X offspring; OR = 0.58, $\chi^2 = 10.85$ ($p = 0.00099$, $p_c = 0.028$).

**Mode of Inheritance of HLA-DRB1*08**

To assess the inheritance pattern of HLA-DRB1*08, we counted offspring in HLA-DRB1*08/X by HLA-DRB1*X/X matings and HLA-DRB1*08/X by HLA-DRB1*X/X matings (where “X” is not HLA-DRB1*08; there were no HLA-DRB1*08/08 parents or affected children). There were 92 HLA-DRB1*08/X offspring and 83 HLA-DRB1*X/X offspring; OR = 1.11 ($p = 0.50$). When HLA-DRB1*15 was removed from the sample in the above mating types, there were 56 HLA-DRB1*08/X offspring and 21 HLA-DRB1*X/X children; OR = 1.7, $\chi^2 = 3.95$ ($p = 0.047$; $p_c = 0.99$).

To examine the relative effects of HLA-DRB1*08 and DRB1*15, matings of HLA-DRB1*08/X by HLA-DRB1*15/X and HLA-DRB1*08/X and HLA-DRB1*15/15 (where “X” is neither HLA-DRB1*15 nor DRB1*08) were assessed. There were 40 HLA-DRB1*08/15, five HLA-DRB1*08/X, 23 HLA-DRB1*15/X, and nine HLA-DRB1*X/X observed genotypes in the offspring. The number of HLA-DRB1*08/15 children was significantly increased from the number of HLA-DRB1*15/X offspring; OR = 1.74 ($p = 0.021$; $p_c = 0.44$).

**Mode of Inheritance of HLA-DRB1*10**

HLA-DRB1*10 is a rare allele in this sample (allele frequency = 0.6%) and numbers and mating types were few. However, in a previous investigation, it was found that HLA-DRB1*10 may have acted to modulate risk [10]. In this new dataset, the number of HLA-DRB1*15/10 to HLA-DRB1*15/X offspring genotypes were compared in HLA-DRB1*10/X by HLA-DRB1*15/X matings (where “X” is neither HLA-DRB1*15 nor DRB1*10); the numbers were one and ten, respectively, OR = 0.09 ($p = 0.0059$, $p_c = 0.09$).

In the absence of HLA-DRB1*15 (HLA-DRB1*10/X by HLA-DRB1*X/X matings, where “X” is not HLA-DRB1*10 or DRB1*15), an opposite trend was observed with 12 HLA-DRB1*10/X offspring to seven HLA-DRB1*X/X offspring found; OR = 1.7 ($p = 0.18$).

To further investigate this contrary finding, we repeated the analysis of our previous investigation [10] using the newly

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**Table 2. The Inheritance of HLA-DRB1*17 in the Absence of HLA-DRB1*15**

| Matings       | Offspring Genotype |
|---------------|--------------------|
| 17/17 versus 17/X | 17/X versus X/X    |
| 17/X by X/X    | NA                 | 94 by 60 (OR = 1.6) |
| 17/X by 17/X   | 14 by 7 (OR = 2.0) | 7 by 4 (OR = 1.8)   |
| 17/17 by X/X   | 9 by 3.5 (OR = 2.6)| NA                 |
| Total          | 23 by 10.5 (OR = 2.2)| 101 by 64 (OR = 1.6)|

$\chi^2$ value (p-value) $(p = 0.018$, $p_c = 0.50)$ 8.30 $(p = 0.0040$, $p_c = 0.11)$

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**Table 4. The Mode of Inheritance of HLA-DRB1*11 in the Absence of HLA-DRB1*15**

| Matings       | Offspring Genotype |
|---------------|--------------------|
| 11/11 versus 11/X | 11/X versus X/X    |
| 11/X by X/X    | NA                 | 58 by 100 (OR = 0.58) |
| 11/11 by X/X   | 2 by 4.5 (OR = 0.44)| 4.5 by 7 (OR = 0.64) |
| Total          | 2 by 4.5 (OR = 0.44)| 62.5 by 107 (OR = 0.58)|

$\chi^2$ value (p-value) $(p = 0.34)$ 11.68 $(p = 0.00063$, $p_c = 0.018)$
ascertained families (n = 559) as a replication dataset (Table 5).

In the new sample, the trend was in the same direction as the original finding in the Dyment et al. investigation [10]. When the results were combined, there was a significant difference in transmission of HLA-DRB1*15 based on the presence or absence of HLA-DRB1*15 in the offspring.

Mode of Inheritance of HLA-DRB1*01

In our previous investigation [10], we saw differences in the transmission of HLA-DRB1*01 based on the presence or absence of HLA-DRB1*15. While HLA-DRB1*01 is not protective in the total transmission disequilibrium test (TDT) from non-HLA-DRB1*15-bearing parents (TR = 185, NT = 195, p = 0.61), further evaluation is warranted based on previous observations.

To examine the relative effects of HLA-DRB1*01 and DRB1*15, matings of HLA-DRB1*01/X by HLA-DRB1*15/X and HLA-DRB1*01/X and HLA-DRB1*15/X (where “X” is neither HLA-DRB1*15 nor DRB1*01) were assessed. There were 50 HLA-DRB1*01/15 and 85 HLA-DRB1*15/X observed genotypes in the offspring. The number of HLA-DRB1*01/15 children was significantly decreased from the number of HLA-DRB1*15/X offspring; OR = 0.59, χ² = 9.1 (p = 0.0026, p = 0.036).

Case and Pseudo-Control Analysis

HLA-DRB1 alleles affecting MS risk were assessed by stepwise model selection, starting with a full model containing effects of each allele observed in the sample with frequency of at least ten across cases and pseudo-controls. Four alleles, HLA-DRB1*15, HLA-DRB1*14, HLA-DRB1*17, and HLA-DRB1*11, are significantly associated with disease. The same model was obtained using forward selection from the null model. The overall fit of the model was assessed by means of the Wald χ² statistic of 258.58 on four degrees of freedom, providing strong evidence of association of HLA-DRB1 alleles with MS (p = 9.2 × 10⁻⁵⁵).

Model selection techniques tend to over fit the data, and result in increased false positive error rates for detecting association with disease. To overcome this problem, we performed 1,000 random permutations of case and pseudo-control status within each matched set. In none of the permutations did the selected model fit as well as in the original data, corresponding to an empirical p-value of less than 0.001 for association of HLA-DRB1 alleles with MS.

Table 6 presents the ORs of MS for each associated allele, relative to all non-associated alleles, denoted HLA-DRB1*X for HLA-DRB1*15, which increases risk of MS (OR = 2.46, p = 1.2 × 10⁻⁴⁷). Allele HLA-DRB1*17 also increases risk of MS (OR = 1.28, p = 0.0021). Alleles HLA-DRB1*14 and HLA-DRB1*11 are protective against MS (ORs = 0.41 and 0.77, respectively; p = 4.9 × 10⁻⁵ and p = 0.0080, respectively).

We next investigated deviations from a multiplicative model of disease risk between associated alleles. We generated a full model including a non-multiplicative effect for each associated allele, and interactions between each pair of associated alleles. Application of stepwise model selection, starting with the full model, dropped all interaction terms and non-multiplicative terms, apart from a dominance effect of the HLA-DRB1*15 allele (OR 0.68, p = 0.0078). Associated alleles act in a multiplicative fashion with respect to risk of MS, except for allele HLA-DRB1*13, which acts in an additive fashion with itself.

Interaction of the HLA-DRB1*15 Allele with Non-associated Alleles

Previous studies have shown that non-associated alleles at the HLA-DRB1 locus interact with the HLA-DRB1*15 allele to modulate disease risk [10]. We investigated this by including interaction effects of each non-associated allele with HLA-DRB1*15 in a full association model. Stepwise model selection dropped all interaction terms, with the exception of the effects of the HLA-DRB1*07/15, HLA-DRB1*08/15, and

| Allele   | OR       | 95% Confidence Interval | p-Value |
|----------|----------|-------------------------|---------|
| HLA-DRB1*15 | 2.461    | 2.179-2.780             | 1.2 × 10⁻⁴⁷ |
| HLA-DRB1*14 | 0.414    | 0.271-0.634             | 4.9 × 10⁻⁵  |
| HLA-DRB1*17 | 1.278    | 1.093-1.493             | 0.0021   |
| HLA-DRB1*11 | 0.768    | 0.632-0.934             | 0.0080   |

Table 6. Allelic ORs of MS for Associated Alleles at the HLA-DRB1 Locus, Relative to All Non-associated Alleles
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**Table 7.** Genotypic ORs of MS Made Up of Associated Alleles at the HLA-DRB1 Locus, Relative to HLA-DRB1*X Homozygotes

| Genotype | Number of Cases | Number of Pseudo-Controls | OR   | 95% Confidence Interval | p-Value |
|----------|----------------|---------------------------|------|------------------------|---------|
| X/X     | 351            | 1490                      | Baseline — —                     | —       |
| X/11    | 83             | 437                       | 0.742 | 0.561–0.983            | 0.038   |
| X/14    | 12             | 105                       | 0.418 | 0.199–0.878            | 0.021   |
| X/15    | 584            | 1291                      | 2.919 | 2.424–3.514            | 1.2 × 10\(^{-29}\) |
| X/17    | 183            | 657                       | 1.343 | 1.057–1.705            | 0.016   |
| 11/11   | 4              | 29                        | 0.457 | 0.184–1.133            | 0.091   |
| 11/14   | 4              | 16                        | 0.731 | 0.234–2.286            | 0.39    |
| 11/15   | 78             | 183                       | 2.440 | 1.762–3.379            | 7.9 × 10\(^{-4}\) |
| 11/17   | 27             | 124                       | 0.976 | 0.586–1.625            | 0.93    |
| 14/14   | 0              | 5                         | —    | —                      | —       |
| 14/15   | 10             | 48                        | 1.064 | 0.558–2.028            | 0.85    |
| 14/17   | 3              | 22                        | 0.588 | 0.169–2.045            | 0.40    |
| 15/15   | 160            | 248                       | 5.428 | 4.116–7.159            | 4.5 × 10\(^{-3}\) |
| 15/17   | 151            | 311                       | 3.269 | 2.503–4.270            | 3.3 × 10\(^{-4}\) |
| 17/17   | 34             | 86                        | 2.109 | 1.287–3.454            | 0.0031  |

**Discussion**

The only consistent genetic association with MS in Northern Europeans has been with extended MHC haplotypes, especially those containing HLA-DRB1*1501 [8]. A previously linked region on Chromosome 5p13 [14] has been shown to harbor the interleukin 7 receptor gene (IL7R), an additional MS susceptibility locus [15], but any effect of IL7R is small (OR = 1.3), and when compared to the MHC (genotypic OR = 5.4, p = 5 × 10\(^{-35}\)), its effect size is clearly too small to influence inheritance patterns. The MS MHC association has been used to add to circumstantial evidence supporting an autoimmune reaction against myelin-related antigens presented to T cells in the restricting context of HLA-DRB1*1501. However, the specific susceptibility gene (e.g., HLA-DR or HLA-DQ) or the specific mechanism of class II determined susceptibility has yet to be convincingly elucidated. Recent scans of the MHC region with a large number of markers do show evidence for the involvement of HLA-DRB1 only [16]. However, the paradigm is more complex than one in which the HLA-DRB1*15 allele acts solely to increase MS risk [10,13]. Our previous investigation showed the presence of HLA-DRB1*14 as a resistance allele, HLA-DRB1*17 as a susceptibility allele, and the more compelling observation that alleles are interacting and that it is the genotype that determines MS risk [10]. This expanded Canadian dataset extends these results. We confirm that HLA-DRB1*15- and HLA-DRB1*17-bearing haplotypes increase risk of MS, and HLA-DRB1*14-bearing haplotypes are protective, and show that HLA-DRB1*11-bearing haplotypes are novel and statistically significant resistance haplotypes (Table 6). To our knowledge, this study provides the most comprehensive analysis to date of HLA susceptibility and resistance alleles for multiple sclerosis.

The protective effect of the HLA-DRB1*14 haplotype is shown to be multiplicative and completely abrogates the risk associated with HLA-DRB1*15 (Table 7). HLA-DRB1*14 homozygotes were not observed in our sample (either affected or unaffected relatives) in order to assess a possible increased protection conferred by HLA-DRB1*14 homozygosity, analogous to the increased susceptibility conferred by HLA-DRB1*15 and DRB1*17 homozygosity. In populations with a high frequency of HLA-DRB1*14, e.g., China [17], where the existence of HLA-DRB1*14 homozygotes may be substantial, the prevalence of MS is extremely low. Thus, HLA-DRB1*14 could influence the distribution of MS and suggests more broadly that the HLA-DRB1 genotype, which reflects more complex interactions, may be a major determinant of the heretofore unexplained geography of this disease.

HLA-DRB1*11 haplotypes also show a multiplicative mode of inheritance (Table 7). The protective effect of HLA-DRB1*11 over HLA-DRB1*15 appears to be weaker than that of HLA-DRB1*14, but both reduce the risk associated with HLA-DRB1*17.

We now show that HLA-DRB1*10-bearing haplotypes are similar to HLA-DRB1*01-bearing haplotypes in that they are both significantly under transmitted in the presence of HLA-DRB1*15, showing a clear protective effect in the presence of HLA-DRB1*15 (Table 5). HLA-DRB1*10 and HLA-DRB1*01 were not under transmitted in the presence of HLA-DRB1*17 (unpublished data).

HLA-DRB1*15 heterozygotes have an increased risk of MS with an OR of 2.9 (Tables 1 and 7). The HLA-DRB1*15/15 offspring genotype also increases risk over HLA-DRB1*X/X by 5.4-fold, and is in keeping with an additive mode of inheritance for HLA-DRB1*15, similar to other studies [12,18–21].

When the relative effects of HLA-DRB1*15 versus HLA-DRB1*17 were compared, HLA-DRB1*15 was found to be preferentially transmitted, most notably when transmissions from HLA-DRB1*17/15 heterozygous parents were counted, thereby ruling out a codominant model for these two risk alleles. This is also in keeping with previous observations [10].
Past studies have reported that HLA-DRB1*17 acts in a recessive manner [12,13,21], but the sample size has been smaller than the one in the present study. In this Canadian sample, HLA-DRB1*17 haplotypes act in an additive fashion (Tables 2 and 7).

HLA-DRB1*08 increases risk significantly with HLA-DRB1*15 in trans. Thus, HLA-DRB1*08 shows a synergistic mode of inheritance with HLA-DRB1*15. HLA-DRB1*17 shows no interaction with HLA-DRB1*08 (unpublished data), further highlighting the difference between HLA-DRB1*15 and HLA-DRB1*17 haplotypes.

As yet, no molecular or functional explanation can be given for the dominant-negative effects of HLA-DRB1*14 and DRB1*11, the complementary effects of HLA-DRB1*08, and the protective nature of HLA-DRB1*01 and DRB1*10 in the presence of HLA-DRB1*15. It has been speculated that poor engagement of the encephalitogenic peptide in the context of HLA-DRB1*14 acts to alter the immune response in a dominant-negative manner and thereby reduce the effect of HLA-DRB1*15 [13]. Another explanation is that HLA-DRB1*14 binds one or more peptides that can delete autoreactive T cells. Alternatively, other risk factors present on HLA-DRB1 haplotypes could be interacting in cis and trans to reduce MS risk. HLA-DRB1 allelic association with MS susceptibility and resistance may reflect linkage disequilibrium with the true disease locus/loci. The identity and location of these putative variants are unknown and will be difficult to identify given the high linkage disequilibrium present within the MHC, although it is likely that these variants, if they exist, will be found in the class II region [16,22]. Susceptibility and resistance haplotypes may need revisiting if/when true or additional MS MHC susceptibility loci are uncovered. As mentioned previously [10], HLA-DRB1*14, DRB1*10, and DRB1*01 share the extended HLA-DQ haplotype HLA-DQA1*01-DQB1*05, which may protect against MS. However, HLA-DRB1*11 haplotypes do not share these HLA-DQ alleles. Interestingly, phylogenetic analysis suggests that HLA-DRB1*10 and DRB1*01 share the same allelic lineage (DR1 group) and HLA-DRB1*11, DRB1*14, and DRB1*12 belong to the DR52 group [23]. Variants on the similar HLA-DRB1*10 and DRB1*01 haplotypes could enable them to be protective in the presence of HLA-DRB1*15. HLA-DRB1*14 and DRB1*11 haplotypes may also possess similar polymorphisms that give them a dominant-protective effect.

It has been shown that low immune responsiveness to certain antigens are HLA-linked dominant traits [24]; this was proposed to be caused by an immune suppression gene present on certain HLA haplotypes that controls the generation of antigen-specific suppressor T cells [25]. Recently, HLA-DRB5*0101 has been shown to reduce the number of autoreactive CD4+ T cells in experimental autoimmune encephalomyelitis models [26]. It is conceivable that dominant variants on HLA-DRB1*14 and HLA-DRB1*11 haplotypes can generate T cells that suppress autoreactive T cells, even those generated by HLA-DRB1*15.

This study highlights the need for a large sample size and systematic methods of analysis (e.g., stratified TDT is a more sensitive measure to detect interactions between alleles than regression) in order to unravel the complexity of HLA associations. Further studies of HLA expression, and/or animal models incorporating the susceptibility and resistance genotypes, are warranted to explain these complex interactions. In the meantime, genotyping for susceptibility and resistance alleles may have some practical value in prospective studies of prevention.

Materials and Methods

Participants. All participants in the study were ascertained through the ongoing CCGPSMS, for which the methodology has been previously described [27].

Genotyping. Total genomic DNA, extracted from whole blood as part of the CCGPSMS, was used to type HLA-DRB1 alleles by an allele-specific PCR amplification method [28]. All genotypes were generated blind to pedigree structure and disease status of the individual. Either 24 (low resolution) or 72 (high resolution) PCRs were carried out to amplify allelotypes corresponding to alleles HLA-DRB1*01, HLA-DRB1*04, HLA-DRB1*07, HLA-DRB1*08, HLA-DRB1*09, HLA-DRB1*10, HLA-DRB1*11, HLA-DRB1*12, HLA-DRB1*13, HLA-DRB1*14, HLA-DRB1*15, HLA-DRB1*16, HLA-DRB1*17, and HLA-DRB1*18, as well as products for the DRB3, DRB4, and DRB5 genes. Each HLA-DRB1 genotype was scored twice by independent observers.

Statistical analyses. TDT was performed using the sib_tdt program of the ASPX 2.3 analysis package (available at http://aspx.sourceforge.net/). The TDT counts the number of times an allele is transmitted to affected offspring from heterozygous parents. For TDTs the chi^2 distribution was used to assess significance, except in TDTs where the sum of transmissions and non-transmissions was less than 50, when the exact binomial test was used. For allele inheritance analysis, a highly conservative Bonferroni correction was applied to correct for the number of statistical tests made for each allele, and for the total number of alleles investigated (n = 7). Corrected p-values are shown as p_adj.
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Note Added in Proof
Reference 31 was added at the proofs stage and is cited out of order.