# The Inheritance of Resistance Alleles in Multiple Sclerosis

Sreeram V. Ramagopalan[1,2☯], Andrew P. Morris[1☯], David A. Dyment[1,2☯], Blanca M. Herrera[1,2], Gabriele C. DeLuca[1,2], Matthew R. Lincoln[1,2], Sarah M. Orton[1,2], Michael J. Chao[1,2], A. Dessa Sadovnick[3], George C. Ebers[1,2*]

**1** Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom, **2** Department of Clinical Neurology, University of Oxford, Oxford, United Kingdom, **3** Department of Medical Genetics and Faculty of Medicine, Division of Neurology, University of British Columbia, Vancouver, Canada

**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.**

Citation: Ramagopalan SV, Morris AP, Dyment DA, Herrera BM, DeLuca GC, et al. (2007) The inheritance of resistance alleles in multiple sclerosis. PLoS Genet 3(9): e150. doi:10.1371/journal.pgen.0030150

## 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.

**Editor:** Gonçalo Abecasis, University of Michigan, United States of America

**Received** March 7, 2007; **Accepted** July 20, 2007; **Published** September 7, 2007

A previous version of this article appeared as an Early Online Release on July 20, 2007 (doi:10.1371/journal.pgen.0030150.eor).



**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;

* To whom correspondence should be addressed. E-mail: george.ebers@clneuro.ox.ac.uk

☯ These authors contributed equally to this work.

## Author Summary

Multiple sclerosis (MS) is a complex neurological disease with a strong genetic component. With the possible exception of a weak association at Chromosome 5p, the major histocompatibility complex is the only locus consistently linked to MS. Because of this the major histocompatibility complex has recently undergone renewed attention. A region at or near the gene *HLA-DRB1* influences the risk of MS. *HLA-DRB1* comes in over 400 different forms (or alleles). A common form in Europe, named *1501*, increases risk of MS by 3-fold. In this paper, to our knowledge the largest-ever analysis of this region in MS, we examine the inheritance of newly discovered *HLA-DRB1* MS resistance alleles, namely *HLA-DRB1*14*, *HLA-DRB1*11*, **10*, and *HLA-DRB1*01*. We show that *HLA-DRB1*14* and *HLA-DRB1*11* are dominantly protective; e.g., *HLA-DRB1*14* significantly reduces the risk associated with *HLA-DRB1*15* when they are inherited together. This may explain, in part, why MS is rare in Asia; there, the *HLA-DRB1*14* allele is frequent. *HLA-DRB1*01* and *HLA-DRB1*10* are protective only in the presence of *HLA-DRB1*15*. *HLA-DRB1*14* and *HLA-DRB1*11* haplotypes and *HLA-DRB1*01* and *HLA-DRB1*10* haplotypes share common ancestral origins and this may be why the alleles can be grouped in terms of their protective nature. Discovery of the mechanism of protection against MS may lead to the discovery of new treatments to make a palpable difference in the lives of those who have been affected by this devastating disease.


### 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; $\chi^2 = 233.99$ ($p = 8.0 \times 10^{-53}$). To avoid confounding results due to the over transmission of *HLA-DRB1*15*, transmissions from only 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, $\chi^2 = 13.36$ ($p = 0.00026$). *HLA-DRB1*14* continues to be protective; TR = 23, NT = 66, OR = 0.35, $\chi^2 = 20.78$ ($p = 5.2\times 10^{-6}$) 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; $\chi^2 = 8.47$ ($p = 0.0036$, $p_c = 0.047$). 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/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; $\chi^2 = 20.24$ ($p = 6.8 \times 10^{-6}$).

**Table 1.** The Inheritance of HLA-DRB1*15

| Mating | Offspring Genotype | |
|---|---|---|
| | 15/15 versus 15/X | 15/X versus X/X |
| 15/X by X/X | NA | 557 by 183 (OR = 3.0) |
| 15/X by 15/X | 107 by 56 (OR = 1.9) | 56 by 17 (OR = 3.3) |
| 15/15 by 15/X | 53 by 33 (OR = 1.6) | NA |
| Total | 160 by 89 (OR = 1.8) | 613 by 200 (OR = 3.1) |
| $\chi^2$ value (*p*-value) | 20.24 ($p = 6.8 \times 10^{-6}$; $p_c = 9.6 \times 10^{-5}$) | 209.8 ($p = 1.5 \times 10^{-47}$; $p_c = 2.1 \times 10^{-46}$) |

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

doi:10.1371/journal.pgen.0030150.t001

### 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 assessing 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 *DRB1*15*, the offspring genotypes of the mating type *HLA-DRB1*15/17* by *HLA-DRB1*X/X* (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; $\chi^2 = 25.25$ ($p = 5.0 \times 10^{-7}$, $p_c = 1.4\text{x}10^{-5}$). 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 analysed 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; $\chi^2 = 29.8$, ($p = 4.8 \times 10^{-8}$, $p_c = 6.2 \times 10^{-7}$). When the over transmission of *HLA-DRB1*15* and *DRB1*17* was controlled for, the OR of *HLA-DRB1*14* was still 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

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

| Mating | 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 17/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 ($p = 0.0040$; $p_c = 0.11$) |

doi:10.1371/journal.pgen.0030150.t002

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

| Mating | 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/X by 11/X | 2 by 4.5 (OR = 0.44) | 4.5 by 7 (OR = 0.64) |
| 11/11 by 11/X | NA | NA |
| 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 ($p = 0.00063$; $p_c = 0.018$) |

doi:10.1371/journal.pgen.0030150.t004

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 ($n = 10/2182$) and unaffected ($n = 30/2126$) siblings, the OR = 0.30, $\chi^2 = 10.63$ ($p = 0.0010$, $p_c = 0.028$). 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 ($n = 4/2,182$) and unaffected ($n = 13/2,126$) 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 *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, $\chi^2 = 10.85$ ($p = 0.00099$, $p_c = 0.028$).

**Table 3.** The Inheritance of *HLA-DRB1*14* in the Absence of *HLA-DRB1*15* and *DRB1*17*

| Mating | Offspring Genotype | |
|---|---|---|
| | 14/14 versus 14/X | 14/X versus X/X |
| 14/X by X/X | NA | 3 by 12 |
| 14/X by 14/X | 0 by 0 | 1 by 3 |
| 14/14 by 14/X | 0 by 0 | NA |
| Total | 0 by 0 | 4 by 15 (OR = 0.27) |
| $\chi^2$ value (*p*-value) | — | ($p = 0.0096$; $p_c = 0.27$) |

doi:10.1371/journal.pgen.0030150.t003

## 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*08/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 36 *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

**Table 5.** Transmission of *HLA-DRB1*10* from non-*HLA-DRB1*15*-Bearing Parents to Offspring Stratified by the Presence or Absence of *HLA-DRB1*15*

| Study | *HLA-DRB1*15*-Positive Children | | *HLA-DRB1*15*-Negative Children | | *p*-Value |
|---|---|---|---|---|---|
| | TR | NT | TR | NT | |
| Dyment et al. [10] | 1 | 6 | 9 | 4 | 0.019 |
| New samples | 0 | 4 | 5 | 4 | 0.057 |
| Combined | 1 | 10 | 14 | 8 | 0.0030 |

doi:10.1371/journal.pgen.0030150.t005

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*10* from non-*HLA-DRB1*15* parents 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*15/15* (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, $\chi^2 = 9.1$ ($p = 0.0026$, $p_c = 0.036$).

## 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 $\chi^2$ statistic of 258.58 on four degrees of freedom, providing strong evidence of association of *HLA-DRB1* alleles with MS ($p = 9.2 \times 10^{-55}$).

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.** Allelic ORs of MS for Associated Alleles at the HLA-DRB1 Locus, Relative to All Non-associated Alleles

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

doi:10.1371/journal.pgen.0030150.t006

Table 6 presents the ORs of MS for each associated allele, relative to all non-associated alleles, denoted *HLA-DRB1*X* forthwith. The strongest effect is observed for allele *HLA-DRB1*15,* which increases risk of MS (OR = 2.46, $p = 1.2 \times 10^{-47}$). 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; $p = 4.9 \times 10^{-5}$ 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*15,* which acts in an additive fashion with itself.

Table 7 presents ORs of MS for each *HLA-DRB1* genotype, relative to *HLA-DRB1*X* homozygotes. As described above, the risk of associated alleles acts in a multiplicative fashion so that, e.g., an individual carrying one copy of the high-risk *HLA-DRB1*15* allele and one copy of the strongly protective *HLA-DRB1*14* allele has the same risk of MS as a *HLA-DRB1*X/X* individual (OR = 1.06, $p = 0.85$). In other words, the effect of the *HLA-DRB1*15* allele is cancelled out by the *HLA-DRB1*14* allele. The *HLA-DRB1*11* allele also provides some protection against the *HLA-DRB1*15* allele, since the risk of the *HLA-DRB1*11/15* genotype is lower than that of the *HLA-DRB1*15/X* genotype (OR = 2.44 and 2.92, respectively). *HLA-DRB1*11* provides protection against the high-risk *HLA-DRB1*17* allele (OR of the *HLA-DRB1*11/17* genotype = 0.98, $p = 0.93$). The *HLA-DRB1*15* allele acts in a nonmultiplicative fashion with itself. Individuals homozygous for the *HLA-DRB1*15* allele are at less risk of MS compared to the *HLA-DRB1*15/X* genotype than would be expected under a multiplicative disease model (OR = 5.43 and 2.92, respectively).

## 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

**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 | $1.2 \times 10^{-29}$ |
| 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 | 0.59 |
| 11/15 | 78 | 183 | 2.440 | 1.762–3.379 | $7.9 \times 10^{-8}$ |
| 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 | $4.5 \times 10^{-33}$ |
| 15/17 | 151 | 311 | 3.269 | 2.503–4.270 | $3.3 \times 10^{-18}$ |
| 17/17 | 34 | 86 | 2.109 | 1.287–3.454 | 0.0031 |

doi:10.1371/journal.pgen.0030150.t007

*HLA-DRB1*10/15* genotypes. The *HLA-DRB1*07/15* and *HLA-DRB1*08/15* genotypes demonstrate increased risk of MS compared to the *HLA-DRB1*X/15* genotype (OR = 1.28 and 1.65, respectively; $p = 0.038$ and $p = 0.016$, respectively). On the other hand, the HLA-*DRB1*10/15* appears protective compared to the *HLA-DRB1*X/15* genotype (OR = 0.12, $p = 0.044$).

## 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, $p = 5 \times 10^{-33}$), 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 CCPGSMS, for which the methodology has been previously described [27].

**Genotyping.** Total genomic DNA, extracted from whole blood as part of the CCPGSMS, 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 ASPEX 2.3 analysis package (available at http://aspex.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 $p_c$.

Association of HLA-DRB1 alleles with MS was assessed by conditional logistic regression with each MS case matched to pseudo-controls, formed from the possible offspring that could have occurred from the parental mating [29]. The genotype of each individual was coded in terms of an indicator variable (0, 1, or 2) for each allele. Association of HLA-DRB1 alleles was then assessed by stepwise model selection, with allelic effects added or removed at each stage if $p < 0.05$. Dominance effects of each allele were coded by additional indicator variables, taking the value 1 if an individual is homozygous for the allele and 0 otherwise. Interaction effects between alleles were generated by introducing further indicator variables that are products of the corresponding allelic effects. To allow for correlations between multiple affected offspring from the same family, cases and matched pseudo-controls were clustered by pedigree and robust Huber-White estimators used to adjust the standard errors of ORs [30].

## Acknowledgments

The authors would like to thank all persons who generously participated in this study, physicians participating in the CCPGSMS, Neil Risch, Katie Morrison, Holly Armstrong, Jordana Tzenova-Bell, and William Valdar. Experiments performed for this investigation comply with current guidelines and ethics. The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and final responsibility for the decision to submit for publication.

**Author contributions.** GCE conceived and designed the experiments. SVR, DAD, BMH, GCD, MRL, SMO, and MJC performed the experiments. SVR, APM, and DAD analyzed the data. SVR and DAD wrote the paper.

**Funding.** This work was funded by the Multiple Sclerosis Society of Canada Scientific Research Foundation. SVR is funded by the Medical Research Council of the United Kingdom. ADS is a Michael Smith Foundation for Health Research Distinguished Scholar.

**Competing interests.** The authors have declared that no competing interests exist.

**References**

1. Sadovnick AD, Baird PA, Ward RH (1988) Multiple sclerosis: Updated risks for relatives. Am J Med Genet 29: 533–541.
2. Ebers GC, Sadovnick AD, Risch NJ (1995) A genetic basis for familial aggregation in multiple sclerosis. Canadian Collaborative Study Group. Nature 377: 150–151.
3. Willer CJ, Dyment DA, Risch NJ, Sadovnick AD, Ebers GC (2003) Twin concordance and sibling recurrence rates in multiple sclerosis. Proc Natl Acad Sci U S A 100: 12877–12882.
4. Dyment DA, Sadovnick AD, Willer CJ, Armstrong H, Cader ZM, et al. (2004) An extended genome scan in 442 Canadian multiple sclerosis-affected sibships: A report from the Canadian Collaborative Study Group. Hum Mol Genet 13: 1005–1015.
5. Jersild C, Svejgaard A, Fog T (1972) HL-A antigens and multiple sclerosis. Lancet 1: 1240–1241.
6. Naito S, Namerow N, Mickey MR, Terasaki PI (1972) Multiple sclerosis: Association with HL–A3. Tissue Antigens 2: 1–4.
7. Winchester R, Ebers G, Fu SM, Espinosa L, Zabriskie J, et al. (1975) B-cell alloantigen Ag 7a in multiple sclerosis. Lancet 2: 814.
8. Fogdell A, Hillert J, Sachs C, Olerup O (1995) The multiple sclerosis- and narcolepsy-associated HLA class II haplotype includes the DRB5*0101 allele. Tissue Antigens 46: 333–336.
9. Dyment DA, Ebers GC, Sadovnick AD (2004) Genetics of multiple sclerosis. Lancet Neurology 3: 104–110.
10. Dyment DA, Herrera BM, Cader MZ, Willer CJ, Lincoln MR, et al. (2005) Complex interactions among MHC haplotypes in multiple sclerosis: Susceptibility and resistance. Hum Mol Genet 14: 2019–2026.
11. Marrosu MG, Murru MR, Costa G, Cucca F, Sotgiu S, et al. (1997) Multiple sclerosis in Sardinia is associated and in linkage disequilibrium with HLA-DR3 and -DR4 alleles. American Journal of Human Genetics 61: 454–457.
12. Modin H, Olsson W, Hillert J, Masterman T (2004) Modes of action of HLA-DR susceptibility specificities in multiple sclerosis. Am J Hum Genet 74: 1321–1322.
13. Barcellos LF, Sawcer S, Ramsay PP, Baranzini SE, Thomson G, et al. (2006) Heterogeneity at the HLA-DRB1 locus and risk for multiple sclerosis. Hum Mol Genet 15: 2813–2824.
14. Ebers GC, Kukay K, Bulman DE, Sadovnick AD, Rice G, et al. (1996) A full genome search in multiple sclerosis. Nat Genet 13: 472–476.
15. Lundmark F, Duvefelt K, Iacobaeus E, Kockum I, Wallstrom E, et al. (2007) Variation in interleukin 7 receptor alpha chain (IL7R) influences risk of multiple sclerosis. Nat Genet. E-pub 29 July 2007.
16. Lincoln MR, Montpetit A, Cader MZ, Saarela J, Dyment DA, et al. (2005) A predominant role for the HLA class II region in the association of the MHC region with multiple sclerosis. Nat Genet 37: 1108–1112.
17. Middleton D, Menchaca L, Rood H, Komerofsky R (2003) New allele frequency database: http://www.allelefrequencies.net. Tissue Antigens 61: 403–407.
18. Barcellos LF, Oksenberg JR, Begovich AB, Martin ER, Schmidt S, et al. (2003) HLA-DR2 dose effect on susceptibility to multiple sclerosis and influence on disease course. American Journal of Human Genetics 72: 710–716.
19. Boon M, Nolte IM, De Keyser J, Buys CH, te Meerman GJ (2004) Inheritance mode of multiple sclerosis: The effect of HLA class II alleles is stronger than additive. Hum Genet 115: 280–284.
20. Rasmussen HB, Kelly MA, Clausen J (2001) Additive effect of the HLA-DR15 haplotype on susceptibility to multiple sclerosis. Mult Scler 7: 91–93.
21. Ebers GC, Paty DW (1980) Studies in familial multiple sclerosis. Trans Am Neurol Assoc 105: 344–347.
22. Chao MJ, Barnardo MC, Bu GZ, Lincoln MR, Ramagopalan SV, et al. (2007) Transmission of class I/II multi-locus MHC haplotypes and multiple sclerosis susceptibility: accounting for linkage disequilibrium. Hum Mol Genet.
23. Andersson G (1998) Evolution of the human HLA-DR region. Front Biosci 3: D739–D745.
24. Sasazuki T, Kaneoka H, Nishimura Y, Kaneoka R, Hayama M, et al. (1980) An HLA-linked immune suppression gene in man. J Exp Med 152: 297S–313S.
25. Nishimura Y, Sasazuki T (1983) Suppressor T cells control the HLA-linked low responsiveness to streptococcal antigen in man. Nature 302: 67–69.
26. Gregersen JW, Kranc KR, Ke X, Svendsen P, Madsen LS, et al. (2006) Functional epistasis on a common MHC haplotype associated with multiple sclerosis. Nature.
27. Sadovnick AD, Risch NJ, Ebers GC (1998) Canadian collaborative project on genetic susceptibility to MS, phase 2: Rationale and method. Canadian Collaborative Study Group. Can J Neurol Sci 25: 216–221.
28. Olerup O, Zetterquist H (1992) HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: An alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. Tissue Antigens 39: 225–235.
29. Cordell HJ, Clayton DG (2002) A unified stepwise regression procedure for evaluating the relative effects of polymorphisms within a gene using case/control or family data: Application to HLA in type 1 diabetes. Am J Hum Genet 70: 124–141.
30. Cordell HJ (2004) Properties of case/pseudocontrol analysis for genetic association studies: Effects of recombination, ascertainment, and multiple affected offspring. Genet Epidemiol 26: 186–205.
31. The International Multiple Sclerosis Genetics Consortium (2007) Risk alleles for multiple sclerosis identified by a genomewide study. N Engl J Med. E-pub ahead of print. 29 July 2007.

**Note Added in Proof**

Reference 31 was added at the proofs stage and is cited out of order.