HLA class I alleles tag *HLA-DRB1*1501* haplotypes for differential risk in multiple sclerosis susceptibility

Michael J. Chao[†‡], Martin C. N. M. Barnardo[§], Matthew R. Lincoln[†‡], Sreeram V. Ramagopalan[†‡], Blanca M. Herrera[†‡], David A. Dyment[†‡], Alexandre Montpetit[¶], A. Dessa Sadovnick[‖], Julian C. Knight[‡], and George C. Ebers[†‡††]

[†]Department of Clinical Neurology, John Radcliffe Hospital, University of Oxford, Oxford OX3 9DU, United Kingdom; [‡]Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford OX3 7BN, United Kingdom; [§]Oxford Transplant Centre, Nuffield Department of Surgery, Churchill Hospital, Oxford OX3 7LJ, United Kingdom; [¶]McGill University and Genome Quebec Innovation Centre, Montreal, QC, Canada H3A 1A4; and [‖]Department of Medical Genetics and Faculty of Medicine, Division of Neurology, University of British Columbia, Vancouver, BC, Canada V6T 1Z4

Edited by Hugh O. McDevitt, Stanford University School of Medicine, Stanford, CA, and approved July 1, 2008 (received for review February 1, 2008)

The major locus for multiple sclerosis (MS) susceptibility is located within the class II region of the Major Histocompatibility Complex (MHC). *HLA-DRB1* alleles, constituting the strongest MS susceptibility factors, have been widely exploited in research including construction of transgenic animal models of MS. Many studies have concluded that *HLA-DRB1*15* allele itself determines MS-associated susceptibility. If this were true, haplotypes bearing this allele would confer equal risk. If *HLA-DRB1*15* bearing haplotypes differed for risk, roles for other loci in this region would be implied and further study of the fine structure of this locus would be compelling. We have tested the hypothesis comparing haplotypes stratified by HLA class I tagging. We show here that *HLA-DRB1*15*-bearing-haplotypes in 1970 individuals from 494 MS families are indeed heterogeneous. Some *HLA-DRB1*15* haplotypes determine susceptibility while others do not. Three groups of class I tagged *HLA-DRB1*15* haplotypes were *not* over-transmitted: (*i*) *HLA-DRB1*15-HLA-B*08* (TR = 25, NT = 23, Odds Ratio = 1.09), (*ii*) *-HLA-B*27* (TR = 18, NT = 17, Odds Ratio = 1.06), and (*iii*) rare *HLA-DRB1*15* haplotypes (frequency <0.02). Rare haplotypes were significantly different from common haplotypes, and transmissions were remarkably similar to those for class-I-matched non-*HLA-DRB1*15* haplotypes. These results unambiguously indicate that *HLA-DRB1*15* is part of a susceptibility haplotype but cannot be the susceptibility allele itself, requiring either epistatic interactions, epigenetic modifications on some haplotypes, or nearby structural variation. These findings strongly imply that differences among *HLA-DRB1*15* haplotypes will furnish the basis for MHC-associated susceptibility in MS and raise the possibility that the MHC haplotype is the fundamental unit of genetic control of immune response.

MHC | class II | transmission

The origins of multiple sclerosis (MS) susceptibility, as with many complex genetic diseases, remain uncertain. It is widely believed that MS is a $CD4^+$ Th1-mediated autoimmune disease (1, 2). This is supported by many studies based on experimental autoimmune encephalomyelitis (EAE) models (3, 4), and by genetic studies showing HLA class II genes represent the strongest risk for MS (5–9). It is often assumed that the MS DR-association is secondary to a role in antigen-presentation to $CD4^+$ T cells responding to a myelin protein (2).

The evidence that familial risk in MS is determined by genes is overwhelming, although broadly acting and as yet unidentified environmental factors are undeniable in accounting for the disease geography (10). Associations between MS and specific HLA alleles are clear, but MS does not display the classical Mendelian inheritance patterns attributable to a single locus (5, 11). Genome scans have shown few consensus linkages (5, 12–15) or associations (16) additional to HLA genes. The highest non-Major Histocompatibility Complex (MHC) lod score previously reported was for markers in chromosome 5p in the initial Canadian cohort (5), which was also positive in a Scandinavian study (17). Zhang and colleagues (2005) found association with the 5p candidate interleukin 7 (IL-7) receptor (15), subsequently confirmed with supporting functional data (18, 19). A whole genome association study has shown few significant effects outside what had been previously highlighted by linkage, and the effect sizes are very small at a population level (16), but larger effects may pertain to individual families. Methods used to estimate the contribution from the MHC may be inappropriate since the assumptions made are not sustainable in MS (20). It has been suggested that some 50% of susceptibility remains to be found (21).

These efforts highlight the MHC as the main susceptibility locus for MS. *HLA-DRB1* allelic subtypes have been widely explored, including the construction of transgenic animal models of MS transgenic for *HLA-DRB1*15*. Complexity in this region clearly indicates an epistatic hierarchy of resistance and susceptibility alleles at *HLA-DRB1*, which interact in *cis* and *trans* to influence overall risk (8). Dense single nucleotide polymorphism (SNP) typing localized association to the immediate region of HLA class II (9). Reports from several case-control studies suggesting an independent association with the HLA class I region (22–24) appear to be secondary to linkage disequilibrium (LD) in family-based material (25). The problems of correcting for LD and for concomitant epistatic interactions, which may be haplotype-specific, are formidable for case-control studies.

Complex *HLA-DRB1* interactions have been demonstrated, and specific susceptibility and resistance alleles and interactions among them in *trans* have been identified (8). The roles of these interactions have been extended in a recent study by evaluating *HLA-DRB1*, *HLA-A*, and *HLA-B* haplotypes requiring the construction of 2-locus haplotypes (25). *HLA-DRB1*15* haplotypes were equally over-transmitted regardless of what common allele was present at *HLA-A* and *-B* in *cis* or in *trans*, illustrating no detectable independent effect of class I on susceptibility. These findings did not rule out a class II-dependent functional interaction for class I indeed the long-range LD surrounding HLA class I and II seen in this study as has characterized many other systems has remained unexplained (25).

Analogies with murine immunogenetic studies imply that *HLA-DRB1* loci themselves are involved directly in autoimmune susceptibility but the data remain circumstantial in the case of MS. Importantly, the probability of haplotype sharing of non-*HLA-DRB1*15* alleles between affected sibling pairs unexpectedly does not differ (26). Based on findings from pooled low

Author contributions: M.J.C. and G.C.E. designed research; M.J.C. performed research; M.J.C., M.C.N.M.B., M.R.L., S.V.R., B.M.H., D.A.D., A.M., A.D.S., J.C.K., and G.C.E. contributed new reagents/analytic tools; M.J.C. and G.C.E. analyzed data; and M.J.C. and G.C.E. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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

Table 1. Two-locus haplotype transmissions of *HLA-DRB1*15–HLA-A* and *HLA-DRB1*X–HLA-A* from *HLA-DRB1*15* positive ($n = 536$) and negative parents ($n = 452$)

*HLA-DRB1*15* Positive Parents ($n = 536$)						*HLA-DRB1*15* Negative Parents ($n = 452$)						Comparison	
Haplotypes	TR	NT	*OR*	χ^2	*P*	Haplotypes	TR	NT	*OR*	χ_2	*P*	χ_2	*P*
15-1	51	26	1.96	8.27	0.0040	*X-1*	119	113	1.05	0.16	0.69	5.21	0.022
15-2	136	55	2.47	34.81	3.64×10^{-9}	*X-2*	145	157	0.92	0.48	0.49	25.67	1.051×10^{-7}
15-3	140	59	2.37	33.95	5.67×10^{-9}	*X-3*	93	73	1.27	2.41	0.12	8.048	0.0046
15-11	41	24	1.71	4.50	0.034	*X-11*	50	42	1.19	0.70	0.40	1.19	0.28
15-23	4	1	4.00	1.93	0.17	*X-23*	13	12	1.08	0.04	0.84	—†	0.22
15-24	92	22	4.18	46.20	1.07×10^{-11}	*X-24*	51	63	0.81	1.26	0.26	31.53	1.96×10^{-8}
15-25	20	5	4.00	9.64	0.0019	*X-25*	13	7	1.86	1.80	0.18	—†	0.14
15-26	11	9	1.22	0.20	0.65	*X-26*	12	27	0.44	5.77	0.016	—†	0.046
15-29	4	16	0.25	7.71	0.0055	*X-29*	14	12	1.17	0.15	0.69	5.44	0.020
15-30	6	20	0.30	7.95	0.0048	*X-30*	9	15	0.60	1.50	0.22	1.24	0.27
15-31	2	4	0.50	0.68	0.41	*X-31*	17	12	1.42	0.86	0.35	—†	0.19
15-32	10	4	2.50	2.66	0.10	*X-32*	12	15	0.80	0.33	0.56	—†	0.071
15-33	2	10	0.20	5.82	0.016	*X-33*	5	11	0.45	2.25	0.13	0.78	0.38
15-68	10	5	2.00	1.70	0.19	*X-68*	24	37	0.65	2.77	0.10	—†	0.039
TOTAL	529	260	2.035	91.71	1.00×10^{-21}	TOTAL	577	596	0.97	0.31	0.58	61.16	5.26×10^{-15}

*HLA-DRB1*X* refers to any allele other than *HLA-DRB1*15*.

†Fisher's exact test was used when the expected transmissions in any of the cells of the table were below 10. Uninformative MS families were excluded in this analysis, which may contain parents homozygous for both class I/II, and/or HLA identical parents for both class I/II, and/or with missing class I/II typing.

frequency haplotypes (25), we hypothesized that constructing HLA class I and II haplotypes might differentiate among *HLA-DRB1*15* alleles, imply MS susceptibility is not solely *HLA-DRB1*15* based and provide a tool for identifying the distinguishing features. Here we show that HLA class I alleles present in *cis* allow construction of haplotypes, which demonstrate marked risk heterogeneity, imply the *HLA-DRB1* alleles do not mediate risk themselves and highlight the potential for regulatory or epigenetic effects in this region.

Results

Two-Locus Haplotype Transmissions of *HLA-DRB1*15-HLA-A* and *HLA-DRB1*X-HLA-A*. Overall, *HLA-DRB1*15-HLA-A* haplotypes were 529 times transmitted and 260 times not transmitted ($P = 1.00 \times 10^{-21}$; Table 1). Most *HLA-DRB1*15-HLA-A* haplotypes, but not all, appeared to be over-transmitted from parents to affected offspring. Haplotypes that were significantly over-transmitted included *HLA-DRB1*15-HLA-A*01* ($P = 0.0040$), *-A*02* ($P = 3.64 \times 10^{-09}$), *-A*03* ($P = 5.67 \times 10^{-09}$), *-A*11* ($P = 0.034$), *-A*24* ($P = 1.07 \times 10^{-11}$), and *-A*25* ($P = 0.0019$) haplotypes (Table 1). Some haplotypes were especially over-transmitted (for example: *HLA-DRB1*15-HLA-A*02*, *-A*03*, *-A*24*), but even the large size of this sample does not allow for definitive comparison among over-transmitted haplotypes given the number of haplotypes and potential comparisons.

Unexpectedly, some haplotypes such as *HLA-DRB1*15-HLA-A*26* ($P = 0.65$) were neutral-transmitted, whereas other haplotypes were significantly under-transmitted including *HLA-DRB1*15-HLA-A*29* ($P = 0.0055$), *-A*30* ($P = 0.0048$), and *-A*33* ($P = 0.016$) haplotypes (Table 1). Increased transmission of *HLA-DRB1*15*-associated *HLA-A* alleles was not present when parents lacked *HLA-DRB1*15*, and no significant transmission distortions were observed for the totalled *HLA-DRB1*X-HLA-A* haplotypes (where X is any *HLA-DRB1* allele other than *HLA-DRB1*15*) ($P = 0.58$) except for *HLA-DRB1*X-HLA-A*26* ($P = 0.016$), which was significantly under-transmitted (Table 1).

Significant differences were found between most of the commonly over-transmitted *HLA-DRB1*15-HLA-A* haplotypes and their paired corresponding *HLA-DRB1*X-HLA-A* haplotypes. Conversely, neutral-transmitted and under-transmitted *HLA-DRB1*15-HLA-A* haplotypes had similar transmission patterns as compared with their paired *HLA-DRB1*X-HLA-A* haplotypes (Table 1).

Two-Locus Haplotype Transmissions of *HLA-DRB1*15-HLA-B* and *HLA-DRB1*X-HLA-B*. Mirroring the transmissions of *HLA-DRB1*15-HLA-A* haplotypes, most *HLA-DRB1*15-HLA-B* haplotypes also appeared to be over-transmitted including *HLA-DRB1*15-HLA-B*07* ($P = 3.19 \times 10^{-17}$), *-B*18* ($P = 5.20 \times 10^{-05}$), *-B*37* ($P = 0.041$), *-B*40* ($P = 0.0030$), *-B*44* ($P = 0.0059$), *-B*49* ($P = 0.039$), *-B*51* ($P = 0.00021$), and *-B*57* ($P = 0.00018$) haplotypes (Table 2).

Haplotypes such as *HLA-DRB1*15-HLA-B*08* ($P = 0.77$) and *-B*27* ($P = 0.87$) were neutral-transmitted, whereas other haplotypes including *HLA-DRB1*15-HLA-B*13* ($P = 0.10$), *-B*38* ($P = 0.15$), and *-B*52* ($P = 0.047$) seemed to be under-transmitted (Table 2). The totalled transmission of *HLA-DRB1*15-HLA-B* haplotypes was 538 times transmitted and 260 times not transmitted ($P = 7.49 \times 10^{-23}$; Table 2).

*HLA-DRB1*X-HLA-B* haplotypes were not over-transmitted from *HLA-DRB1*15* negative parents, and there were no significant transmission distortions observed for the totalled *HLA-DRB1*X-HLA-B* haplotypes ($P = 0.35$) except for *HLA-DRB1*X-HLA-B*39* ($P = 0.0090$) and *-B*49* ($P = 0.022$) haplotypes, which were significantly under-transmitted (Table 2).

Significant differences were found between the common *HLA-DRB1*15-HLA-B* haplotypes and their paired *HLA-DRB1*X-HLA-B* haplotypes. On the other hand, as with *HLA-A* haplotypes, the rare *HLA-DRB1*15* bearing haplotypes had similar transmission patterns as compared with their corresponding *HLA-DRB1*X-HLA-B* haplotypes (Table 2).

Two-Locus Haplotype Transmissions of HLA-DRB1*15-HLA-A/*B* and *HLA-DRB1*X-HLA-A/B*: Categorized by Their HLA Class I Broad Serological Equivalents. In the *HLA-A* broad serological equivalent analyses, *HLA-A9* (with closely related *HLA-A*23*, and *-A*24* allele groups; $P = 2.20 \times 10^{-11}$), and *-A10* (with closely related *HLA-A*25*, and *-A*26* allele groups; $P = 0.011$) carrying *HLA-DRB1*15* were significantly over-transmitted, whereas *HLA-A19* haplotypes carrying *HLA-DRB1*15* (with closely related *HLA-A*29*, *-A*30*, *-A*31*, *-A*32*, and *-A*33* allele groups; $P = 0.00068$) were significantly under-transmitted. No significant transmission

Table 2. Two-locus haplotype transmissions of *HLA-DRB1*15*–*HLA-B* and *HLA-DRB1*X*–*HLA-B* from *HLA-DRB1*15* positive (n = 536) and negative parents (n = 452)

*HLA-DRB1*15* Positive Parents (n = 536)						*HLA-DRB1*15* Negative Parents (n = 452)						Comparison	
Haplotypes	TR	NT	*OR*	χ^2	*P*	Haplotypes	TR	NT	*OR*	χ^2	*P*	χ^2	*P*
15-7	263	104	2.53	71.22	3.19×10^{-17}	*X-7*	60	58	1.03	0.03	0.85	17.39	3.044×10^{-5}
15-8	25	23	1.09	0.08	0.77	*X-8*	68	61	1.11	0.38	0.54	0.0056	0.94
15-13	4	10	0.40	2.66	0.10	*X-13*	11	16	0.69	0.93	0.34	0.59	0.44
15-14	6	4	1.50	0.40	0.53	*X-14*	14	12	1.17	0.15	0.69	—†	0.28
15-15	6	2	3.00	2.09	0.15	*X-15*	29	26	1.12	0.16	0.69	—†	0.16
15-18	31	7	4.43	16.37	5.20×10^{-5}	*X-18*	31	21	1.48	1.92	0.17	—†	0.016
15-27	18	17	1.06	0.03	0.87	*X-27*	33	23	1.43	1.79	0.18	0.49	0.48
15-35	19	11	1.73	2.16	0.14	*X-35*	67	82	0.82	1.51	0.22	3.37	0.066
15-37	12	4	3.00	4.19	0.041	*X-37*	11	15	0.73	0.62	0.43	—†	0.031
15-38	2	6	0.33	2.09	0.15	*X-38*	3	10	0.30	3.77	0.052	—†	0.39
15-39	3	0	0.00	4.16	0.041	*X-39*	9	24	0.38	6.82	0.0090	—†	0.031
15-40	21	6	3.50	8.83	0.0030	*X-40*	14	17	0.82	0.29	0.59	—†	0.0090
15-41	2	4	0.50	0.68	0.41	*X-41*	5	4	1.25	0.11	0.74	—†	0.29
15-44	47	24	1.96	7.59	0.0059	*X-44*	71	66	1.08	0.18	0.67	3.94	0.047
15-49	20	9	2.22	4.28	0.039	*X-49*	6	17	0.35	5.26	0.022	—†	0.0020
15-51	22	4	5.50	13.72	0.00021	*X-51*	26	26	1.00	0.00	1.00	—†	0.0022
15-52	1	6	0.17	3.96	0.047	*X-52*	1	1	1.00	0.00	1.00	—†	0.39
15-55	3	0	0.00	4.16	0.041	*X-55*	2	8	0.25	3.60	0.058	—†	0.035
15-56	1	4	0.25	1.93	0.17	*X-56*	2	0	0.00	2.00	0.16	—†	0.14
15-57	20	3	6.67	14.07	0.00018	*X-57*	19	15	1.27	0.47	0.49	—†	0.011
15-58	6	4	1.50	0.40	0.53	*X-58*	3	7	0.43	1.60	0.21	—†	0.15
15-60	1	4	0.25	1.93	0.17	*X-60*	16	18	0.89	0.12	0.73	—†	0.22
15-62	3	0	0.00	4.16	0.041	*X-62*	27	24	1.13	0.18	0.67	—†	0.16
15-64	2	4	0.50	0.68	0.41	*X-64*	14	22	0.64	1.78	0.18	—†	0.34
TOTAL	538	260	2.069	96.85	7.49×10^{-23}	TOTAL	542	573	0.95	0.86	0.35	55.46	9.54×10^{-14}

*HLA-DRB1*X* refers to any allele other than *HLA-DRB1*15*.

†Fisher's exact test was used when the expected transmissions in any of the cells of the table were below 10. Uninformative MS families were excluded in this analysis, which may contain parents homozygous for both class I/II, and/or HLA identical parents for both class I/II, and/or with missing class I/II typing.

distortions of non-*HLA-DRB1*15* haplotypes were observed (Table 3).

In the *HLA-B* broad serological equivalent analyses, *HLA-B15* (with closely related *HLA-B*51*, and *-B*52* allele groups; P = 0.024) and *-B17* (with closely related *HLA-B*57*, and *-B*58* allele groups; P = 0.00094) were significantly over-transmitted in the presence of *HLA-DRB1*15*, whereas *HLA-B16* (with closely related *HLA-B*38*, and *-B*39* allele groups; P = 0.76) and *-B22* (with closely related *HLA-B*55*, and *-B*56* allele groups; P = 1.00) were neutral-transmitted with *HLA-DRB1*15*. No significant transmission distortions of non-*HLA-DRB1*15* haplotypes were observed, except for *HLA-DRB1*X-HLA-B16* (with closely related *HLA-B*38*, and *-B*39* allele groups; P = 0.0012), which was significantly under-transmitted (Table 3).

Transmission of Common and Rare *HLA-DRB1*15* Haplotypes. Two-locus (*HLA-DRB1*15-HLA-A* and *HLA-DRB1*15-HLA-B*) haplotypes were separated into 2 groups: (*i*) common, and (*ii*) rare, based on their haplotype frequency (greater or less than 0.02). The common *HLA-DRB1*15-HLA-A* and *HLA-DRB1*15-HLA-B* haplotypes were significantly different as compared with their corresponding rare *HLA-DRB1*15* bearing haplotypes ($P = 1.60 \times 10^{-10}$, $P = 1.12 \times 10^{-08}$, respectively). The common *HLA-DRB1*15* positive haplotypes were found to be significantly over-transmitted, whereas the rare *HLA-DRB1*15* positive haplotypes were mostly neutral-transmitted or under-transmitted (Table 4).

Discussion

A strong association between the *HLA-DRB1*15* allele and MS has been shown in studies of northern Europeans and their descendants. MS susceptibility has been variably attributed to alleles at the *HLA-DR* (27, 28) and *HLA-DQ* loci (29), but epistasis (gene-gene interactions) in this region is demonstrably strong (8, 29). Unexpectedly, sharing of haplotypes between affected sibling pairs from non-*HLA-DRB1*15* bearing families is not less than in *HLA-DRB1*15* bearing families (26). This finding suggested heterogeneity among *HLA-DRB1*15* haplotypes. The concept that risk is dependent on factors peculiar to some but not all haplotypes thereby directs attention away from the structural elements of *HLA-DRB1* gene itself.

This study has incorporated family data with haplotype transmission disequilibrium test (TDT), which simultaneously assesses linkage and association. Haplotypes produce more definitive transmissions than do the alleles encompassing them, and this tends to increase power. However, the larger number of haplotypes relative to alleles at individual loci tends to decrease power due to the additional degrees of freedom required for the analysis (30) and may account for why the findings reported here have not previously been detected.

To overcome these limitations, the current study included an exceptionally large sample of MS families, focusing analysis on particular haplotypes and groups of haplotypes. *HLA-DRB1*15* haplotypes have been assembled into their *HLA-A* or *HLA-B* broad serological equivalents (see Table 3), and have also been assigned as either common or rare based on their transmission frequencies (see Table 4).

In the analyses of *HLA-A* broad serological equivalents, we found that the closely related *HLA-A*23* and *-A*24* allele groups (*HLA-A9*), and *HLA-A*25* and *-A*26* allele groups (*HLA-A10*) carrying *HLA-DRB1*15* were significantly over-transmitted, whereas *HLA-A19* haplotypes (with closely related *HLA-A*29*, *-A*30*, *-A*31*, *-A*32*, and *-A*33* allele groups) carrying *HLA-DRB1*15* was significantly under-transmitted. For the *HLA-B*

Table 3. Two-locus haplotype transmissions of *HLA-DRB1*15/X−HLA-A* and *HLA-DRB1*15/X−HLA-B* from *HLA-DRB1*15* positive (n = 536) and negative parents (n = 452) pooled by their HLA class I broad serological equivalents

Broad Serological Equivalents	*HLA-DRB1*15* Positive Parents (n = 536)						*HLA-DRB1*15* Negative Parents (n = 452)						Comparison	
	Haplotypes	TR	NT	*OR*	χ^2	*P*	Haplotypes	TR	NT	*OR*	χ^2	*P*	χ^2	*P*
HLA-A9	*15-23*	4	1	4.00	1.93	0.17	*X-23*	13	12	1.08	0.04	0.84	—†	0.22
	15-24	92	22	4.18	46.20	1.07×10^{-11}	*X-24*	51	63	0.81	1.26	0.26	31.53	1.96×10^{-8}
	Total	96	23	4.17	44.78	2.20×10^{-11}	Total	64	75	0.85	0.87	0.35	32.64	1.11×10^{-8}
HLA-A10	*15-25*	20	5	4.00	9.64	0.0019	*X-25*	13	7	1.86	1.80	0.18	—†	0.14
	15-26	11	9	1.22	0.20	0.65	*X-26*	12	27	0.44	5.77	0.016	—†	0.046
	Total	31	14	2.21	6.42	0.011	Total	25	34	0.74	1.37	0.24	7.22	0.0072
HLA-A19	*15-29*	4	16	0.25	7.71	0.0055	*X-29*	14	12	1.17	0.15	0.69	5.44	0.020
	15-30	6	20	0.30	7.95	0.0048	*X-30*	9	15	0.60	1.50	0.22	1.24	0.27
	15-31	2	4	0.50	0.68	0.41	*X-31*	17	12	1.42	0.86	0.35	—†	0.19
	15-32	10	4	2.50	2.66	0.10	*X-32*	12	15	0.80	0.33	0.56	—†	0.071
	15-33	2	10	0.20	5.82	0.016	*X-33*	5	11	0.45	2.25	0.13	0.78	0.38
	Total	24	54	0.44	11.54	0.00068	Total	57	65	0.88	0.53	0.47	5.024	0.025
HLA-B15	*15-51*	22	4	5.50	13.72	0.00021	*X-51*	26	26	1.00	0.00	1.00	—†	0.0022
	15-52	1	6	0.17	3.96	0.047	*X-52*	1	1	1.00	0.00	1.00	—†	0.39
	Total	23	10	2.30	5.12	0.024	Total	27	27	1.00	0.00	1.00	3.25	0.071
HLA-B16	*15-38*	2	6	0.33	2.09	0.15	*X-38*	3	10	0.30	3.77	0.052	—†	0.39
	15-39	3	0	0.00	4.16	0.041	*X-39*	9	24	0.38	6.82	0.0090	—†	0.031
	Total	5	6	0.83	0.091	0.76	Total	12	34	0.35	10.52	0.0012	—†	0.13
HLA-B17	*15-57*	20	3	6.67	14.07	0.00018	*X-57*	19	15	1.27	0.47	0.49	—†	0.011
	15-58	6	4	1.50	0.40	0.53	*X-58*	3	7	0.43	1.60	0.21	—†	0.15
	Total	26	7	3.71	10.94	0.00094	Total	22	22	1.00	0.00	1.00	—†	0.0068
HLA-B22	*15-55*	3	0	0.00	4.16	0.041	*X-55*	2	8	0.25	3.60	0.058	—†	0.035
	15-56	1	4	0.25	1.93	0.17	*X-56*	2	0	0.00	2.00	0.16	—†	0.14
	Total	4	4	1.00	0.00	1.00	Total	4	8	0.5	1.33	0.25	—†	0.28

*HLA-DRB1*X* refers to any allele other than *HLA-DRB1*15*.

†Fisher's exact test was used when the expected transmissions in any of the cells of the table were below 10. *HLA-A*23* and *-A*24* are serological equivalents of *HLA-A9*. *HLA-A*25* and *-A*26* are serological equivalents of *HLA-A10*. *HLA-A*29*, *-A*30*, *-A*31*, *-A*32*, and *-A*33* are serological equivalents of *HLA-A19*. *HLA-B*51* and *-B*52* are serological equivalents of *HLA-B15*. *HLA-B*38* and *-B*39* are serological equivalents of *HLA-B16*. *HLA-B*57* and *-B*58* are serological equivalents of *HLA-B17*. *HLA-B*55* and *-B*56* are serological equivalents of *HLA-B22*.

broad serological equivalent analyses, *HLA-B15* (with closely related *HLA-B*51*, and *-B*52* allele groups) and *-B17* (with closely related *HLA-B*57*, and *-B*58* allele groups) were significantly over-transmitted in the presence of *HLA-DRB1*15*, whereas *HLA-DRB1*15* bearing *HLA-B16* and *-B22* haplotypes were neutral-transmitted (including closely related *HLA-B*38*, *-B*39* allele groups, and *HLA-B*55*, *-B*56* allele groups respectively; see Table 3).

We have shown in MS that alleles at *HLA-DRB1*, *HLA-A* and *HLA-B* are not randomly transmitted, instead alleles tend to be associated with other alleles in a set or cassette of common haplotypes. This long range and discontinuous LD between HLA class I and II has already been described previously (25). Since transmissions to affected offspring among parental *HLA-DRB1*15* haplotypes are significantly different, it is plausible that *HLA-DRB1*15* bearing *HLA-A9* (*HLA-A23*, and *-A*24*), *-A10* (*HLA-A*25*, and *-A*26*), *-B15* (*HLA-B*51*, and *-B*52*) and *-B17* (*HLA-B*57*, and *-B*58*) haplotypes had different functional properties under selection as compared with *HLA-DRB1*15* bearing *HLA-A19* (*HLA-A*29*, *-A*30*, *-A*31*, *-A*32*, and *-A*33*), *-B16* (*HLA-B*38*, and *-B*39*) and *-B22* (*HLA-B*55*, and *-B*56*) haplotypes. These observations may well reflect more general characteristics of this region, which may be present in many other autoimmune related diseases.

General over-transmission of *HLA-DRB1*15* positive 2-locus haplotypes have been observed for most of the common haplotypes (see Table 4). However there were notable exceptions, with *HLA-DRB1*15-HLA-B*08* and *HLA-DRB1*15-HLA-B*27*, 2 of the common haplotypes, along with pooled rare haplotypes taken together. Not only were the transmissions of rare haplotypes significantly different from the common haplotypes, but their transmissions were also remarkably similar as compared with non-*HLA-DRB1*15* haplotypes matched at HLA class I. For example, when parents are positive for *HLA-DRB1*15*, the transmission of *HLA-DRB1*15* haplotypes carrying *HLA-B*07* was significantly increased, but the transmission of *HLA-DRB1*15* haplotypes bearing *HLA-B*08* was neutral. Furthermore, the transmission of *HLA-DRB1*15-HLA-B*08* (Odds Ratio [OR] = 1.09) was no different from its paired *HLA-DRB1*X-HLA-B*08* haplotype (OR = 1.11). This differential

Table 4. A comparison of common and rare *HLA-DRB1*15−HLA-A* and *HLA-DRB1*15−HLA-B* haplotypes transmission

Haplotypes	Common Haplotypes					Rare Haplotypes					Comparison	
	TR	NT	*OR*	χ^2	*P*	TR	NT	*OR*	χ^2	*P*	χ^2	*P*
*HLA-DRB1*15−HLA-A*	480	191	2.51	124.47	6.64×10^{-29}	49	69	0.71	3.39	0.066	40.90	1.60×10^{-10}
*HLA-DRB1*15−HLA-B*	470	184	2.55	125.07	4.91×10^{-29}	68	76	0.89	0.44	0.50	32.63	1.12×10^{-8}

Two-locus (*HLA-DRB1*15−HLA-A,* and *HLA-DRB1*15−HLA-B*) haplotypes were separated into two groups: 1) Common haplotypes with haplotype frequency greater than 0.02, and 2) Rare haplotypes with haplotype frequency less than 0.02.

transmission was also evident among other 2-locus *HLA-DRB1*15/X* haplotypes.

These observations indicate unequivocally that *HLA-DRB1*15* haplotypes are heterogeneous and not all *HLA-DRB1*15* haplotypes are associated with MS susceptibility. This contradicts expectations of disease-related antigen-specific *HLA-DRB1* allele restriction as the basis for MHC association. TDT analysis of HLA class I and class II haplotypes reveals at least 3 populations of *HLA-DRB1*15* haplotypes, including those that are over-transmitted (susceptible), neutral-transmitted/under-transmitted (non-susceptible). The differential transmissions of these susceptible and non-susceptible *HLA-DRB1*15* haplotypes were confirmed by the contingency table analysis for common and rare haplotypes (see Table 4). This is not accounted for by differences in the alleles themselves and places focus on adjacent variation in regulatory regions and on the importance of extended haplotypes containing alleles in LD.

In this study we have demonstrated that *HLA-DRB1*15* is part of a susceptibility haplotype, but cannot be viewed as a MS susceptibility allele itself. The alleles or haplotypes can be considered as markers for differential susceptibility carried by these haplotypes, which would generate a ready approach to identify haplotype-specific variation responsible for disease risk in a region of the genome characterized by extraordinary polymorphism. Furthermore, increased haplotype sharing accompanied by the absence of distorted haplotype transmission in the *HLA-DRB1*15* negative families highlights the possibility of epigenetic modification of *HLA-DRB1* haplotypes.

These novel findings strongly imply that differences between susceptible (over-transmitted) and non-susceptible (neutral-transmitted/under-transmitted) *HLA-DRB1*15* haplotypes in *HLA-DRB1*15* positive families, complemented by increased haplotype sharing in the absence of increased haplotype transmission in *HLA-DRB1*15* negative families will contain the basis for MHC-associated risk in MS and may shed light on the nature of HLA class I−II LD. We also believe that these findings will be a general phenomenon, with wider relevance for other MHC-associated complex disease phenotypes, and animal model construction.

The difference between common and rare haplotypes is intriguing and suggests the differences among them in terms of disease risk may relate to evolutionary age and the timing of divergence of the rarer haplotypes from common ancestral trees. Additionally, contributions from selection might reasonably be expected. The findings in this study suggest that the fundamental unit of immunogenetics may be a haplotype-based functional cassette rather than a single histocompatibility allele and its ability to bind specific peptides as has been widely believed. Although the findings are derived from a single autoimmune disease they further raise the possibility that epistatic interactions of HLA class II alleles with as yet unidentified linked variation (such as seen in the HLA class I loci) determine immune responses more generally.

Methods

Subjects. We selected 1970 individuals from 494 MS families as part of an ongoing Canadian Collaborative Project on the Genetic Susceptibility to MS (CCPGSMS), for which the methodology has been described previously (31). Informed consent was obtained from all subjects and the experiments performed for this investigation comply with current guidelines and ethics. All families were Canadian and of European descent. The family types included are type 1 [families with both parents positive for *HLA-DRB1*15* (either heterozygous or homozygous for *HLA-DRB1*15*)], type 2 [families with one parent positive for *HLA-DRB1*15* (either heterozygous or homozygous for *HLA-DRB1*15*), and one parent negative for *HLA-DRB1*15*], and type 3 [families with both parents negative for *HLA-DRB1*15*].

This study used an expanded cohort of families from a previous study (25). In that previous study, a total of 1258 individuals from 294 MS families were included. In this current study, an additional independent cohort consisting 712 individuals from 200 MS families were included, which confirms the findings from the previous study (25). When comparing the 2 cohorts, we found no differences for the HLA class I/II haplotype transmissions, and the haplotype TDT patterns were very similar between the 2 datasets (data not shown). Therefore, we have decided to pool the 2 cohorts together for this study. The initial dataset (25) generated the hypotheses tested in this study, which asks if there are any *HLA-DRB1*1501* haplotypes tagged by HLA class I that are different for risk. By using this enlarged cohort of 1970 individuals from 494 MS families, we were able to adequately address this question.

HLA Typing. The genotyping for the *HLA-DRB1* was performed using either low- or high-resolution allele-specific PCR amplification method (8). Low-resolution *HLA-DRB1* genotypes were obtained by a combination of 24 PCR reactions, and high-resolution *HLA-DRB1* genotypes were obtained with an additional 48 PCR reactions. In each individual reaction, positive control primers were designed to amplify a second non-polymorphic genomic control segment. Amplified products were separated by electrophoresis in 2% agarose gels containing ethidium bromide after the addition of loading buffer, and visualized them using UV illumination.

The genotyping for the *HLA-A* and *HLA-B* were performed using a low-resolution allele-specific PCR amplification method (32). Low-resolution *HLA-A* and *HLA-B* genotypes were obtained by a combination of 96 PCR reactions. PCR products were electrophoresed in 1% agarose gels containing ethidium bromide after the addition of loading buffer and visualized using UV illumination.

Statistical Methods. The family pedigree files were first tested using the PEDCHECK program (33) for the presence of errors in Mendelian transmission.

Two-locus (*HLA-DRB1-HLA-A*, and *HLA-DRB1-HLA-B*) haplotypes were constructed. Transmissions of *HLA-DRB1*15* haplotypes from *HLA-DRB1*15* positive parents, and transmissions of *HLA-DRB1*X* haplotypes (where X refers to any allele other than *HLA-DRB1*15*) from *HLA-DRB1*15* negative parents were analyzed. To eliminate the main affect of *HLA-DRB1*15*, transmissions of *HLA-DRB1*X* haplotypes from *HLA-DRB1*15* heterozygous parents (for example: 15/X, where X refers to any allele other than *HLA-DRB1*15*) were not counted. Transmissions of *HLA-DRB1*X* haplotypes were only counted from *HLA-DRB1*15* negative parents (for example: X/X, where X refers to any allele other than *HLA-DRB1*15*).

Parents positive for *HLA-DRB1*15* can either be heterozygous or homozygous for *HLA-DRB1*15*, whereas *HLA-DRB1*15* negative parents can bear any *HLA-DRB1* allele except *HLA-DRB1*15*. The ''Total *HLA-DRB1*15* Positive Parents'' includes all parents from Type 1 families plus the *HLA-DRB1*15* positive parents from Type 2 families, whereas the ''Total *HLA-DRB1*15* Negative Parents'' includes all parents from Type 3 families plus the *HLA-DRB1*15* negative parents from Type 2 families (see *Subjects* section for details).

TDT was performed for each locus individually and also to multilocus haplotypes using the UNPHASED program (ref. 34 and www.hgmp.mrc.ac.uk). Since this study reports no independent associations of HLA class I alleles, correction for multiple testing was not applied, and all *P*-values in tables were presented as uncorrected *P*-values ($P_{uncorrected}$).

Two-locus *HLA-DRB1*15* bearing haplotypes (*HLA-DRB1*15-HLA-A* and *HLA-DRB1*15-HLA-B*) were categorized by their *HLA-A* and *HLA-B* broad serological equivalents, and the totals of the allele/haplotype groups were calculated. Furthermore, 2-locus *HLA-DRB1*15* bearing haplotypes (*HLA-DRB1*15-HLA-A* and *HLA-DRB1*15-HLA-B*) were then separated into 2 groups: (*i*) *common* haplotypes with frequency >0.02, and 2) *rare* haplotypes with frequency <0.02. This threshold of 0.02 used to assign common or rare haplotypes is only arbitrary and was considered based on the sample size and the haplotypes transmission probabilities.

ACKNOWLEDGMENTS. We thank our colleagues at the Wellcome Trust Centre for Human Genetics and the Oxford Transplant Centre for their help and support. We would also like to thank all patients who generously participated in this study, and physicians participating in the CCPGSMS including: J. J-F. Oger, D. W. Paty, S. A. Hashimoto, V. Devonshire, J. Hooge, J. P. Smythe, and T. Traboulsee (Vancouver); L. Metz (Edmonton); S. Warren (Calgary); W. Hader (Saskatoon); R. Nelson and M. Freedman (Ottawa); D. Brunet (Kingston); J. Paulseth (Hamilton); G. Rice and M. Kremenchutzky (London); P. O'Connor, T. Gray, and M. Hohol (Toronto); P. Duquette and Y. Lapierre (Montreal); J-P. Bouchard (Quebec City); T. J. Murray, V. Bhan, and C. Maxner (Halifax); W. Pryse-Phillips and M. Stefanelli (St. Johns). This work was funded by the Multiple Sclerosis Societies of Canada and Great Britain and Northern Ireland. This study was made possible by the Canadian Collaborative Project on the Genetic Susceptibility to MS (CCPGSMS).

1. Martin R, McFarland HF, McFarlin DE (1992) Immunological aspects of demyelinating diseases. *Annu Rev Immunol* 10:153–187.
2. Hafler DA (2004) Multiple sclerosis. *J Clin Invest* 113:788–794.
3. Zamvil SS, Steinman L (1990) The T lymphocyte in experimental allergic encephalomyelitis. *Annu Rev Immunol* 8:579–621.
4. Gregersen JW, *et al.* (2006) Functional epistasis on a common MHC haplotype associated with multiple sclerosis. *Nature* 443:574–577.
5. Ebers GC, *et al.* (1996) A full genome search in multiple sclerosis. *Nat Genet* 13:472–476.
6. Haines JL, *et al.* (1998) Linkage of the MHC to familial multiple sclerosis suggests genetic heterogeneity. *Hum Mol Genet* 7:1229–1234.
7. Dyment DA, Ebers GC, Sadovnick AD (2004) The genetics of MS. *Lancet Neurol* 3:104–110.
8. Dyment DA, *et al.* (2005) Complex interactions among MHC haplotypes in multiple sclerosis: Susceptibility and resistance. *Hum Mol Genet* 14:2019–2026.
9. Lincoln MR *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.
10. Ebers GC (2008) Environmental factors and multiple sclerosis. *Lancet Neurol* 7:268–277.
11. Ebers GC, Sadovnick AD (1994) The role of genetic factors in multiple sclerosis susceptibility. *J Neuroimmunol* 54:1–17.
12. Hains JL, *et al.* (1996) A complete genomic screen for multiple sclerosis underscores a role for the major histocompatability complex. *Nat Genet* 13:469–471.
13. Sawcer S, *et al.* (1996) A genome screen in multiple sclerosis reveals susceptibility loci on chromosome 6p21 and 17q22. *Nat Genet* 13:464–468.
14. Kuokkanen S, *et al.* (1997) Genomewide scan of multiple sclerosis in Finnish multiplex families. *Am J Hum Genet* 61:1379–1387.
15. Zhang Z, *et al.* (2005) Two genes encoding immune-regulatory molecules (LAG3 and IL7R) confer susceptibility to multiple sclerosis. *Genes Immun* 6:145–152.
16. Sawcer S, *et al.* (2002) A whole genome screen for linkage disequilibrium in multiple sclerosis confirms disease associations with regions previously linked to susceptibility. *Brain* 125:1337–1347.
17. Oturai A, *et al.* (1999) Linkage and association analysis of susceptibility regions on chromosome 5 and 6 in 106 Scandinavian sibling pair families with multiple sclerosis. *Ann Neruol* 46:612–616.
18. Gregory SG, *et al.* (2007) Interleukin 7 receptor α chain (IL7R) shows allelic and functional association with multiple sclerosis. *Nat Genet* 39:1083–1091.
19. Lundmark F, *et al.* (2007) Variation in interleukin 7 receptor α chain (IL7R) influences risk of multiple sclerosis. *Nat Genet* 39:1108–1113.
20. Risch N, Merikangas K (1996) The future of genetic studies of complex human disease. *Science* 273:1516–1517.
21. Peltonen L (2007) Old suspects found guilty—the first genome profile of multiple sclerosis. *N Engl J Med* 357:927–929.
22. Fogdell-Hahn A, Ligers A, Gronning M, Hillert J, Olerup O (2000) Multiple sclerosis: A modifying influence of HLA class I genes in an HLA class II associated autoimmune disease. *Tissue Antigens* 55:140–148.
23. Harbo HF, *et al.* (2004) Genes in the HLA class I region may contribute to the HLA class II-associated genetic susceptibility to multiple sclerosis. *Tissue Antigens* 63:237–247.
24. Yeo TW, *et al.* (2007) A second major histocompatibility complex susceptibility locus for multiple sclerosis *Ann Neurol* 61:228–236.
25. Chao MJ, *et al.* (2007) Transmission of class I/II multi-locus MHC haplotypes and multiple sclerosis susceptibility: Accounting for linkage disequilibrium. *Hum Mol Genet* 16:1951–1958.
26. Ligers A, *et al.* (2001) Evidence of linkage with HLA-DR in DRB1*15-negative families with multiple sclerosis. *Am J Hum Genet* 69:900–903.
27. Ghabanbasani MZ, *et al.* (1995) Importance of HLA-DRB1 and DQA1 genes and of the amino acid polymorphisms in the functional domain of DR beta 1 chain in multiple sclerosis. *J Neuroimmunol* 59:77–82.
28. Haegert DG, Swift FV, Benedikz J (1996) Evidence for a complex role of HLA class II genotypes in susceptibility to multiple sclerosis in Iceland. *Neurology* 46:1107–1111.
29. Spurkland A, *et al.* (1997) The HLA-DQ (alpha 1*0102, beta 1*0602) heterodimer may confer susceptibility to multiple sclerosis in the absence of HLA-DR (alpha 1*01, beta 1*1501) heterodimer. *Tissue Antigens* 50:15–22.
30. Seltman H, Roeder K, Devlin B (2001) Transmission/disequilibrium test meets measure haplotype analysis: Family-based association analysis guided by evolution of haplotypes. *Am J Hum Genet* 68:1250–1263.
31. Sadovnick AD, Risch NJ, Ebers GC, The Canadian Collaborative Study Group (1998) Canadian collaborative project on genetic susceptibility to MS, phase 2: Rationale and method. *Can J Neurol Sci* 25:216–221.
32. Bunce M, *et al.* (1995) Phototyping: Comprehensive DNA typing for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5 and DQB1 by PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP). *Tissue Antigens* 46:355–367.
33. O'Connel JR, Weeks DE (1998) PedCheck: A program for identifying genotype incompatibilities in linkage analysis. *Am J Hum Genet* 63:259–266.
34. Dudbridge F (2003) Pedigree disequilibrium tests for multilocus haplotypes. *Genet Epidemiol* 25:115–221.