# Epistasis among *HLA-DRB1*, *HLA-DQA1*, and *HLA-DQB1* loci determines multiple sclerosis susceptibility

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**Multiple sclerosis (MS), a common central nervous system inflammatory disease, has a major heritable component. Susceptibility is associated with the MHC class II region, especially *HLA-DRB5*0101–HLA-DRB1*1501–HLA-DQA1*0102–HLA-DQB1*0602* haplotypes (hereafter DR2), which dominate genetic contribution to MS risk. Marked linkage disequilibrium (LD) among these loci makes identification of a specific locus difficult. The once-leading candidate, *HLA-DRB1*15*, localizes to risk, neutral, and protective haplotypes. *HLA-DRB1*15* and *HLA-DQB1*0602*, nearly always located together on a small ancestral chromosome segment, are strongly MS-associated. One intervening allele on this haplotype, viz. *HLA-DQA1*0102*, shows no primary MS association. Two Canadian cohorts ($n = 830$ and $n = 438$ trios) genotyped for *HLA-DRB1*, *HLA-DQA1* and *HLA-DQB1* alleles were tested for association using TDT. To evaluate epistasis involving *HLA-DRB1*15*, transmissions from *HLA-DRB1*15*-negative parents were stratified by the presence/absence of *HLA-DRB1*15* in affected offspring. All 3 alleles contribute to MS susceptibility through novel epistatic interactions. *HLA-DQA1*0102* increased disease risk when combined with *HLA-DRB1*1501 in trans*, thereby unambiguously implicating *HLA-DQ* in MS susceptibility. Three-locus haplotypes demonstrated that *HLA-DRB1*1501* and *HLA-DQB1*0602* each influence risk. Transmissions of rare morcellated DR2 haplotypes showed no interaction with *HLA-DQA1*0102*. Incomplete haplotypes bearing only *HLA-DRB1*1501* or *HLA-DQB1*0602* did not predispose to MS. Balanced reciprocal transmission distortion can mask epistatic allelic association. These findings implicate epistasis among HLA class II alleles in human immune responses generally, provide partial explanation for intense linkage disequilibrium in the MHC, have relevance to animal models, and demonstrate key roles for DR2-specific interactions in MS susceptibility. MHC disease associations may be more generally haplotypic or diplotypic.**

genetics | MHC | linkage disequilibrium

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system characterized by myelin loss, axonal pathology, and progressive neurological dysfunction (1). With a prevalence of approximately 1/1,000 in Canada, MS is the most common cause of acquired neurological disability in young adults. Although the etiology of MS remains largely unknown, it is clear that both genetic and environmental components play important roles in pathogenesis (2). It is widely believed that MS is a $CD4^+$ $T_H1$-mediated autoimmune disorder. Support for this view comes from a variety of studies involving both murine models (3, 4) and human genetic studies (5–8).

The major histocompatibility complex (MHC) dominates the genetic influences on MS risk (7). While the association of HLA class II alleles with MS susceptibility has been long established, the involvement of these alleles is complex. Within this region, multiple genes and several of their individual alleles have been implicated (9, 10); these alleles appear to influence MS risk through a variety of complex interactions. Considerable uncertainty remains over which of these alleles may be primarily involved (11).

The central question of whether *HLA-DRB1* or *HLA-DQB1* is the primary MS susceptibility gene has not been resolved due to intense linkage disequilibrium (LD). *HLA-DRB1*1501* and *HLA-DQB1*0602* are in tight LD on a common ancestral haplotype and in northern Europeans are almost always transmitted together. In other populations, where MS is less common, greater haplotypic diversity is frequently observed. Admixture studies have used this diversity to evaluate the effects of *HLA-DRB1* and *HLA-DQB1* in these populations. An early study involving a small cohort of Afro-Brazilians suggested that *HLA-DQB1*0602* may be the primary locus (12). Subsequently, a larger study of African-Americans implicated *HLA-DRB1*15* (13). While admixture studies have provided important insights, haplotypic differences between Africans and northern Europeans mean that the primacy of neither *HLA-DRB1*15* nor *HLA-DQB1*0602* has been established in northern Europeans.

Analysis of Canadian families lacking *HLA-DRB1*1501* has demonstrated additional *HLA-DRB1* alleles which confer susceptibility and resistance but not additional MHC loci (14). Associations at class I suggested by case-control studies to be independent of HLA class II (15) were not sustained once account was taken of linkage disequilibrium (8, 16). In addition to the MHC, modest associations with other non-MHC loci have been found for *IL7R* (17, 18), *IL2R* (19), *EVI5* (20) and *KIF1B* (21). The latter is the largest of these with an odds ratio (OR) of 1.34 in sporadic MS and 1.74 in multiplex families (22).

The LD obstacle is compounded by complex interactions between *HLA-DRB1* alleles/haplotypes. The full *HLA-DRB1* genotype or diplotype (the 2 haplotypes in combination) largely determine genetic risk (7, 23). Multiple *HLA-DRB1* alleles are MS-associated, some dependent on the presence/absence of *HLA-DRB1*15 in trans* (7). The HLA class II alleles have been implicated in MS animal models (24, 25), and epistasis between HLA class II alleles influences susceptibility and clinical course in experimental autoimmune encephalomyelitis (3, 26). Recently it has been shown that HLA class I alleles tag haplotypes differential for MS risk with *HLA-DRB1*1501* present on susceptibility, neutral, and protective haplotypes (11). These data clearly show that this locus does not carry risk alone and completely changes the perspective on the *HLA-DRB1* vs. *HLA-DQB1* controversy; indeed, the effect of

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*HLA-DRB1*1501* can be completely changed in polarity by adjacent variation.

In light of the complexity of these findings, systematic examination of potential epistatic interactions between *HLA-DRB1*, *HLA-DQA1*, and *HLA-DQB1* was undertaken. In this, the largest family-based investigation of the HLA class II loci in MS, alleles at each of these loci were found to influence MS susceptibility through novel epistatic interactions. In particular, *HLA-DQA1*0102*, which instructively showed no independent association, was found to interact strongly with *HLA-DRB1*15 in trans*, increasing MS risk in the presence of *HLA-DRB1*15* and playing a protective role in its absence.

## Results

**Association of HLA Class II Alleles.** *HLA-DRB1*, *HLA-DQA1* and *HLA-DQB1* were each assessed for association with MS using the transmission disequilibrium test (TDT) [supporting information (SI) Table S1]. *HLA-DRB1*15* was positively associated with MS with an OR of 2.4 ($\chi_1^2 = 88$; uncorrected $P = 5.3 \times 10^{-21}$). Transmissions from *HLA-DRB1*15*-negative parents were assessed to identify additional HLA class II associations independent of HLA-DRB1*15 (7). In this analysis, 9 HLA class II alleles showed significant MS-association (Table S2).

**Epistatic Interactions Between *HLA-DRB1* Alleles.** Epistasis was first defined by Bateson (27) as an extension of the concept of dominance, whereby an allele at one locus may mask the effect of variation at a second locus. The term is used here broadly as an interaction between 2 alleles (*in cis* or *trans*) in which the risk associated with a particular allele depends on the presence or absence of a second allele. Because interactions between *HLA-DRB1* alleles/haplotypes determine MS risk (7, 23), we assessed each of the HLA class II alleles for epistatic interactions with *HLA-DRB1*15*. To assess these alleles for interaction with *HLA-DRB1*15*, transmissions of HLA class II alleles from *HLA-DRB1*15*-negative parents were stratified by the presence or absence of *HLA-DRB1*15* (transmitted from the other parent) in affected offspring (7). Alleles which exhibit different odds ratios in *HLA-DRB1*15*-positive and -negative offspring may be said to interact with *HLA-DRB1*15* or another locus in strong LD with *HLA-DRB1*15*. Interactions of *HLA-DRB1*01* and *HLA-DRB1*10* with *HLA-DRB1*15* observed previously (23, 28) were noted when the 2 cohorts were pooled (Table S3). *HLA-DRB1*17* also showed weak nominal significance. Consistent with and extending previous studies (7, 28), *HLA-DRB1*15* was strongly overtransmitted in the presence of *HLA-DRB1*08* (T/NT = 22/3; $\chi_1^2 = 14$; $P = 0.00015$).

**Epistasis Between *HLA-DRB1*15* and *HLA-DQA1*0102*.** Alleles of *HLA-DQA1* were examined for epistasis with *HLA-DRB1*15* by similar stratification. Three alleles of *HLA-DQA1* were differentially transmitted to *HLA-DRB1*15*-positive and *HLA-DRB1*15*-negative cases in the primary cohort (Table 1). Evidence for epistasis with *HLA-DRB1*15* was strongest for *HLA-DQA1*0102* (comparison $\chi_1^2 = 7.4$; $P = 0.0063$). With *HLA-DRB1*15* present, *HLA-DQA1*0102* was overtransmitted (OR = 2.1; $\chi_1^2 = 3.9$; $P = 0.048$) and in its absence, undertransmitted (OR = 0.64; $\chi_1^2 = 4.0$; $P = 0.047$). Similarly, *HLA-DQA1*0201* transmission was reciprocally distorted, trending toward overtransmission to *HLA-DRB1*15*-positive cases and toward undertransmission in *HLA-DRB1*15*-negative cases. *HLA-DQA1*0101* and *HLA-DQB1*0501* were protective with *HLA-DRB1*15* present and neutral in its absence.

In a secondary cohort, we posed the specific question generated in the first cohort. We replicated the proposed *HLA-DRB1*15–HLA-DQA1*0102* epistasis (comparison $\chi_1^2 = 5.3$; $P = 0.022$; Table 1). Reciprocal transmission distortion of *HLA-DQA1*0102* was proportional to that seen in the primary cohort when stratified by the presence/absence of *HLA-DRB1*15*.

The magnitude of the *HLA-DRB1*15–HLA-DQA1*0102* interaction was estimated by pooling the 2 cohorts. *HLA-DQA1*0102* showed no overall transmission distortion (T/NT = 85/98; $\chi_1^2 = 0.92$; $P = 0.34$) but significant overtransmission from *HLA-DRB1*15*-negative parents to *HLA-DRB1*15*-positive (OR = 2.0; $\chi_1^2 = 6.0$; $P = 0.014$) and significant undertransmission to *HLA-DRB1*15*-negative offspring (OR = 0.61; $\chi_1^2 = 7.4$; $P = 0.0063$). This reciprocal distortion (comparison $\chi_1^2 = 13$) remained significant after robust permutation correction for multiple testing (corrected $P = 0.0080$). Transmissions of HLA class II alleles from parents negative for both *HLA-DRB1*15* and *HLA-DQA1*0102* showed no transmission distortion (data not shown).

We next asked if these results were independent of *HLA-DRB1*15* interactions, and no influence of these results was detected. *HLA-DQA1*0102* from parents negative for alleles previously confirmed to interact specifically with *HLA-DRB1*15* (7, 28) (*HLA-DRB1*01*, *HLA-DRB1*08*, and *HLA-DRB1*10*) was still significantly overtransmitted to *HLA-DRB1*15*-positive (OR = 2.1; $\chi_1^2 = 5.8$; $P = 0.016$) and significantly undertransmitted to *HLA-DRB1*15*-negative offspring (OR = 0.59; $\chi_1^2 = 6.44$; $P = 0.011$). Comparison of the 2 strata revealed epistasis (comparison $\chi_1^2 = 12$), which remained significant after permutation correction for multiple comparisons (corrected $P = 0.0095$), demonstrating that *HLA-DRB1*15–HLA-DQA1*0102* epistasis is independent of potential *HLA-DRB1* allelic interactions.

Inversely, transmissions from parents to offspring for the rare *HLA-DQA1*0102*-negative but *HLA-DRB1*15*-positive haplotypes stratified by the presence of *HLA-DQA1*0102* in affected offspring (received *in trans*) were as predicted by the epistatic interaction observed above in pooled data: *HLA-DRB1*15* trends toward overtransmission (T/NT = 8/3) with *HLA-DQA1*0102* present and toward undertransmission (T/NT = 1/5) in the absence of *HLA-DQA1*0102*. Comparison of these strata reveals a marginally significant interaction (Fisher's exact test; comparison $P = 0.049$). More clearly, in larger numbers *HLA-DQA1*0102* transmission to 338 unaffected siblings stratified by the presence/absence of *HLA-DRB1*15* was neutral for both *HLA-DRB1*15*-positive (T/NT = 8/7) and *HLA-DRB1*15*-negative (T/NT = 19/18) individuals.

Demonstration that epistasis between *HLA-DRB1*15* and *HLA-DQA1*0102* is independent of *HLA-DRB1* allelic interactions very strongly implicates an independent role for HLA-DQ in susceptibility.

Given that there is no known physical interaction between *HLA-DQA1* and *HLA-DRB1*, *HLA-DQB1* was targeted because it forms a functional dimer with *HLA-DQA1*. Problematically, *HLA-DQB1*0602* is not prevalent on non-DR2 haplotypes, whereas *HLA-DQA1*0102* is highly so. Three-locus class II haplotypes were assessed for transmission distortion (Table 2). Expectedly, *HLA-DRB1*15–HLA-DQA1*0102–HLA-DQB1*0602* haplotypes were significantly overtransmitted (OR = 2.6; $\chi_1^2 = 120$; $P = 1.2 \times 10^{-27}$). Uncommon to rare *incomplete* haplotypes lacking any one or 2 of these loci (Table 2) were neutrally transmitted (T/NT = 146/153; $\chi_1^2 = 0.16$; $P = 0.69$), suggesting that susceptibility is attenuated or absent on incomplete haplotypes. In particular, those lacking either *HLA-DRB1*15* or *HLA-DQB1*0602* were neutral (T/NT = 114/129 and T/NT = 139/145, respectively).

Among haplotypes simultaneously *HLA-DRB1*15*-positive and *HLA-DQB1*0602*-negative, it became apparent that the single haplotype accounting for overtransmission contained the closely-related *HLA-DQB1*0603*, reportedly overtransmitted in MS (29) and similarly protective against type I diabetes (30). Among *HLA-DRB1*15*-positive, *HLA-DQB1*0602/*0603*-negative haplotypes, transmission was neutral (T/NT = 14/13). Similarly, among very rare *HLA-DRB1*15*-negative, *HLA-DQB1*0602*-positive haplotypes, transmission was also neutral (T/NT = 7/6). Further complexity is suggested by comparison of the transmission of morcel-

**Table 1. Transmission of *HLA-DQA1* and *HLA-DQB1* alleles from *HLA-DRB1*15*-negative parents to offspring stratified by the presence or absence of *HLA-DRB1*15* in the primary and secondary cohorts**

| Cohort | *HLA-DRB1*15*-Positive Children | | | *HLA-DRB1*15*-Negative Children | | | Comparison | |
|---|---|---|---|---|---|---|---|---|
| Primary | | | | | | | | |
| *HLA-DQA1* | T | NT | OR | T | NT | OR | $\chi_1^2$ | *P* |
| **0101* | 23 | 48 | 0.48 | 60 | 62 | 0.97 | 5.2 | 0.023 |
| **0102* | 21 | 10 | 2.1 | 32 | 50 | 0.64 | 7.4 | 0.0063 |
| **0103* | 8 | 16 | 0.5 | 27 | 36 | 0.75 | 0.66 | 0.42 |
| **0201* | 51 | 37 | 1.38 | 52 | 68 | 0.76 | 4.3 | 0.037 |
| **03* | 35 | 30 | 1.2 | 66 | 61 | 1.1 | 0.061 | 0.81 |
| **04* | 19 | 6 | 3.2 | 15 | 13 | 1.2 | 2.9 | 0.089 |
| **0501* | 37 | 32 | 1.2 | 81 | 44 | 1.8 | 2.3 | 0.13 |
| **0505* | 26 | 40 | 0.65 | 53 | 53 | 1 | 1.8 | 0.17 |
| **06* | 0 | 1 | 0 | 2 | 1 | 2 | —[a] | 1 |
| *HLA-DQB1* | | | | | | | | |
| **0201* | 38 | 31 | 1.2 | 82 | 41 | 2. | 2.5 | 0.11 |
| **0202* | 44 | 33 | 1.3 | 41 | 50 | 0.82 | 2.4 | 0.12 |
| **0301* | 36 | 54 | 0.67 | 70 | 78 | 0.90 | 1.2 | 0.27 |
| **0302* | 24 | 15 | 1.6 | 46 | 43 | 1.1 | 1.1 | 0.30 |
| **0303* | 9 | 6 | 1.5 | 19 | 20 | 0.95 | 0.55 | 0.46 |
| **0304* | 0 | 0 | — | 1 | 0 | — | —[a] | 1. |
| **0305* | 0 | 0 | — | 0 | 1 | 0 | —[a] | 1. |
| **0402* | 19 | 6 | 3.2 | 16 | 14 | 1.1 | 3.0 | 0.082 |
| **0501* | 19 | 37 | 0.51 | 51 | 50 | 1.0 | 4.0 | 0.045 |
| **0502* | 4 | 4 | 1. | 6 | 8 | 0.75 | —[a] | 1. |
| **050301* | 4 | 13 | 0.31 | 9 | 15 | 0.6 | 0.90 | 0.34 |
| **0601* | — | — | — | — | — | — | — | — |
| **0602* | 1 | 1 | 1. | 2 | 1 | 2. | —[a] | 1. |
| **0603* | 8 | 16 | 0.5 | 23 | 36 | 0.64 | 0.23 | 0.63 |
| **060401* | 14 | 8 | 1.75 | 17 | 24 | 0.71 | 2.8 | 0.093 |
| **0605* | 6 | 2 | 3. | 9 | 11 | 0.82 | —[a] | 0.22 |
| Secondary | | | | | | | | |
| *HLA-DQA1* | | | | | | | | |
| **0101* | 12 | 23 | 0.52 | 24 | 25 | 0.96 | | |
| **0102* | 15 | 8 | 1.9 | 17 | 30 | 0.57 | 5.3 | 0.022 |
| **0103* | 5 | 8 | 0.62 | 14 | 20 | 0.7 | | |
| **0201* | 25 | 26 | 0.96 | 27 | 26 | 1.0 | | |
| **03* | 29 | 26 | 1.1 | 36 | 38 | 0.95 | | |
| **04* | 4 | 10 | 0.4 | 6 | 8 | 0.75 | | |
| **0501* | 20 | 19 | 1.0 | 36 | 28 | 1.3 | | |
| **0505* | 30 | 19 | 1.6 | 51 | 33 | 1.5 | | |
| **06* | 0 | 1 | 0 | 0 | 1 | 0 | | |
| *HLA-DQB1* | | | | | | | | |
| **0201* | 22 | 20 | 1.1 | 36 | 24 | 1.5 | | |
| **0202* | 27 | 25 | 1.1 | 27 | 22 | 1.2 | | |
| **0203* | 1 | 0 | — | 0 | 0 | — | | |
| **0301* | 35 | 37 | 0.95 | 48 | 44 | 1.1 | | |
| **0302* | 26 | 16 | 1.6 | 30 | 23 | 1.3 | | |
| **0303* | 8 | 7 | 1.1 | 5 | 8 | 0.62 | | |
| **0402* | 7 | 10 | 0.7 | 6 | 11 | 0.55 | | |
| **0501* | 15 | 22 | 0.68 | 30 | 26 | 1.2 | | |
| **0502* | 7 | 4 | 1.8 | 4 | 4 | 1. | | |
| **050301* | 0 | 3 | 0 | 0 | 4 | 0 | | |
| **0602* | 1 | 0 | — | 0 | 1 | 0 | | |
| **0603* | 6 | 10 | 0.6 | 14 | 22 | 0.64 | | |
| **060401* | 9 | 11 | 0.82 | 5 | 19 | 0.26 | | |
| **0605* | 1 | 0 | — | 4 | 1 | 4. | | |

[a]Fisher's exact test (two-tailed) was used if any of the expected cell counts were <5.

lated haplotypes that contain or do not contain *HLA-DQA1*0102* (Table 2). Comparison of transmission of pooled haplotypes negative for one of either *HLA-DRB1*15* or *HLA-DQB1*0602* with transmission for intact DR2 haplotypes was marginally significant ($\chi_1^2 = 4.3$; $P = 0.038$) and comparison of transmissions of the complete DR2 haplotype to the entire pool of incomplete haplotypes was also significant ($\chi_1^2 = 46$; $P = 9.2 \times 10^{-12}$).

Furthermore, in MS offspring selected for having *HLA-DRB1*X–HLA-DQA1*0102–HLA-DQB1*Z* haplotypes (where **X* and **Z* represent any non-DR2 allele), we saw overtransmission of archetypical DR2 3-locus haplotypes from the other parent (T/NT = 35/7) which differed from that of *HLA-DRB1*15–HLA-DQA1*Y–HLA-DQB1*Z* (T/NT = 6/6), (comparison $P = 0.027$) implying *trans* interactions of *HLA-DQA1*0102* specific to DR2 haplotypes. This argues for interaction between *DQA1*0102* and *DQB1*0602*.

Heterogeneity among *HLA-DRB1*15*-bearing haplotypes prompted examination of other alleles/haplotypes. As the most "neutral" control (T/NT = 150/151 in a previous study of a northern European-derived population; ref. 7), *HLA-DRB1*13* was selected.

**Table 2. Transmission of *HLA-DRB1*15—HLA-DQA1*0102—HLA-DQB1*0602* and morcellated haplotypes lacking *HLA-DRB1*15*, *HLA-DQA1*0102* or *HLA-DQB1*0602***

| Complete vs. incomplete haplotypes | T | NT | OR |
|---|---|---|---|
| *HLA-DRB1*15—HLA-DQA1*0102—HLA-DQB1*0602* | 431 | 165 | 2.6 |
| *HLA-DRB1*15*-positive, *HLA-DQB1*0602*-negative[a] | 14 | 13 | 1.1 |
| *HLA-DRB1*15—HLA-DQA1*04—HLA-DQB1*0402* | 1 | 1 | 1 |
| *HLA-DRB1*15—HLA-DQA1*06—HLA-DQB1*0301* | 1 | 2 | 0.5 |
| *HLA-DRB1*15—HLA-DQA1*0102—HLA-DQB1*0303* | 0 | 2 | 0 |
| *HLA-DRB1*15—HLA-DQA1*0102—HLA-DQB1*0501* | 2 | 2 | 1 |
| *HLA-DRB1*15—HLA-DQA1*0102—HLA-DQB1*0502* | 2 | 2 | 1 |
| *HLA-DRB1*15—HLA-DQA1*0102—HLA-DQB1*0603* | 18 | 8 | 2.2 |
| *HLA-DRB1*15—HLA-DQA1*0102—HLA-DQB1*05031* | 1 | 1 | 1 |
| *HLA-DRB1*15—HLA-DQA1*0103—HLA-DQB1*0601* | 3 | 3 | 1 |
| *HLA-DRB1*15—HLA-DQA1*0505—HLA-DQB1*0301* | 4 | 0 | 4 |
| *HLA-DRB1*15—HLA-DQA1*0505—HLA-DQB1*0603* | 0 | 1 | 0 |
| *HLA-DRB1*15*-negative, *HLA-DQB1*0602*-positive | 7 | 6 | 1.2 |
| *HLA-DRB1*08—HLA-DQA1*03—HLA-DQB1*0602* | 1 | 0 | — |
| *HLA-DRB1*08—HLA-DQA1*04—HLA-DQB1*0602* | 1 | 1 | 1 |
| *HLA-DRB1*08—HLA-DQA1*0102—HLA-DQB1*0602* | 0 | 1 | 0 |
| *HLA-DRB1*12—HLA-DQA1*0102—HLA-DQB1*0602* | 2 | 0 | — |
| *HLA-DRB1*13—HLA-DQA1*0102—HLA-DQB1*0602* | 0 | 2 | 0 |
| *HLA-DRB1*13—HLA-DQA1*0103—HLA-DQB1*0602* | 2 | 1 | 2 |
| *HLA-DRB1*17—HLA-DQA1*0102—HLA-DQB1*0602* | 1 | 1 | 1 |
| *HLA-DRB1*15*-positive, *HLA-DQB1*0602*-positive | 0 | 2 | 0 |
| *HLA-DRB1*15—HLA-DQA1*0101—HLA-DQB1*0602* | 0 | 2 | 0 |
| *HLA-DRB1*15*- and *HLA-DQB1*0602*-negative | 107 | 123 | 0.87 |

[a]The total for *HLA-DRB1*15*-positive, *HLA-DQB1*0602*-negative haplotypes excludes *HLA-DQB1*0603* already implicated in MS susceptibility, and supported here.

Unexpectedly, this neutrality similarly masks several balanced reciprocal transmissions to affected , much as is seen for *HLA-DQA1*0102* (Table 3). *HLA-DRB1*13–HLA-DQA1*0505–HLA-DQB1*0301* showed marked overtransmission (T/NT = 76/41; $\chi_1^2 = 10$; $P = 0.0012$), while *HLA-DRB1*13–HLA-DQA1*0103–HLA-DQB1*0603* was undertransmitted (T/NT = 52/107; $\chi_1^2 = 19$; $P = 1.3 \times 10^{-5}$) and *HLA-DRB1*13–HLA-DQA1*0102–HLA-DQB1*06041* trended toward undertransmission (T/NT = 46/64; $\chi_1^2 = 2.9$; $P = 0.086$).

These results find independent confirmation in Israel. There, MS is not *HLA-DRB1*1501*- but *HLA-DRB1*13*-associated, the same *HLA-DRB1*13–HLA-DQA1*0505–HLA-DQB1*0301* haplotype coherently unmasked here (30). Similarly, of the 2 common *HLA-DRB1*04*-bearing haplotypes, only *HLA-DRB1*04–HLA-DQA1*03–HLA-DQB1*0301* was undertransmitted; *HLA-DRB1*04–HLA-DQA1*03–HLA-DQB1*0302* showed no evidence for transmission distortion (Table 3). These haplotypes differ only at *HLA-DQB1*, but the difference in transmission was statistically significant ($\chi_1^2 = 16$; $P = 5.3 \times 10^{-5}$), illustrating the clear advantages of MHC *haplotypes* to dissect associations.

Epistasis involving *HLA-DQA1* and the haplotype interactions involving *HLA-DQB1* implicate HLA-DQ in MS susceptibility. To examine whether *HLA-DRB1* exerts effects independent of HLA-DQ or is secondarily associated, haplotypes sharing *HLA-DQA1*0505* and *HLA-DQB1*0301* alleles were assessed for transmission distortion (Table 4). The *HLA-DRB1*11*-bearing haplotype was significantly undertransmitted (OR = 0.68; $\chi_1^2 = 9.8$; $P = 0.0017$), whereas that bearing *HLA-DRB1*13* was overtransmitted (OR = 1.9; $\chi_1^2 = 10$; $P = 0.0012$). These results cohere with the incomplete haplotype data, implying roles for *HLA-DRB1* independent of HLA-DQ loci.

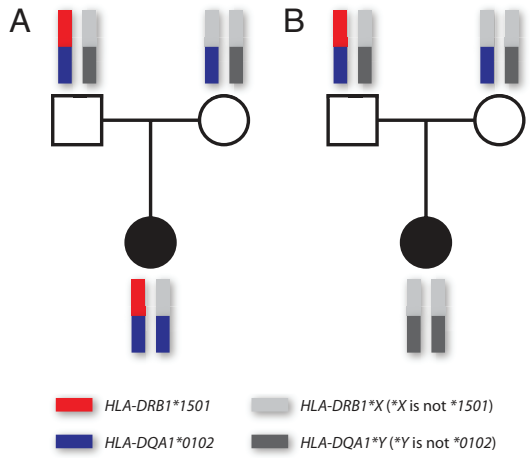


**Fig. 1.** *Trans* Epistasis between *HLA-DRB1*1501* and *HLADQA1*0102*. (*A*) *HLA-DQA1*0102* (blue) is overtransmitted (OR = 2.1) when *HLADRB1*1501* (red) is also present in affected offspring. (*B*) When *HLADRB1*1501* is absent, *HLA-DQA1*0102* is undertransmitted (OR = 0.64).

## Discussion

Searches for the primary MHC association in multiple sclerosis have inconclusively implicated *HLA-DRB1*1501* or *HLA-DQB1*0602*. The central problem is one common to other HLA-associated disorders: more generally the dilemma is how to identify a disease allele (say, a relatively common variant) in a region of tight linkage disequilibrium when one allele almost completely predicts the adjacent alleles. In the case of MS, the relevant candidate alleles are common in both MS patients and unaffected family members.

Emphasis on *HLA-DRB1* and *HLA-DQB1* has shifted focus away from other functional loci in the HLA class II region, including *HLA-DQA1*0102*. Understandably this locus had been discounted since *HLA-DQA1*0102* has not been shown to have a primary association with MS.

The presence of *HLA-DQA1*0102* on *HLA-DRB1*15*-negative haplotypes crucially enabled investigation for epistasis. *Trans* interactions were demonstrated by a modification of the TDT, where transmissions of *HLA-DQA1*0102* from *HLA-DRB1*1501*-negative parents were shown to depend on the *HLA-DRB1*1501* status of the offspring (Fig. 1). *HLA-DQA1*0102* was overtransmitted if *HLA-DRB1*1501* was the homologous allele present and undertransmitted if *HLA-DRB1*1501* was absent. Interactions *in cis* were demonstrated by examination of 3 locus haplotypes (Fig. 2). Observed *cis* interactions between *HLA-DRB1*15* and *HLA-DQA1*0102* and between *HLA-DRB1*15* and *HLA-DQB1*0602* may reflect either functional relationships between these alleles or additional haplotype-specific effects. The availability of a large

**Table 3. Common 3-locus HLA Class II haplotypes in MS: transmission heterogeneity**

| Haplotype | T | NT | OR | $\chi_1^2$ | *P* |
|---|---|---|---|---|---|
| *HLA-DRB1*13—HLA-DQA1*0505—HLA-DQB1*0301* | 76 | 41 | 1.9 | 10 | 0.0012 |
| *HLA-DRB1*13—HLA-DQA1*0102—HLA-DQB1*06041* | 46 | 64 | 0.72 | 2.9 | 0.086 |
| *HLA-DRB1*13—HLA-DQA1*0103—HLA-DQB1*0603* | 52 | 107 | 0.49 | 19 | $1.3 \times 10^{-5}$ |
| *HLA-DRB1*04—HLA-DQA1*03—HLA-DQB1*0301* | 53 | 103 | 0.51 | 16 | $6.2 \times 10^{-5}$ |
| *HLA-DRB1*04—HLA-DQA1*03—HLA-DQB1*0302* | 150 | 127 | 1.2 | 1.9 | 0.17 |

**Table 4. Haplotypes sharing *HLA-DQA1*0505* and *HLA-DQB1*0301* in MS: the effect of varying *HLA-DRB1* on transmission**

| Haplotype | T | NT | OR | $\chi_1^2$ | *P* |
|---|---|---|---|---|---|
| *HLA-DRB1*01—HLA-DQA1*0505—HLA-DQB1*0301* | 11 | 8 | 1.4 | 0.47 | 0.49 |
| *HLA-DRB1*04—HLA-DQA1*0505—HLA-DQB1*0301* | 0 | 1 | 0 | —[a] | 1 |
| *HLA-DRB1*11—HLA-DQA1*0505—HLA-DQB1*0301* | 107 | 158 | 0.68 | 9.8 | 0.0017 |
| *HLA-DRB1*12—HLA-DQA1*0505—HLA-DQB1*0301* | 16 | 27 | 0.59 | 2.8 | 0.093 |
| *HLA-DRB1*13—HLA-DQA1*0505—HLA-DQB1*0301* | 76 | 41 | 1.9 | 10 | 0.0012 |
| *HLA-DRB1*14—HLA-DQA1*0505—HLA-DQB1*0301* | 1 | 1 | 1 | —[a] | 1 |
| *HLA-DRB1*15—HLA-DQA1*0505—HLA-DQB1*0301* | 4 | 0 | — | —[a] | 0.12 |
| *HLA-DRB1*16—HLA-DQA1*0505—HLA-DQB1*0301* | 1 | 0 | — | —[a] | 1 |
| *HLA-DRB1*17—HLA-DQA1*0505—HLA-DQB1*0301* | 0 | 2 | 0 | —[a] | 0.5 |
| *HLA-DRB1*18—HLA-DQA1*0505—HLA-DQB1*0301* | 1 | 1 | 1 | —[a] | 1 |

[a]Binomial test (two-sided) was used if T + NT <10.

sample of MS families allowed this effect to be separated from previously identified interactions at *HLA-DRB1*. *HLA-DQA1 cis* and *trans* effects, independent of *HLA-DRB1*, strongly implicated HLA-DQ in the MS immune response, perhaps through formation of *cis* or *trans HLA-DQA1*0102–HLA-DQB1*0602* encoded dimers. However, the morcellated haplotypes studied here appear to implicate the intact *HLA-DRB1–HLA-DQA1–HLA-DQB1* haplotype at a minimum. Intriguingly, DR2 haplotypes exhibit the strongest LD among HLA class II haplotypes (3, 31), implying functional requirements under selection (3).

Epistasis among alleles at different HLA class II loci which exhibit reciprocal transmission distortion warrants consideration in future association studies. Concentration of epistatic alleles on the same susceptibility haplotype supports complex functional relationships between *HLA-DR* and *HLA-DQ* loci determining MS susceptibility. Genetic interactions between alleles at *HLA-DRB1*, *HLA-DQA1*, or *HLA-DQB1* have been reported in narcolepsy (32) and diabetes (33) and functional epistasis between *HLA-DRB1* and *HLA-DRB5* has been demonstrated in murine models of MS (3). In addition to the allelic interactions reported here, the differential susceptibility associated with different *HLA-DRB1*15*-bearing haplotypes (11) may imply structural and epigenetic influences. The latter may explain the reciprocal distortion seen for *HLA-DQA1*.

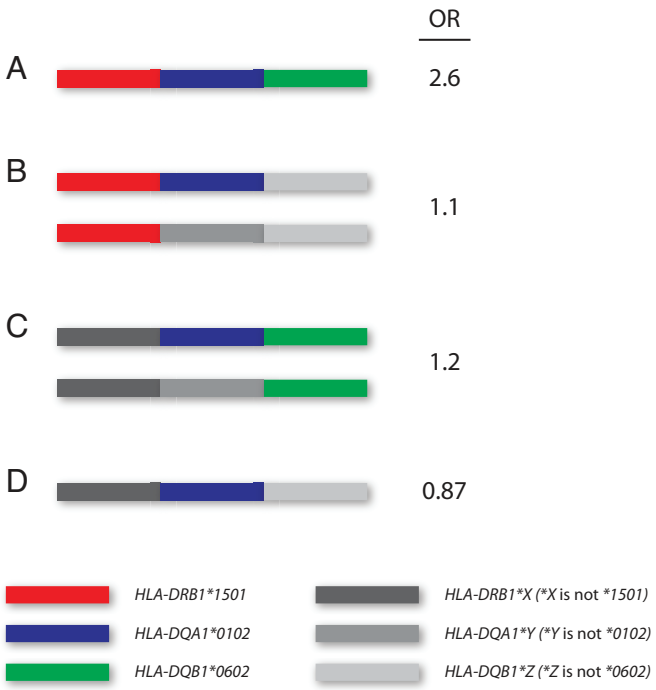


**Fig. 2.** Transmission of morcellated DR2 haplotypes reveals *cis* epistasis in the MHC class II region. (*A*) The classical MS susceptibility haplotype bearing *HLA-DRB1*1501, HLA-DQA1*0102*, and *HLA-DQB1*0602* confers susceptibility to MS with an OR of 2.6. (*B*) Haplotypes bearing *HLA-DRB1*1501* in the absence of *HLA-DQB1*0602* confer no susceptibility (OR = 1.1). (*C*) Haplotypes bearing *HLA-DQB1*0602* in the absence of *HLA-DRB1*1501* are also neutral (OR = 1.2). (*D*) *HLA-DQA1*0102*-bearing haplotypes which lack *HLA-DRB1*1501* and *HLA-DQB1*0602* are protective (OR = 0.87).

These findings may be relevant to other aspects of the immune response (34) and to finding genes for complex traits. We have previously shown how false positives can result from LD (16). Here we show how balanced reciprocal transmission distortion masks association and envision similar limitations in detecting epigenetic effects which have now been shown to be important in MS at the same loci (35) and may be responsible for this phenomenon. Reciprocal distortion suggests that pairings of *HLA-DQA1* molecules with *HLA-DQB1* are selected, not necessarily independent of HLA-DR–HLA-DQ epistasis. The variable effect of *HLA-DRB1* on DQ pairings may have analogies for *HLA-DQA1*0102–HLA-DQB1*0602* haplotypes, explaining populations in which the *HLA-DQB1*0602* association exceeds that for *HLA-DRB1*1501*.

Epistatic interactions between *HLA-DRB1*, *HLA-DQA1*, and *HLA-DQB1* appear to play defining roles in MS susceptibility. Analysis stringently restricted to parents negative for any *HLA-DRB1* allele having nominally significant interactions showed that the epistatic effect of *HLA-DQA1*0102* was undiminished, demonstrating independence from *HLA-DRB1*. Previously described "dose effects" of *HLA-DRB1*1501* may reflect, at least in part, epistasis between HLA-DR and HLA-DQ loci.

Strategic accounting for allelic associations was essential since haplotypic transmission probability is influenced by alleles on a parent's other haplotype. Large samples with available parents enabled the obligate haplotype construction. Assumptions made (36) in measuring individual contributions of loci using sib-pair haplotype sharing may not apply in complex circumstances. Haplotypic associations in MS, different by population, suggest additional yet unidentified haplotype-specific features and 2 recent papers indicate that there may be additional haplotypic-specific influences within the MHC (35, 37), as improbable as this may have seemed a priori. However, the results presented here illuminate how MS risk is influenced by the DR-DQ region, strongly implying that allelic variations in all 3 genes in the interval localized by dense SNP mapping (8) are operative. This paradigm may be more generally applicable to human immune and autoimmune responses. It is possible that MHC-disease associations will more generally be haplotypic rather than allelic in nature. Furthermore, the recent observation that vitamin D may influence *HLA-DRB1*1501* expression via a vitamin D response element which is largely specific to *HLA-DRB1*1501* haplotypes shows that gene-environment interactions may act within the MHC (37), potentially adding a fourth element to an archetypic risk haplotype in MS.

## Materials and Methods

**Subjects.** To minimize the number of false positive test results, a 2-stage replication design was chosen in advance. The primary cohort consists of 2,024 individuals (830 trios) derived from 394 Canadian multiplex MS families. The secondary cohort similarly consists of 982 individuals (438 trios) derived from 295 families. Both cohorts were ascertained through the Canadian Collaborative Project on Genetic Susceptibility to MS (CCPGSMS). Ascertainment methodology has been described in detail previously (38). Informed consent was obtained from all subjects and the experiments performed for this investigation comply with current guidelines and ethics.

**HLA Typing.** Each sample was genotyped for *HLA-DRB1* with either a low- (7, 39) or a high-resolution allele-specific PCR amplification (8). Before analysis, all *HLA-DRB1* genotypes were converted to 2-digit precision. *HLA-DQA1* and *HLA-DQB1* were genotyped using the same protocol. Fifteen allele-specific PCR reactions were used to type *HLA-DQA1* and 23 were used to characterize *HLA-DQB1*. For each Mendelian error, the entire family was regenotyped for each locus. Where Mendelian errors remained, the family was eliminated from subsequent analyses.

**Statistical Methods.** Individuals for whom consistent genotypes could not be obtained for each of the loci under study were removed from the study. Missing parental genotypes were reconstructed from the genotypes of unaffected offspring where possible. Hardy-Weinberg equilibrium was assessed at each locus, with none showing significant deviation among founder genotypes.

HLA class II alleles were assessed for association with the TDT (40). Tests for interaction between alleles were performed on the raw transmission counts as described previously (7). To correct for multiple testing, permutation tests were performed ($10^6$ permutations). Within each family, the transmission status of each allele was permuted randomly in a fashion that preserved the IBD status of affected offspring and the haplotypic relationships between alleles.

Three-locus haplotypes of HLA class II alleles were constructed using an E-M algorithm (8). Only haplotypes which could be inferred unambiguously were included in these analyses.

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