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Report

Evidence of Linkage with HLA-DR in DRB1*15-Negative Families with Multiple Sclerosis

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The importance of the HLA-DR locus to multiple sclerosis (MS) susceptibility was assessed in 542 sib pairs with MS and in their families. By genotyping 1,978 individuals for HLA-DRB1 alleles, we confirmed the well-established association of MS with HLA-DRB1*15 (*HLA-DRB1*1501* and *HLA-DRB5*0101*), by the transmission/disequilibrium test ($\chi^2 = 138.3$; $P < .0001$). We obtained significant evidence of linkage throughout the whole data set (mlod = 4.09; 59.9% sharing). Surprisingly, similar sharing was also observed in 58 families in which both parents lacked the DRB1*15 allele (mlod = 1.56; 62.7% sharing; $P = .0081$). Our findings suggest that the notion that HLA-DRB1*15 is the sole major-histocompatibility-complex determinant of susceptibility in northern-European populations with MS may be incorrect. It remains possible that the association of MS with HLA-DRB1*15 is due to linkage disequilibrium with a nearby locus and/or to the presence of disease-influencing allele(s) in DRB1*15-negative haplotypes.

Studies of twins and of recurrence rates in families have shown that the risk for multiple sclerosis (MS [MIM 142860]), an inflammatory disease of the CNS, is genetically influenced (Dyment et al. 1997; for review, see Sadovnick et al. 1997). A large number of studies have confirmed the association of MS with a haplotype carrying the major histocompatibility complex (MHC) class II HLA-DR15 and HLA-DQ6 alleles (*HLA-DRB1*1501, HLA-DRB5*0101, HLA-DQA1*0102,* and *HLA-DQB1*0602*). However, since the original report, ~26 years ago, of an MHC class II association (Winchester et al. 1975), the finding has contributed relatively little to the understanding of disease mechanisms (Olerup and Hillert 1991).

Association analysis of HLA genes is complicated by the presence of many highly polymorphic genes and by strong linkage disequilibrium, thus requiring large numbers of individuals. Recently, a case-control analysis of 948 Swedish patients with MS confirmed the importance that DRB1*15 has for susceptibility to MS but showed no influence on either disease course or disease severity (Masterman et al. 2000). Because the frequency of DRB1*15-negative families is relatively low, it has not previously been possible to adequately evaluate evidence of linkage in DRB1*15-negative families with MS. The intent of this study was to extend the analysis to a large number of families with MS, thereby providing an opportunity to assess the influence, independent from the already-well-established DRB1*15 association, of the HLA gene region.

The total study group was composed of 441 white Canadian families—comprising 542 sib pairs with MS. The types and quantity of families and individuals studied are shown in table 1. Patients with MS who were studied herein met the criteria for "clinically probable" or "clinically definite" MS (Sadovnick et al. 1998). Of the probands, 88% had clinically definite MS, and 12% had clinically probable MS. Of the affected siblings, 90.5% were clinically definite, and 9.5% were clinically probable. Patients who had a relapsing/remitting onset and patients who had a primary progressive onset were

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Table 1

Details of Families and Individuals Studied

Group	No.
Families:	
Affected sib pairs	399
Affected trios	39
Four affected individuals	1
Five affected individuals	2
Total	441
Affected sib pairs	542
Families with two parents available	162
Families with one parent available	144
Families with neither parent available	135
Unaffected sib pairs (families)	569 (244)
Individuals	1,978

included in this sample. A natural history study of a Canadian population with MS provided support for the notion that these two clinical types of MS share a common genetic background (Ebers et al. 2000). Genotyping of *HLA-DRB1* and *HLA-DRB5* was performed by PCR with sequence-specific primers (Olerup SSP AB) in a nested fashion, with PCR preamplification of a 270-bp fragment of the DRB genes. The analysis consisted of 24 parallel amplifications with unique primer pairs and with internal amplification controls for each reaction.

Identity-by-descent (IBD) sharing and identity-by-state (IBS) sharing were assessed by the sib_ibd and sib_phase programs of the ASPEX-2.3 statistical package, respectively. Allele frequencies were estimated from parental alleles that were not transmitted to affected individuals (Terwilliger and Ott 1992; Risch and Teng 1998). IBS analysis was performed, as well as IBD analysis, because IBS analysis uses information from families in which parental genotypes are unavailable (see table 1). Transmission/disequilibrium testing (TDT) was performed by the sib_tdt program of ASPEX-2.3. Unaffected siblings were included to reconstruct missing parental information. TDT was performed only in families that were fully informative, by either genotyping or genotype reconstruction.

The mlod of DRB1 for the entire data set of 441 families was 4.09, with 59.9% sharing and $P = .000017$ (table 2). In addition, 58 families in which neither parent carried the DRB1*15-susceptibility allele were identified. In the DRB1*15-negative subset of families, borderline evidence of linkage was observed (mlod = 1.56; 62.7% sharing; $P = .0081$) (table 2).

We then investigated the transmission of DRB1 alleles to those individuals in the total study group who were affected (table 3). This confirmed the importance of DRB1*15 (69% rate of transmission to affected individuals, $\chi^2 = 138.3$; $P < .00001$). The transmission of DRB1*15 was also assessed in the unaffected offspring. In this sample, DRB1*15 was transmitted 140 times and was not transmitted 147 times ($\chi^2 = 0.13$; P value was not significant). The lack of a significant DRB1*1501 transmission deficit in this group may be explained by the low relative risk of DRB1*1501. All other alleles also showed a lack of distortion in transmission to unaffected siblings (data not shown).

To determine whether other DRB1 alleles influence risk, we reanalyzed the data in table 3, after removing DRB1*15 (table 4), and also examined the results of a TDT of the 58 families in which neither parent carries DRB1*15 (table 5). To do so, we calculated odds ratios (ORs) for each allele versus all other alleles and tested the significance of deviation from an OR of 1.0, by a likelihood-ratio χ^2 test for the derived 2 × 2 table. The ORs are given in tables 4 and 5. From the total study group, it is clear that, in frequency among patients, some alleles are increased and others are decreased; specifically, DRB1*14 and DRB1*11 are decreased (i.e., protective), whereas DRB1*8 is increased. The uncorrected P value for DRB1*14 is .00355, which, after a conservative (Bonferroni) correction for 13 tests, yields a corrected P value of <.05. When this allele is removed from

Table 2

Sharing of HLA-DRB1 Alleles in Siblings with MS, Determined by IBD Analysis or IBS Analysis

Analysis	Families	Sib Pairs	Sharing (%)	χ^2	z_0	z_1	z_2	λ_{locus}	mlod
IBD:									
DRB1*15 positive	167	204	59.0	11.58	.17	.48	.35	1.47	2.54
DRB1*15 negative	58	63	62.7	7.00	.14	.47	.39	1.78	1.56
Total	441	542	59.9	18.56	.16	.36	.36	1.56	4.09
IBS:									
DRB1*15 positive			58.9	.36	.17	.48	.35	...	2.53
DRB1*15 negative			62.1	2.95	.14	.47	.39	...	1.48
Total			57.0	10.39	.18	.49	.32	...	3.65

NOTE.—Families are subdivided according to presence or absence, in parents, of the MS-associated allele DRB1*15; only families in which both parents were available were stratified in this way. z values indicate the most likely frequencies of sharing zero, one, or two chromosomes. Statistical analyses were performed by the ASPEX-2.3 program.

Table 3

Transmission of DRB1 Alleles, in Total Study Group

Allele	T:NT[a]	χ^2	$P_{\text{uncorrected}}$[b]
1	83:134	11.9	.00057
4	128:136	.24	NS
7	111:139	3.14	NS
8	37:26	5.41	.02
9	8:17	6.00	.0143
10	5:5	.09	NS
11	51:91	4.25	.0392
12	8:19	2.29	NS
13	118:126	.68	NS
14	13:39	8.0	.0047
15	330:148	138.3	<.00001
16	13:17	.31	NS
17	114:120	4.84	.0278
18	1:3	.14	NS

NOTE.—Analysis is of HLA-DRB1 alleles HLA-DRB1*1–HLA-DRB1*18. HLA-DRB1*15 and HLA-DRB1*16 are splits of DR2, whereas HLA-DRB1*17 and HLA-DRB1*18 are splits of DR3.

[a] T = transmitted; NT = not transmitted.

[b] NS = not significant.

analysis and after correction for multiple testing, none of the alleles are formally significant; however, it is likely that the predispositional effect of DRB1*8 and the protective effect of DRB1*11 are also present, given the similar ORs obtained both in the total study group and in the DRB1*15-negative families (tables 4 and 5). We calculated the correlation, in ORs, between the two groups of families (after excluding DRB1*10 and DRB1*18, which were observed fewer than five times in the DRB1*15-negative families). For the remaining 11 ORs, the correlation was .79. Thus, it is possible that alleles other than DRB1*15 confer different risks, albeit not as deviant—except, possibly, for DRB1*14, which appears to have an OR of >.40—as those conferred by DRB1*15, which had an OR of 2.23. Furthermore, DRB1*17, the common split of DR3, which has occasionally been reported to be increased in patients with MS (Olerup and Hillert 1991; Masterman et al. 2000), also was increased in our TDT (tables 4 and 5); however, the ORs were not extremely elevated (e.g., 1.24 and 1.42 in the families in tables 4 and 5, respectively).

From the OR estimates (for DRB1*15 and for other alleles), it is possible to predict the amount of allele sharing expected in affected sib pairs (Risch 1987). Assuming a multiplicative model for DRB1*15, an OR of 2.23, and an allele frequency of .15, we calculated an expected HLA-allele sharing, across all families, of 53.2%. Including the other alleles increased the expected sharing only marginally and, in fact, in DRB1*15-negative families, did not predict allele sharing much in excess of 50%. Thus, it appears that the excess allele sharing of 62.7% in DRB1*15-negative families cannot be accounted for by predispositional or protective effects of other DRB1 alleles. It appears more likely that another locus, possibly in linkage disequilibrium with DRB1, is involved.

Our discovery of linkage in DRB1*15-negative families contrasts with data reported by Haines et al. (1998). In their study of an American population, evidence of linkage was obtained in families containing parental DRB1*15-bearing (i.e., DRB1*15-positive) chromosomes, whereas the non–DR1*15-bearing (i.e., DRB1*15-negative) families showed little to no evidence of linkage. However, the DRB1*15-negative families were few ($n = 19$). In addition, several extended pedigrees were included, increasing the likelihood, owing to genetic heterogeneity, that a few DRB1*15-negative families strongly influenced the outcome. It is possible that large extended pedigrees may differ from sib pairs' families, and results of comparisons therefore should be interpreted with caution.

Using the equation $z_0 = 0.25/\lambda_S$ (Risch 1987) and assuming zero recombination between HLA and MS susceptibility, we obtained a DRB1-specific λ_S of 1.56, in all families. This clearly supports the hypothesis that the HLA region confers a minority of the genetic etiology in MS (14.8%; range 7%–34.3% given a LOD-score interval of 1), since the overall λ_S is suggested to be ~20 (Sadovnick et al. 1997). The similarity between the λ_S observed in DRB1*15-positive families ($\lambda_S = 1.56$) and that observed in DRB1*15-negative families ($\lambda_S = 1.78$) indicates a similar strength of effect (table 2).

In conclusion, we have found evidence of linkage to the MHC, in the absence of DRB1*15, as well as evidence that the 62.7% sharing observed cannot be accounted for by secondary DRB1 associations (e.g.,

Table 4

Reanalysis of Total Study Group, after Removal of DRB1*15

Allele	T:NT	OR	χ^2	$P_{\text{uncorrected}}$
1	83:134	.75	3.62	NS
4	128:136	1.23	2.38	NS
7	111:139	1.01	.01	NS
8	37:26	1.84	5.59	.18
9	8:17	.59	1.57	NS
10	5:5	1.27	.14	NS
11	51:91	.68	4.39	.036
12	8:19	.53	2.45	NS
13	118:126	1.22	2.05	NS
14	13:39	.41	8.50	.00355
15	...	...	...	...
16	13:17	.97	.01	NS
17	114:120	1.24	2.29	NS
18	1:3	.42	.63	NS

NOTE.—Data are as described in the footnotes to table 3.

Table 5

Transmission of DRB1 Alleles, in Affected Offspring in DRB1*15-Negative Families

Allele	T:NT	OR	χ^2	$P_{\text{uncorrected}}$
1	34:34	1.00	.00	NS
4	36:37	.97	.02	NS
7	33:27	1.26	.70	NS
8	9:7	1.30	.26	NS
9	2:4	.50	.69	NS
10	3:1	3.03	1.06	NS
11	9:21	.40	5.30	.021
12	5:10	.49	1.76	NS
13	42:29	1.56	2.88	NS
14	1:7	.14	5.15	.024
15	…	…	…	…
16	3:5	.59	.52	NS
17	34:27	1.42	.94	NS
18	0:2	.00	2.78	NS

NOTE.—Data are as described in the footnotes to table 3.

DRB1*17, DRB1*8, DRB1*14, and DRB1*11). This is compatible with the presence of either a second locus, within the HLA region, independent of DR15,DQ6 and acting to increase MS risk or a single, as-yet-unidentified primary (i.e., non-DRB1) MS-susceptibility locus in very strong disequilibrium with the DR15-DQ6 haplotype.

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Electronic-Database Information

The accession number and URLs for data in this article are as follows:

ASPEX Package, The: Affected Sib-Pair Exclusion Mapping, ftp://lahmed.stanford.edu/pub/aspex/doc/usage.html (for the programs of Hinds and Risch's ASPEX-2.3 statistical package)
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for MS [MIM 142860])

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