

Reappraisal of HLA in Multiple Sclerosis: Close Linkage in Multiplex Families

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Abstract

Although association between multiple sclerosis (MS) and HLA-DR2,DQw6 has been well documented, family studies have not established linkage to HLA. Here we have (1) carried out an HLA-DQA1, -DQB1 association study in unrelated patients and controls, and (2) analyzed linkage between MS and HLA in multiplex families using both nonparametric and parametric methods. The subjects and families were derived from the genetically homogeneous Finnish population, and 14 of the 21 families came from a high-risk area with exceptional familial clustering of cases. In the association study, the frequencies of the alleles DQA1*0102 and DQB1*0602 (encoding DR2-associated DQw6 antigen) were significantly increased in MS patients compared to controls. In the families, we observed that the segregation of MS with DQA1*0102 and DQB1*0602 was not HLA haplotype specific, i.e., these alleles were frequently transmitted to MS relatives on different parental haplotypes. Consequently, we found strong evidence for linkage between MS and HLA only when the haplotype-independent segregation of the MS-associated alleles was controlled. This observation may partially explain the lack of linkage evidence in previous family studies. The highest LOD scores were obtained to the DQA1 locus ($LOD_{max} = 6.43$, $\theta = 0.00$). The linkage analyses suggest that both the patients' HLA haplotypes may contribute to MS susceptibility. In one of a patient's haplotypes, the susceptibility locus was closely associated with DQA1*0102 and DQB1*0602, whereas in the other haplotype no association with any of the individual candidate loci was found. These results demonstrate, for the first time, a close linkage between MS and HLA, and raise the possibility of distinct HLA-linked susceptibility genes in MS.

Key Words

Multiple sclerosis
Genetics
HLA
Linkage analysis
Family study

Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system characterized by loss of the myelin sheath and gliosis. Although both humoral and cell-mediated immune abnormalities have been observed in MS patients, their relation to the demyelination process is not understood. Both genetic and environmental factors have been implicated in the etiology of MS [1]. The evidence for genetic predisposition is mainly based on the higher concordance rate observed in monozygotic compared to dizygotic twins and other siblings [2], and on associations between MS and HLA. The most prominent HLA association has been demonstrated with DR2,DQw6 in populations of northern European ancestry [reviewed in ref. 3]. These populations show the highest incidence of the disease, whereas considerably lower incidences are found in other racial and ethnic groups [4]. The mode of inheritance has not been established, and the susceptibility is probably conferred by several interacting loci. In addition to HLA, a role for the T cell receptor β germline genes has been proposed [5, 6], although conflicting results have also been published [7, 8]. Apart from abnormalities in the immune response, defects in the myelin-forming system have been suspected in MS. Recent association [9, 10] and linkage [10] data by us and others suggest close linkage between MS and the myelin basic protein (MBP) gene, located on chromosome 18.

The highly polymorphic HLA region is located on chromosome 6 (6p21.3) covering about 4 Mb of genomic DNA. HLA genes are subdivided into class I (HLA-A, -B and -C) and class II (HLA-DR, -DQ, -DP) genes. The gene products are heterodimeric cell surface glycoproteins required for the recognition of both self and foreign antigens by T lymphocytes. A hallmark of the HLA gene complex is

linkage disequilibrium between alleles at different loci, which greatly complicates any attempt to pinpoint disease susceptibility to a specific locus.

A striking inconsistency exists between HLA association and linkage studies in MS. Despite the associations observed in several population studies, family linkage studies have not unequivocally established linkage between MS and HLA. Some affected sib-pair studies have reported increased sharing of HLA haplotypes among affected sib-pairs [11, 12], whereas others have found no deviation from random inheritance of HLA haplotypes [13–15]. LOD score analyses have provided evidence for linkage between MS and HLA [16–18], but the maximum LOD scores were obtained at recombination distances $\theta = 0.10$ – 0.20 , which are incompatible with the association maintained at the population level.

On the basis of shared HLA-DQ antigen residues in DR2,DQw6 and non-DR2, non-DQw6 patients, it has been suggested that the DQA1 and DQB1 genes coding for α and β chains of the DQ antigens, respectively, could be the primary HLA genes in MS susceptibility [19]. In the present study, therefore, we have analyzed linkage between MS and HLA-A,C,B,DR,DQA1,DQB1 haplotypes and also, for the first time, between MS and HLA candidate loci (DQA1 and DQB1) in 21 Finnish multicase families. We have also carried out an HLA-DQA1, -DQB1 association study. Since MS may be a genetically heterogeneous disease, it is advantageous to focus the analyses on a homogeneous population such as the Finns [20]. Furthermore, most of the families came from a high-risk area in western Finland demonstrating exceptional familial clustering of MS [21, 22].

Subjects and Methods

Subjects and Families

Seventy-two nonrelated Finnish patients with definite [23] MS (both relapsing-remitting and progressive) and 85 Finnish control subjects were studied in the association analysis. Twenty-one patients had living family members with MS; these were probands of the 21 families (with 2–6 MS cases per family), which were studied in the linkage analysis [for more details of the patients and families see ref. 10]. Fourteen of the families were from a high-risk area in western Finland, where up to 30% of MS cases are familial [21]. Five of the families have been partially analyzed in a previous HLA-A,C,B,DR haplotype study [12]; all the members of these families were retyped for this study. There were also 2 patients with monosymptomatic optic neuritis (ON), a condition which usually leads to MS [24], and these subjects were classified as affected in the linkage analysis. Since very mild and subclinical forms of MS may occur, attempts were made to identify such cases. Both the patients and the asymptomatic siblings of 9 families were examined using cranial magnetic resonance imaging (MRI) [25]. Three of the asymptomatic siblings showed lesions strongly suggestive of MS, and they were classified either as 'affected' or 'unknown' in the linkage analyses.

HLA Typing

Serological HLA-A,B,C and DR typing was performed in 15 of the 21 families on peripheral blood lymphocytes, using the antibody-mediated cytotoxicity test [26]. The reagents used define 14 A, 22 B, 7 C and 9 DR locus specificities. All families, nonrelated patients and controls were analyzed for DQA1 and DQB1. Six DQA1 alleles were determined using the AmpliType™ Forensic DQ α DNA Typing Kit (Perkin-Elmer Cetus) [27]. The alleles DQA1*04,*05, and *06 [28] could not be discriminated, and alleles typed as DQA1*04-05-06 were assigned as two different alleles in those families where they were present both on a DQB1*0201 and on a DQB1*0402 haplotype. The DQB1 alleles were determined using an oligotyping method essentially identical to the one described in the 11th HLA Workshop protocols [29]. The main deviation from the protocol was the use of PCR primers GLPDQB1 and GAMPDQX β [30]. The prehybridization (30 min at 56°C) and hybridization (1.5 h at 56°C) were carried out in 3.0 M tetramethylammonium chloride, 50 mM Tris HCl pH 8.0, 2 mM EDTA pH 8.0, 5 × Denhardt's solution, 0.1% SDS, and 100 μ l/ml denatured herring DNA. The oligonucleotide probes were:

DQB230N, 5'-AACGGGACCGAGCGCGTG-3'
DQB2602, 5'-CGTTATGTGACCAGATAC-3'
DQB2603, 5'-CGTCTTGTGACCAGATAC-3'
DQB2604, 5'-CGTCTTGTAAACCAGACAC-3'
DQB3701, 5'-AGGAGTACGTGCGCTTCG-3'
DQB4501, 5'-GACGTGGAGGTGTACCGG-3'
DQB5701, 5'-GCGGCCTGTTGCCGAGTA-3'
DQB5702, 5'-GCGGCCTAGCGCCGAGTA-3'
DQB5704, 5'-GCGGCCTGATGCCGAGTA-3'
DQB5705, 5'-GGCTGCCTGCCGCCGAGT-3'
DQB5707, 5'-GGCCGCCTGCCGCCGAGT-3'
DQB5708, 5'-GCGGCTTGACGCCCGAGTA-3'
with specificity for DQB1 alleles 0303+0402 (DQB2302N), 0601+0301 (DQB2602), 0602+0302+0303 (DQB2603), 0603+0604 (DQB2604), 0501+0502+0503 (DQB3701), 0301 (DQB4501), 0501+0604+0605 (DQB5701), 05032+0602+0603 (DQB5704), 0201 (DQB5705), 0302 (DQB5707), and 0401+0402 (DQB5708). The final wash was at 59°C in the presence of 3 M tetramethylammonium chloride.

Statistical Analyses

The HLA-DQA1 and -DQB1 allele frequencies in nonrelated MS patients, in the unaffected parents of the MS families, and in controls were compared using the χ^2 method. The allele frequencies were calculated by gene counting. Those unaffected parents who were typed for DQA1 and DQB1, or for whom both alleles could be deduced, were chosen for this analysis. Relative risk (RR) was calculated by the method of Woolf using the formula $(A \times D)/(B \times C)$. When one element of the equation was zero, RR was calculated with the formula of Haldane:

$$[(2A + 1)(2D + 1)] / [(2B + 1)(2C + 1)].$$

Linkage analysis between MS and HLA was carried out using both nonparametric and parametric methods. The transmission disequilibrium test was used to analyze the transmission of the MS-associated allele DQA1*0102 in the families by combining all affected and nonaffected offspring in the data [31]. In the sib-pair analysis, the sharing of HLA haplotypes was determined in affected siblings and the distortion from random segregation was evaluated using the χ^2 method. Two-point linkage analyses using the LOD score method were carried out using the computer program MLINK (version 5.10) of the LINKAGE package [32]. Equal recombination fractions were used for males and females. Autosomal dominant, intermediate and recessive models with reduced age-dependent penetrance [10] ($f = 0.05, 0.38$ and 0.76 , which cover the reasonable penetrance estimates in MS) were applied

Table 1. Comparison of HLA-DQA1 and -DQB1 alleles between patients and controls and between MS patients and unaffected parents of MS patients

| | MS ^a (n = 144) | Controls ^a (n = 170) | Parents ^a (n = 98) | χ^2 (MS vs. controls) | χ^2 (MS vs. parents) |
|-------------|------------------------------|------------------------------------|----------------------------------|----------------------------------|---------------------------------|
| <i>DQA1</i> | | | | | |
| 0101 | 21 (15) | 37 (22) | 14 (14) | 2.18 | 0.00 |
| 0102 | 53 (37) | 28 (16) | 35 (36) | 12.50* | 0.02 |
| 0103 | 3 (2) | 11 (6) | 6 (6) | 3.36 | 2.56 |
| 0201 | 6 (4) | 8 (5) | 5 (5) | 0.05 | 0.12 |
| 0301 | 24 (17) | 35 (21) | 10 (10) | 0.64 | 1.73 |
| 04-05-06 | 36 (25) | 50 (29) | 28 (29) | 0.55 | 0.28 |
| | | | | $\Sigma = 19.28$ | $\Sigma = 4.71$ |
| <i>DQB1</i> | | | | | |
| 0501 | 22 (15) | 34 (20) | 15 (15) | 0.98 | 0.00 |
| 0502 | - | 1 (0.5) | - | NI | NI |
| 0503 | - | 2 (1) | - | NI | NI |
| 0602 | 50 (35) | 22 (13) | 30 (31) | 16.13** | 0.30 |
| 0603 | 3 (2) | 11 (6) | 7 (7) | 3.36 | 3.61 |
| 0604 | 3 (2) | 6 (4) | 4 (4) | 0.57 | NI |
| 0605 | - | 1 (0.5) | - | NI | NI |
| 0201 | 20 (14) | 27 (16) | 12 (12) | 0.20 | 0.12 |
| 0301 | 14 (10) | 14 (8) | 9 (9) | 0.19 | 0.02 |
| 0302 | 17 (12) | 29 (17) | 6 (6) | 1.47 | 1.98 |
| 0303 | 1 (1) | 6 (4) | 3 (3) | 2.81 | NI |
| 0402 | 12 (8) | 17 (10) | 12 (12) | 0.24 | 0.90 |
| | | | | $\Sigma = 25.95$ | $\Sigma = 6.93$ |

Values in parentheses are percentages.

* $p < 0.0005$, ** $p < 0.0001$. NI = Not included in the χ^2 .

^a One patient and one control were query homozygotes for DQA1, and two patients for DQB1 – in these cases only one allele was included. n = Number of chromosomes.

in the linkage analyses. An intermediate model was utilized because dose effect or interaction of HLA susceptibility determinants have been demonstrated in HLA-associated diseases [33–36]. Because three genetic models and three penetrance estimates were tested, the LOD score significance level was corrected, conservatively, for 9 tests [37]: $Z_0 + \log 9 = 3 + 0.95 = 3.95$. Statistical tests for heterogeneity were carried out with the admixture test as implemented in the HOMOG program [32]. The estimate of the MS-susceptibility-gene frequency (p) was based on the prevalence data [21], $Q = 1/1000$, using the formula $f[p^2 + 2p(1-p)] = Q$ in the autosomal dominant model, $fp^2 + 1/10 \times f2p(1-p) = Q$ in the intermediate model, which allows a 10-fold increased penetrance when both chromosomes carry the susceptibility gene, and $fp^2 = Q$ in the recessive

model. For the linkage analysis, the HLA haplotypes were assigned as seven different alleles without any decrease in informativeness. Linkage disequilibrium (LD) between MS and DQA1 was adapted to the linkage analyses and was calculated using the association analysis data (table 1): the allele frequencies in the MS population were multiplied by the susceptibility-gene frequency (p), and the allele frequencies in the control population were multiplied by the wild-type gene frequency ($1-p$). When LD is allowed for the linkage analysis, the susceptibility gene is transmitted with an increased probability on HLA haplotypes carrying DQA1*0102. In the analyses not allowing for LD, the gene frequencies for HLA haplotypes, DQA1, DQB1 and DR were extrapolated from the DQA1 allele frequencies in the control population (table 1).

Results

Association with DQA1 and DQB1

The frequencies of both DQA1*0102 and DQB1*0602 were significantly increased in MS patients, when compared to controls (table 1). To gain sensitivity in the detection of other MS-associated alleles, the allele frequencies were also analyzed after exclusion of DQA1*0102- and DQB1*0602-positive subjects, but no significant differences were found between patients and controls in these comparisons (data not shown). Interestingly, when the allele frequencies in MS patients were compared to those observed in the unaffected parents of MS families, there was no significant deviation, and the frequencies of both DQA1*0102 and DQB1*0602 were almost identical (table 1).

In order to detect possible genetic heterogeneity, the group of MS patients was subdivided with respect to five determinants: (1) familial versus nonfamilial MS; (2) males versus females; (3) ON versus other presenting symptoms; (4) relapsing-remitting versus progressive (both primarily and secondarily progressive) disease course, and (5) birth place in the high-risk area versus other regions of Finland. No statistically significant differences in the DQA1 and DQB1 allele frequencies were found in these subdivisions ($p > 0.1$ in all comparisons, data not shown).

The DQA1*0102 phenotype was found in 46 (63.8%) of the patients versus 27 (31.8%) of the controls [RR = 3.8, 95% confidence interval (CI) = 1.9–7.2], and the DQB1*0602 phenotype in 42 (58.3%) of the patients versus 22 (25.9%) of the controls (RR = 4.0, 95% CI = 2.0–7.6). Genotype-wise the highest RRs were associated with DQA1*0102, DQB1*0602 homozygotes (7 patients versus 0 controls: RR = 19.6, 95% CI = 2.3–160) and DQA1*0102/04-05-06, DQB1*0602/0201 heterozygotes (10 patients versus 2 controls:

RR = 6.7, 95% CI = 1.5–21.0). The frequency of the inferred haplotype DQA1*05, DQB1*0201, included in the latter genotype, was similar in patients (11%) and in controls (12%). Spurkland et al. [19] have reported that 96% of Norwegian MS patients and 60% of controls carry one of the DQA1 alleles (0102, 0103, 0401, 0501) encoding glutamine at residue 34 and one of the specific DQB1 alleles (0602, 0603, 0604, 0302, 0303), conferring a RR of 13. In our study, the above-mentioned allele combinations were found in 67% of the patients and in 55% of the controls, the RR being only 1.6. We analyzed the combinations of a number of polymorphic amino acid residues on the hypervariable domain of DQ α and DQ β chains, encoded in cis or in trans [34]. However, we did not find any combinations (other than those observed in DQA1*0102, DQB1*0602 homozygotes and DQA1*0102/04-05-06, DQB1*0602/0201 heterozygotes), which would have conferred a higher RR than DQA1*0102 and DQB1*0602 alone (analysis not shown).

HLA Haplotypes in the MS Families

We analyzed whether there would be any predominant antigen combinations in the HLA-A,B,DR,DQA1,DQB1 haplotypes found in the familial patients, most of whom come from the high-risk area in western Finland. The MS-associated alleles DQA1*0102 and DQB1*0602 appeared to be found in heterogeneous haplotypic backgrounds. DQA1*0102 was transmitted to the patients on 15, and DQB1*0602 on 12 different HLA-A,B,DR,DQA1,DQB1 haplotypes in the 15 families typed for these HLA loci. All DR2 haplotypes carried DQA1*0102 and DQB1*0602, the common Caucasian haplotype B7,DR2,DQA1*0102, DQB1*0602 being the most frequent. This haplotype constituted 41% of all individual DR2, DQA1*0102,DQB1*0602 haplotypes, which

Table 2. Transmission of HLA-DQA1*0102 from heterozygous parents to MS patients and to unaffected siblings

| MS patients | | Unaffected siblings | |
|------------------|-------|---------------------|-------|
| DQA1*0102 | other | DQA1*0102 | other |
| 28 | 5 | 18 | 11 |
| $\chi^2 = 16.03$ | | $\chi^2 = 1.69$ | |
| $p < 0.0001$ | | $p > 0.1$ | |

$\chi^2 = (b-c)^2/(b+c)$, where b = number of transmissions of DQA1*0102, and c = number of transmissions of other alleles.

is close to the frequency observed in European Caucasians [38], and in the general population of Finland [J. Partanen, unpubl. data]. DQA1*0102 was also found on three different DRw6, DQA1*0102, DQB1*0604 haplotypes. In three families, DQA1*0102 was found in patients either on a DRw6,DQA1*0102, DQB1*0604, or on a DR2,DQA1*0102, DQB1*0602 haplotype. As to the non-DQA1*0102, non-DQB1*0602 haplotypes transmitted to the patients, they were also heterogeneous with no predominant A, B or DR locus antigens or DQA1, DQB1 alleles (data not shown).

Nonparametric Linkage Analysis

To analyze the segregation of the MS-associated allele DQA1*0102 in the families, we applied the transmission test for LD [31]. The transmission of DQA1*0102 from heterozygous parents to both affected and nonaffected offspring was analyzed. In these meioses, the transmission of DQA1*0102 to MS patients was significantly increased, while its transmission to unaffected offspring showed no deviation (table 2). Then we carried out an affected sib-pair analysis, first, in a conventional way, in families fully informative for

HLA haplotypes, and second, by excluding families with a parent homozygous for the associated allele $\overline{DQA1*0102}$, analogous to a study on diabetes-associated insulin gene polymorphisms by Julier et al. [39]. In 15 families, sibships were fully informative for HLA haplotypes. These included 10 affected sib-pairs, 3 trios, 1 quartet and 1 quintet. The remaining 6 families were not included in the sib-pair analysis because they only included affected relative-pairs other than sib-pairs (5 families), or because they were not informative (in 2 sibships only 3 haplotypes could be unequivocally assigned). Some of the parents were deceased and therefore not available for typing, but the typing of patients' children helped to assign the haplotypes in these families. Since there were many sibships with more than two affected sibs, the sib-pairs formed in these families were weighted [40]. The sharing of HLA haplotypes was slightly increased in the MS sib-pairs (table 3). When the transmission of DQA1*0102 was analyzed in each pair, we observed that of the 23 pairs (weighted), 7.59 pairs (33%) inherited this allele on different HLA haplotypes. Consequently, when the families with DQA1*0102 homozygous parents were excluded, the evidence for linkage was much more significant (table 3). When the observed haplotype sharing was compared to expected values under recessive and dominant models with different disease gene frequencies, the minimum χ^2 values were 0.05 and 1.86 for recessive and dominant models, respectively. Thus, none of the models could be rejected, although the observed values fit slightly better with a recessive model.

LOD Score Analysis

Three models of inheritance (dominant, intermediate and recessive) with three penetrance estimates (0.05, 0.38 and 0.76) were tested. Two intra-HLA recombinations were

Table 3. HLA haplotype sharing in MS sib-pairs^a

| Shared haplotypes | Observed | Random expected | Dominant expected | Recessive expected |
|---|----------|-----------------|-------------------|--------------------|
| <i>All informative families</i> | | | | |
| 2 | 9.77 | 5.75 | 9.84 | 10.93 |
| 1 | 12.07 | 11.50 | 11.29 | 9.84 |
| 0 | 1.17 | 5.75 | 1.86 | 2.21 |
| χ^2 (2 d.f.) | | 6.49 | 0.31 | 1.13 |
| p | | <0.05 | 0.86 | 0.57 |
| Gene frequency ^b | | | 0.10 | 0.45 |
| <i>As above but excluding families with a parent homozygous for DQA1*0102</i> | | | | |
| 2 | 8.33 | 3.25 | 5.98 | 8.32 |
| 1 | 4.00 | 6.50 | 6.44 | 4.16 |
| 0 | 0.67 | 3.25 | 0.59 | 0.52 |
| χ^2 (2 d.f.) | | 10.95 | 1.86 | 0.05 |
| p | | <0.005 | 0.39 | 0.98 |
| Gene frequency ^b | | | 0.05 | 0.25 |
| Subjects with abnormal MRI were classified as 'affected'. | | | | |
| ^a Sib-pairs weighted: number of pairs $s - 1$, where s = number of affected sibs. | | | | |
| ^b Disease gene frequency with which the lowest χ^2 value was obtained. | | | | |

observed in unaffected relatives, one between A and B, the other between B and DR. In these cases, the DR-DQ segment was considered as the segregating HLA haplotype. Pairwise LOD scores for linkage using either whole HLA haplotypes or DQA1 alleles as segregating markers are presented in table 4. Adapting the LD observed between MS and DQA1, we obtained significant LOD scores in the linkage analyses between MS and DQA1 (table 4). The LOD scores obtained between MS and the DQA1 locus ($\text{LOD}_{\text{max}} = 6.43$, $\theta = 0.00$, $f = 0.05$, intermediate model) were considerably higher than those obtained between MS and HLA haplotypes ($\text{LOD}_{\text{max}} = 3.48$, $\theta = 0.10$, $f = 0.05$, recessive model). The LOD scores between MS and DQA1 were also slightly higher than those between MS and DQB1 ($\text{LOD}_{\text{max}} = 5.39$, $\theta = 0.00$, $f = 0.05$,

intermediate model). Recessive and intermediate models yielded consistently higher LOD scores than dominant models. When the 3 asymptomatic siblings with abnormal MRI were classified as 'unknown', the LOD scores slightly increased (MS vs. HLA: $\text{LOD}_{\text{max}} = 3.73$, $\theta = 0.10$, $f = 0.05$, recessive model; MS vs. DQA1: $\text{LOD}_{\text{max}} = 6.68$, $\theta = 0.00$, $f = 0.05$, intermediate model) but the differences between models were retained as presented in table 4. No evidence for genetic heterogeneity was obtained with the family admixture test (HOMOG program).

To facilitate comparison with previously published linkage analyses we also performed linkage analyses without allowing for LD. In these analyses (table 4), all LOD scores were nonsignificant when HLA haplotypes were used as markers, $\text{LOD}_{\text{max}} = 1.54$ ($\theta = 0.10$),

Table 4. Pairwise LOD-scores for linkage between MS and HLA haplotypes, and between MS and $\bar{D}Q\bar{A}1$

| Penetrance | | LD allowed | | | LD not allowed | | |
|------------------|------|------------|-------|------|----------------|-------|-------|
| | | 0.00 | 0.10 | 0.20 | 0.00 | 0.10 | 0.20 |
| <i>MS - HLA</i> | | | | | | | |
| Dominant | 0.05 | 0.59 | 2.20 | 1.93 | -0.73 | 1.02 | 0.83 |
| | 0.38 | -6.27 | 0.32 | 0.80 | -7.05 | -0.50 | -0.03 |
| | 0.76 | -14.58 | -0.77 | 0.83 | -15.81 | -1.92 | -0.12 |
| Intermediate | 0.05 | 3.26 | 3.48 | 2.90 | 1.21 | 1.54 | 1.20 |
| | 0.38 | 1.11 | 2.90 | 2.70 | -0.70 | 1.11 | 1.09 |
| | 0.76 | -0.84 | 2.37 | 2.34 | -2.21 | 0.78 | 0.85 |
| Recessive | 0.05 | 1.15 | 3.21 | 2.99 | 0.10 | 1.37 | 1.23 |
| | 0.38 | -5.51 | 2.72 | 3.48 | -7.07 | 0.59 | 1.47 |
| | 0.76 | -18.14 | 1.05 | 3.18 | -19.88 | -0.63 | 1.44 |
| <i>MS - DQA1</i> | | | | | | | |
| Dominant | 0.05 | 4.93 | 4.06 | 2.92 | 3.26 | 2.50 | 1.56 |
| | 0.38 | 1.53 | 2.10 | 1.68 | 0.13 | 0.99 | 0.65 |
| | 0.76 | -3.46 | 0.57 | 1.33 | -5.30 | -0.22 | 0.34 |
| Intermediate | 0.05 | 6.43 | 5.43 | 4.06 | 3.84 | 3.01 | 1.95 |
| | 0.38 | 6.23 | 5.49 | 4.08 | 3.65 | 3.13 | 2.08 |
| | 0.76 | 5.00 | 4.82 | 3.58 | 2.62 | 2.62 | 1.74 |
| Recessive | 0.05 | 4.54 | 5.14 | 4.08 | 3.21 | 2.97 | 2.04 |
| | 0.38 | 1.59 | 6.11 | 5.36 | 0.17 | 3.33 | 2.72 |
| | 0.76 | -4.87 | 5.48 | 5.35 | -6.35 | 2.82 | 2.85 |

The level of significance is corrected for 9 tests [37]: $Z_0 = 3 + \log 9 = 3.95$. Significant maximum LOD scores are italicized. Subjects with abnormal MRI and ON are classified as 'affected'.

and the LOD scores were mostly negative at $\theta = 0.00$. This LOD score profile is similar to many previous reports [see especially ref. 18]. In the analyses between MS and DQA1 the highest LOD scores peaked at $\theta = 0.00$, and were very close to the conservative significance level of 3.95 ($\text{LOD}_{\max} = 3.95$, $\theta = 0.00$, when subjects with abnormal MRI were classified as 'unknown').

Discussion

The finding of an increased frequency of HLA-DQA1*0102 and DQB1*0602 (encoding the DR2-associated DQw6 antigen) in MS patients appears to be remarkably consistent between different studies in populations of northern European descent [3, 19, 41]. Other findings have been controversial. Paradoxically, family studies have not established linkage between MS and HLA despite this well-documented association. The increased fre-

quency of specific DQA1 and DQB1 allele combinations observed in Norwegian patients has not been confirmed in a Swedish [3], a British [41], or our present study. Mainly on the basis of association studies, three major theories have emerged to explain the connection between MS and HLA: (1) a rare, as yet unidentified, susceptibility gene exists in the HLA region in linkage disequilibrium with DR2, DQW6; (2) susceptibility is conferred by certain allele combinations in specific haplotypes, such as B7,DR2,DQW6, not only by one locus, and (3) the DR or DQ loci are themselves relevant in MS susceptibility. The two latter hypotheses are not mutually exclusive. Linkage strategies utilized to date based on HLA- A, B or HLA-A,B,DR haplotype segregation are most powerful on the assumption of a rare susceptibility gene and have satisfactorily tested only the first hypothesis, with controversial results [11–18]. Several factors may have confounded previous analyses, including biased assumptions of the susceptibility-gene frequency [15, 42] and locus heterogeneity. An additional crucial open question is the contribution of individual HLA candidate loci in MS, which has not been addressed in previous family studies. In the results of the present study, there are several overlapping aspects to be discussed.

First, in the linkage analyses, the strongest evidence for linkage was obtained when the haplotype-independent segregation of the MS-associated alleles was controlled. This was accomplished in the nonparametric analyses by excluding families in which a parent was homozygous for DQA1*0102 (and in most cases also for DQB1*0602 and DR2), and in the LOD score analysis by using DQA1 and DQB1 loci as segregating markers. It is of note that the LOD score analysis did not provide statistically significant evidence for linkage on the basis of haplotype segregation when not allowing for LD ($LOD_{max} = 1.54, \theta =$

0.10). In these analyses, the negative contribution to the LOD scores came mainly from families in which the MS relatives inherited different HLA haplotypes carrying the MS-associated alleles. The haplotype-independent transmission of DR2, DQB1*0602 and, most prominently, of DQA1*0102 in this data set, may partially explain the absence of close linkage on the basis of previous haplotype segregation data [13–18]. This finding emphasizes the importance of estimating LD between the disease and specific alleles by performing association analysis along with linkage analysis. This is probably especially important in polygenic diseases, in which the susceptibility gene frequencies may be relatively high. An analogous situation has been described by Julier et al. [39] who studied the segregation of insulin IGF2 haplotypes in insulin-dependent diabetes mellitus (IDDM) families. They found that when a parent carried fully informative haplotypes but was homozygous for a candidate site, both haplotypes were equally transmitted to the affected offspring providing no evidence for linkage. Evidence for linkage was obtained only after several family selection criteria were applied, including parental heterozygosity for candidate sites.

Second, the MS-associated alleles were present on a heterogeneous group of HLA haplotypes, which showed variation not only at the A and B loci but in some cases also at the DR and DQB1 loci. There were MS relatives, who inherited DQA1*0102 on different parental chromosomes, but did not carry the same DQB1 alleles and DR antigens. Whether this indicates that DQA1*0102 is an HLA-linked susceptibility factor by itself, rather than being in linkage disequilibrium with an allele at another locus, cannot be solved in this context due to the small number of observations. DQA1*0102 was most frequently found on the haplotype DR2,DQA1*0102,

DQB1*0602, and there were only three exceptions to this in our families (DRw6, DQA1*0102, DQB1*0604 haplotype). The question of the primary role of the individual alleles on the DR2, DQA1*0102, DQB1*0602 haplotype has been very difficult to clarify in association studies. Therefore, family studies, as here, and the identification of susceptibility haplotypes with only one common denominator may provide an alternative approach to this question. The overall heterogeneity of HLA haplotypes in these families demonstrated that the HLA-linked susceptibility was not restricted to only a few haplotypes such as B7, DR2, DQA1*0102, DQB1*0602. This divergence of HLA haplotypes among patients conforms with the results of Hauser et al. [43], and suggests that the DR2, DQA1*0102, DQB1*0602-associated susceptibility determinants in MS may be quite common instead of being present only on a limited set of A, B, DR, DQA1, DQB1 haplotypes.

Third, although the mode of inheritance of the HLA-linked susceptibility determinants cannot be clearly defined, both the sib-pair analysis, and the LOD score analysis, to some extent, suggest that both HLA chromosomes of an individual contribute to MS susceptibility. In the sib-pair analysis, an excess of MS sib-pairs shared both haplotypes when the DQA1*0102 homozygous parents were excluded. In the LOD score analysis, the recessive and intermediate models consistently provided the strongest evidence for linkage. DQA1*0102 and DQB1*0602 were almost invariably present on one HLA haplotype in the familial patients, while in the case of the patient's other haplotype not only the DQA1 and DQB1 alleles but the whole haplotype from A to DQB1 was typically the same among the patients within a family. No predominant DQA1 or DQB1 alleles were present on the other haplotype, a situation also observed in the nonfamilial patients and

which could not be explained by any preferential DQ α , DQ β chain amino acid combinations as proposed by Spurkland et al. [19] for MS and, analogously, by Khalil et al. [34] for IDDM. These observations do not support any simple recessive one-locus model for the HLA susceptibility factors, and moreover, population data indicate that the DR2, DQA1*0102, DQB1*0602 haplotype has a dominant effect [3, 15]. The question of the contribution of the other haplotype is intriguing, and based on our data, another susceptibility locus, distinct from the DQA1*0102-associated locus, may exist. The heterogeneous DQA1, DQB1 genotypes and the absence of any MS-associated DQ alleles other than DQA1*0102 and DQB1*0602 indicate that the other putative susceptibility locus does not show linkage disequilibrium with DQA1 or DQB1. Additional complexity is provided by the DQA1*0102, DQB1*0602 homozygotes since this genotype, although relatively infrequent (10%) among the patients, conferred the highest RR for MS, which may indicate a dose effect.

Available data do not provide many clues for the localization of the other putative HLA-linked susceptibility locus, given the lack of any consistent associations other than those with DR2, DQA1*0102 and DQB1*0602. The increased frequency of DQA1*0102/04-05-06, DQB1*0602/0201 heterozygous patients in our series suggests a possible role for the DQA1*05, DQB1*0201 haplotype (DR3 associated) in MS susceptibility. This haplotype has an increased frequency among patients in several HLA-associated diseases, and slightly increased frequencies of this haplotype have also been occasionally observed in MS patients [3]. In our series, its frequency was increased only in combination with DQA1*0102, DQB1*0602. In a few HLA-class-II-associated diseases it has been suggested that both HLA chromosomes contrib-

ute to the disease, as in the case of IDDM, celiac disease and systemic lupus erythematosus (SLE) [32–35]. In IDDM and in celiac disease, specific α and β chain combinations of the DQ $\alpha\beta$ heterodimer encoded in cis or in trans have been proposed, and an interplay between distinct loci within HLA has been suggested in SLE (DQ and the complement genes) and in celiac disease (DQ and DP).

Finally, it is of interest that in the same set of Finnish families, MS shows linkage both to HLA on chromosome 6 and to the MBP gene on chromosome 18 [10]. Further investigation on the interaction of the genes within HLA, and their joint inheritance with the MBP-linked predisposing gene and perhaps with still other genes, such as those coding for T cell receptors which functionally interact with HLA antigens, may help to identify the

components of genetic susceptibility in MS. Such an approach will eventually provide insight into the pathological mechanisms in MS, yield tools to identify high-risk individuals for therapeutic or preventive strategies, and aid the development of a genetically susceptible animal model.

Acknowledgements

We are indebted to the patients and their families for their assistance and cooperation, to Ms. Kaarina Lindberg for her technical assistance, and to Professor Kimmo Aho, MD, for his valuable help in the preparation of the manuscript. This study was supported by grants from the Paulo Foundation, the Sigrid Juselius Foundation, the Orion Inc. Research Foundation, the Finnish Neurology Foundation, the Finnish Medical Society Duodecim, the Oskar Öflund Foundation, and the University of Helsinki 350 Years Foundation.

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