Linkage analysis conditional on HLA status in a large North American pedigree supports the presence of a multiple sclerosis susceptibility locus on chromosome 12p12

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Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system with a probable immune-mediated pathogenesis. Strong evidence supports the hypothesis that MS is determined by genetic and environmental factors, but these factors remain largely undefined. The genetic component is suggested by a higher concordance rate in monozygotic (28%) versus dizygotic (5%) twins as well as familial recurrence risk. Several studies have shown association of MS with the histocompatibility leukocyte antigen (HLA) class II region, specifically DR15, DQ6. However, there is no convincing evidence of a common susceptibility locus. We have identified a pedigree of Pennsylvania Dutch extraction, in which MS segregates with an autosomal dominant inheritance pattern. We have collected blood samples from 18 family members, seven of whom show typical signs of MS lesions by magnetic resonance imaging. The 18 individuals were serotyped for HLA class I and II and analyzed by a genome-wide screen for linkage analysis. We have found evidence for suggestive linkage to markers on 12p12 with a maximum multipoint LOD score of 2.71, conditional on the presence of DR15, DQ6. Contingency table analysis showed that all MS affected individuals have both the DR15, DQ6 allele and the 12p12 haplotype whereas the unaffected individuals have either one or neither of these markers (P = 0.00011). Our data suggests that both HLA DR15, DQ6 and a novel locus on chromosome 12p12 may be necessary for development of MS in this family.

INTRODUCTION

Multiple sclerosis (MS) is presumed to be an autoimmune mediated demyelinating disease of the central nervous system (CNS) caused by interactions between genetic and environmental factors (1,2). Genetic factors are known to be important in the development of this disorder and several lines of evidence suggest that the mode of transmission is complex including multiple, possibly interacting genes, along with genetic heterogeneity and incomplete penetrance. The importance of genetic factors in MS has been demonstrated by familial, twin, adoption and half-sib studies (3–5). Familial aggregation occurs in MS with an increased recurrence risk in siblings of 20–40 times compared to the general population prevalence of 0.1%. A higher rate of concordance for MS has been consistently observed in monozygotic (28%) versus dizygotic (5%) twins in different populations (6). Additionally, studies on adoptees have shown an increased risk of MS only in biological relatives of adopted MS patients. Half-siblings have a reduced risk compared with that of full siblings (5). Several linkage analysis and association studies have shown that susceptibility to MS is associated with the histocompatibility leukocyte antigen genes (HLA) class II region, specifically DR15, DQ6, although several genome-wide screens have not yet identified any consistent susceptibility gene with a major influence on familial risk of MS (7–11).

In order to investigate the genetics of MS we studied an unusual pedigree involving multiple affected individuals identified over three generations. Our results suggest that HLA...
DR15, DQ6 and a novel locus on chromosome 12p12 are both necessary for development of MS in this family.

RESULTS

HLA class I and II serotyping, genome-wide screening and linkage analysis

All 18 individuals in the present study were HLA class I and II serotyped. The HLA-A, B, DR and DQ haplotypes are illustrated in Figure 1. All seven MS patients (132, 130, 122, 125, 123, 121) had the DR15, DQ6 allele. The DR15, DQ6 allele was also found in three family members (134, 133 and 124) exhibiting neither clinical nor magnetic resonance imaging (MRI) (134 and 124) evidence of MS. This observation suggested that presence of DR15, DQ6 may be necessary but not sufficient for development of MS in this family.

Based on this finding we hypothesized that an additional genetic locus was necessary to explain the occurrence of the disease in this family. This second locus would be shared by all affected individuals, and could be present in the DR15, DQ6 negative unaffected ones, but not in the DR15, DQ6 positive unaffected individuals. In order to identify this new genetic locus, we performed LOD score linkage analysis using a parametric model and a screening mapping set as described in Materials and Methods. Our analysis conditional on HLA status resulted in maximum LOD scores (MLS) of 2.25, 2.48 and 2.28 at a recombination fraction of 0 from markers D12S373, D12S1042 and D12S1292, which are located at relative distances of 12.64 and 2.2 cM on chromosome 12p12. Under the same parametric model, all other markers gave LOD scores of <1.6.

Results of two-point linkage analysis obtained after typing additional markers in the 12p12 region are shown in Table 1.
Multipoint linkage analysis including markers D12S373, D12S1066, GATA91H01, D12S1034, D12S1042 and D12S1292 gave a MLS of 2.71 over the entire interval between D12S1034 and D12S1292. All the available individuals from this family were analyzed. A simulated linkage analysis by means of the SLINK program (12) showed that this is the MLS achievable in this family with the available individuals and under the specific genetic model we hypothesized.

By comparing the haplotypes in all affected and unaffected individuals, markers D12S1715 (recombinant in affected individual 132) and GATA63D01 (recombinant in unaffected individual 134), located 18 cM apart, can be identified as the distal and proximal flanking markers of our candidate locus region on 12p12 (Fig. 1).

Significance of association between HLA DR15, DQ6/12p12 and MS

The significance of the association of MS with both the HLA DR15 allele and the chromosome 12p12 haplotype in this family was further tested by contingency table analysis. All seven MS affected individuals have both the DR15 allele and the chromosome 12 haplotype (Table 2). In contrast, of the nine at-risk unaffected individuals, three (134, 133 and 124) have the DR15 allele but not the chromosome 12 haplotype, three (135, 137 and 138) have the chromosome 12 haplotype but not the DR15 allele, and the remaining three (139, 140 and 168) have neither one. This results in a chi-square of 16 and a \( P \)-value of 0.0001 by simulation analysis (13).

**DISCUSSION**

The major histocompatibility complex (MHC) has been consistently associated with susceptibility to MS (7–11). These studies have focused primarily on Caucasians of Northern European descent where predisposition to sporadic MS has been associated with the HLA-A3, B7, DR2 extended haplotype (14). Allelic association has been found specifically with haplotype DR15, DQ6 (15,16). Using the transmission-disequilibrium test approach, significant allelic association to this haplotype was also found in familial cases, confirming the existing biological association with a locus in this region and MS (9,15,17).

All seven affected individuals in our family show the DR15, DQ6 haplotype. However, the same haplotype is also present in three unaffected individuals, suggesting that the association to DR15, DQ6 alone is not sufficient to explain the occurrence of the disease, and that additional genetic loci may be involved in susceptibility to MS in this family. Our parametric linkage analysis supports the hypothesis of linkage to the chromosome 12p region with a multipoint LOD score of 2.71, conditional on the presence of the HLA DR15 haplotype. In particular, all affected individuals shared not only the DR15, DQ6 haplotype but also an additional haplotype defined by several markers located on chromosome 12p. None of the nine unaffected individuals has the same two-locus genotype, suggesting a variable expressivity from a common genetic cause. Such heterogeneity in disease state within a family is not unusual since even in monozygotic twins concordant for MS markedly different phenotypes can be found.

Our data are consistent with several genetic models (18) recently presented involving interactions between HLA and additional as yet unidentified genes. Among the genes proposed to play a role in MS pathogenesis, CD4 is located on 12p but it is not within the critical interval that we have identified. A potentially interesting gene in the critical region

**Table 1. Results of two-point linkage analysis for chromosome 12 markers**

<table>
<thead>
<tr>
<th>Marker</th>
<th>cM</th>
<th>LOD scores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( \theta = 0.0 )</td>
</tr>
<tr>
<td>D12S1303</td>
<td>3.04</td>
<td>-3.49</td>
</tr>
<tr>
<td>D12S1715</td>
<td>0.54</td>
<td>-2.28</td>
</tr>
<tr>
<td>D12S373</td>
<td>5.49</td>
<td>2.25</td>
</tr>
<tr>
<td>D12S1066</td>
<td>0.55</td>
<td>0.76</td>
</tr>
<tr>
<td>GATA91H01</td>
<td>1.28</td>
<td>1.69</td>
</tr>
<tr>
<td>D12S1591</td>
<td>2.57</td>
<td>0.21</td>
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<td>D12S1034</td>
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<td>2.41</td>
</tr>
<tr>
<td>D12S1042</td>
<td>2.2</td>
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</tr>
<tr>
<td>D12S1292</td>
<td>2.74</td>
<td>2.28</td>
</tr>
<tr>
<td>GATA63D01</td>
<td>1.86</td>
<td>-( \infty )</td>
</tr>
<tr>
<td>GATA123B12</td>
<td>5.84</td>
<td>-( \infty )</td>
</tr>
<tr>
<td>D12S85</td>
<td>-( \infty )</td>
<td>-0.29</td>
</tr>
</tbody>
</table>

*Genetic distance in cM from next marker according to Marshfield Clinic’s Center for Medical Genetics sex-average map (http://research.marshfieldclinic.org/genetics/).
is GD3 synthase gene SIAT8 \((\text{Sialytransferase8}(\alpha-N\text{-acetylneuramininate}:\alpha-2,8\text{-sialytransferase, GD3 synthase})A)\). GD3 synthase is part of the sialyltransferase family (19,20) characterized by having the sialyl motif and a key regulatory domain that controls the ganglioside biosynthesis pathway. It is well known that gangliosides are neuronal ligands for myelin-associated glycoprotein (21,22).

Mice lacking this key enzyme in complex ganglioside biosynthesis exhibit decreased CNS myelination and axonal degeneration (23). It is intriguing that syntenic regions between human chromosome 12p12, rat chromosome 4 and mouse chromosome 6 (C6) exist, thus underlining the presence of non-MHC loci controlling inflammatory disease. A recent study of MS in a Swedish population points to linkage with the syntenic region to the rat Oia2 locus and tests several markers from the human syntenic region, which corresponds to a segment mapping to 12p13–12 (24) which is consistent with our finding of linkage on 12p12. The C6 region in mice is known to be associated with experimental autoimmune encephalomyelitis (25). The clustering of loci in both experimental animal models and human disease suggests a common gene or multiple genes within an interval influencing autoimmune disease susceptibility.

A previous linkage analysis study done on a British population (10) identified 12p as one of the loci having a MLS at or above the level associated with a nominal significance of 5%. Taken together these results point to a new line of research focused on genes located on 12p12 as key regulators of this pathogenic autoimmune response.

### MATERIALS AND METHODS

**Patient collection**

We studied 18 members from a three-generation family (Figure 1), seven of whom (five females and two males) were diagnosed with MS [six clinically definite (CDMS), 132, 130, 122, 125, 123, 121; one clinically probable (CPMS), 131] after neurologic evaluation (history, neurological examination, laboratory testing). The phenotypic expression and temporal profile of the disease are quite varied with regard to age of onset (24–33 years), clinical course (four patients are relapsing-remitting, two are secondary progressive, one is primary progressive) and anatomical location of the lesions [cerebral (six patients), optic nerve (five), cerebellum (five), brain stem (three) and spinal cord (six)]. All had abnormal MRI scans and four of them also had abnormal cerebrospinal fluid (CSF) profiles consistent with MS (26) (Table 2). A pertinent history and neurological examination performed on the unaffected family members showed that they did not...
exhibit any signs or symptoms that could be associated with MS. Additionally, MRI scans performed on four of the unaffected family members (124, 134, 135 and 137) revealed that they did not have MS.

HLA serotyping analysis

HLA typing was performed using the carboxyfluorescein diacetate (CFDA) lymphoctyotoxicity method on nylon wool column purified T and B cell suspensions (27). CFDA-labeled T and B cells were dispensed into each well of either a HLA Class I or II typing tray, respectively (One Lambda, Canoga Park, CA). Complement was added, followed by an ethidium bromide solution and the trays were scored using a fluorescence microscope. One hundred HLA antigens defined at the 11th Histocompatibility Workshop were studied: 23-A, 45-B, 8-C, 15-DR and 9-DQ (28).

Genotyping analysis

Blood was collected under an approved Institutional Review Board protocol, in accordance with regulations mandated by the Department of Health and Human Services. Genomic DNA was extracted from whole blood or cell lines (lymphoblast or fibroblast) with the QIAGENE DNA Isolation Kit according to the manufacturer’s protocol. Amplifications were performed on HYBAID Omnigene thermocyclers under the following conditions: 95°C for 2 min, 1 cycle; 94°C for 45 s, 57°C for 45 s, 72°C for 60 s, 30 cycles; 72°C for 60 s, 1 cycle. The genome-wide screen was performed using 613 markers from Marshfield Center of Medical Genetics maps (http://research.marshfieldclinic.org/genetics/) that we arranged based on a single chromosome scan set. The markers consist of short-tandem-repeats with an average heterozygosity of 0.76. The largest interval between two adjacent markers is 16.84 cM, on chromosome 17, and the average sex-equal spacing between markers is 5.6 cM.

Statistical analysis

Linkage analysis was performed assuming autosomal dominant transmission with a disease allele frequency of 0.001 and two liability classes defined conditional on HLA haplotype. In the first class, we included all individuals who were carriers of the HLA DR15 haplotype. In this class, we assumed complete penetrance of the disease (probability of being affected given presence of the disease allele = 1). In the other liability class, we included all individuals who did not have the HLA DR15 haplotype, and we assumed 0 penetrance (probability of being affected given presence of the disease allele = 0). In both cases, we also assumed the absence of phenocopies. Individuals who were not available for phenotyping were included in the first (complete penetrance) class. Two-point and multipoint LOD score analyses were carried out using the MLINK program of the LINKAGE package (29) and the VITESSE program (30), respectively.

Significance of the association between the disease and the DR15 allele and chromosome 12 haplotype was evaluated by means of the CLUMP program (13). In this way, the significance of the chi-square test was assessed using a Monte Carlo approach, by performing 500 000 simulations to generate tables having the same marginal totals as the one we observed, and counting the number of times that a chi-square value equal or greater than the observed one was achieved by the randomly simulated data. This means that the significance levels assigned are unbiased and that no special account needs to be taken of continuity corrections or small expected values.

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