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A Second-Generation Genomic Screen for Multiple Sclerosis

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Multiple sclerosis (MS) is a debilitating neuroimmunological and neurodegenerative disorder. Despite substantial evidence for polygenic inheritance of the disease, the major histocompatibility complex is the only region that clearly and consistently demonstrates linkage and association in MS studies. The goal of this study was to identify additional chromosomal regions that harbor susceptibility genes for MS. With a panel of 390 microsatellite markers genotyped in 245 U.S. and French multiplex families (456 affected relative pairs), this is the largest genomic screen for MS conducted to date. Four regions met both of our primary criteria for further interest (heterogeneity LOD [HLOD] and *Z* scores >2.0): 1q (HLOD = 2.17; *Z* = 3.38), 6p (HLOD = 4.21; *Z* = 2.26), 9q (HLOD; *Z* = 2.71), and 16p (HLOD = 2.64; *Z* = 2.05). Two additional regions met only the *Z* score criterion: 3q (*Z* = 2.39) and 5q (*Z* = 2.17). Further examination of the data by country (United States vs. France) identified one additional region demonstrating suggestive linkage in the U.S. subset (18p [HLOD = 2.39]) and two additional regions generating suggestive linkage in the French subset (1p [HLOD = 2.08] and 22q [HLOD = 2.06]). Examination of the data by human leukocyte antigen (HLA)–DR2 stratification identified four additional regions demonstrating suggestive linkage: 2q (HLOD = 3.09 in the U.S. DR2 − families), 6q (HLOD = 3.10 in the French DR2 − families), 13q (HLOD = 2.32 in all DR2+ families and HLOD = 2.17 in the U.S. DR2+ families), and 16q (HLOD = 2.32 in all DR2+ families and HLOD = 2.13 in the U.S. DR2+ families). These data suggest several regions that warrant further investigation in the search for MS susceptibility genes.

Introduction

Multiple sclerosis (MS [MIM 126200]) is a debilitating neuroimmunological and neurodegenerative disorder. Plaques and lesions formed in the CNS of patients with the disease impair saltatory conduction along axons that is necessary for normal functioning of nerve impulses. MS is a clinically heterogeneous disease with a range of symptoms that present as episodic or progressive neurological impairments. The disease is common in young adults and affects females two to three times more frequently than males. Despite the devastating impairment and deterioration seen in MS, lifespan of affected individuals is only slightly shortened—creating a significant impact on the quality of life for patients and on national healthcare systems.

Although little is known about the underlying etiology of the disease, MS is physiologically an inflammatory disorder resulting from an autoimmune response directed against CNS antigens, particularly myelin proteins. MS exhibits several characteristics common to autoimmune disorders, including polygenic inheritance, evidence of disease-associated environmental exposure, and partial susceptibility conferred by a human leukocyte antigen (HLA)–associated gene (Barcellos et al. 2002).

Perhaps it is not surprising that the strongest and most consistent finding for linkage to MS is at chromosome 6p21, the location of the major histocompatibility complex (MHC) containing HLA. To date, the MHC is the only region that clearly and consistently demonstrates linkage and association with MS (Haines et al. 1996, 1998; Sawcer et al. 1996; Yaouanq et al. 1997). The MHC has been estimated to account for 10%–50% of the genetic component of MS susceptibility, at least

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Table 1

Description of the Data Set

Families	No. of Families	No. of Affected			No. of Families Designated	
		Individuals	Sib Pairs	Other Relative Pairs	HLA-DR2+	HLA-DR2−
U.S.	151	383	242	88	83	31
French	94	204	102	24	28	35
All	245	587	344	112	111	66

Note.—Families were designated HLA-DR2+ if every affected individual carried at least one HLA-DR2 allele and were designated HLA-DR2− if no affected individuals carried an HLA-DR2 allele.

in white populations of northern European descent (Haines et al. 1998; Dyment et al. 2004). It appears that the association with the HLA-DR2 allele largely explains this linkage signal (Haines et al. 1998; Barcellos et al. 2002). However, the exact mechanism by which a gene or genes in the MHC increase disease risk has yet to be determined.

The clinical heterogeneity and complex etiology of MS have been confounding factors in studies of the disease. Despite these complexities, it is clear that genes play a vital role in the susceptibility to MS. In an attempt to identify regions that harbor MS loci, numerous research groups have conducted genomic screens (Ebers et al. 1996; Haines et al. 1996; Sawcer et al. 1996,2002; Kuokkanen et al. 1997; Broadley et al. 2001; Coraddu et al. 2001; Akesson et al. 2002; Ban et al. 2002; Eraksoy et al. 2003; Hensiek et al. 2003; Dyment et al. 2004). These studies have identified >60 genomic regions with potential disease loci, but little overlap has been seen between studies. These results suggest that the existence of genes with strong individual effects is unlikely.

One major complication for linkage analysis is genetic heterogeneity—the presence of different loci that produce similar disease phenotypes. Underlying genetic heterogeneity in MS has likely masked the effects of true loci in many linkage studies. An approach that attempts to overcome loss of power due to heterogeneity is conditional analysis with covariates. By accounting for regions of known linkage (e.g., the MHC) with stratification or weighting schemes, conditional analysis can identify additional regions of interest. Conditional analysis can also provide evidence for interactive effects of loci, potentially providing increased power for detection of epistatic genes in complex diseases such as MS.

The goal of this study was to identify chromosomal regions that harbor MS susceptibility genes. We conducted a collaborative genomic screen using an expanded data set of U.S. and French families. With 245 multiplex families (456 affected relative pairs) and 390 microsatellite markers, this is the largest genomic screen for MS conducted to date.

Material and Methods

Families

The data set used in this study consisted of U.S. families from a genomic screen published elsewhere (Haines et al. 1996), 66 additional U.S. families, and 94 French families. The full data set included 245 multiplex families, consisting of 587 affected individuals, 344 affected sib pairs, 112 other affected relative pairs, and a total of 1,085 samples (table 1).

U.S. families were ascertained by the University of California at San Francisco (UCSF). All affected U.S. family members were examined or had their medical records reviewed by one of the authors (S.L.H.). Families were extended through all affected first-degree relatives, if possible. French families were collected through a national network of university and community hospitals and private practitioners. All affected French family members were examined by a clinician from one of three centers (Paris, Rennes, or Toulouse).

All protocols were approved by the appropriate institutional review boards, and all individuals provided informed consent before participating in the study. Positive family histories were investigated by direct contact with other family members, by request for medical records, and by clinical examination, laboratory testing, or paraclincial studies (magnetic resonance imaging [MRI] scanning and evoked-response testing). Consistent and stringent clinical criteria were applied as described elsewhere (Goodkin et al. 1991; Haines et al. 1996). Individuals were placed into one of four categories: definite MS, probable MS, possible MS, and no evidence of MS. Only individuals categorized as definite MS cases were considered as affected in the analysis.

To account for possible heterogeneity, the data were examined for differences by country (United States vs. France) and HLA-DR2 genotype (HLA-DR2+ vs. HLA-DR2−). Families were designated HLA-DR2+ if every affected individual carried at least one HLA-DR2 allele and were designated HLA-DR2− if no affected individuals carried an HLA-DR2 allele (table 1).

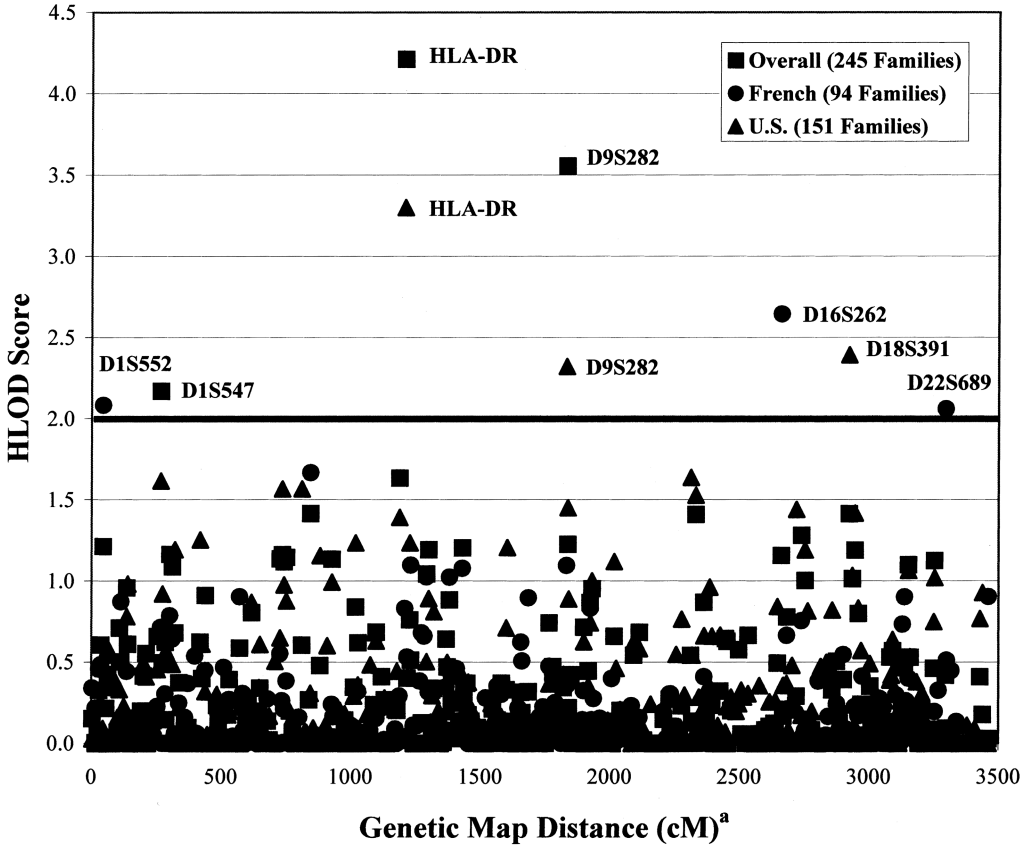

Figure 1 Two-point HLOD scores for the overall data set. [a]Marshfield genetic map (see Marshfield Web site).

Molecular Analysis

After informed consent was obtained, blood samples were collected from each individual. Genomic DNA was extracted from blood, by use of standard procedures as described elsewhere (Vance 1998). All samples were coded and stored at 4°C until used.

Marker primer sequences were obtained from the Genome Database (see Genome Database Web site) or were designed with Primer3 software (see Primer3 Web site) and were synthesized by Invitrogen Life Technologies. Amplification was performed in a PCR Express machine (ThermoHybaid) under the following conditions: 94°C for 4 min; 94°C for 15 s, annealing temperature for 30 s, and 72°C for 45 s (35 cycles); and 72°C for 4 min. PCR products were denatured for 3 min at 95°C and were run on a 6% polyacrylamide gel (Sequagel-6 [National Diagnostics]) for ~1 h at 75 W. Gels were stained with a SybrGold rinse (Molecular Probes) and were scanned with the Hitachi Biosystems FMBIOII laser scanner. Genotypes for HLA-DR in the U.S. families were determined at UCSF by use of nonradioactive PCR-sequence–specific oligonucleotide probes (PCR-SSOP). Genotypes for HLA-DR in the French families were determined using reverse dot blot hybridization.

Marker order and intermarker distance were obtained from linkage reference maps (see Marshfield and deCODE Web sites). The average intermarker distance for the screen was <10 cM. The Vanderbilt and Duke laboratories each genotyped a subset of markers on the complete set of DNA samples. Laboratory personnel were blinded to pedigree structure, affected status, and location of quality-control samples. Duplicate quality-control samples (three unblinded CEPH individuals and four blinded controls) were placed both within and across plates, and equivalent genotypes were required to ensure accurate genotyping.

Allele frequencies were calculated from the genotyped founders in each family. Hardy-Weinberg calculations were performed for each marker, and Mendelian inconsistencies were identified using PedCheck (O'Connell and Weeks 1998). Suspect genotypes were reread and/or rerun. All microsatellites were required to have >85% of possible genotypes. Verification of relationships between pairs of samples within families was performed using RELPAIR (Epstein et al. 2000). Markers and

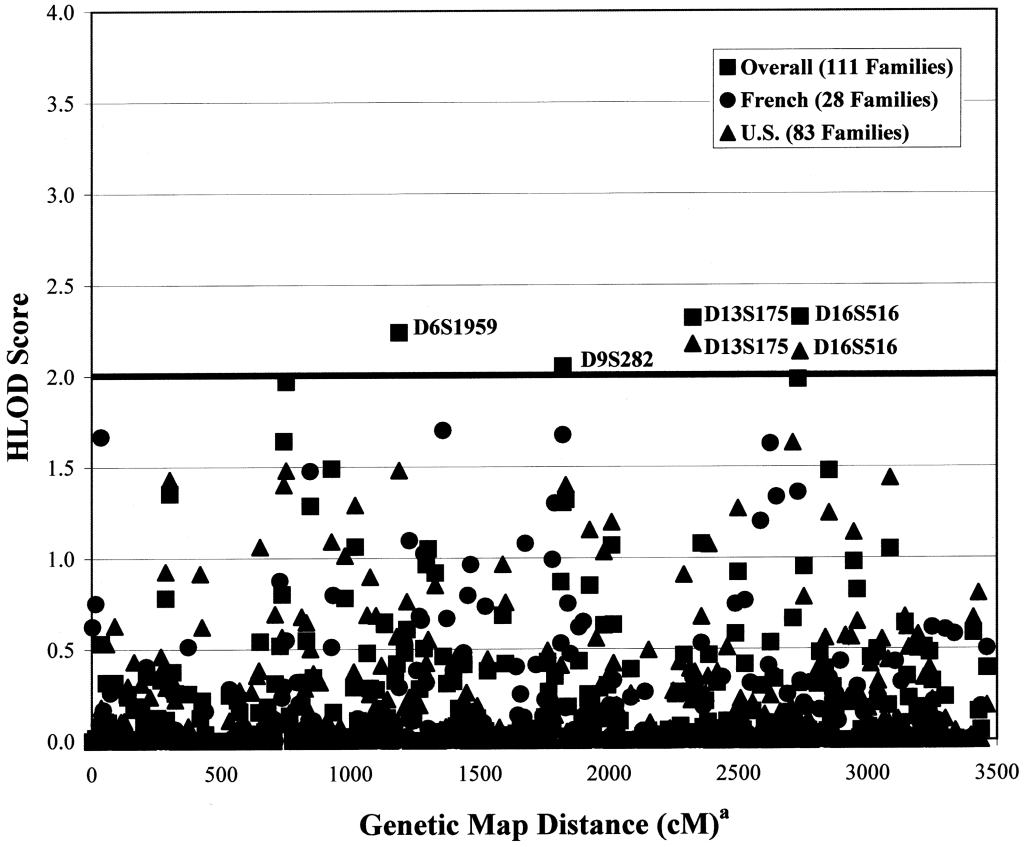

Figure 2 Two-point HLOD scores for the HLA-DR2+ subsets. [a]Marshfield genetic map (see Marshfield Web site).

samples failing to pass quality-control measures were dropped from the analysis.

Statistical Analysis

Both model-based and model-free analyses were performed. Parametric (model-based) analyses were conducted using autosomal dominant and autosomal recessive models with disease-allele frequencies of 0.01 and 0.20, respectively, to model a common susceptibility allele. A penetrance value of 0.95 was used for both dominant and recessive models, and individuals with no evidence of MS were coded as normal for these analyses. Two-point LOD scores were calculated by FASTLINK (Cottingham et al. 1993; Schaffer et al. 1994), and heterogeneity LOD (HLOD) scores were calculated by HOMOG (Smith 1963; Ott 1986). Two-point HLOD scores for the overall data set, the HLA-DR2+ subsets, and the HLA-DR2− subsets are provided in figures 1, 2, and 3, respectively.

Multipoint model-free analyses were performed using the "score pairs" option and the exponential model (Kong and Cox 1997) in Allegro (Gudbjartsson et al. 2000). Multipoint results are reported as *Z* scores. Because the HLA-DR2 allele is known to be associated with MS, the interaction between HLA and other regions was tested by correlation between family nonparametric linkage (NPL) values in the 236 nuclear families with at least one affected sib pair.

The criterion to consider a region as interesting was at least one marker with a maximum HLOD >2.0 or a multipoint *Z* score >2.0. Because other research groups have advocated the use of more liberal criteria in genomic screens, we also report markers generating HLOD or *Z* scores >1.5 and have made the complete set of scores available at the Vanderbilt Center for Human Genetics Research Web site.

We performed a number of statistical tests on microsatellite markers, disease models, and subsets, raising concern about multiple comparisons. The level of correction necessary to account for these factors is a topic of substantial debate, and it is not currently clear how to select an appropriate level of corrections for this study design. We therefore have chosen to present the results of this study without correction for multiple tests. To gain some idea of a significance level for our data set, we performed a simulation using the observed family structures. The value of the threshold for HLOD scores

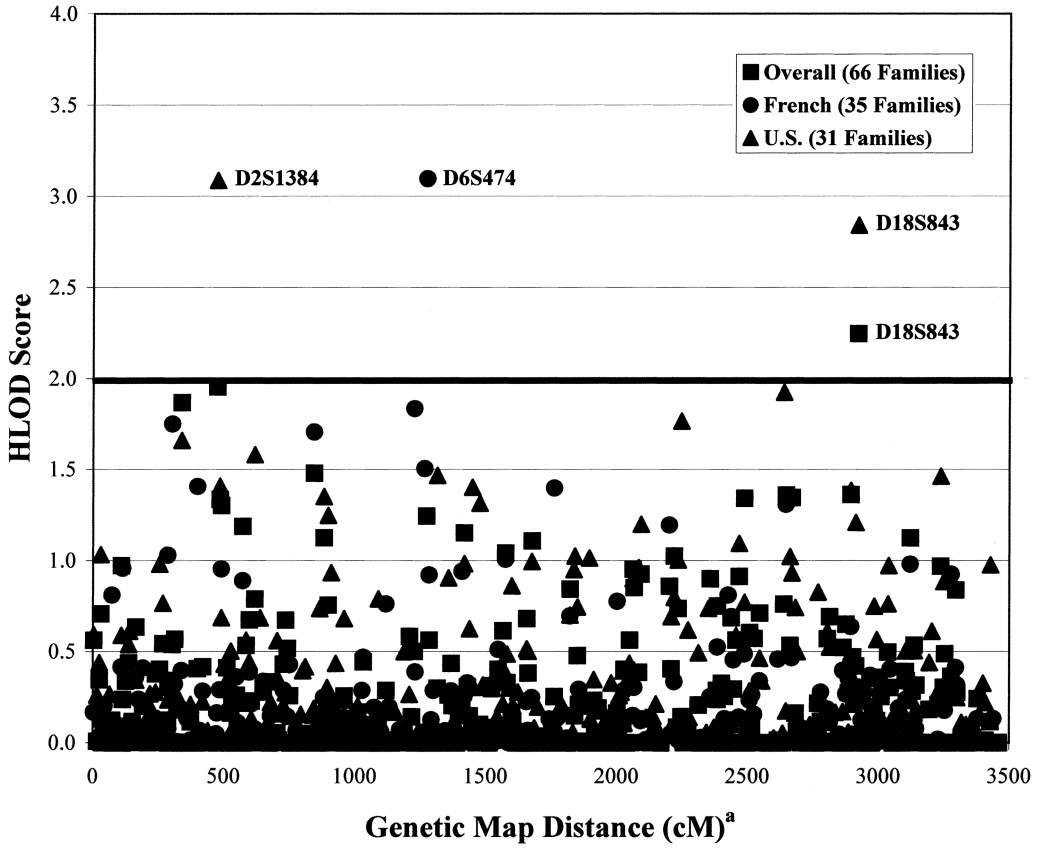

Figure 3 Two-point HLOD scores for the HLA-DR2− subsets. [a]Marshfield genetic map (see Marshfield Web site).

for a genomewide type I error of 1% was 1.86, under the hypothesis of no linkage. The value of the threshold for the model-free statistic Z for a genomewide type I error of 5% was 3.56, under the hypothesis of no linkage.

Results

Overall Analysis

Four regions met the primary criterion for further interest in both two-point and multipoint calculations (HLOD and Z scores >2.0): 1q (HLOD = 2.17; Z = 3.38), 6p (HLOD = 4.21; Z = 2.26), 9q (HLOD = 3.55; Z = 2.71), and 16p (HLOD = 2.64; Z = 2.05) (tables 2 and 3). Seven regions (1p, 2q, 6q, 13q, 16q, 18p, and 22q) generated only HLOD scores >2.0, and two regions (3q and 5q) generated only Z scores >2.0. The use of a more liberal criterion of an HLOD score >1.5 identified eight additional regions in the two-point analysis (2p, 3p, 3q, 4p, 4q, 7p, 12q, and 15q), whereas a more liberal criterion of a Z score >1.5 identified three additional regions in the multipoint analysis (2p, 10q, and 18p).

Site Stratification

Further examination of the data by country (United States vs. France) identified three regions demonstrating suggestive linkage in the U.S. subset: 6p (HLOD = 3.30), 9q (HLOD = 2.32), and 18p (HLOD = 2.39) (table 2). Two of these three regions (6p and 9q) were also identified in examination of the overall data set, as discussed above. Site stratification also identified three regions generating suggestive linkage in the French subset only: 1p (HLOD = 2.08), 16p (HLOD = 2.64), and 22q (HLOD = 2.06).

HLA-DR2 Stratification

Further examination of the data identified markers generating suggestive HLOD scores after HLA-DR2 stratification in seven regions: 2q (HLOD = 3.09 in the U.S. DR2− families), 6p (HLOD = 2.24 in all DR2+ families), 6q (HLOD = 3.10 in the French DR2− families), 9q (HLOD = 2.05 in all DR2+ families), 13q (HLOD = 2.32 in all DR2+ families and HLOD = 2.17 in the U.S. DR2+ families), 16q (HLOD = 2.32 in all DR2+ families and HLOD = 2.13 in the U.S. DR2+ families), and 18p (HLOD = 2.25 in all DR2−

Table 2

Regions with Two-Point HLOD Scores >2.0

Chromosome and Location[a] (cM)	Marker	Data Set[b]	HLOD[c]
1p:			
45	*D1S552*	3	***2.08***[d]
1q:			
268	*D1S547*	1	***2.17***[d]
2q:			
200	*D2S1384*	7	***3.09***[e]
6p:			
34	*D6S1959*	4	***2.24***[d]
44	*HLA-DR*	1, 2	***4.21***[e], 3.30[d]
6q:			
119	*D6S474*	8	***3.10***[d]
9q:			
136	*D9S282*	1, 2, 4	***3.55***[d], 2.32[d], 2.05[d]
13q:			
6	*D13S175*	4, 5	***2.32***[d], 2.17[d]
16p:			
8	*D16S2622*	3	***2.64***[d]
16q:			
100	*D16S516*	4, 5	***2.32***[d], 2.13[d]
18p:			
19	*D18S391*	2	***2.39***[e]
28	*D18S843*	6, 7	2.25[e], ***2.84***[e]
22q:			
29	*D22S689*	3	***2.06***[d]

[a] Marshfield genetic map (see Marshfield Web site).

[b] 1 = All families; 2 = only U.S. families; 3 = only French families; 4 = all DR2+ families; 5 = U.S. DR2+ families; 6 = all DR2− families; 7 = U.S. DR2− families; 8 = French DR2− families.

[c] The highest HLOD scores are indicated in bold italics.

[d] Calculated under a recessive model.

[e] Calculated under a dominant model.

families and HLOD = 2.84 in the U.S. DR2− families) (table 2). Four of these regions (2q, 6q, 13q, and 16q) were identified in only HLA-DR2–stratified subsets.

Discussion

Genetic linkage analysis has proven to be successful in locating Mendelian disease genes, but whole-genome screens have been less successful in locating genes for complex genetic diseases such as MS. Rarely does any region reach a single-stage significance level, which suggests that a two-stage design requiring confirmation in at least one additional data set is necessary to declare linkage. Our results hold true with this general pattern, but several of the regions identified in this screen do recapitulate significant linkage suspected by other groups. Our large data set and stringent criteria for identifying regions of interest (HLOD and/or Z scores >2.0) suggest several regions of linkage for MS.

Not surprisingly, a marker in the HLA-DR region on chromosome 6p21 generated the highest two-point LOD score (HLOD = 4.21) and one of the highest multipoint LOD scores (Z = 2.26) for the entire screen, confirming the overwhelming evidence of a risk factor in this region (Haines et al. 1996; Sawcer et al. 1996; Kuokkanen et al. 1997; Akesson et al. 2002).

The strongest evidence of linkage to a non-MHC region in the overall data set was for 9q33 (HLOD = 3.55; Z = 2.71). The initial Multiple Sclerosis Genetics Group (MSGG) screen, MSGG follow-up study, and recent screens in Nordic sib pairs and Turkish families also demonstrate moderate support for 9q, highlighting the need for further investigation of this region (Haines et al. 1996, 2002; Akesson et al. 2002; Eraksoy et al. 2003).

Another region of interest from this screen that is supported by several lines of evidence is 1q. Marker *D1S547* in 1q43 met the criterion for further interest in both two-point and multipoint calculations (HLOD = 2.17; Z = 3.38). Nearby markers have also demonstrated suggestive linkage and/or association in several other MS screens using a variety of study populations (Broadley et al. 2001; Ban et al. 2002; Goedde et al. 2002; Sawcer et al. 2002; Laaksonen et al. 2003). Another compelling piece of evidence for 1q is linkage to this region in the autoimmune disorders rheumatoid arthritis (Jawaheer et al. 2001) and systemic lupus erythematosus (Shai et al. 1999), suggesting the presence of a gene for general autoimmune processes. The 1q region is also orthologous to a region suggested to contain a risk factor for experimental autoimmune encephalomyelitis (EAE) in the rat (Roth et al. 1999).

Chromosome 5q is another region of interest from the screen that is supported by several lines of evidence. Marker *D5S816* in the 5q31 region met the multipoint criterion for further interest, with a Z score of 2.17. Linkage to the 5q region has also been suggested in another recent MS screen (Broadley et al. 2001). Like region 1q, 5q has been investigated for a risk factor in other inflammatory and autoimmune disorders, including Crohn disease (Rioux et al. 2001), type 1 diabetes (Morahan et al. 2001), celiac disease (Greco et al. 1998; Naluai et al. 2001), and asthma and allergy (Cookson and Moffatt 2000). The 5q region is also orthologous

Table 3

Regions with Multipoint *Z* Scores >2.0

Chromosome	Location[a] (cM)	Marker	*Z*
1q	268	*D1S547*	3.38
3q	216	*D3S2418*	2.39
5q	139	*D5S816*	2.17
6p	34	*D6S1959*	2.26
9q	136	*D9S282*	2.71
16p	8	*D16S2622*	2.05

[a] Marshfield genetic map (see Marshfield Web site).

to a region suggested to contain a risk factor for EAE in the rat (Roth et al. 1999).

The initial MSGG screen on 52 families identified 19 regions that potentially harbor MS susceptibility loci (Haines et al. 1996). Follow-up of these regions with an expanded data set of 96 families continued to provide the strongest support for five regions: 6p21, 6q27, 12q23-24, 16p13, and 19q13 (Haines et al. 2002). Three of these five regions continue to generate HLOD scores >1.5 in this second-generation genomic screen of 245 families (6p21, 12q23-24, and 16p13), providing consistent support for these regions in three of our studies to date.

Although evidence for region 19q13 has been consistently seen in our families, there is decreased evidence for this region in the current study. Despite this decreased evidence, 19q13 remains interesting for several reasons. Outside of the MHC, 19q13 is the region most consistently observed for linkage and/or association with MS. At least five genomic screens, including the initial MSGG screen and the present screen (HLOD = 1.44), support at least moderate linkage to an MS risk locus at 19q13 (Ebers et al. 1996; Haines et al. 1996; Sawcer et al. 1996; Kuokkanen et al. 1997; Coraddu et al. 2001). Numerous allelic association studies also provide evidence of a risk locus in this region. In addition, there is substantial evidence that the ApoE gene in this region modulates the severity and/or progression of MS (Chapman et al. 1999, 2001; Evangelou et al. 1999; Fazekas et al. 2000, 2001; Hogh et al. 2000; Schmidt et al. 2002; Enzinger et al. 2004). Further investigation will be necessary to confirm and identify a specific 19q13 disease locus.

As suggested in the literature, stratification yielded substantial increases in our linkage signals in several defined data sets (Leal and Ott 2000). In addition, four regions yielded HLOD scores >2.0 only when HLA-DR2 stratification was performed: 2q was identified in the U.S. DR2− families, 6q was identified in the French DR2− families, and 13q and 16q were identified in both the U.S. DR2+ families and the overall set of DR2+ families. Results in the 2q and 6q regions suggest effects independent of HLA-DR, whereas results in the 13q and 16q regions suggest potential interactive effects with HLA-DR. However, formal tests of correlations between NPL scores in families with at least one affected sib pair ($n = 236$) and NPL scores for regions on chromosomes 1, 3, 5, 9, and 16 failed to detect any significant correlations.

It will be important to examine the regions identified in this genomic screen in more detail by use of both larger data sets and denser maps of markers. The advent of high-density SNP maps now allows for very rapid and accurate genotyping of large numbers of SNPs in small regions, thus promoting high levels of information extraction. Finer mapping studies with additional families are currently underway to further localize linkage signals identified in this screen.

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Electronic-Database Information

The URLs for data presented herein are as follows:

deCODE Genetics, http://www.decode.com/
Genome Database, http://www.gdb.org/
Marshfield Human Genetic Linkage Map, http://research.marshfieldclinic.org/genetics/Map_Markers/maps/IndexMapFrames.html
Primer3, http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/
Vanderbilt Center for Human Genetics Research, http://chgr.mc.vanderbilt.edu/publications.html

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