

Genomewide Scan of Multiple Sclerosis in Finnish Multiplex Families

Satu Kuokkanen,^{1,*} Michele Gschwend,^{3,*} John D. Rioux,³ Mark J. Daly,³ Joseph D. Terwilliger,⁵ Pentti J. Tienari,² Juhani Wikström,² Jorma Palo,² Lincoln D. Stein,³ Thomas J. Hudson,³ Eric S. Lander,^{3,4} and Leena Peltonen¹

¹Department of Human Molecular Genetics, National Public Health Institute and Institute of Biomedicine, and ²Department of Neurology, University of Helsinki, Helsinki University of Helsinki, Helsinki; ³Whitehead Institute for Biomedical Research and ⁴Department of Biology, Massachusetts Institute of Technology, Cambridge; and ⁵Department of Psychiatry and Columbia Genome Center, Columbia University, New York

Summary

Multiple sclerosis (MS) is a neurological, demyelinating disorder with a putative autoimmune etiology. It is thought to be a multifactorial disease with a complex mode of inheritance. Here we report the results of a two-stage genomewide scan for loci predisposing to MS. The first stage of the screen, with a low-resolution map, was performed in a selection of 16 pedigrees collected from an isolated Finnish population. Multipoint, non-parametric linkage analysis of the 328 markers did not reveal statistically significant results. However, 10 slightly interesting regions ($P = .1-.15$) emerged, including our previous findings of the HLA complex on 6p21 and a putative locus on 5p14-p12. Eight of these novel regions were further analyzed by use of denser marker maps, in the second stage of the scan. For the chromosomal regions 4cen, 11tel, and 17q, the statistical significance increased, but not conclusively; for 2q32 and 10q21, the statistical significance did not change. Accordingly, genotyping of the high-density markers in these regions was performed, and the data were analyzed by use of two-point, parametric linkage analysis using the complete pedigree information of the 21 Finnish multiplex families. We detected suggestive evidence for a predisposing locus on chromosomal region 17q22-q24. Several markers on 17q22-q24 yielded positive LOD scores, with the maximum LOD score (Z_{\max}) occurring with D17S807 ($Z_{\max} = 2.8$, $\theta = .04$; dominant model). Interestingly, a suggestive linkage between MS and the markers on 17q22-q24 was also revealed by a recent genomewide scan in MS families from the United Kingdom.

Received June 9, 1997; accepted for publication September 23, 1997; electronically published December 5, 1997.

Address for correspondence and reprints: Prof. Leena Peltonen, Department of Human Molecular Genetics, National Public Health Institute, Mannerheimintie 166, FIN-00300 Helsinki, Finland. E-mail: Leena.Peltonen@kth.fi

*These authors contributed equally to this paper.

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Introduction

Multiple sclerosis (MS) is an inflammatory, demyelinating disease of the CNS. The prevalence of this putative autoimmune disorder is 0.1% in Caucasians of northern European origin. MS has an obvious genetic component, as indicated by the higher concordance rate of 25%–30% in MZ twins and by the 20–40-times greater risk in siblings compared with the general population (Ebers et al. 1986; Sadovnick et al. 1988). Adoption and half-sib studies show evidence for genetic determinants but also implicate the importance of environmental factors in the etiology (Ebers et al. 1995; Sadovnick et al. 1996). The candidate-gene approach has not been very encouraging: positive findings in one data set have usually not been replicated in other studies (Beall et al. 1989; Seboun et al. 1989; Hillert et al. 1991; Lynch et al. 1991). Thus far, only association with the HLA complex on 6p21 has been a well-established finding in most populations (Olerup and Hillert 1991).

Recently, three publications reported the results of genomewide screens of MS in families collected from the United Kingdom, Canada, and the United States, but no major susceptibility locus could be detected (Ebers et al. 1996; The Multiple Sclerosis Genetics Group 1996; Sawcer et al. 1996). Each study reported several provisional sites, but only two such genomic regions, 6p21 (HLA complex) and 5p, were positive in more than one data set. Together, these genome screens support previous assertions of a polygenic and heterogenic etiology of MS. Nonetheless, the number and the character of genes predisposing to MS remains unknown.

We have focused our genetic studies of MS on the isolated Finnish population, which originates from a limited number of founders (de la Chapelle 1993). This population structure should be highly useful in genetic studies of a complex disease, because of the likely restricted number of founder mutations. Using a candidate gene approach, we have previously found evidence for the contribution of three distinct chromosomal regions to the genetic susceptibility of MS in 21 Finnish families:

the region containing the Golli (for gene expressed in the oligodendrocyte lineage)-MBP (for myelin basic protein) gene on 18q22-q23, the HLA complex on 6p21, and a region on 5p14-p12 (Tienari et al. 1992b, 1993; Kuokkanen et al. 1996).

Here we present the results of a genomewide scan performed in the same Finnish multiplex MS families, utilizing a semiautomated mapping technique. The study was conducted in a two-stage approach, and statistical analyses were performed by use of both multipoint, nonparametric-linkage analysis and two-point, parametric-linkage analysis. The results suggest evidence for linkage on 17q22-q24—in exactly the same region that showed suggestive linkage to MS in two British data sets (Sawcer et al. 1996).

Families, Material, and Methods

Pedigrees and Affection Status

The complete study material consisted of 21 MS families with two to six affected cases per pedigree (fig. 1). These families are identical to those previously used in our genetic-mapping studies (Tienari et al. 1992b, 1993; Kuokkanen et al. 1996). Fourteen of them originated from the province of Vaasa in western Finland, where the prevalence of MS is twice as high as elsewhere in Finland and where the familial occurrence of MS is increased as much as 30% (Wikström 1975; Kinnunen et al. 1983). Diagnosis of MS in affected individuals strictly followed Poser's diagnostic criteria (Poser et al. 1983). In magnetic-resonance imaging (MRI), three asymptomatic siblings showed lesions typical for MS, and their affection status was regarded as unknown in the analysis (Tienari et al. 1992a). Two patients with a diagnosis of optic neuritis were members in the families with multiple MS cases originating from the high-risk region of Vaasa, and thus, in the present study, we regarded these individuals as affected (Ebers et al. 1981).

A two-stage genomewide screen with increasing marker resolution was performed on 104 individuals from the 16 most informative families of the 21 families, including 11 from the Vaasa region. For the screens, we included all affected individuals of the 16 families, as well as either their parents, if available, or two or three unaffected siblings. With this selection, individuals to be genotyped were chosen on the basis of either their affection status or their ability to facilitate phase determination.

For the final phase of the study, we selected chromosomal regions in which addition of the denser set of markers either led to an increase in statistical significance or did not change it. In an effort to extract complete pedigree information, we extended the number of families from 16 to 21, genotyped all 191 available indi-

viduals, and performed two-point linkage analysis. At this stage, we included all individuals for the study, since two-point linkage analysis using FASTLINK resulted in the utilization of information from all individuals in the pedigree; however, a parametric inheritance model needed to be specified. In contrast, multipoint, nonparametric linkage analysis using GENEHUNTER resulted in the utilization of information from all markers, but some individuals of the larger pedigrees were excluded because of computational constraints of the program. Seven (44%) of the 16 pedigrees used in our scan were sufficiently large to require trimming in this fashion, for the GENEHUNTER multipoint analysis.

Low- and High-Resolution Maps

A low-resolution map of 328 polymorphic, fluorescently labeled markers from the Cooperative Human Linkage Center (CHLC) human screening set/Weber version 6.0 were used for the initial stage of our study. The order and sex-averaged distance of the scan markers were based on the published CHLC map (Murray et al. 1994; <http://www.chlc.org/ChlcMaps.html/>). Markers had an average heterozygosity of .76 and an average spacing of 12 cM.

For follow-up of the low-resolution genetic screen, we selected those regions with a nonparametric linkage (NPL_{all}) score >1 (corresponding to $P \approx .16$). This criterion is clearly well below the threshold for statistical significance but provided a convenient cutoff for identification of regions meriting higher-density genotyping. The high-density genotyping proceeded as follows: six markers on chromosomal region 2q, six markers on 3q, seven markers on 4cen, four markers on 10q, three markers on 11tel, seven markers on 17q, three markers on 18tel, and four markers on 19tel. Microsatellite markers for the high-resolution screen were selected from the CHLC and Génethon marker maps (Murray et al. 1994; Dib et al. 1996). The marker order and distance were determined according to published integrated maps (Genome Database and Marshfield Medical Research Foundation). The reported marker order was consistent with the recombination data observed in the Finnish MS families. The average marker spacing for the fine-mapping regions was 4.5 cM (range 0.01–10 cM). For 17q22-q24, we selected the same markers as were used in the previously published British study, to aid in a direct comparison of the results (Sawcer et al. 1996). The marker order on 17q22-q24 was confirmed by use of radiation hybrid mapping (Stanford version G3). According to the physical map of the 17q22-q24 region, the positions of all other markers were in agreement with the published genetic maps, except for D17S1882, which is located distal to D17S807 (Whitehead Institute/MIT Genome Center [<http://www-genome.wi.mit.edu/>]

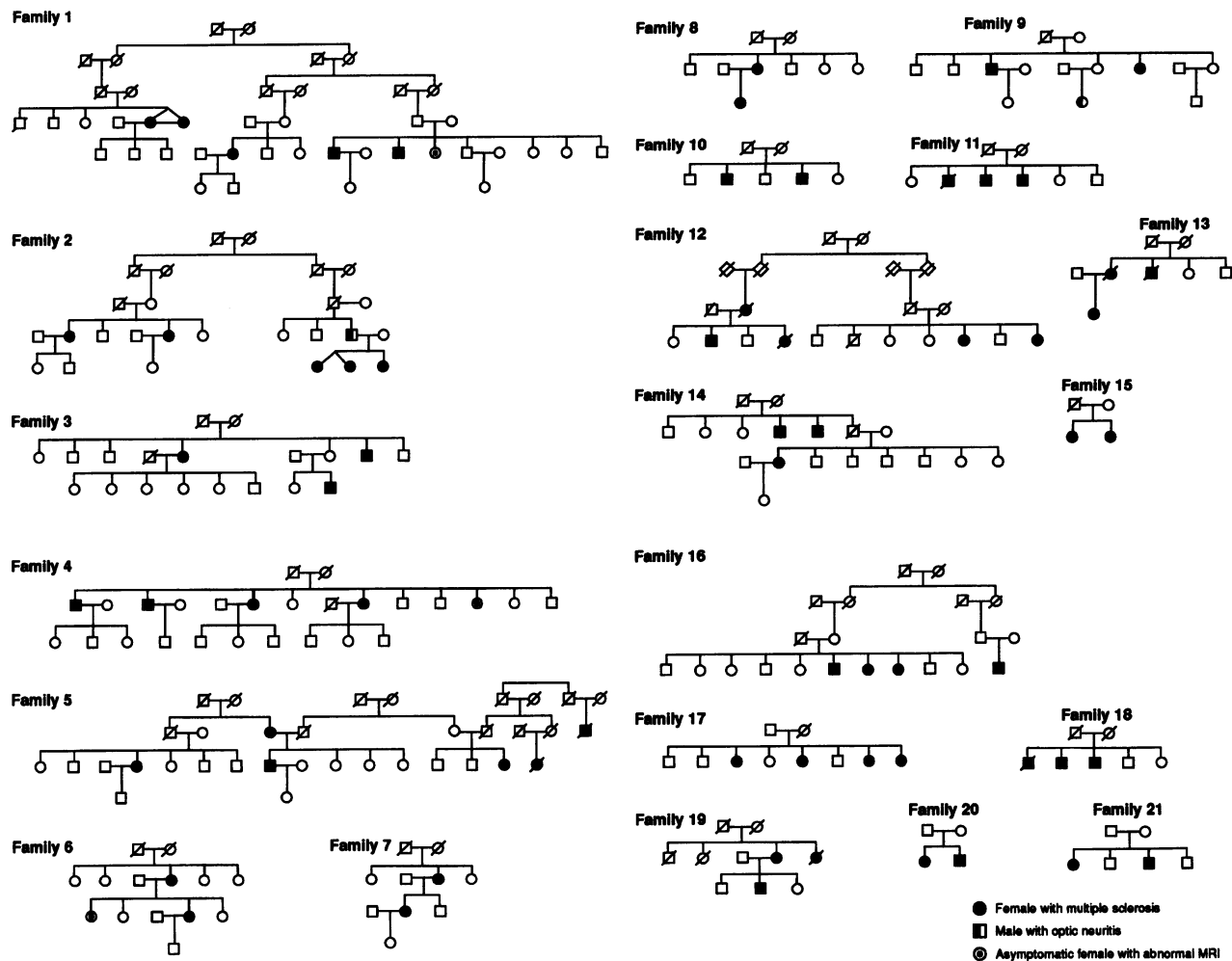


Figure 1 Pedigrees of 21 Finnish MS families. Families 1–7, 9, 11–15, and 21 originate from the Vaasa region, a high-risk region in western Finland. Families 1–5, 9–12, 14–18, 20, and 21 were included in the initial stage of the genomewide scan. Three pedigrees, families 2, 5, and 16, have previously been published by our group (Tienari et al. 1992b).

cgi-bin/contig/phys_map]). Here we used the marker order based on the physical map.

Determination of the Haplotypes

For the low-resolution genome scan, we used fluorescently labeled markers, which were detected by use of an ABI 377 sequencer (Perkin-Elmer). Gels were processed by use of BASS/GRACE (L. D. Stein, unpublished data; also see <http://www-genome.wi.mit.edu/ftp/distribution/software/>); size standards and alleles were determined automatically by use of in-home allele-calling software (M. J. Daly, unpublished data), and genotypes were stored in a LABBASE database (<http://www-genome.wi.mit.edu/ftp/distribution/software/>). Segregation was checked and linkage-formatted files were produced by use of PEDMANAGER (M. P. Reeve-Daly, personal communication). Markers for the high-

resolution mapping were genotyped by use of either the aforementioned protocol or radioactively labeled markers as described elsewhere (Kuokkanen et al. 1996).

Statistical Analysis

Multipoint, nonparametric linkage analysis of the low- and high-resolution genome screens was performed by use of the GENEHUNTER computer package (Kruglyak et al. 1996). The analysis was performed on a panel of 16 pedigrees and a subpanel of 11 Vaasa pedigrees, in an attempt to enrich for possible genetic homogeneity.

To incorporate complete pedigree information in statistical analysis, we also used two-point linkage analysis for the high-resolution mapping. Two-point linkage analysis was performed by use of the MLINK program of the LINKAGE package, FASTLINK version 2.2 (Lathrop et al. 1984; Cottingham et al. 1993; Schäffer et

al. 1994). Genetic heterogeneity was tested by use of the admixture test of the HOMOG program (Ott 1991). Since the mode of inheritance of MS is unknown, LOD-score analysis was performed by use of four different modes of inheritance: dominant and recessive, each with reduced penetrance of either $f = .05$ or $f = .76$. In the dominant model with penetrance value of either $f = .05$ or $f = .76$, we used disease allele-frequency estimates of $P = .01$ and $P = .0006$, respectively, as explained elsewhere (Kuokkanen et al. 1996); and in the recessive model with penetrance value of either $f = .05$ or $f = .76$, we used disease allele-frequency estimates of $P = .014$ and $P = .035$, respectively. Because of a relatively late onset of symptoms, we used five age-adjusted penetrance classes. Phenocopy penetrance of .001 was used throughout. For each marker, allele frequencies were determined by an allele-counting method using pedigree data themselves. To see whether misspecification of allele frequencies had an effect on the obtained results, LOD-score analysis for the high-density markers on 4cen and 17q22-q24 was also performed under the assumption of equal allele frequencies.

To test for linkage disequilibrium with each marker locus of the high-density maps, the data were analyzed by the method of Terwilliger (1995). Affected-sib-pair analysis was performed by a LOD score-based algorithm (Knapp et al. 1994; Kuokkanen et al. 1996).

Results

Our initial low- and high-resolution screens consisted of 104 individuals in 16 pedigrees selected from 21 MS families. Five of the 21 pedigrees were not included in the genome scan, because of their small size. These five families were included in the final phase of the study, in which we genotyped a total of 191 individuals in 21 families for 34 high-resolution markers mapping to the most interesting regions (2q, 4cen, 10q, 11tel, and 17q) identified in the genome scan. For these markers, we performed two-point linkage analysis to allow the incorporation of all available pedigree information in the analysis.

For the initial low-resolution scan, we genotyped 328 polymorphic markers at an average spacing of 12 cM. The information content for the genome scan averaged 60%. Multipoint, nonparametric linkage analysis for the low-resolution genome scan was performed by use of two data sets: all pedigrees ($n = 16$) and a subset of pedigrees from the Vaasa region ($n = 11$). The latter data set is composed of families from a high-risk area in western Finland (Wikström 1975; Kinnunen et al. 1983). In both cases, the initial genome screen revealed no statistically significant ($P < 2 \times 10^{-5}$; $NPL_{all} > 4.1$) or suggestive evidence ($P < 1 \times 10^{-3}$; $NPL_{all} > 3.0$) for susceptibility loci predisposing to MS (Lander and Krug-

lyak 1995). However, we observed slightly positive NPL scores in several regions (fig. 2). Ten chromosomal areas—on 2q, 3q, 4cen, 5p, 6p, 10q, 11tel, 17q, 18tel, and 19tel—were identified as having $NPL_{all} > 1$ ($P < .16$), the criterion that we had set for further examination. The regions with $NPL_{all} > 1$ on chromosomes 5 and 6 did not require follow-up, since we previously had published our findings from the high-resolution mapping of 5p14-p12 and of the HLA complex on chromosome 6 in the same MS families (Tienari et al. 1993; Kuokkanen et al. 1996). Accordingly, high-resolution mapping was focused on the other regions with $NPL_{all} > 1$ (2q, 3q, 4cen, 10q, 11tel, 17q, 18tel, and 19tel). In each region, additional markers were genotyped and analyzed separately for all MS families and for Vaasa families, by multipoint, nonparametric linkage analysis. In all regions, genotyping of additional markers increased the information content to $>70\%$, which we considered adequate for the present purpose.

The NPL scores from the high-resolution screen of 16 pedigrees are illustrated, as solid red lines, in figure 2. In three chromosomal regions an increase of the maximum NPL score was obtained: on 4cen the NPL score increased from $NPL_{all} = 1.17$ ($P = .12$) to $NPL_{all} = 2.23$ ($P = .02$), on 11tel from $NPL_{all} = 1.02$ ($P = .15$) to $NPL_{all} = 2.0$ ($P = .03$), and on 17q22-q24 from $NPL_{all} = 1.33$ ($P = .1$) to $NPL_{all} = 2.34$ ($P = .01$). The NPL score for markers on 2q23 and 10q21 did not change; and for markers on 3q, 18tel, and 19tel the NPL score declined. No evidence for linkage disequilibrium was observed with any of the marker loci of the denser marker maps.

Since, even with the high-resolution map, we did not find suggestive evidence of linkage to any locus, we extended our analysis to all individuals from the 21 multiplex MS families, on the peak chromosomal areas on 2q32, 4cen, 10q21, 11cen, and 17q22-q24. In these regions, we genotyped the markers of our high-resolution map with 191 DNAs and performed two-point linkage analysis, using four different modes of inheritance (see Families, Material, and Methods section). Detailed LOD-score results for the high-resolution markers are available from our Website at <http://www.ktl.fi/molbia/ms>. As before, pairwise linkage analysis was performed separately for the complete pedigree material and for the Vaasa pedigrees.

Chromosomes 2 and 10

In the regions of 2q32 and 10q21, the LOD-score results were not impressive for either chromosomal site: near D2S1391 the maximum LOD score (Z_{max}) was 0.73 ($\theta = .22$; dominant model, $f = .76$), and near D10S1220 Z_{max} was 0.95 ($\theta = .06$; dominant model, $f = .05$).

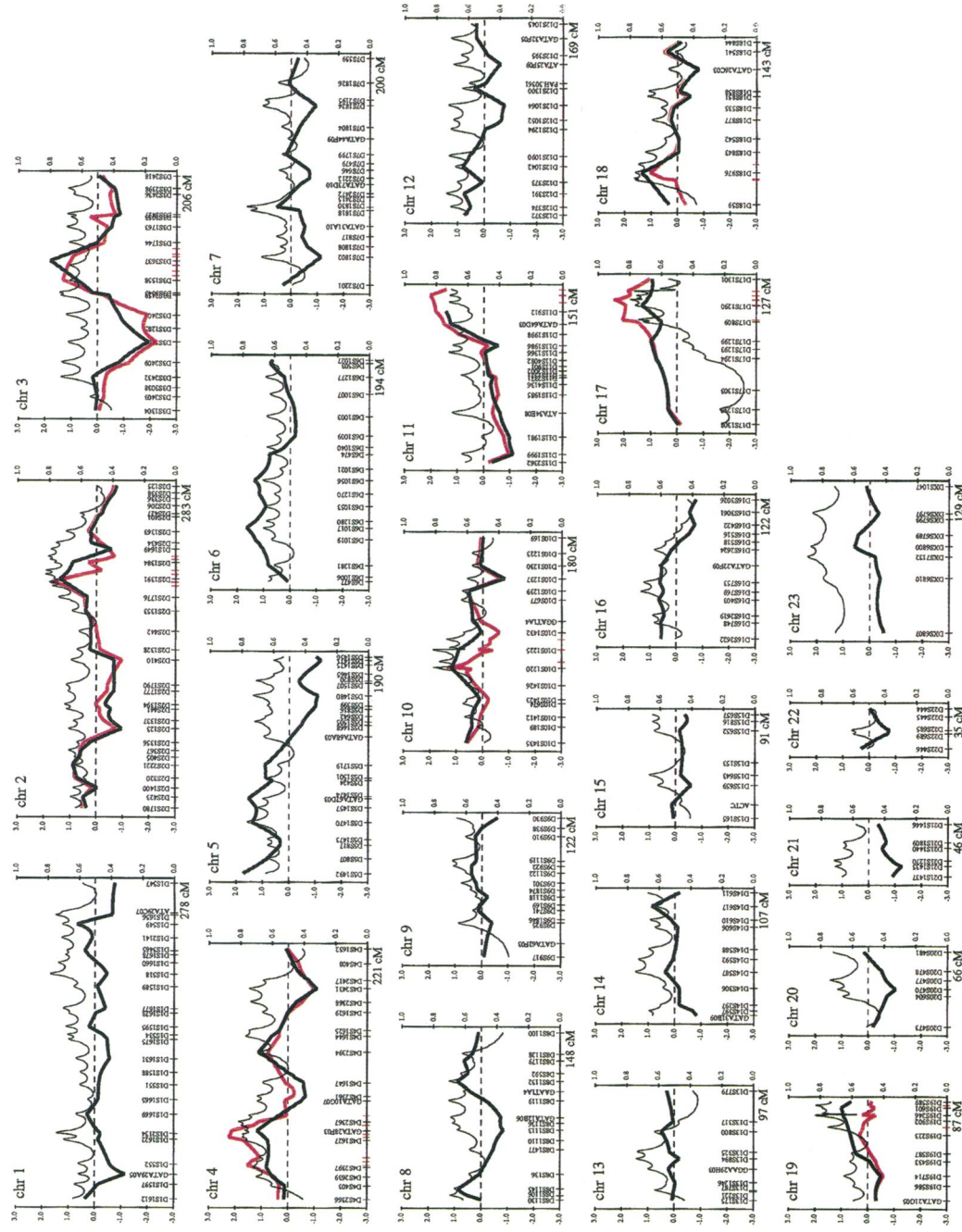


Figure 2 NPL_{all} statistic (*thinner line*1-axis) and information content (*thinner line*2-axis) for each chromosome analyzed in the genome scan of 16 families genotyped with 328 low-resolution markers (x-axis). The red line represents the NPL_{all} statistic produced from the analysis of 16 families for low-resolution markers plus 40 additional high-resolution markers (indicated by vertical red ticks on the x-axis). The names (in map-position order) of the added high-resolution markers are as follows: chromosome 2—D252188, D2S389, D2S2318, D2S155, and D2S2321; chromosome 3—D3S3576, D3S1589, D3S1290, D3S3554, D3S3694, and D3S1550; chromosome 4—D4S3244, D4S2408, D4S2629, D4S2379, D4S2432, and D4S2393; chromosome 10—D10S196, D10S1790, D10S1652, and D10S1672; chromosome 11—D11S910, D11S969, and D11S968; chromosome 17—D17S787, D17S794, D17S807, D17S1882, D17S795, D17S2059, and D17S949; chromosome 18—D18S54, D18S52, and D18S62; and chromosome 19—APOC2, D19S867, D19S907, and D19S206.

Chromosome 4

For chromosome 4 markers we did not detect suggestive evidence of linkage in two-point LOD-score analysis (fig. 3). The Z_{\max} was obtained with GATA28F03 ($Z_{\max} = 0.96$, $\theta = .16$) under a dominant model with low penetrance ($f = .05$). For the Vaasa pedigrees the Z_{\max} of 1.35 ($\theta = .08$; dominant model, $f = .05$) was detected with GATA28F03. The magnitude of the LOD scores remained similar in two-point linkage analysis when equal allele frequencies were applied to the markers (data not shown). Affected-sib-pair analysis with GATA28F03 showed increased allele sharing IBD, both for all pedigrees ($P = .005$) and for Vaasa pedigrees ($P = .0005$). No evidence for locus heterogeneity could be detected.

Chromosome 11

For 11qter, two-point LOD score analysis provided $Z_{\max} = 1.1$ ($\theta = .12$), with D11S910, under a recessive model with low penetrance ($f = .05$). Two other, more telomeric markers, D11S969 and D11S968, also produced slightly positive LOD scores—0.8 and 0.7, respectively. Additionally, increased allele sharing among affected sib pairs was detected with D11S910 ($P = .001$), with D11S969 ($P = .008$), and with D11S968 ($P = .008$).

Chromosome 17

The results of two-point LOD-score analysis for the high-resolution markers on 17q22-q24 are shown in figure 3. We show only the LOD-score results for a dominant model with low penetrance ($f = .05$), since this mode of inheritance systematically revealed the highest LOD scores. Linkage analysis with all pedigrees yielded a two-point Z_{\max} of 2.8 ($\theta = .04$) with D17S807, providing suggestive evidence for linkage. A few adjacent markers, both proximal and distal to D17S807, also produced positive LOD scores. Similarly, in the panel of the Vaasa pedigrees, two-point-LOD-score linkage analysis provided a Z_{\max} of 3.5 ($\theta = .0$) at D17S807, with flanking markers also providing suggestive LOD scores. As expected, a small increase in two-point LOD scores occurred for all markers on 17q22-q24 when equal allele frequencies were applied to markers. For D17S807, the LOD score increased from 2.8 to 3.6 in the complete pedigree material and from 3.5 to 4.2 in the subset of pedigrees from Vaasa. This would further indicate that our result at the D17S807 locus could not have been dramatically influenced by incorrect allele-frequency estimates. Evidence for locus heterogeneity could not be detected, and allelic association was not found, for any of the markers on 17q22-q24.

Chromosome 19

The distal region of chromosome 19 is of particular interest, since it harbors a gene coding for apolipoprotein C-2 (APOC2). Some evidence for the role of this gene in MS susceptibility has been reported in different populations (Ebers et al. 1996; The Multiple Sclerosis Genetics Group 1996; Sawcer et al. 1996). Although the NPL scores decreased in our high-resolution multipoint analysis, we analyzed the intragenic APOC2 marker in 21 MS pedigrees and performed two-point linkage analysis, using four different modes of inheritance. No evidence of linkage or allelic association was observed.

Discussion

Given the evidence for genetic heterogeneity of MS, we have focused the genetic studies of MS on the isolated Finnish population. To further minimize genetic heterogeneity, a majority of our pedigrees were collected from the high-risk region of Vaasa, in western Finland (Wikström 1975; Kinnunen et al. 1983). In the present study, we have performed a genomewide scan for genes predisposing to MS in the Finnish multiplex families. This strategy aimed to systematically identify potential linkages for MS susceptibility, throughout the genome. The results obtained by a two-stage mapping strategy of 354 markers suggest one additional vulnerability locus for MS, on 17q22-q24, in our study material.

Multipoint, nonparametric linkage analysis of the low- and high-resolution screens in 16 pedigrees provided hints for the involvement of the chromosomal region 17q22-q24. When all individuals from the complete family material ($n = 21$) were included, several markers on 17q22-q24 produced positive two-point LOD scores under the dominant model, with a maximum with D17S807 ($Z_{\max} = 2.8$, $\theta = .04$). In the subset of Vaasa pedigrees, the pairwise Z_{\max} for D17S807 was even higher ($Z_{\max} = 3.5$, $\theta = .0$), indicating that the role of this putative susceptibility locus may be more pronounced in the families originating from this subisolate in western Finland.

One needs to be cautious when interpreting the linkage results of markers on 17q22-q24, since none of the loci meet the most stringent level of statistical significance for linkage (Lander and Kruglyak 1995). However, it is of special interest that a recent genomewide scan of MS in British families also showed evidence for linkage on 17q22-q24 ($P = .0004$), with exactly the same markers (D17S807 and D17S795) where our strongest results occur (Sawcer et al. 1996).

It is highly encouraging that, in a relatively small number of families originating from a population subisolate, perhaps representing a rare familiar form of MS, it is

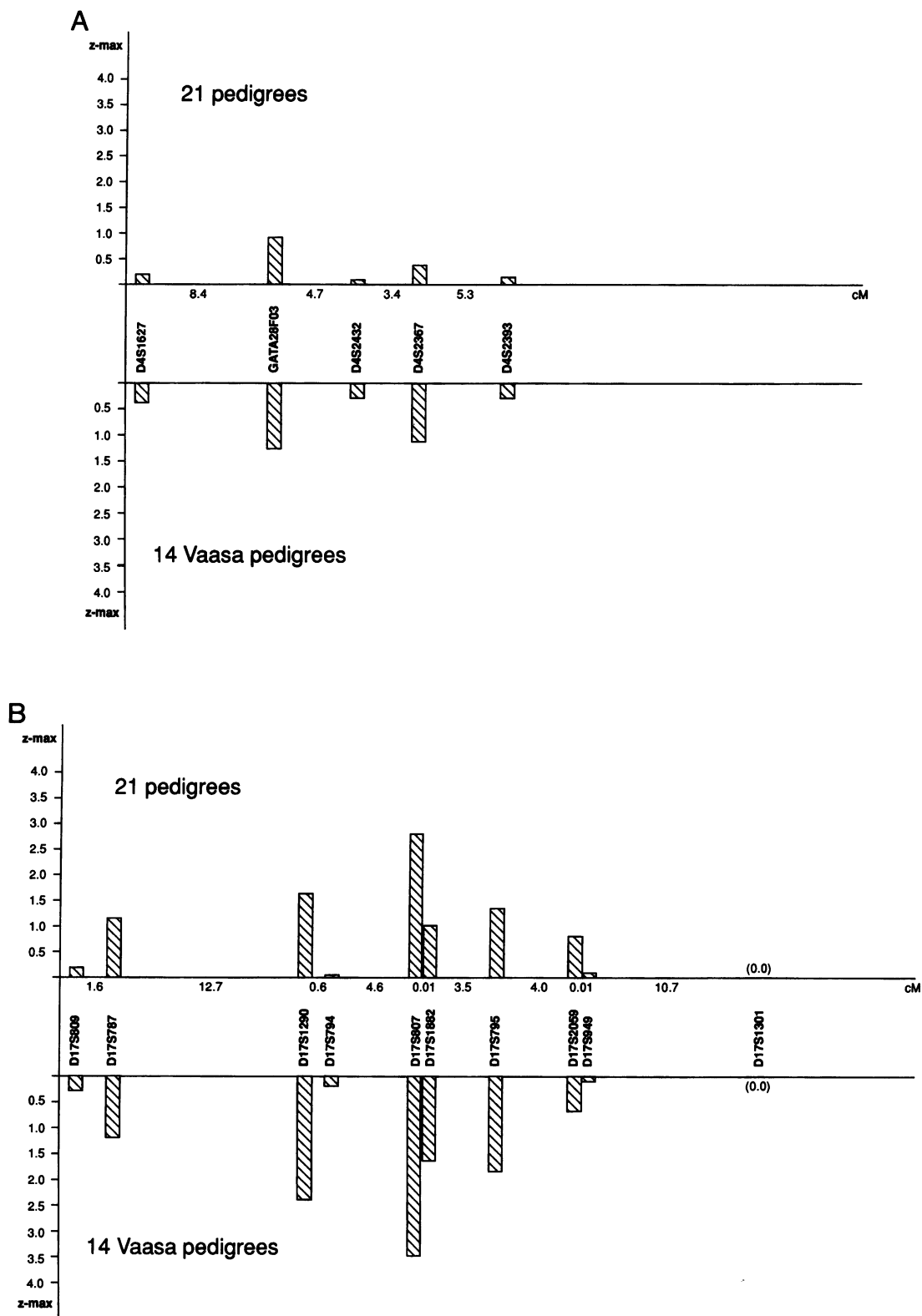


Figure 3 Two-point Z_{max} values of the high-resolution markers on chromosome 4cen and 17q22-q24, obtained by use of MLINK, FASTLINK version 2.2. In both panel A (4cen) and panel B (17q22-q24), the upper part of the diagram demonstrates Z_{max} for 21 Finnish families, and the lower part of the diagram shows the results for 14 families from the province of Vaasa. Z_{max} values are shown on the vertical axis. The horizontal axis shows the marker names and the sex-average distances between the adjacent markers (in centimorgans [cM]). The chromosome 4 map was obtained from CHLC (version 6.0), and the chromosome 17 map was obtained from Marshfield Medical Research Center integrated marker maps. All Z_{max} scores were calculated under a dominant mode of inheritance with low penetrance ($P = .05$).

possible to identify suggestive linkage to the same chromosomal region where it also was detected in an independent genomewide screen in a more mixed population (Sawcer et al. 1996). This may suggest that data from families from genetic isolates not only reveal rare loci that do not contribute to genetic susceptibility in other populations but can aid in the identification of loci that have a more general impact.

Allelic association could not be detected with any markers on 17q22-q24, in either the present study or the British study. One explanation may be that the markers are still too distant from the susceptibility gene itself or alternatively, that, even in the Finnish genetic isolate, there may be multiple ancestral predisposing alleles for this relatively common disease. In the future, the finding of allelic association either in the Finnish population or in the Vaasa subpopulation would greatly facilitate the cloning of this putative susceptibility gene on 17q22-q24.

In addition to the suggestive evidence that we have presented here for linkage to 17q22-q24, our previous studies, based on a candidate-gene approach in the same MS families, have provided both statistically significant evidence of linkage of MS to the HLA complex and suggestive evidence of linkage of MS to the Golli-MBP gene (Tienari et al. 1992*b*, 1993). The role of MBP in MS may be either of minor effect or population specific, since this candidate gene has not shown positive evidence of linkage or association in several other populations (Rose et al. 1993; Eoli et al. 1994; Vandervyver et al. 1994; Wood et al. 1994). Furthermore, we have found suggestive evidence for linkage to a locus on 5p14-p12 by performing screening of human chromosomal regions syntenic to the susceptibility loci of murine EAE (Sundvall et al. 1995; Kuokkanen et al. 1996). Interestingly, a genomewide scan in Canadian families also has provided suggestive evidence for linkage between MS and markers on the proximal limit of 5p14-p12 (Ebers et al. 1996). Thus, one of the genes predisposing to MS may represent a homologue of Eae2.

In the present study, the regions 5p14-p12 and 6p21 met our criterion for follow-up in the initial, low-resolution genome scan. By contrast, chromosome 18qter did not reveal any hint of linkage in the genome scan, most likely because of the fact that the markers on 18q22-q23 were not located close enough to the MBP locus. The other sites chosen for follow-up in the present study were not consistent with any regions of interest detected in previously published genomewide searches for MS linkage.

The finding of evidence for linkage of multiple loci in the same limited set of MS families can be explained by a selection bias: we have selected exceptional MS families with multiple affected cases. In such families, enrichment of several predisposing genes is highly possible,

especially since most of the families originate from a subisolate from western Finland.

In summary, a genomewide scan was performed in Finnish multiplex MS families. In addition to the loci identified by us before, evidence suggestive of a novel predisposing locus was found in the chromosomal region of 17q22-q24. The same region recently has been implicated as showing a highly suggestive linkage to MS, by a genomewide scan in British families. Thus, our positive results provide further support for this region's contribution to MS susceptibility.

Acknowledgments

We thank Anne Wikman, Linda Cutone, and Rose Koujomjian for excellent technical assistance, and we thank Andrew Kirby for writing the program for presenting the genome-scan statistics. Above all, we wish to thank the participating Finnish MS families. This work was financially supported in part by National Institute of Health grant HG00098 (to E.S.L.), by support from the Academy of Finland and European Commission contract BMH4-CT97-2522 (both to L.P.), and by grants from the Cultural Foundation of Finland, the Maud Kuistilas's Foundation, the Finnish Medical Society Duodecim, The Farnos and Orion Research Foundation, and the Multiple Sclerosis Foundation of Finland (all to S.K.). J.D.T. is a Hitchings-Elion Fellow (Burroughs-Wellcome Foundation).

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