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A whole genome screen for linkage in Turkish multiple sclerosis

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Abstract

Factors exerting recessive effects on susceptibility to complex traits are expected to be over-represented in communities having a higher frequency of consanguineous marriage. Multiple sclerosis, a typical complex trait, is relatively common in Turkey where cultural factors also determine a high rate of consanguineous marriage. Previous genetic studies of multiple sclerosis in Turkey have been confined to the search for associations with candidate genes. In order to exploit the special genetic features of the Turkish population, we performed a whole genome screen for linkage in 43 Turkish multiplex families employing 392 microsatellite markers. Two genomic regions where maximum lod score (MLS) values were suggestive of linkage were identified (chromosomes 13q and 18q23) along with a further 14 regions of potential linkage. Parametric analysis of these data using a recessive model, appropriate for populations with a high frequency of consanguinity, increased the LOD scores in four regions.

Keywords: Multiple sclerosis; Turkey; Genome screen; Linkage

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1. Introduction

To date, six whole genome screens for linkage have been completed in multiple sclerosis (Sawcer et al., 1996; Haines et al., 1996; Ebers et al., 1996; Kuokkanen et al., 1997; Broadley et al., 2001; Corraddu et al., 2001). The majority of these are based on populations of Northern European descent and relatively few have considered Southern Europeans, such as the Turkish population. Turkey lies at the geographical boundary between Northern Europe, where the risk of developing multiple sclerosis is high (>100/100,000), and Asia where the disease is considerably less frequent (Compston et al., 1998). Although the frequency of multiple sclerosis in Turkey has never been formally assessed, a prevalence of 24/100,000 has been observed in Turkish individuals migrating to Cyprus (Dean et al., 1997), indicating an intermediate risk of the disease in the Turkish population. Previous studies in Turkey have demonstrated that the disease clusters in families, having a familial recurrence rate of around 3% (Eraksoy et al., 1998, 2002) (this is lower than most other series—around 20%). Association with HLA alleles and haplotypes is well established, being strongest with the class II haplotype DRB1*1501–DQA1*0102–DQB1*0602 (the same haplotype associated with multiple sclerosis in northern Europe (Hauser et al., 1989; Olerup and Hillert, 1991; Allen et al., 1994; Coraddu et al., 1998), although an additional weaker association with DRB1*04 has also been suggested (Saruhan-Direskeneli et al., 1997). The high rate of consanguineous marriages in Turkey (Şimşek et al., 1999) makes it likely that genetic studies in this population will have enhanced power to identify susceptibility factors acting in a recessive manner and this social feature makes Turkey a particularly interesting population to study in the context of genetic susceptibility to multiple sclerosis and other complex traits. We have therefore systematically screened the genome for linkage in Turkish multiplex families.

2. Methods and materials

2.1. Families

All the families included in our study were recruited in Turkey and are of Turkish descent. All gave written informed consent for genetic analysis. Ethical approval was obtained from local ethics committees and the Turkish Ministry of Health. A total of 43 multiplex families was considered including: 27 with affected second or third degree relatives (aunts, uncles, cousins, nieces and nephews) and 16 with ≥ 2 affected siblings; together, these provided a total of 92 affected (61 females and 31 males) and 78 unaffected individuals (46 females and 32 males). Consanguinity was observed in 13 families (30%). All affected individuals fulfilled the Poser criteria (Poser et al., 1983) for clinically definite (89%), laboratory supported definite (7%) or clinically probable multiple sclerosis (4%). Magnetic resonance imaging (MRI) of the brain was performed in all affected individuals and fulfilled radiological criteria for multiple sclerosis (Paty et al., 1998). Lumbar puncture was performed in 60 affected individuals of whom 58 (97%) were found to have unmatched oligoclonal IgG bands in the cerebrospinal fluid. As expected, there was an excess of females (1.9 F/M). Demographic details of the probands are unremarkable with the mean age of 38.7 years and mean duration 10.0 years. Eighty-three percent of patients had relapsing–remitting disease, 13% were in the secondary progressive stage and 4% had primary progressive multiple sclerosis.

2.2. Markers

A total of 392 microsatellite markers from the Applied Biosystem Medium Density Linkage Mapping Set were genotyped in the study, providing an average marker separation of approximately 10 cM across the genome. These microsatellite markers were highly informative with an observed mean heterozygosity of 78%.

2.3. Genotyping

DNA was extracted from venous blood by standard methods. Each marker was amplified by polymerase chain reaction (PCR) using either a Touchdown PCR in 10 μl reaction volumes, as previously described, (Sawcer et al., 1996) or True Allelle (Applied Biosystems) PCR in 15 μl reaction volumes using the manufacturer's recommended conditions. PCR products were pooled into pre-determined panels prior to slab electrophoresis on a 373A sequencing Machine (Applied Biosystems) using 6% acrylamide gels or capillary electrophoresis on a 3700 DNA Analyser (Applied Biosystems) using POP6 polymer.

2.4. Statistical methods

Allele frequencies were estimated from the data using the SPLINK program (version 1.07) (Lander and Krugylak, 1995; Holmans and Clayton, 1995). Multipoint nonparametric linkage analysis was performed using the GENHUNTER-PLUS program (Kong and Cox, 1997) which also calculates the proportion of the potential genetic information extracted from the families by the markers typed. GENHUNTER-PLUS calculates a maximum lod score (MLS) value at each point in the genome on the basis of all the available genotypes, and thereby generates MLS profiles along each chromosome.

In view of the consanguineous nature of the Turkish population, recessive alleles are expected to play a more important part in determining disease susceptibility. Therefore, we also performed a parametric analysis of these data

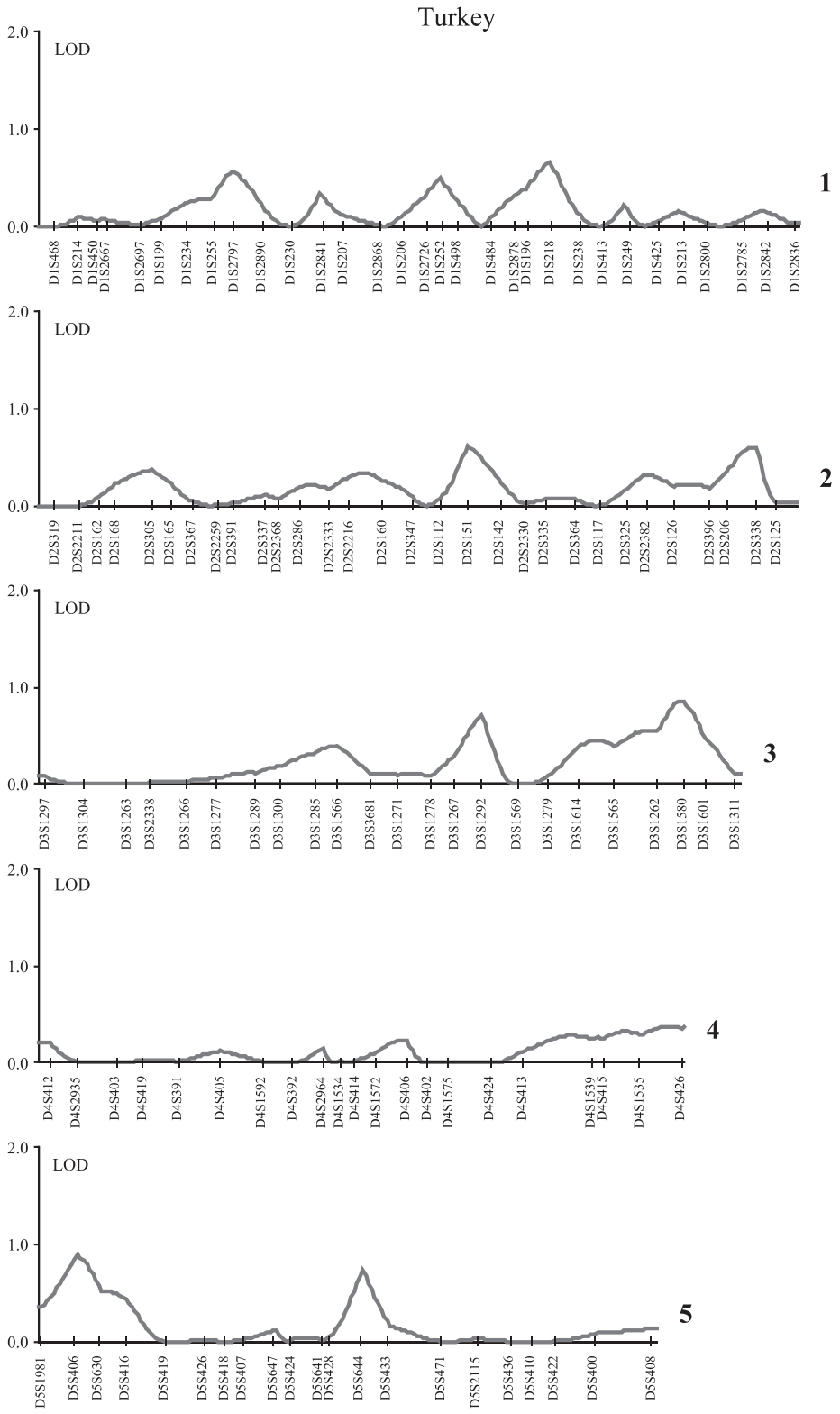
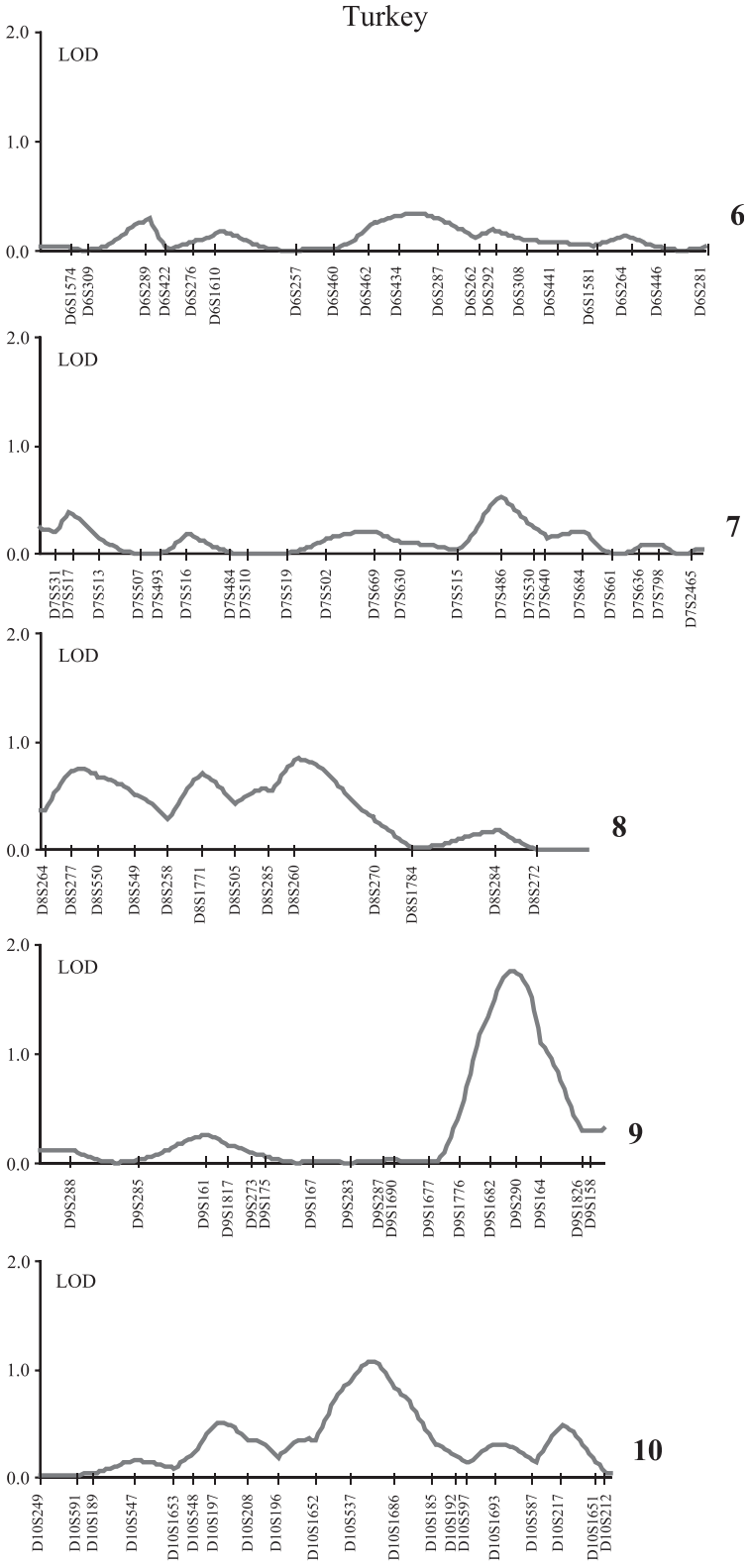


Fig. 1. Maximum lod score profiles obtained in stage 1 of the linkage screen are shown in the figure which is composed of 23 graphs. In each graph, the length of the *x*-axis is proportional to the genetic length of the chromosome and the position of the markers typed on that chromosome is indicated by the tick. Marker names are listed in map order under the *x*-axis as close as possible to the corresponding tick mark. The *y*-axis is scaled from 0 to 2.0 in each case.



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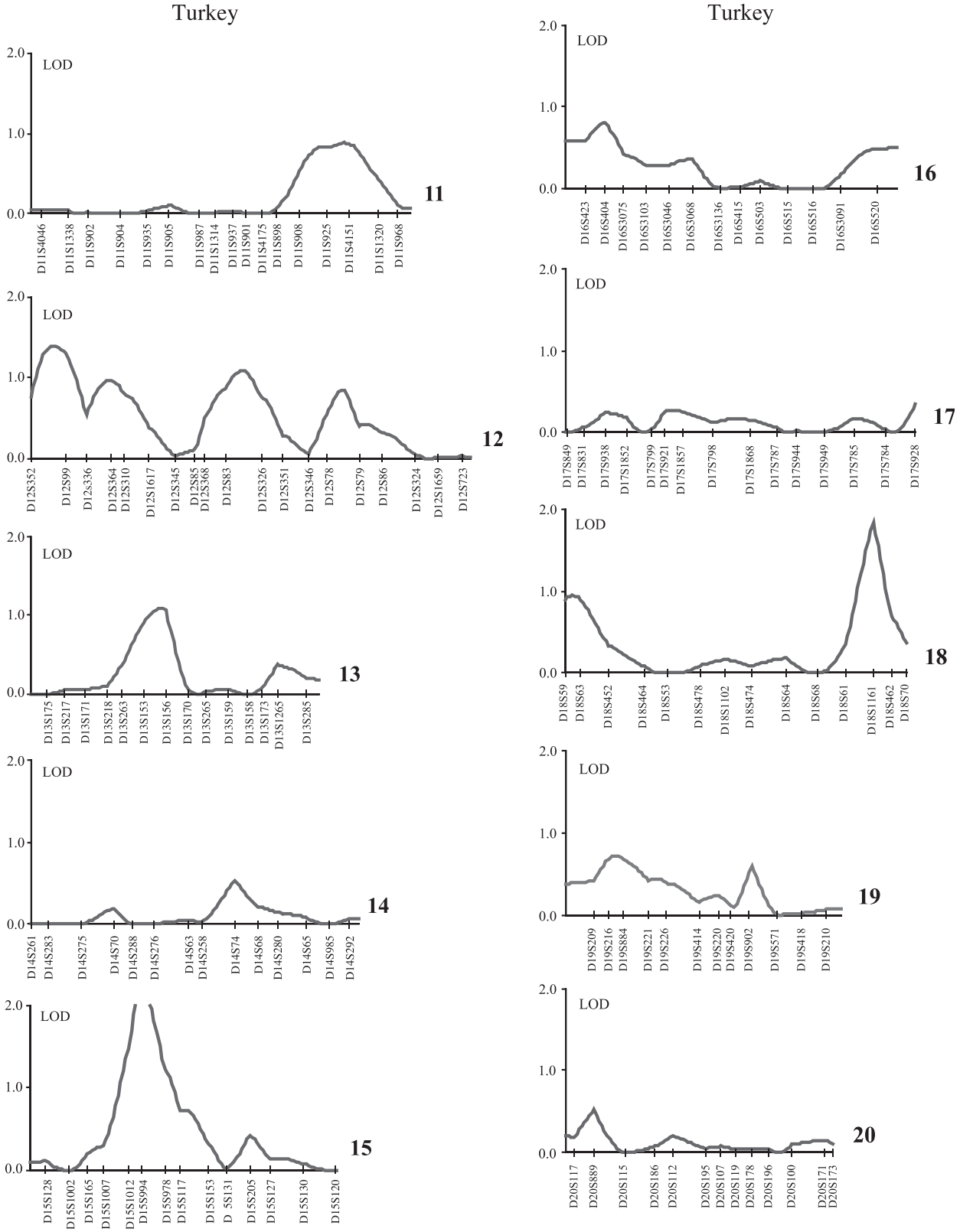

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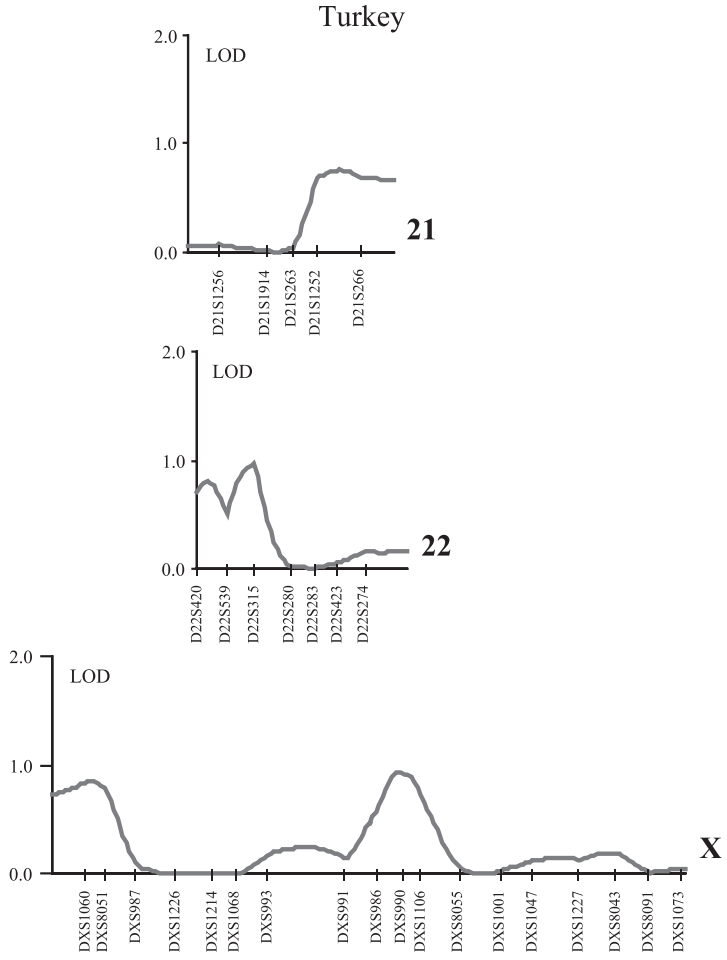

Fig. 1 (*continued*).

assuming a recessive model with disease allele frequency of 20% and incomplete penetrance.

3. Results

A two-stage approach was employed to search the genome for genes that predispose to multiple sclerosis. In the first screening stage, only the affected members ($n = 92$) from each family were genotyped; in the second stage, unaffected family members were also typed for markers from the most promising regions.

The results of multipoint non-parametric linkage analysis are presented in Fig. 1. Stage 1 of the analysis revealed 25 regions showing maximum lod score (MLS) peaks at or above the nominal 5% significance level (MLS $\geq$ 0.7) (Holmans, 1993). This is significantly more than would be expected for a marker map of this density, where only 10 such regions would have been expected by chance alone (Lander and Krugylak, 1995; Sawcer et al., 1997). None of these regions achieved lod scores indicating linkage with genome-wide statistical significance (Lander and Krugylak, 1995); however, three regions (9q, 15q and 18q) demonstrated MLS values exceeding the threshold suggestive of linkage (MLS $\geq$ 1.8) (Lander and Krugylak, 1995). Parametric analysis of these data using a recessive model resulted in an increase of the lod scores on 8p (lod = 1.20), 5p (lod = 1.10), 19p (lod = 0.89) and 18p (lod = 1.84), whereas all other peaks were reduced compared to non-parametric analysis.

In the second stage of the screen, the available unaffected family members were genotyped for 45 markers from the most promising regions identified in stage 1, in order to increase the information extracted from these regions. The MLS values were increased on chromosomes 3q21, 5p15, 5q15, 8q, 13q, 19p13, Xp22 but reduced on chromosomes 9q12, 15q, 22q and Xq. After the second stage typing, 16 regions retained an MLS >0.7 and two regions (chromosomes 13q and 18q23) continued to show MLS values exceeding the suggestive linkage threshold.

4. Discussion

This study represents the first systematic screen for linkage to multiple sclerosis performed in the Turkish

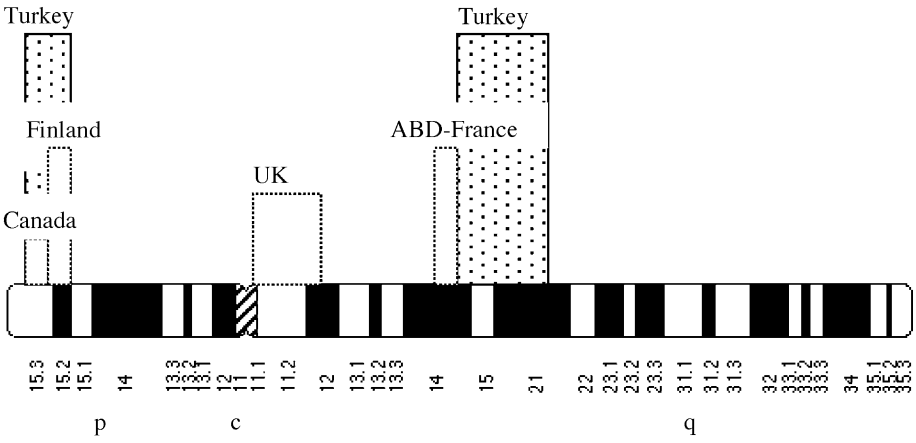

Fig. 2. This ideogram of chromosome 5 indicates the regions of peak linkage identified in the linkage genome screens from Finland, Canada, USA–France as compared with our own Turkish study.

population. A total of 43 multiplex families and 392 microsatellite markers were considered. More than 20 regions of potential linkage are identified with 2 regions exceeding the threshold suggestive of linkage. Four regions show linkage fitting a recessive model of inheritance and could encode genes acting in this manner.

The power to detect linkage is profoundly influenced by the frequency of susceptibility alleles in the population studied (Risch and Merikangas, 1996), a factor which is unknown a priori but is expected to be more favourable in some populations than others. Since Turkey is an intermediate risk population for multiple sclerosis, it is conceivable that the frequency of some alleles may be more favourable in this population than in Northern Europeans where polymorphisms determining susceptibility are relatively over-represented, perhaps exceeding the crucial frequency needed to demonstrate linkage. In addition, the high consanguinity rate of the Turkish population is expected to enhance the power to identify regions containing susceptibility genes acting in a recessive manner. These favourable factors encouraged us to screen the modest number of available families despite the obvious low power compared to existing studies clearly showing that non-parametric screens have only limited power to detect susceptibility genes of modest effect.

Our failure to identify any region of linkage with genome-wide significance is thus not unexpected but more regions achieved nominal significance than expected by chance alone (Lander and Krugylak, 1995; Sawcer et al., 1997), confirming the importance of genetic factors in Turkish multiple sclerosis patients and making it likely that some results at least are true positives. Two regions showed peak MLS values suggestive of linkage (>1.8). The result on chromosome 18q23 is particularly intriguing since this encodes the gene for myelin basic protein. Evidence for association and linkage of the myelin basic protein locus to multiple sclerosis has been reported in the Finnish population (Tienari et al., 1998), which has historic links with Turks that date back to the prehistoric period (Haywood et al., 1997; Comrie et al., 1997). It is interesting to note that the MLS values were also increased (MLS = 1.0) on chromosomes 5p15 and 5q15. These are close to the regions revealed by Finnish, Canadian and American studies (Fig. 2). D5S676 is located on chromosome 5p15, close to the region syntenic for a murine susceptibility locus in experimental allergic encephalomyelitis (Kuokkanen et al., 1996). Our results add to the accumulating data supporting the possibility that chromosome 5 contains a gene determining susceptibility to multiple sclerosis. Conversely, failure to see significant linkage in the HLA region is not unexpected given the limited power of linkage studies compared to those based on association, and the modest genetic effect attributable to HLA. In studies involving nuclear families, typing parents and unaffected siblings generally adds relatively little additional information. In our screen, this typing was more productive because of the more complex nature of the included pedigrees.

In conclusion, we have identified two genomic regions suggestive of linkage (chromosomes 13q and 18q23), four regions showing predominantly recessive effects (chromosomes 8p, 5p, 19p and 18p) and a further 14 regions of potential linkage. Many of the best supported regions identified in this Turkish screen have not previously been identified in whole genome linkage screens in multiple sclerosis suggesting that effects, which may be restricted to this population, have been identified.

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