Linkage Mapping of a Novel Susceptibility Locus for Behçet’s Disease to Chromosome 6p22-23

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Objective. The etiology of Behçet's disease is unknown; however, familial aggregation studies indicate a strong genetic background and a complex inheritance model. Association of HLA–B51 with Behçet’s disease is regarded as being the strongest evidence of genetic contribution described to date. A low rate of recombination was observed within the telomeric end of the major histocompatibility complex up to the HFE gene, which causes hereditary hemochromatosis. We therefore hypothesized that the telomere of 6p may harbor a susceptibility gene for Behçet's disease.

Methods. A series of 28 multicase families of Turkish origin was ascertained, and 78 of the 183 available family members were diagnosed as having Behçet’s disease. For the analysis of the telomeric region adjacent to HLA–B, we used a panel of 20 highly polymorphic microsatellite markers between D6S273 and D6S470, covering a region of ~36 cM.

Results. Multipoint nonparametric linkage analysis using GeneHunter 2.0 software revealed a broad peak of linkage, with the highest Z score of 4.11 at position D6S285, which is ~17 cM telomeric to HLA–B.

Conclusion. This significant linkage finding may indicate a second susceptibility locus in the telomere of chromosome 6p. Identification of this putative susceptibility gene could help to further understand the pathogenesis of Behçet’s disease.

Behçet’s disease is a chronic inflammatory disorder characterized by recurrent oral aphthous ulceration, genital ulceration, uveitis, and skin lesions. It is now recognized as a systemic vasculitis that also affects the joints, all sizes and types of blood vessels, the lungs, the central nervous system, and the gastrointestinal tract (1).

The etiology of Behçet’s disease is unknown; however, it has been suggested that some environmental agents, such as certain strains of streptococci or herpes simplex virus, can trigger the disease manifestations in genetically susceptible individuals (2). The prevalence of Behçet’s disease is considerably higher in countries from the Mediterranean region to Japan, along the Silk Road. The highest incidence rate, 8–37/10,000, occurs in the population of Turkey (3). It has been supposed that the disease susceptibility gene might have been spread along this trading route by nomadic tribes or immigrating Turks, or that this geographic predilection may be a reflection of the increased frequency of HLA–B51 in the healthy population (3,4).

The majority of Behçet’s disease cases occur sporadically in families, and the parents are typically asymptomatic; however, clustering of cases in families has long been recognized. A recent study revealed a sibling recurrence risk of 4.2% and, hence, a λs value between 11.4 and 52.5 for Behçet’s disease in Turkey (5). Analysis of a small group of families suggested no particular Mendelian inheritance pattern, a finding that supports a complex genetic background for the disease (6). A genetic anticipation in the form of earlier disease onset among the affected children of patients with Behçet’s disease has also been reported in Turkish families (7).

Association of HLA–B51 with Behçet’s disease is regarded as being the strongest evidence of genetic contribution described to date (4,8,9). However, whether HLA–B51 is itself a Behçet’s disease susceptibility gene or if this strong association reflects linkage disequilibrium with another gene has yet to be elucidated. Since association studies are subject to error based on the selection of cases and healthy controls with
population stratification, we recently used the transmission disequilibrium test (TDT) in a preliminary study of 12 multicase families and demonstrated linkage of the HLA–B locus to Behçet’s disease (10).

A lower rate of recombination has been observed within the extended major histocompatibility complex (MHC) region up to the HLA gene, which causes hereditary hemochromatosis, and strong linkage disequilibrium is a feature of this part of the genome (11,12). We therefore hypothesized that the telomere of chromosome 6p may harbor a susceptibility gene for Behçet’s disease that may be in linkage disequilibrium with the HLA–B locus. We directed our investigation toward the telomeric region adjacent to HLA–B and used highly polymorphic microsatellite markers to evaluate families with multiple cases of Behçet’s disease.

PATIENTS AND METHODS

Families. A series of 28 multicase families of Turkish origin, including the previously studied 12 families (10), was ascertained at the Behçet’s Disease Outpatient Clinic (Division of Rheumatology, Istanbul Faculty of Medicine) from patients who had an affected first-degree relative. All available family members were examined by one of us (AG). Of the 183 family members, 78 (46 males, 32 females) were diagnosed as having Behçet’s disease according to the criteria of the International Study Group for Behçet’s disease (13). A further 42 family members had isolated Behçet’s disease–related manifestations, such as recurrent oral aphthous ulcers or positive findings on pathergy testing. These individuals could not be classified as having Behçet’s disease based on the International Study Group criteria, and were therefore grouped as “unknown” cases for the purposes of the genetic analyses.

Thus, the 78 Behçet’s disease patients were from 28 families. Twelve families contained 28 affected individuals who were from a single sibship (9 pairs, 2 trios, and 1 quadruple), and 16 families had a more complex structure, with affected parents or 3-generation extended families.

The study was approved by the Ethics Committee of the Istanbul Faculty of Medicine. Blood samples were collected from all individuals after their informed consent was obtained. Samples were maintained at −20°C, packed in dry ice, and transferred to the Arthritis Research Council Epidemiology Unit, Manchester, UK. Genomic DNA was extracted from whole blood using the DNace MaxiBlood Purification System (Bioline, London, UK).

Microsatellite analysis. For the analysis of the telomeric region adjacent to HLA–B, we used a panel of highly polymorphic microsatellite markers evenly distributed every ≤1 cM (0.3–1 cM) between centromeric D6S273 and telomeric D6S276 (Figure 1). We also added 4 more telomeric markers (D6S422, D6S259, D6S1721, D6S470), which helped to cover an ~36-cM region in the telomere of 6p. After the initial analysis, 6 more markers (D6S1665, D6S285, D6S274, D6S288, D6S289, D6S1267) were included to increase the information content between markers D6S422 and D6S259. The mean heterozygosity of the 20 microsatellite markers was 0.78 (range 0.69–0.83).

Fluorescently labeled (with 6-FAM, HEX, or TET) oligonucleotide primers were used for amplification of genomic DNA. Pooled polymerase chain reaction products were electrophoresed on denaturing polyacrylamide gels using a model 373A automated DNA sequencer (PE Applied Biosystems, Foster City, CA). Gels were analyzed with GeneScan 2.0 software, and genotypes were determined using GenoTyper 2.1 software (PE Applied Biosystems).

Statistical analysis. Multipoint nonparametric linkage (NPL) analysis was done with the use of the GeneHunter (version 2.0 beta) software package (14). GeneHunter provides a unified framework for both parametric and nonparametric (model-free) analysis and helps to extract all available inheritance information from general pedigrees of moderate size, such as the ones in this study. It has been reported that the NPL statistic is robust to uncertainty about the mode of inheritance, and superior to many commonly used parametric methods in the analysis of such families (14).

Allele frequencies were calculated from the data. The map distances used for the analysis are shown in Figure 1. NPL analysis was carried out with the “score all” function of the GeneHunter 2.0 software.

Parametric linkage was also evaluated under the dominant and recessive models of inheritance. Penetrance of the disease susceptibility gene was assumed to be 0.50. Since the prevalence of Behçet’s disease ranges between 0.0008 and 0.0037 in Turkey, the disease allele frequency was set at 0.003 for the dominant and 0.05 for the recessive disease models.

RESULTS

Multipoint NPL analysis with the first 14 microsatellite markers examined gave a broad peak of linkage between D6S422 and D6S259, with the highest Z score of 3.99 (P = 0.00015) at position D6S422 (Figure 1). Parametric linkage analysis with genetic heterogeneity also revealed a logarithm of odds (LOD) score of 2.91 for the dominant disease model, with 77% heterogeneity at the same position. An LOD score of 3.39 was obtained under the recessive model at a position 5.1 cM telomeric to D6S422, with 61% heterogeneity.

After this initial analysis, we added 6 more polymorphic microsatellite markers to increase the information content in the linkage region. Repeated multipoint NPL analysis using GeneHunter 2.0 revealed a higher Z score of 4.11 at position D6S285, located 1.7 cM telomeric to D6S422 (Figure 1 and Table 1). A second peak (Z score 3.78) was also observed at the telomeric marker D6S259, possibly reflecting the fluctuation in the information content (data not shown).

Linkage analysis under the dominant model revealed an LOD score of 2.64, with 69% heterogeneity, at D6S1665, and under the recessive model, the LOD score
was 2.82, with 46% heterogeneity, at position D6S285 (Table 1).

The maximum information content in the region of D6S422 giving the highest Z score in the first analysis was increased from 84% to 93% after the addition of 6 microsatellite markers. Similarly, there was an increase in the mean information content across the ~36-cM region, from 89.7% to 91.5%.

**DISCUSSION**

We present evidence that a novel susceptibility locus for Behçet’s disease maps to chromosome 6p22-23. Multipoint NPL analysis revealed a maximum Z score of 4.11 at position D6S285, ~17 cM telomeric to HLA-B.

Familial aggregation studies indicate a strong genetic background for Behçet’s disease, with a complex pattern of inheritance (5,6,15). Although its pathogenic role remains to be determined, HLA–B51 is the only genetic susceptibility factor for Behçet’s disease that has been confirmed in many different ethnic groups. In a preliminary study with 12 multicase families, we confirmed the linkage of the HLA–B locus with Behçet’s disease using the TDT, and we estimated the highest contribution of this locus to the overall genetic susceptibility to Behçet’s disease to be 19%, using the zero allele-sharing method of Risch in affected sibling pairs.

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**Table 1. Results of the repeated multipoint NPL analysis Z scores and LOD scores under the dominant and recessive models for loci in the chromosome 6p linkage region***

<table>
<thead>
<tr>
<th>Locus</th>
<th>NPL analysis</th>
<th>Linkage analysis LOD score</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Z score</td>
<td>P</td>
</tr>
<tr>
<td>D6S276</td>
<td>2.64</td>
<td>0.005</td>
</tr>
<tr>
<td>D6S1665</td>
<td>3.85</td>
<td>0.0002</td>
</tr>
<tr>
<td>D6S422</td>
<td>3.94</td>
<td>0.0002</td>
</tr>
<tr>
<td>D6S428</td>
<td>4.11</td>
<td>0.0001</td>
</tr>
<tr>
<td>D6S274</td>
<td>3.70</td>
<td>0.0003</td>
</tr>
<tr>
<td>D6S288</td>
<td>3.64</td>
<td>0.0004</td>
</tr>
<tr>
<td>D6S289</td>
<td>3.07</td>
<td>0.002</td>
</tr>
<tr>
<td>D6S1267</td>
<td>3.75</td>
<td>0.0003</td>
</tr>
<tr>
<td>D6S259</td>
<td>3.78</td>
<td>0.0003</td>
</tr>
<tr>
<td>D6S1721</td>
<td>3.01</td>
<td>0.002</td>
</tr>
</tbody>
</table>

* LOD = logarithm of odds; NPL = nonparametric linkage; D50 = dominant model; R50 = recessive model; het. = heterogeneity.
(see ref. 10). Alloallelic association, genotypic differentiation, and stratification analyses in different ethnic groups have supported the direct role of HLA-B51 in the disease pathogenesis, and all other associations with neighboring genes or markers, including MICA (MHC class I chain–related gene A), could be explained by linkage disequilibrium with HLA-B51 (9).

It is generally accepted that linkage disequilibrium extends <300–500 kb in large outbred populations; however, there are reports of linkage disequilibrium spanning very long distances in special populations or in particular regions of the genome (11,12,16). For example, hereditary hemochromatosis is associated with HLA–A3, but because of a reduced rate of recombination, the HFE gene was positionally cloned in a region ~4 cM telomeric to the HLA–A locus (11,12). Similarly, we first hypothesized that a susceptibility gene for Behçet’s disease was located in this extended MHC region and in linkage disequilibrium with the HLA–B locus. However, linkage studies in 28 families revealed a highly significant NPL score in a region nearly 17 cM telomeric to HLA–B. Since a normal rate of recombination was observed beyond the HFE gene (12), an association between HLA–B51 and D6S285 is unlikely.

Our finding of NPL scores <3 in the HLA–B region of 28 families does not contradict our previous results confirming the linkage of the HLA–B locus to Behçet’s disease with the use of the TDT. Allelic association studies, including family-based methods such as TDT, have much more power compared with linkage analysis, especially when the marker is a candidate gene itself. The broad peak of linkage observed in this study can also be interpreted as an extension of the positive linkage finding for the HLA–B locus. However, we think that the maximum NPL score at D6S285 indicates a second susceptibility gene in the telomere of chromosome 6p. The genes already mapped to the genomic segment close to these markers include some transcription and nuclear factors with immunologic importance, such as SOX4, ID4, and DEK. One of the putative inflammatory bowel disease susceptibility genes (IBD3) has also been mapped to this telomeric region (17).

We believe that the identification of this novel susceptibility gene will help in further understanding the pathogenesis of Behçet’s disease. This, in turn, might enable us to develop new treatment modalities and better diagnostic tests for the disease.

REFERENCES