A major susceptibility locus for leprosy has recently been mapped on chromosome 10 (10p13) by genome-wide linkage analysis. Microsatellite markers from this genome screen that showed suggestive evidence of linkage to leprosy were evaluated in an additional 140 families with affected sib pairs. A second region of linkage has thus been identified on chromosome 20 (20p12). The peak of linkage lies at marker D20S115, which has a significant single-point maximum logarithm of odds score of 3.48 ($P < .00003$). Transmission disequilibrium testing of the microsatellite markers in 20p12 showed that the marker D20S835 is associated with protection against leprosy ($P = .021$), which suggests that a locus controlling susceptibility lies close to this marker.

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*. Globally, there were ~720,000 cases in the year 2000, with the majority of these occurring in India [1]. It is a disease that has long been recognized to occur in families. Twin studies in India demonstrated higher concordance rates for leprosy in monozygotic twins (60%–85%) than in dizygotic twins (5%–20%) [2, 3]. Complex segregation analyses have found models consistent with a single major codominant or recessive gene and perhaps several modifying genes controlling susceptibility to leprosy in some populations [4, 5]. We have previously carried out a genome scan and, despite the polygenic nature of leprosy susceptibility, identified a major susceptibility locus on chromosome 10p13 (maximum logarithm of odds score [MLS], 4.09) [6]. Because of the likelihood that several genes are relevant to leprosy susceptibility, we decided to extend this work. We did this by examining additional microsatellite markers that showed suggestive evidence of linkage to leprosy in the first round of the genome screen but that had not previously been analyzed further because, in the absence of more–finely spaced markers, strong evidence of another locus was not apparent.

We have thus identified a region on chromosome 20 (20p12) that is both linked to and associated with leprosy susceptibility.

Methods

**Sample collection.** Indian families in which ≥2 siblings were affected by leprosy were identified from patient records [6]. One hundred seventy-five families (which included 185 independent affected sib pairs) were found in Sakthi Nagar and Kumbakonam in Tamil Nadu. Fifty-eight other families (including 71 independent sib pairs) were found in Vizag and Andhra Pradesh. Both parents were available for study participation in all but 5 families. All siblings were affected by paucibacillary leprosy, except for 10 families in which 1 sibling was affected by multibacillary leprosy. In no families did all affected siblings have multibacillary leprosy. Diagnosis was made using World Health Organization guidelines [http://www.who.int/lep/disease/disease.htm] for all patients.

**Study design.** Microsatellite typing of families was done in 2 stages. The first stage included 93 families (103 independent sib pairs), all of which were from Tamil Nadu, India. The second stage included 140 more families (153 independent sib pairs), 82 (82 independent sib pairs) of which were from Tamil Nadu and 58 (71 independent sib pairs) of which were from the Andhra Pradesh region of India. Regions of the genome were considered to have suggestive evidence of linkage with leprosy in the first set of families if the marker attained a significance level of $P < .1$ or an MLS ≥1. Eleven markers (D1S2800, D2S347, D3S2338, D3S3681, D3S1278, D3S1292, D6S446, D7S661, D7S2465, D18S478, and D20S115) that met either of these criteria in the first round were typed in the second-stage families. Eight more markers on chromosome 20p12 were typed in all families to increase the marker density around D20S115.

**Genotyping.** Semiautomated fluorescent genotyping was used to type the microsatellite markers [6]. Standard polymerase chain reaction conditions were used, and the pooled reaction products
were analyzed by capillary electrophoresis, using an ABI Prism 3700 DNA Analyzer (Applied Biosystems). Genotype analysis was carried out using Genotyper software (Applied Biosystems).

Statistical analysis. Genotypes were analyzed using the SIBPAIR ANALYZE (ftp://ftp.ebi.ac.uk/pub/software/linkage_and_mapping/linkage_cpmc_columbia/analyze/) and MAPMAKER/SIBS (ftp://ftp-genome.wi.mit.edu/distribution/software/sibs/) program, as described elsewhere [6]. The position of each marker in centimorgans is based on the Genethon map (ftp://ftp.genethon.fr/pub/Gmap). Transmission disequilibrium testing was carried out using the TRANSMIT program (http://www-gene.cimr.cam.ac.uk/clayton/software/) [7]. A single offspring was selected at random from the families included the analysis, to detect association independent of linkage. To assess the interaction between 2 regions of linkage, the program TWOLOC (ftp://ftp.well.ox.ac.uk/pub/genetics/twoloc/) was used [8]. This is a maximum likelihood-based multilocus linkage test that evaluates the support for an interaction between constituent susceptibility loci. The program computes MLS statistics for various user-defined 2-locus models.

Results

We evaluated 11 microsatellites that showed weak evidence of linkage to leprosy in the first stage of a genome scan [6]. We typed these markers in 140 more families (153 independent sib pairs). Significant evidence for linkage was detected at marker D20S115, which had a single-point MLS of 3.39 ($P = 0.0003$) and a multipoint MLS of 2.17. The 2 markers flanking D20S115 (D20S97 and D20S163) were genotyped in the second-stage families, and, to increase the marker density in the region, 8 additional microsatellite markers were also analyzed in the first and second stage (table 1). After inclusion of these marker data, the peak of linkage on chromosome 20 became more significant when only the 175 families (185 independent sib pairs) from Tamil Nadu were included. The peak of linkage at marker D20S115 in these families had a single-point MLS of 3.48 and a multipoint MLS of 3.16 (figure 1).

Because the sib-pair samples collected from both regions were mainly from siblings with the paucibacillary form of leprosy, we examined the effect of removing the small number of multibacillary cases from the analysis. We found that there were no significant differences in either the location or the strength of linkage when siblings with multibacillary leprosy were excluded (data not shown). These data suggest that the region of linkage on chromosome 20 may be linked more strongly to paucibacillary leprosy susceptibility in the Tamil Nadu population than in the Andhra Pradesh population. This finding reflects a recognized problem in carrying out genome scans in mixed populations, in which loci with susceptibility alleles more frequent in one population may dilute the overall strength of linkage in the combined populations.

Because linkage in the region was only observed in the families from Tamil Nadu, only this group was included in testing for association. Transmission disequilibrium testing of D20S115 and its 8 flanking markers was carried out using the TRANSMIT program. An allele of the microsatellite marker D20S835 was found to be associated with protection against leprosy ($P = 0.021$).

Several genes are likely to be relevant to leprosy susceptibility. Linkage to a region of chromosome 10 has previously been identified in this population [6], and therefore we asked whether the genes controlling susceptibility in these chromosomal regions interact or act independently, as if they represent 2 independent biological pathways leading to disease. The TWOLOC program was used to test for interactions between marker D20S115 and the 6 markers within the chromosome 10 region of linkage

Table 1. Single-point and multipoint maximum logarithm of odds score (MLS) values for families from Tamil Nadu and Andhra Pradesh, India.

| Microsatellite marker | Position, cM | MLS values for all families | | | | MLS values for families from Tamil Nadu | | | | MLS values for families from Andhra Pradesh | | |
|-----------------------|--------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                       |              | Single point | Multipoint | Single point | Multipoint | Single point | Multipoint | Single point | Multipoint |
| D20S97                | 12.1         | 0.036         | 0.13        | 0.66         | 0            | 0            | 0            |
| D20S835               | 14.8         | 0.13          | 1.4         | 0            | 0            | 0            | 0            |
| D20S905               | 16.9         | 0.25          | 0.659       | 1.956        | 0            | 0            | 0            |
| D20S194               | 18.0         | 0.29          | 1.75        | 2.06         | 0            | 0            | 0            |
| D20S846               | 18.5         | 0.45          | 0.494       | 2.325        | 0            | 0            | 0            |
| D20S115               | 20.9         | 1.29          | 3.48        | 3.16         | 0.38         | 0            | 0            |
| D20S900               | 20.9         | 1.29          | 0.585       | 3.16         | 0            | 0            | 0            |
| D20S851               | 23.8         | 0.7           | 0.51        | 1.898        | 0            | 0            | 0            |
| D20S117               | 25.0         | 0.31          | 0.808       | 1.338        | 0            | 0            | 0            |
| D20S160               | 27.7         | 0.11          | 0.044       | 0.92         | 0            | 0            | 0            |
| D20S163               | 31.4         | 0.005         | 0.147       | 0.398        | 0            | 0            | 0            |

NOTE. Single-point MLS values were calculated using SIBPAIR ANALYZE; multipoint MLS values were calculated using MAPMAKER/SIBS.
(D10S1763, D10S1661, D10S548, D10S1662, D10S582, and D10S1660). No significant interaction was observed between the regions of linkage on chromosomes 10 and 20 \( (P > .05) \) in the families from Tamil Nadu. This suggests that the genes at these loci act independently of each other.

**Discussion**

A genome scan in South India has identified a region of linkage for susceptibility to leprosy on chromosome 10 [6]. Because >1 gene is likely to control leprosy susceptibility, we examined additional markers that showed suggestive evidence of linkage in our initial study. A region of linkage on chromosome 20 (20p12) was identified as a susceptibility locus in the Tamil Nadu region of South India. However, this chromosome region does not appear to be linked among families from Andhra Pradesh. Fewer families from Andhra Pradesh than from Tamil Nadu were included in the present study (58 vs. 175), and therefore we would not expect the evidence of linkage to be as strong. However, the observation may indicate real differences between the 2 populations, such as differences in the relative importance of various susceptibility loci, in disease manifestations, or in the prevalent *M. leprae* strains. Because standard diagnostic criteria were used in the classification of leprosy type and because the paucibacillary form was predominant in both geographic regions, it is less likely that differences in disease diagnosis are responsible.

Although all the families in this study were from India, it is recognized that many different populations with different origins and migration histories have contributed to the complex genetic structure of this country. Also, the many caste populations may not be uniform over the entire country. Studies of Y chromosome markers, mDNA, and HLA reiterate this diversity and have traced the migration routes and diversity of the caste populations of India [9–11]. It is not uncommon to find that a region of linkage or association shows heterogeneity between populations. Several genome scans of complex diseases, such as type 2 diabetes, have been carried out in a wide range of populations. There have been some overlaps in the loci identified in these studies; however, several chromosome regions appear to be population specific and have yet to be replicated in other studies. Genetic evidence for a population-specific allele-dependent predisposition has also been obtained for South Indian pulmonary tuberculosis and psoriasis [12, 13].

Analysis of the region of linkage on chromosome 10p13 suggested that it contributes substantially to the total genetic component of leprosy susceptibility [6]. However, as is true of other complex diseases, the number of genes controlling susceptibility to leprosy is unknown. The identification of a second susceptibility locus in this study highlights the importance of following up chromosomal regions that show evidence of linkage, even though data may not reach a significant threshold. It is also likely that some of the susceptibility loci will be dependent on the genotype at another locus. Important biological information could be gained by knowing whether such genetic interactions exist. Two susceptibility loci have now been identified by a leprosy genome scan in a South Indian population. We did not find any evidence of a genetic interaction occurring between the 2 regions, which suggests that they represent 2 independent biological pathways leading to disease. However, there would not have been sufficient power to detect a minor interaction in this dataset.

Transmission disequilibrium testing of the microsatellites in and around the region of linkage found that one of the markers is associated with protection against leprosy \( (D20S835) \). There are no known genes in this region that appear to be strong
positional candidates [14]. However, at present, there are a num-
ber of expressed-sequence tags and predicted exons with un-
known functions. The association detected in the present study
provides a useful starting point for further fine-mapping and
association studies aimed at identifying the gene.

It is likely that the pathways affecting many autoimmune/
inflammatory diseases may be the same, even though the clinical
phenotypes may be distinct. Linkage and association to the
major histocompatibility complex region has been reported for
many immune diseases, although the actual genes and alleles
responsible for susceptibility are different. The region of chro-
mosome 20 found to be linked to leprosy in this study has been
found elsewhere to contain a region of susceptibility for atopic
dermatitis [15] and psoriasis [16, 17]. Atopic dermatitis, psori-
asis, and leprosy are all skin diseases caused by inappropriate
immune responses to an environmental stimulus. The region of
linkage shared on chromosome 20 may, therefore, indicate the
presence of a gene or multiple genes involved in the regulation
of immune responses.

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