

FULL PAPER

Genome scan for loci linked to mite sensitivity: the Collaborative Study on the Genetics of Asthma (CSGA)

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Mite sensitivity has been reported to be a major risk factor for asthma. As part of the Collaborative Study on the Genetics of Asthma (CSGA), a genome scan using mite reactivity (*Dermatophagoides Pteronyssinus* (*Der p*) and *Dermatophagoides farinae* (*Der f*)) as the phenotype was conducted. In 287 CSGA families, 122 were informative for linkage. Evidence supporting linkage was observed for regions on chromosome 19 (D19S591, lod = 2.43, $P = 0.0008$; D19S1037, lod = 1.57, $P = 0.007$) and chromosome 20 (D20S473/D20S604, lod = 1.41, $P = 0.01$). All three ethnic groups appeared to contribute to the evidence for linkage on chromosome 20. African-American families gave strongest support for linkage on chromosomes 3 (D3S2409, lod = 1.33, $P = 0.01$), 12 (D12S373, lod = 1.51, $P = 0.008$) and 18 (ATA82B02, lod = 1.32, $P = 0.01$). Caucasian families showed strong evidence for linkage on chromosome 19 (D19S591, lod = 3.51, $P = 0.00006$). Hispanic families supported linkage on chromosomes 11 (D11S1984, lod = 1.56, $P = 0.007$), 13 (D13S787, lod = 1.30, $P = 0.01$) and 20 (D20S470, lod = 1.71, $P = 0.005$). These results suggest that multiple genes may be involved in controlling skin reactivity to *Dermatophagoides*.

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Introduction

The importance of genetic factors in the development of atopy has been noted since its initial descriptions.¹ Atopy or allergic skin reaction to specific allergens has been shown to cluster in families, and its prevalence is increased among first-degree relatives of atopic subjects. Concordance for atopy-associated phenotypes in monozygotic (MZ) twins is significantly greater than that observed for dizygotic (DZ) twins.² Reviews of heritability estimates have been reported for total serum IgE (47–84%), for specific skin test reactivity (8–21%) and for specific serum IgE levels against house dust mites (14–34%),^{2,3} further suggesting that a genetic component is important in the development of both atopy and specific mite responses. In addition, sensitization to mite allergens is associated with asthma,⁴ thus motivating the search for genes governing mite skin test reactivity.

The Collaborative Study of the Genetics of Asthma (CSGA) has completed a genome screen to study genetic control of asthma and its associated phenotypes.

Families evaluated by the CSGA were recruited through an asthmatic sib pair, drawing from three ethnic groups (Caucasian, African-American and Hispanic-American). Genome-wide linkage analyses to identify genes influencing the risk to asthma,⁵ total IgE levels⁶ and generalized atopy (defined as the presence of at least one positive skin test to a set of common allergens)⁷ have been reported previously. Here, we present the analysis of the genome-wide scan using positive skin test response for two asthma-associated perennial allergens, *Der pteronyssinus* (*Der p*) and *Der farinae* (*Der f*), as the phenotype in a genome-wide scan for susceptibility genes.

Results

Subject characteristics

The proportion of our sample that was positive to mite allergens is shown in Table 1. In the total CSGA sample, 1284 of the 1793 subjects (71.6%) who underwent skin prick testing were atopic (positive for at least one allergen) and 672 (52.3%) of these subjects were positive for mites. For the 960 subjects who had clinical pulmonary testing and were diagnosed with asthma, 422 (44.0%) had a positive response to mite allergen. In the 750 subjects who have both asthma and atopy ('atopic asthma'), 422 (56%) were positive for mites.

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Table 1 Proportion of mite-sensitive subjects within phenotype groups, by ethnicity

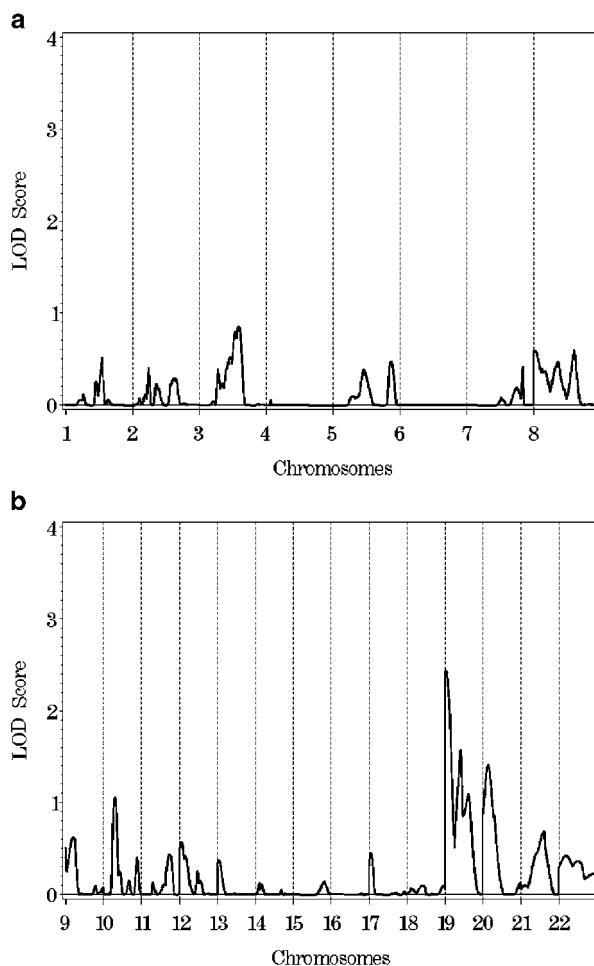
Ethnic group	Phenotype groups			Sample size	
	Atopy	Asthma	Atopic asthma	Total CSGA families	Families informative for linkage
African American	209/413 (50.6%)	141/369 (38.2%)	141/275 (51.3%)	115	33
European American	394/715 (55.1%)	232/494 (47.0%)	232/384 (60.4%)	138	74
Hispanic American	69/156 (44.2%)	49/97 (50.5%)	49/91 (53.8%)	34	15
Total	676/1284 (52.3%)	422/960 (44%)	422/750 (56%)	287	122

Overall, approximately half of these subjects were positive for mites, and these proportions were quite similar across ethnic groups (Table 1). Among the 287 families (115 African-American, 138 Caucasian and 34 Hispanic-American), 122 were informative for mite sensitivity (two or more relatives positive for either *Der p* or *Der f*) (33 African-Americans, 74 Caucasians and 34 Hispanic-American) (Table 1).

Of the 112 families informative for mite sensitivity (either *Der p*, *Der f* or both), there were 105 families informative for *Der f* (29 African-Americans, 64 Caucasian and 12 Hispanic-Americans) and 105 families informative for *Der p* (25 African-Americans, 69 Caucasian and 11 Hispanics); most of these families were positive for both allergens. No significant differences in their clinical variables were seen across ethnic groups. The Caucasians were slightly older (probably due to the recruitment of larger, and older, families recruited in Minnesota). Mean log serum IgE levels were slightly higher in African-Americans (2.22–2.29) compared to Caucasians (1.96–1.98) and Hispanics (2.07–2.14). Hispanics had higher pulmonary compared to African-Americans and Caucasians (87–92%). PC20 was higher in Caucasians (6.15–6.29) compared with either Hispanics or African-Americans (4.16–4.90). Skin test results were similar across ethnic groups, with the exception in Hispanics (mean positivity of 6.2) due to testing for three additional New Mexico-specific allergens in this ethnic group.

Genome-wide screen

The results for the genome screen for mite sensitivity in the CSGA families are shown in Figures 1a (chromosomes 1–8) and 1b (chromosomes 9–22). Among all informative families for mites (positive reaction in two or more relatives for either *Der p* or *Der f*), evidence for linkage was strongest on chromosome 19 (D19S591, lod = 2.43, $P = 0.0008$; D19S1037, lod = 1.57, $P = 0.007$) and on chromosome 20 (D20S473/D20S604, lod = 1.41, $P = 0.01$). When subdivided by ethnic group (Figures 2a and b), the African-American families gave the strongest support for linkage on chromosome 3 (D3S2409, lod = 1.33, $P = 0.01$), chromosome 12 (D12S373, lod = 1.51, $P = 0.008$) and chromosome 18 (ATA82B02, lod = 1.32, $P = 0.01$). Caucasian families showed significant evidence for linkage on chromosome 19 (D19S591, lod = 3.51, $P = 0.00006$). Hispanic families supported linkage to genes controlling mite reactivity on chromo-

**Figure 1** Genome scan for mite sensitivity, all families: (a) chromosomes 1–8 and (b) chromosomes 9–22.

some 11 (D11S1984, lod = 1.56, $P = 0.007$), chromosome 13 (D13S787, lod = 1.30, $P = 0.01$) and chromosome 20 (D20S470, lod = 1.71, $P = 0.005$).

A summary of linkage results for *Der p* and *Der f*, stratified by individual chromosomes, is presented in Table 2. The evidence for linkage on chromosome 19 is maintained, with the Caucasian families most strongly supporting linkage for sensitivity to both *Der p* and *Der f*, as seen within the combined mite sensitivity phenotype. Linkage on chromosome 20 was influenced primarily by

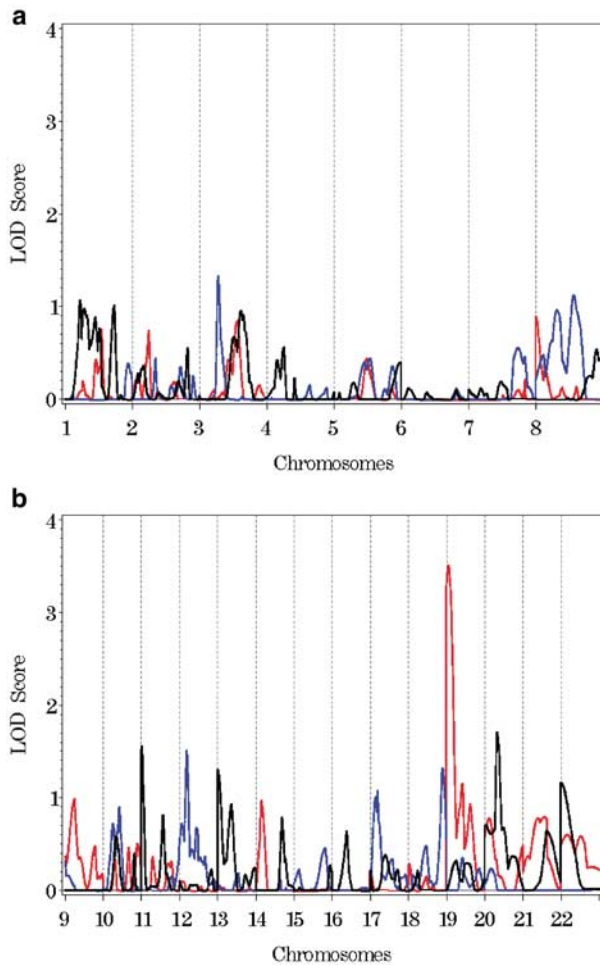


Figure 2 Genome scan for mite sensitivity: (a) African-American (black lines), Caucasian (blue lines) and Hispanic families (red lines) chromosomes 1–8 and (b) African-American, Caucasian and Hispanic families chromosomes 9–22.

the sensitivity to *Der p* (within all three ethnic groups). Evidence supporting linkage to the *Der f* phenotype was apparent on chromosome 20 in Caucasians. Hispanic families strongly supported linkage near D20S470 for both *Der p* and *Der f*. Support for linkage to chromosome 12 in African-Americans was primarily due to the evidence for linkage using sensitivity to *Der p* as the phenotype, while both *Der p* and *Der f* sensitivity contributed to linkage signals on chromosomes 3 and 18. There was little evidence supporting differences in the evidence for linkage using either *Der p* or *Der f*, suggesting that a common set of genes may control sensitivity to these common mite allergens.

Discussion

Few genome scans have been performed using the phenotype of specific skin test reactivity, although several have been performed using the phenotype of skin test reactivity to any allergen as reviewed by Blumenthal.² Investigation of the general phenotype of skin sensitivity to one or more common aeroallergens has been reported previously for this CSGA data.⁶ A complex pattern of the evidence for linkage to several different regions on chromosomes 11q and 20p was observed in this analysis. Multilocus modeling suggested that, in addition to these two chromosomal locations, two other regions (on 10p and 17q) accounted for the majority of evidence for linkage to atopic responses in these CSGA families. There was also evidence supporting gene–gene interaction, based on findings in the subset of pedigrees that provided positive evidence for linkage on chromosome 11q also supporting linkage to other chromosomal locations. In this subset of families, the evidence for linkage increased significantly for regions on chromosome 5q, 8p, 12p and 14q. Since this previous analysis could reflect a generalized reactivity to multiple allergens, further refinement of the atopic phenotype could

Table 2 Maximum lod scores and positions for linkage to mite sensitivity by chromosome for the entire CSGA sample and partitioned by ethnic group

Chrom	Total sample lod score (position)	Caucasian lod score (position)	African-American lod score (position)	Hispanic lod score (position)
1	0.51 (151.7)	0.75 (152.2)	0.39 (258.7)	1.06 (65.2)
2	0.40 (64.5)	0.74 (64.0)	0.44 (90.5)	0.55 (215.5)
3	0.85 (141.5)	0.84 (134.5)	1.33 (70.5)	0.96 (147.0)
4	0.05 (25.9)	0.01 (25.9)	0.15 (129.9)	0.56 (59.4)
5	0.47 (152.0)	0.44 (85.0)	0.44 (93.5)	0.40 (172.5)
6	0.00 (166.2)	0.00 (9.2)	0.11 (154.7)	0.12 (26.7)
7	0.41 (154.7)	0.21 (154.7)	0.55 (136.2)	0.19 (94.2)
8	0.60 (101.3)	0.89 (4.3)	1.12 (94.3)	0.54 (148.8)
9	0.61 (36.7)	0.99 (42.7)	0.32 (14.2)	0.00 (14.2)
10	1.06 (56.3)	0.50 (156.3)	0.90 (75.3)	0.58 (62.8)
11	0.43 (97.6)	0.36 (40.1)	0.34 (131.1)	1.56 (3.6)
12	0.57 (14.4)	0.10 (17.9)	1.51 (35.9)	0.22 (135.9)
13	0.37 (13.4)	0.03 (93.9)	0.18 (63.9)	1.30 (8.9)
14	0.12 (26.0)	0.97 (30.5)	0.11 (91.5)	0.78 (96.0)
15	0.14 (89.7)	0.01 (88.2)	0.46 (89.7)	0.27 (100.7)
16	0.01 (95.2)	0.01 (87.7)	0.02 (101.2)	0.64 (50.7)
17	0.45 (6.6)	0.21 (0.6)	1.07 (23.6)	0.39 (50.6)
18	0.09 (47.5)	0.28 (2.0)	1.32 (104.0)	0.21 (28.0)
19	2.43 (11.8)	3.51 (13.8)	0.35 (47.8)	0.32 (32.8)
20	1.41 (21.0)	0.78 (19.0)	0.24 (23.5)	1.71 (37.0)
21	0.68 (36.5)	0.79 (33.5)	0.00 (3.0)	0.65 (37.0)
22	0.42 (12.1)	0.60 (10.6)	0.02 (45.6)	1.16 (4.1)

clarify as to which of these linkage signals, if any, reflect reactivity to specific mite allergens.⁶

In our study, mite sensitivity for the CSGA families gave the strongest support for linkage on chromosomes 19 and 20. In the genome scan for specific atopy, evidence supporting linkage ($\text{lod} \sim 1.0$) occurred on chromosome 19p at D19S591 in Caucasians, which is the same location where we observed evidence of linkage for the mite reactivity phenotype. In addition, the region identified on chromosome 20q for atopy (between D20S473 and D20S604) was the same region observed here for specific mite reactivity. Among African-American families, there was previous support for linkage to genes contributing to atopy for chromosome 12p (D12S373),⁶ the same location that provided evidence for linkage using only mite reactivity. In Hispanic families, the region on chromosome 20 that provided strong support for mite reactivity (D20S470) was also implicated in linkage analysis using a general atopy phenotype.⁶

The results from the CSGA genome scan fail to provide evidence supporting a quantitative trait loci (QTL) contributing to mite sensitivity in the HLA region on human chromosome 6p21.3. There have been a large number of studies in the literature demonstrating an association of specific HLA antigens with an allergic response, including *Der p* and *Der f*.² As noted here, few studies have been able to examine the evidence for linkage of HLA loci with the allergic response. In the CSGA, the genome scan did have markers near (but not within) the HLA region on 6p21.3; however, there was little evidence for linkage of HLA with mite sensitivity. This does not exclude the possibility that certain HLA alleles are associated with mite sensitivity.

Three other genome screens using a phenotype for mite-immediate sensitivity and mite-sensitive asthma have previously been investigated. In a founder population, Ober *et al*⁸ reported evidence of linkage to mite sensitivity on chromosome 2 (D2S2944) and chromosome 20 (D20S470), the latter also being observed in our CSGA Hispanic families. Others have also identified the region on 20p (20p12–p11.23) as potentially involved in asthma and atopy-related phenotypes. Linkage to asthma was found in Caucasian families to the 20p13 region that contained ADAM33, a putative asthma susceptibility locus.^{9,10} One marker that defines the ADAM33 region (D20S906) is within 2 cM of our telomeric marker (D20S473), which support the evidence of linkage to mite sensitivity.^{5,11} In a founder population, Ober *et al*⁸ reported that the phenotype of skin test reactivity to house dust mites was linked to a site on chromosome 20, but at 39 cM from pter, somewhat proximal to the region of interest from this analysis.

Koppleman *et al*¹² reported linkage using skin test reactivity in a Dutch population to chromosomal regions on 5p15, 8p23, 11q22, 12p13, 17q21 and 22q11. When specific IgE in the serum was used as the phenotype, linkage was reported with regions on chromosome 3q25–q26, 18p11 and 22q11. A common area on chromosome 12p may occur between our population using specific *Der p* and *Der f* phenotypes with those identified in the Dutch study using phenotypes based on a combination of *Der p* and *Der f* allergen. A study of mite-sensitive asthma by Yokouchi *et al*¹³ demonstrated linkage on chromosome 5q31–33 as well as suggestive evidence of linkage to 4q35 and 13q11 (where our Hispanic families

yielded some evidence of linkage on 13p). Lastly, a genome-wide candidate region search in a multiethnic European population observed a maternal imprinting effect for 8p23 (MOD score, 4.76) in a British population (Kurz *et al*¹⁴); however, there was suggestive evidence for linkage on chromosome 19 (D19S922, NPL = 1.95, $P = 0.02$) but not on chromosome 20. Evidence for linkage observed in these CSGA Caucasian families was at D19S591 ($\text{lod} = 3.51$), approximately 15 cM distal to the site reported by the European study. Thus, it appears that skin test reactivity is a complex phenotype, one that most likely involves control by a variety of genetic as well as environmental factors.

Materials and methods

Family ascertainment

The recruitment and the clinical characteristics of these multiplex asthmatic families ascertained by the CSGA have been reported previously.^{15,16} Briefly, families were ascertained at four centers—Johns Hopkins University (Baltimore, USA) with Howard University (Washington, DC, USA); the University of Chicago; the University of Maryland (Baltimore, USA) with the University of New Mexico; and the University of Minnesota. Families were ascertained through two asthmatic siblings, and were expanded to include other relatives either by extending the family through asthmatic relatives or through no more than one unaffected relative (to include other asthmatic relatives). Each Institutional Review Board approved CSGA protocols, and informed consent for all diagnostic procedures was obtained from all subjects.

All subjects were evaluated using a standardized CSGA protocol.¹⁵ To be included in these studies, each of the two asthmatic siblings had to have met the following criteria: (1) at least 6 years of age; (2) either bronchial hyper-responsiveness, defined as a fall in baseline FEV1 greater than 20% in 1 s after inhalation of less than 25 mg/ml methacholine, or reversibility, defined as a greater than 15% increase in baseline FEV1 after inhaled bronchodilator (albuterol) for those with reduced baseline FEV1; (3) presence of two or more of the symptoms of coughing, wheezing and shortness of breath; (4) less than three pack-years of cigarette smoking; and (5) physician's diagnosis of asthma with no conflicting pulmonary disease. All family members were evaluated for asthma using identical protocols.¹⁶ Clinical characteristics of these subjects have been described elsewhere.¹⁵

Phenotyping

A standardized, interviewer-administered questionnaire was given to all subjects. The American Thoracic Society respiratory questionnaire¹⁷ was modified to include questions about the frequency, severity and duration of asthma and atopy symptoms in order to clarify conflicting diagnoses, and to document current and prior therapy. Pulmonary function studies, including methacholine challenges, were performed as described previously.¹⁵ Serum IgE levels were measured using standard methods.¹⁵

Skin testing

Skin prick testing was performed using standardized allergens *Der p*, *Der f*, 12 other standardized allergens (*Cladosporium herbarum*, *Aspergillus fumigatus*, *Alternaria tenuis*, *Poa pratensis*, *Ambrosia artemisiifolia*, *Artemisia vulgaris*, *Betula nigra*, *Quercus alba*, *Felis domesticus*, *Canis familiaris*, *Gistlia germana*, *Periplaneta americana*), as well as a glycerol saline (negative) and histamine (positive) control. Reactions were recorded by tracing the perimeter of the wheal that occurred 15 min after the puncturing of the skin on transparent surgical tape (Transpore; 3M, Minneapolis, MN, USA). This tracing was transferred to paper as a permanent record. The area from skin prick wheal reactions was estimated from the paper tracings or by calculating the elliptical area of the widest diameter measured with the diameter of the perpendicular axis. Significant skin reactivity was defined as a wheal area greater than 9 mm² greater than that observed for the negative control.

Genotyping

DNA was extracted from whole blood, cell pellets or EBV-transformed lymphoblastoid cell lines at each of the four centers using standard methods. Genotyping was performed at the Mammalian Genotyping Service (MGS) in Marshfield, Wisconsin (http://research.marshfieldclinic.org/genetics/Genotyping_Service/mgsver2.htm) using a scanning fluorescence detector system developed for high-throughput genotyping. Data used in these analyses included 323 highly polymorphic markers of the human autosomes. The original screening set included a mixture of dinucleotide, trinucleotide and tetranucleotide polymorphisms, with average distance between markers of approximately 10 cM. In all tables and figures, location (position) is given in centiMorgan (cM) distance from the most distal marker on the *P*-chromosomal arm (pter). Sex-averaged distances from this point of origin are based on the MGS map.

Prior to genetic analysis, each genome scan marker was evaluated using a CSGA quality control procedure. A set of blind duplicate samples were included in those subjected to the genome scan. As the CSGA samples were genotyped in two batches, a set of identical quality control samples was genotyped in each of two batches. Markers that were judged by the CSGA to have high rates of inconsistent results within and across batches were deleted from formal analysis and replaced by alternate markers in the same location. A set of 48 markers was deleted from the original sample (as a result of the CSGA quality control procedures) and gaps over 20 cM (resulting from deletion of markers) were filled using non-MGS markers. These 'gap' (non-MGS) markers were genotyped by each center using appropriate CEPH standards to allow for each laboratory to calibrate standards and to ensure comparability of allele sizes.

Statistical methods

A series of preliminary analyses were performed on the genetic marker data, including checks for Mendelian consistency and analyses of likely pedigree structure. In the latter, each relative pair has an expected sharing of alleles identical by descent, so the observed sharing (from the marker data) can be used to determine the likelihood of the reported relationship. For all significant deviations from the reported relationship, pedigree

structures were corrected to provide the most likely relationship. All analyses were performed on these corrected pedigree structures.

For linkage analyses, Marshfield map distances were used. Estimates of allele sharing were based on the marker allele frequencies estimated from each ethnic group. As parents were available for most of these families, the impact of missing parents (and, therefore, marker allele frequencies) was negligible. The estimated likelihood within each ethnic group was pooled to obtain the likelihood for the combined (total) sample. This process was performed so ethnic-specific marker allele frequencies could be used. Evidence for linkage over the genome was obtained by nonparametric multipoint linkage analysis using the S(pairs) statistic of the computer program GeneHunter-plus.^{18,19} One-parameter allele-sharing model lod scores were calculated based on the distribution of test statistics under the null hypothesis of 'no linkage' and conditional on the data using the ancillary software (asm, Kong and Cox¹⁹) for each ethnic group separately and for the three ethnic groups combined. For the initial genome screen, each family was given equal weighting in the analysis.

For the 'pooled' analysis (in which all families are used to provide evidence for QTLs across ethnic groups), the estimated identity-by-descent statistics for the pooled data were based on individual ethnic-specific marker allele frequencies. At each position in the genome, for each family (within each ethnic group), likelihoods were computed. The 'pooled analysis', therefore, represented the combination of the likelihoods from each ethnic group in which ethnic-specific frequencies were used. The final 'pooled analysis' consisted of comparisons of likelihoods that were composites of the ethnic-specific data, not a simple average of marker allele frequencies over all families.

Evidence for linkage is reported for lod scores greater than 1.18, corresponding to a nominal $P < 0.01$. The criterion for presenting linkage data remains a matter of debate, including the description of evidence in support of a region as 'significant', 'strongly supportive', 'supportive' or 'promising' for linkage. The Lander-Kruglyak²⁰ criterion (lod > 3.3) has been described as being too conservative. In contrast, an lod > 1.75 ($P < 0.0023$)²¹ would be equivalent to 1 false-positive region for each genome scan given a set marker density. Others suggest lod > 1.18 ($P < 0.01$) as one that should encourage further investigation. As it is becoming apparent in the detection of QTLs that contribute to either variation in a phenotype or to susceptibility is based on many unknown factors, the less stringent criterion of lod > 1.18 (nominal $P < 0.01$) is used in this report to indicate linkage worthy of further investigation.

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