

A genome-wide search for quantitative trait loci contributing to variation in seasonal pollen reactivity

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Background: Asthma and atopy represent complex traits for which genetic predisposition has been demonstrated. Pollen sensitivity, whether seasonal or chronic, appears to be a major contributor to the asthmatic phenotype.

Objective: Regions of the genome contributing to skin test reactivity to 5 seasonal allergens are to be identified in a genome-wide scan. These regions may be distinct from those contributing to risk for asthma and/or atopy.

Methods: In the Collaborative Study on the Genetics of Asthma, 4 sites collected 287 families with 2 or more members with asthma. Reactivity to individual pollens were determined on all family members. A genome scan was performed at 9-centiMorgan intervals, and skin test reactivity to 5 seasonal allergens was the focus of nonparametric genetic linkage analysis.

Results: Chromosomal regions that exhibited suggestive linkage (logarithm of the odds >1.18; $P < .01$) to seasonal pollen reactivity were identified on chromosomes 13q34, 20p12, and 21q21. Evidence of ethnic differences in linkage to seasonal allergens was demonstrated, with support for linkage in African American subjects on chromosomes 8, 10, and 12, in European American subjects on chromosomes 14, 19, 20, and 22, and in Hispanics on chromosome 21. In all families, evidence for linkage of skin test reactivity for *Betula*, *Lolium*, and *Artemisia* was strongest in a region on chromosome 21 that contained the candidate gene, A Disintegrin And Metalloprotease domain 33 (*ADAM33*).

Conclusion: These results suggest both substantial genetic overlap and extensive heterogeneity in the genetic basis for the allergic response to seasonal allergens. (J Allergy Clin Immunol 2006;117:79-85.)

Key words: Genome scan, seasonal pollen sensitivity, asthma, atopy, linkage

Asthma and its associated phenotypes represent a complex trait for which a strong genetic predisposition has been demonstrated. Specific genes contributing to risk for asthma have begun to emerge, although there are continuing issues of replication of results, novel gene discovery, and biological pathways.¹⁻⁵ A major component of the asthma phenotype is atopy. The definition of atopy is composed of symptoms of asthma, rhinitis and eczema, immediate skin test reactivity to an allergen, and a family history of allergy.⁶ Many of these individual features of atopy tend to run in families, thus implicating a heritable component to risk here also.

Atopy is characterized by an adverse immune reaction involving increased production of IgE antibodies to environmental allergens. The immediate allergen skin reaction depends, in part, on the level of specific IgE and its affinity to a particular allergen. Other factors that influence skin reaction are the ability of IgE to attach to the cell containing the mediating substances, the particular mediating substances, the ability of the mediators to be released from cells, and the ability of the skin to respond to the mediator. The development of seasonal allergic rhinitis results from a combination of genetic risk and environmental exposure to specific pollen. The IgE antibody response to pollen exposure is likely to be controlled by multiple genes.⁷

The Collaborative Study on the Genetics of Asthma (CSGA) has studied the genetics of several of these phenotypes in families with asthma,^{8,9} including total serum IgE level,¹⁰ generalized atopy,¹¹ and the specific immediate skin test reactivity to house dust mites (*Der p* and *Der f*).¹² In this report, we present results on the analysis of a genome scan to identify regions that may harbor genes contributing to skin test reactivity to 5 seasonal pollen allergens: *Betula verrucosa* (*Bet v*), *Quercus alba* (*Que a*), *Lolium perenne* (*Lol p*), *Artemisia vulgaris* (*Art v*), and *Ambrosia artemisiifolia* (*Amb a*).

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Abbreviations used

ADAM33: A Disintegrin And Metalloprotease domain 33
cM: centiMorgan
CSGA: Collaborative Study on the Genetics of Asthma
LOD: Logarithm of the odds
MGS: Mammalian Genotyping Service

METHODS**Family ascertainment**

The CSGA recruitment sites were the Johns Hopkins University (with Howard University), the University of Chicago, the University of Maryland (with the University of New Mexico), and the University of Minnesota. These sites collected families with 2 or more members affected with asthma, as previously reported.^{13,14} Briefly, families were ascertained through 2 siblings with asthma. The families were expanded to permit recruitment of other relatives by either (1) extending the families through asthmatic relatives or (2) including no more than 1 unaffected relative to permit a lineage to incorporate other relatives with asthma. The inclusion criteria for each family required that the 2 siblings with asthma met the following criteria for the proband: (1) being at least 6 years of age; (2) having either bronchial hyperresponsiveness, defined as a fall in baseline FEV₁ greater than 20% in 1 second 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) having 2 or more of the symptoms of coughing, wheezing, and shortness of breath; (4) having less than 3 pack-years of cigarette smoking; and (5) having a physician's diagnosis of asthma with no conflicting pulmonary disease. All probands were evaluated for asthma by using a common protocol. Clinical characteristics of these subjects have been described elsewhere. The Institutional Review Board of each institution approved the CSGA protocol, and informed consent for all diagnostic procedures was obtained from all subjects.

Procedures

All family members underwent a standardized CSGA protocol consisting of an interviewer-administered questionnaire, pulmonary function studies including a methacholine challenge and/or reversibility studies, blood drawing for serum IgE levels, and skin prick testing using standardized allergens.^{13,14} Skin prick testing was performed in all subjects by using 14 standardized allergens (*Cladosporium herbarum*, *Aspergillus fumigatus*, *Alternaria tenuis*, *L. perenne*, *A. artemisiifolia*, *A. vulgaris*, *Betula nigra* and *Betula verrucosa*, *Q. alba*, *Der pteronyssinus*, *Der farinae*, *Felis domesticus*, *Canis familiaris*, *Gistlia germana*, *Periplaneta americana*) as well as a glycerol saline (negative) and a histamine (positive) control. The Hispanic American families were also tested with Bermuda grass, Russian thistle, and mountain cedar. The area from skin prick wheal reactions (15 minutes after the puncturing of the skin) was estimated from graph paper tracings on transparent surgical tape (Transpore; 3M, Minneapolis, Minn) 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 more than 9 mm² greater than that observed for the negative control. Allergens evaluated for this report were *A. artemisiifolia* (*Amb a*, ragweed), *A. vulgaris* (*Art v*, mugwort), *B. verrucosa* (*Bet v*, or *B. nigra*, *Bet n*, silver birch), *L. perenne* (*Lol p*, rye grass), and *Q. alba* (*Que a*, white oak).

Genotyping

DNA was extracted from whole blood, cell pellets, or EBV-transformed cell lines at each of the 4 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 autosomal markers. The original screening set included a mixture of dinucleotide, trinucleotide, and tetranucleotide polymorphisms, with average distance between markers of approximately 9 centiMorgans (cM). In all tables and figures, location (position) is given in centiMorgan 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.

Before genetic analysis, each genome scan marker was evaluated by using a CSGA quality control procedure. A set of blind duplicate samples was included in those sent to MGS for the genome scan. As the genome scan of CSGA samples was performed in 2 batches of samples, a set of identical quality control samples was genotyped in each of the 2 batches. There were 48 markers judged by the CSGA to have high rates of inconsistent results within and across batches. Any gap in the genetic map greater than 20 cM (resulting from deletion of markers) was filled by using both MGS and non-MGS markers. These markers were genotyped by each center by using Centre d'Etude du Polymorphisme Humain (CEPH) standards to allow each laboratory to calibrate allele sizes and to ensure comparability of allele calls.

Statistical methods

Preliminary analyses were performed on the genetic marker data for determining mendelian consistency and likely pedigree structure.^{15,16} Genotypes that generated sporadic inconsistencies (no consistent evidence for relationship errors) were coded as unknown for that marker in the individual. For relative pairs with significant deviations from the reported relationship, pedigree structures were corrected to reflect the most likely relationship. Analyses were repeated to assure most likely pedigrees were used for subsequent linkage analyses.

Estimates of allele sharing were based on allele frequencies obtained from within each ethnic group. Because parents were available for most of these families, the effect of missing parents (and, therefore, marker allele frequencies) was negligible. To use the ethnic-specific marker allele frequencies, the estimated likelihood for linkage within each ethnic group was pooled to obtain a likelihood estimate for the combined (total) sample. Evidence for linkage was determined by nonparametric multipoint linkage analysis using the S(pairs) statistic in GeneHunter-plus.^{17,18} The 1-parameter allele-sharing model (asm) logarithm of the odds (LOD) scores were calculated based on the distribution of test statistics under the null hypothesis of no linkage. For the initial linkage analysis, each family was given equal weighting. Here evidence for linkage is reported for LOD scores greater than 1.18, corresponding to a nominal $P < .01$, and considered as worthy of further investigation. In this report, no result reached the Lander-Kruglyak¹⁹ criteria for significant support for linkage ($P < 2.6 \times 10^{-5}$).

Genome scan microsatellite markers on chromosomes 20 and 21 (corresponding to suggestive evidence for linkage) were examined for association by using a GEE1 modeling (SAS GENMOD; SAS Institute, Cary, NC). Both allelic and genotypic associations were examined, in which the analytic models accounted for familial correlation (family structure) using a sandwich estimator of the variance under exchangeable correlation assumption. Each model was adjusted for age and sex. For each marker, a $P < .05$ was used as significance for global association. In tests of allelic association,

TABLE I. CSGA families informative for linkage using skin test reactivity to seasonal pollens as a phenotype

Families	Number ascertained	Informative* for pollen	<i>Amb a</i>	<i>Art v</i>	<i>Bet v</i>	<i>Lol p</i>	<i>Que a</i>
African American	115	48	31	11	11	31	19
European American	138	94	72	34	50	54	48
Hispanic	34	29	19	20	20	22	14
Total	287	171	122	65	81	107	81

*Reactivity to any of the 5 seasonal pollens would cause a family member to be considered affected; presence of multiple family members affected with pollen reactivity would make a family informative for linkage.

TABLE II. Pollen sensitivity in CSGA family members

Family ethnic group	Number of subjects	Pollen sensitivity in subjects with the following:		
		Atopy	Asthma	Atopic asthma
African American	612	260/413 (63.0%) n = 600	173/369 (46.9%) n = 592	173/275 (62.9%) n = 369
European American	1152	528/714 (74.0%) n = 1090	277/493 (56.2%) n = 1074	277/383 (72.3%) n = 493
Hispanic	183	130/156 (83.3%) n = 179	81/97 (83.5%) n = 176	81/91 (89.0%) n = 97
Total	1947	918/1283 (71.6%) n = 1869	531/959 (55.4%) n = 1842	531/749 (70.9%) n = 959

n = Number of individuals with measurements for pollen sensitivity and the atopy and/or asthma phenotypes.

the number of alleles was reduced so that allele frequency was greater than 5%. For D20S604, 11 alleles were reduced to 5 common alleles (frequency > 0.05) and 1 pooled allele (frequency = 0.0762). For D21S1270, 13 alleles were reduced to 8 common alleles and 1 pooled allele (frequency = 0.0418). In tests of genotypic association, the number of genotypes was reduced so that the smallest genotypic class would have at least 6 observations. For D20S604, the original 44 genotypes were reduced to 29 (28 and 1 pooled); for D21S1270, the 68 genotypes were reduced to 46. Similar reductions in allele and genotypic levels were performed for other markers on chromosomes 20 and 21.

RESULTS

Clinical characteristics

Two hundred eighty-seven families were evaluated in this study. Only a portion of these families were informative for linkage analysis—48 African American families, 94 European American families, and 29 Hispanic families—using any reactivity from the 5 pollens surveyed as the affected phenotype (Table I). Reactivity to *Amb a* (ragweed) and *Lol p* (rye grass) was most prevalent in African American families, followed by *Que a* (white oak). In European American families, there was high prevalence of reactivity to *Amb a*, followed by *Lol p*, *Bet v* (silver birch), and *Que a*. Reactivity to each of the 5 pollens was of similar prevalence in Hispanic family members.

There was a consistent trend across ethnic groups with respect to presence of atopy, asthma, and atopic asthma in the family members with reactivity to any one of these 5 seasonal pollens (Table II). In the CSGA families, 71.6% of family members who were atopic showed reactivity to at least 1 of the 5 seasonal pollens, with prevalence in African American family members the least percentage

of positive responses (63.0%), European American family members intermediate (74.0%), and Hispanic family members the highest (83.3%). The frequency of reactivity to seasonal pollens was similar in those with atopic asthma (African Americans, 62.9%; European Americans, 72.3%; Hispanics, 89.0%). The same pattern is maintained for pollen reactivity in those with asthma (African Americans, 46.9%; European Americans, 56.2%; Hispanics, 83.5%). Thus, there is extensive reactivity to pollen in members with atopy, atopic asthma, and asthma, although the prevalence differs by ethnicity.

Clinical characteristics of individuals from families of the different ethnic groups were similar with respect to age, age of onset, having asthma, serum IgE levels, skin test reactivity, and pulmonary function studies (Table III). The mean age at ascertainment for family members was equivalent in the African American (25.9 ± 15.6 years) and Hispanic (24.2 ± 16.1 years) and younger than the European American (34.2 ± 19.3 years) family members. This age difference was a result of the extended families with older participants identified at the Minnesota site, whereas the other sites focused on a predominantly pediatric population. This pattern is also observed in the age at onset for asthma, with the African American (11.0 ± 11.8 years) and Hispanic (10.2 ± 12.0 years) family members younger at onset compared with the European American (14.7 ± 14.7 years) family members. Several of the other clinical characteristics exhibited similar patterns, with non-European American family members more asthmatic (58.0% vs 42.2%), with a higher serum IgE level (2.21 vs 1.96), lower PC₂₀ FEV₁ (5.21 vs 6.05), and proportionally greater number of reactions to skin tests (33% vs 25%). African Americans had lower

TABLE III. Clinical characteristics for subjects in informative families for presence of any individual pollen reactivity (*Amb a*, *Art v*, *Bet v*, *Lol p*, or *Que a*)

	African American	European American	Hispanic	Total
Age (mean $\pm$ SD) (n)	25.9 $\pm$ 15.6 (304)	34.2 $\pm$ 19.3 (809)	24.2 $\pm$ 16.1 (156)	31.0 $\pm$ 18.6 (1269)
Age at first symptom (n)	11.0 $\pm$ 11.8 (257)	14.7 $\pm$ 14.7 (651)	10.2 $\pm$ 12.0 (127)	13.2 $\pm$ 13.9 (1035)
% With asthma (n)	59.5% (296)	42.2% (775)	55.0% (151)	48.0% (1222)
Serum IgE (n)	2.22 $\pm$ 0.72 (298)	1.96 $\pm$ 0.69 (795)	2.19 $\pm$ 0.67 (152)	2.05 $\pm$ 0.71 (1245)
FEV ₁ (% predicted)* (n)	87.0 $\pm$ 17.4 (298)	92.4 $\pm$ 16.6 (774)	94.5 $\pm$ 18.2 (152)	91.4 $\pm$ 17.2 (1224)
FEV ₁ /forced vital capacity (n)	0.82 $\pm$ 0.09 (293)	0.81 $\pm$ 0.09 (771)	0.80 $\pm$ 0.09 (150)	0.81 $\pm$ 0.09 (1214)
PC ₂₀ FEV ₁ (n)	5.22 $\pm$ 7.46 (155)	6.05 $\pm$ 7.30 (377)	5.18 $\pm$ 6.20 (77)	5.73 $\pm$ 7.21 (609)
Number of positive skin tests† (n)	4.2 $\pm$ 3.7 (299)	3.5 $\pm$ 3.4 (773)	6.5 $\pm$ 4.4 (154)	4.1 $\pm$ 3.8 (1226)

*Adjusted for age and race.

†Maximum of 14 skin tests, except in Hispanics (maximum of 17).

FEV₁ (% predicted) than the European American and Hispanic members (87.0 vs 92.7), whereas there was no difference in FEV₁/forced vital capacity (FVC).

Linkage analysis—seasonal pollen reactivity

The analysis of pollen sensitivity over all ethnic groups (Fig 1) identified 3 chromosomal regions with LOD > 1.18 (equivalent to a nominal *P* value < .01). These regions of linkage occurred on chromosomes 13q24 (LOD = 1.24 at D13S285; *P* = 8.4×10^{-3}), 20p12 (LOD = 2.39 at D20S604; *P* = 4.6×10^{-4}), and 21q21 (LOD = 2.58 at D21S1270; *P* = 2.8×10^{-4}). It should be noted that only the regions on 20p12 and 21q21 reach the Lander-Kruglyak suggestive level of significance for linkage (*P* < 7.4×10^{-4}). Other regions in the genome had LOD > 1.0, but less than the criteria of LOD < 1.18. Specifically, chromosomes 3q13.3 (LOD = 1.15 at D3S2460), 11q22 (LOD = 1.16 at D11S2000), and 22q11.2 (LOD = 1.05 at D22S1174) showed some evidence of linkage.

Each region with LOD > 1.18 was further investigated for support for linkage within ethnic groups. The region on 13q24 (Fig 2, A) had a LOD = 1.24, and the support for linkage was strongest in the European American families (LOD = 0.73). Hispanic and African American families provided weaker support for linkage at the point of maximum LOD on 13q24; however, the non-European American families supported linkage on 13q, albeit at different chromosomal locations (African American LOD = 0.58 at D13S317 [13q31]; Hispanic LOD = 0.90 at D13S788 [13q14]). Thus, there appears to be some support for a locus contributing to seasonal pollen reactivity on 13q in all ethnic groups, in or near 13q24. The region on 20p12 (Fig 2, B) was supported by families in all 3 ethnic groups, although the exact point of maximum evidence for linkage varied. The region on 21q21 (Fig 2, C) was also supported in all 3 ethnic groups, although the strongest level of support came from the Hispanic families.

There was evidence for ethnic-specific differences in the degree of support for linkage for seasonal pollen reactivity. Although these regions need to be considered with caution, given the smaller number of families within

each group, the location of ethnic-specific linkage may suggest avenues for future exploration or confirmation of existing findings. In African American families, evidence supporting linkage was observed on chromosomes 8q21.2 (LOD = 1.53 at D8S1119; *P* = 4.0×10^{-3}), 10q22 (LOD = 1.45 at D10S1432; *P* = 4.9×10^{-3}), and 12p11.2 (LOD = 1.49 between D12S373 and D12S1042; *P* = 4.4×10^{-3}). In European American families, evidence supporting linkage was observed on chromosomes 14q12 (LOD = 1.42 at D14S297; *P* = 5.3×10^{-3}), 19q13.2 (LOD = 1.22 at D19S198; *P* = 8.9×10^{-3}), and 2 locations on chromosome 22q12 (LOD = 1.70 at D22S1176; *P* = 2.6×10^{-3} ; LOD = 1.71 at D22S445; *P* = 2.5×10^{-3}). In Hispanic families, the only evidence suggestive of linkage was that observed on chromosome 21q21 (LOD = 2.76; *P* = 1.8×10^{-4}).

Linkage analysis—individual seasonal pollen reactivity

In the analyses of reactivity for each seasonal pollen, the evidence for linkage was somewhat clustered, with the best evidence for linkage focused on chromosome 21q21. Individual pollens supporting linkage to 21q21 are *Bet v* (LOD = 1.85), *Lol p* (LOD = 2.30), and *Art v* (LOD = 1.34). Linkage support with *Amb a* was observed on chromosomes 9q33 (LOD = 1.59 at D9S934; *P* = 3.4×10^{-3}) and 20p13 (LOD = 1.37 at D20S473; *P* = 6.1×10^{-3}); *Art v*, in addition to 21q21, linkage support was on chromosomes 3p13 (LOD = 1.36 at D3S2406; *P* = 6.2×10^{-3}) and 16p13.1 (LOD = 1.56 at D16S2619; *P* = 3.7×10^{-3}); *Bet v*, in addition to 21q21, chromosome 12p13 (LOD = 1.44 at D12S372; *P* = 5.0×10^{-3}); *Lol p*, in addition to 21q21, chromosomes 1p11 (LOD = 1.30 at D1S534; *P* = 7.3×10^{-3}) and 10p12 (LOD = 1.92 at D10S1423; *P* = 1.5×10^{-3}); *Que a* supported linkage to 1q31 (LOD = 1.23 at D1S518; *P* = 8.8×10^{-3}) and 3q23 (LOD = 1.20 at D3S1764; *P* = 9.4×10^{-3}). Further details of the clinical characteristics of families informative for linkage with individual seasonal pollens and the genome scan results can be found in the Online Repository (see this article's Tables E1-E5 and Figs E1-E5 at www.jacionline.org).

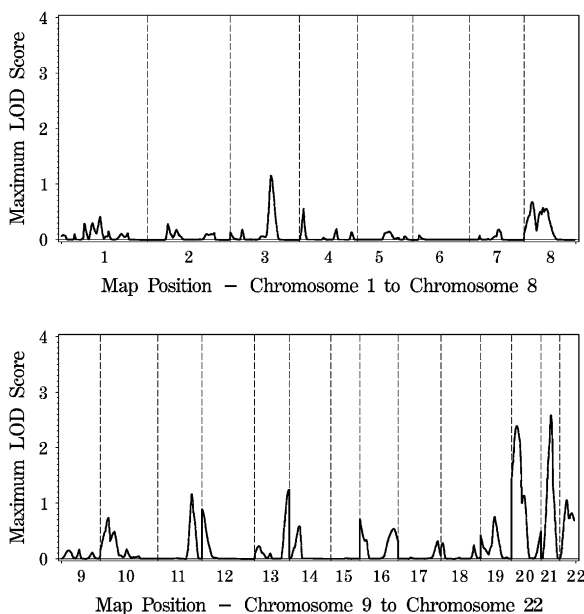


FIG 1. Genome scan for reaction to any of 5 pollens.

Association analysis—seasonal pollen reactivity

Although the microsatellite markers in the linkage scan do not reside in genes, evidence of association of alleles or genotypes was determined with the markers that exhibited linkage (D20S604 and D21S1270) as well as other markers on chromosomes 20 and 21. For D20S604, the global test of allelic association failed to achieve significance ($\chi^2_5 = 5.63$; $P = .34$), and there was no evidence for genotypic association with reactivity to seasonal pollen exposure ($\chi^2_{28} = 25.20$; $P = .62$). Tests of allelic and genotypic association with other microsatellite markers on chromosome 20 (D20S473, D20S470, D20S477, D20S481, D20S196, D20S171) failed to reach statistical significance. For D21S1270, the global test of allelic association did not reach significance ($\chi^2_8 = 7.91$; $P = .44$), and there was no evidence of genotypic association with reactivity to seasonal pollen exposure ($\chi^2_{46} = 33.61$; $P = .91$). For other markers on chromosome 21 (D21S1432, D21S1437, D21S1440, D21S156, D21S1446), only marker D21S1446 exhibited a marginally significant allelic association (global $\chi^2_7 = 14.02$; $P = .05$), with alleles D21S1446*7 (odds ratio, 1.25; $P = .05$) and D21S1446*12 (odds ratio, 1.50; $P = .02$) having increased risk. For genotypic association, only marker D21S1432 had a marginally significant association (global $\chi^2_{20} = 31.78$; $P = .05$) with genotype. However, these analyses are exploratory and require adjustments for multiple comparisons. Because each would be considered a separate test for allelic and genotypic associations, the critical P values for markers on chromosome 20 (critical $P = .008$) and chromosome 21 (critical $P = .01$) should be adjusted. Under these conditions, no additional tests of association achieve statistical significance. In addition, for the alleles

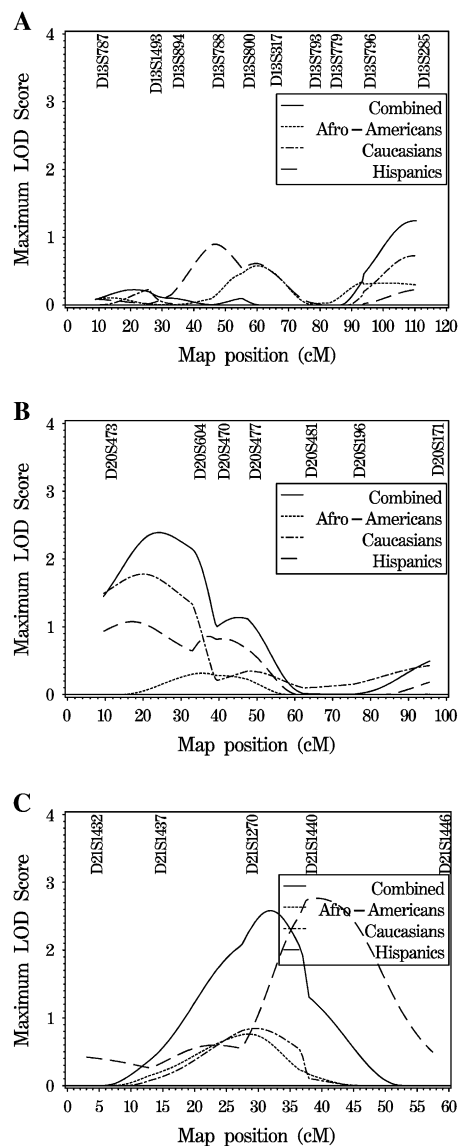


FIG 2. Genome scan for reaction to any of 5 pollens. A, Chromosome 13. B, Chromosome 20. C, Chromosome 21.

that are associated for D21S1446 (*7 and *12), there is no increased genotypic association; similarly, for the genotype associated for D21S1432*3/4, the individual alleles are not associated. Thus, the analyses for the linked markers (D20S604 and D21S1270) and the markers on chromosomes 20 and 21 that did not exhibit linkage suggest an absence of association.

DISCUSSION

The CSGA has previously described evidence supporting linkage for asthma,^{8,9} serum IgE levels,¹⁰ atopy,¹¹ and skin test reactivity to mites.¹² This report provides evidence for linkage to specific regions in the genome for seasonal pollen reactivity (a global measure of response to

TABLE IV. Summary of genome scan results using reactivity to any of 5 seasonal pollens as the phenotype

Population	Marker (position)	LOD (P value)	LOD-1 interval	Comments
Combined	D13S285 (110 cM)	1.24 (.0084)	(91-110) cM	Asthma, ⁸ atopy, ²⁰ eosinophilia ²¹
	D20S604 (24 cM)	2.39 (.0005)	(10-38) cM	Atopy ¹¹ (<i>ADAM33</i>) ³¹
	D21S2170 (32 cM)	2.58 (.0003)	(22-38) cM	Asthma ²⁵
African American	D8S1119 (101 cM)	1.53 (.0040)	(87-115) cM	
	D10S1432 (94 cM)	1.45 (.0049)	(76-106) cM	
	D12S1042 (52 cM)	1.33 (.0067)	(28-69) cM	
European American	D14S297 (38 cM)	1.42 (.0053)	(17-50) cM	
	D19S198 (66 cM)	1.22 (.0089)	(10-90) cM	Asthma, ^{8,25} bronchial hyperresponsiveness, ²¹ mites ¹²
	D20S473 (20 cM)	1.78 (.0021)	(10-37) cM	Atopy ¹¹ (<i>ADAM33</i>), ³¹ mites ¹²
	D22S445 (41 cM)	1.71 (.0025)	(5-46) cM	
Hispanic	D21S156 (39 cM)	2.76 (.0002)	(32-49) cM	Asthma ²⁵

seasonal pollen exposure) as well as individual seasonal pollen reactivity (pollen-specific exposure). The families included in this report are a subset of those in previous CSGA reports, and the exposure (seasonal pollens) represents a more homogeneous subset of allergens used in the definition of atopy; thus, the similarities and differences in regions supporting linkage can be defined (Table IV).

Results of the genome scan for pollen sensitivity have identified 2 chromosomal regions that support evidence for loci controlling seasonal pollen reactivity on chromosomes 20p12 and 21q21. Inherent in the analyses for specific pollens is the assumption that different ethnic groups and different geographic areas have equivalent qualitative exposures. Thus, evidence for reactivity to any of the seasonal pollens may provide support for a common gene involved in response, whereas ethnic-specific support may reflect heterogeneity of exposures.

Although not attaining significant support for linkage in this study, the 13q24 region has previously been shown to support linkage to asthma (CSGA⁸), atopy,²⁰ and eosinophilia.²¹ A strong candidate gene for asthma and IgE level, plant homeodomain finger protein 11 (*PHF11*), also maps to the long arm of chromosome 13³; however, the location of PHD finger protein 11 is 13q14.1, far from 13q24. Thus, there are suggestive and multiple lines of evidence that a quantitative trait locus contributing to asthma, atopy, and reactions to environmental allergens may reside in the 13q24 region.

The 20p12 region, which does attain suggestive evidence of linkage for seasonal pollen reactivity, has been identified previously in the CSGA for atopy.¹¹ The 20p12 region has yielded one of the initial asthma susceptibility genes, A Disintegrin And Metalloprotease domain 33 (*ADAM33*).² Polymorphisms in the *ADAM33* gene have been shown to be associated with susceptibility to allergic rhinitis caused by Japanese cedar pollen (*Cryptomeria japonica*).²² *ADAM33* appears to be expressed in multiple tissues and, within the lung, its expression is greatest in bronchial smooth muscle cells and lung fibroblasts.²³ The current theory is that *ADAM33* plays some role in lung inflammatory response, airway hyperresponsiveness, and remodeling.²⁴

The strongest support for linkage to seasonal pollen reactivity in these CSGA families was observed for the 21q21 region. This region of the genome was also identified in a scan for asthma susceptibility loci in the Hutterites, a founder population of European ancestry.²⁵ The support for linkage in the Hutterite families was to a strict definition of asthma, was originally reported by the CSGA in a set of Hispanic families,⁸ and was confirmed in the Hispanic sample in this analysis.

In addition to the CSGA, other investigators have reported suggestions of linkage for nonspecific skin test reactivity and allergen specific IgE response. These included mite sensitivity (showing evidence for linkage to chromosome 2, 5q, 4q, 12q 13q, and 20p²⁶⁻²⁹) and non-specific skin test reactivity (where evidence of linkage has been reported on chromosomes 2, 3q, 4q, 5q, 7p, 7q, 8q, 10p, 11q, 12p, 13q, 17q, 18p, 20, and 22q^{2,20,21,26,27,30}).

Many association studies of reactivity phenotypes with genes in the HLA complex have been reported. These associations have implicated this region of chromosome 6 as controlling reactivity to many purified antigens (including *Amb a V*, *Amb VI*, *Bet v I*, *Lol p I*, *Lol p II*, *Lol p III*, *Der p*, and *Der f*). The CSGA has generally failed to demonstrate linkage for multiple atopic phenotypes (pollen, mite reactivity, skin test reactivity, or specific immune responses to allergens) to the HLA region on chromosome 6p21. This may be a result of the crude allergens used or reliance on skin test response to purified allergens rather than specific IgE antibody levels. Furthermore, the genome scan data presented here did have markers near (but not within) the MHC complex; thus, the CSGA may have had low power to detect evidence of association (linkage disequilibrium, not linkage). These results do not exclude the possibility that certain HLA antigens are associated with seasonal pollen reactivity. Rather, it may be that the production of allergen-specific IgE is not an obligatory inherited result in families with the atopic/asthmatic predisposition. Members of multiplex families may exhibit an atopic phenotype because of other genetic factors that do not increase allergen-specific IgE. Other factors may impinge on the immune system to direct different clinical outcomes. Both genetic and environmental factors

clearly influence the clinical phenotype examined here (skin test reactivity).

In conclusion, this study demonstrates that there are several potential chromosomal regions yielding evidence of linkage for a seasonal pollen reactivity phenotype. Suggestive evidence for linkage was found on chromosomes 20p12 and 21q21. Although not reaching statistical significance, a region on 13q24 was implicated over all populations, as were sites in African American (8q21.2, 10q22, and 12p11.2) and European American (14q12, 19q13.2, and 22q12) families. Many of these same chromosomal regions have also shown linkage to asthma and asthma-related phenotypes in other studies. On the basis of these results, there appears to be evidence for both heterogeneity and pleiotropy in regulation of reactivity to seasonal pollen exposure.

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REFERENCES

- Yoshinaka T, Nishii K, Yamada K, Sawada H, Nishiwaki E, Smith K, et al. Identification and characterization of novel mouse and human ADAM33s with potential metalloprotease activity. *Gene* 2002;282:227-36.
- Van Eerdewegh P, Little RD, Dupuis J, Del Mastro RG, Falls K, Simon J, et al. Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. *Nature* 2002;878:1-5.
- Zhang Y, Leaves NI, Anderson GG, Ponting CP, Broxholme J, Holt R, et al. Positional cloning of a quantitative trait locus on chromosome 13q14 that influences immunoglobulin E levels and asthma. *Nat Genet* 2003;34:181-6.
- Laitinen T, Polvi A, Rydman P, Vendelin J, Pulkkinen V, Salmikangas P, et al. Characterization of a common susceptibility locus for asthma-related traits. *Science* 2004;304:300-4.
- Oguma T, Palmer LJ, Birben E, Sonna LA, Asano K, Lilly CM. Role of prostanoïd DP receptor variants in susceptibility to asthma. *N Engl J Med* 2004;351:1752-63.
- Cooke RA, Vander Veer A. Human sensitization. *J Immunol* 1916;1:201-305.
- Blumenthal M, Marcus-Bagley D, Awdeh Z, Johnson B, Yunis EJ, Alper CA. HLA-DR2, [HLA-B7, SC31, DR2], and [HLA-B8, SC01, DR3] haplotypes distinguish subjects with asthma from those with rhinitis only in ragweed pollen allergy. *J Immunol* 1992;148:411-6.
- CSGA. A genome-wide search for asthma susceptibility loci in ethnically diverse populations. The Collaborative Study on the Genetics of Asthma (CSGA). *Nat Genet* 1997;15:389-92.
- Xu J, Meyers DA, Ober C, Blumenthal MN, Mellen B, Barnes KC, et al. Collaborative Study on the Genetics of Asthma. Genome wide screen and identifying gene-gene interactions for asthma susceptibility loci in three U.S. populations: Collaborative Study on the Genetics of Asthma (CSGA). *Am J Hum Genet* 2001;68:1437-46.
- Mathias RA, Freidhoff LR, Blumenthal MN, Meyers DA, Lester L, King R, et al. CSGA (Collaborative Study of the Genetics of Asthma). Genome-wide linkage analyses of total serum IgE using variance components analysis in asthmatics families. *Genet Epidemiol* 2001;20:340-55.
- Blumenthal MN, Langefeld CD, Beaty TH, Bleecker ER, Ober C, Lester L, et al. A genome-wide search for allergic response (atopy) genes in three ethnic groups: Collaborative Study on the Genetics of Asthma (CSGA). *Hum Genet* 2004;114:157-64.
- Blumenthal MN, Ober C, Beaty TH, Bleecker ER, Langefeld CD, King RA, et al. CSGA. Genome scan for loci linked to mite sensitivity: the Collaborative Study on the Genetics of Asthma (CSGA). *Genes Immun* 2004;5:226-31.
- Lester LA, Rich SS, Blumenthal MN, Togias A, Murphy S, Malveaux F, et al. Collaborative Study on the Genetics of Asthma. Ethnic differences in asthma and associated phenotypes: Collaborative Study on the Genetics of Asthma. *J Allergy Clin Immunol* 2001;108:357-62.
- Blumenthal MN, Banks-Schlegel S, Bleecker ER, Marsh DG, Ober C. Collaborative Studies on the Genetics of Asthma—National Heart, Lung, and Blood Institute. *Clin Exp Allergy* 1995;26:892-6.
- O'Connell JR, Weeks DE. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *Am J Hum Genet* 1998;63:259-66.
- McPeck MS, Sun L. Statistical tests for detection of misspecified relationships by use of genome screen data. *Am J Hum Genet* 2000;66:1076-94.
- Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES. Parametric and non-parametric linkage analysis: a unified multipoint approach. *Am J Hum Genet* 1996;58:1347-63.
- Kong A, Cox NJ. Allele-sharing models: LOD scores and accurate linkage tests. *Am J Hum Genet* 1997;61:1179-88.
- Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet* 1995;11:241-7.
- Daniels SE, Bhattacharya S, James A, Leaves NI, Young A, Hill MR, et al. A genome-wide search for quantitative trait loci underlying asthma. *Nature* 1996;383:247-50.
- Dizier MH, Besse-Schmittler C, Guilloud-Bataille M, Annesi-Maesano I, Boussaha M, Bousquet J, et al. Genome screen for asthma and related phenotypes in the French EGEA study. *Am J Respir Crit Care Med* 2000;162:1812-8.
- Cheng L, Enomoto T, Hirota T, Shimizu M, Takahashi N, Akahoshi M, et al. Polymorphisms in ADAM33 are associated with allergic rhinitis due to Japanese cedar pollen. *Clin Exp Allergy* 2004;34:1192-201.
- Holloway JW, Keith TP, Davies DE, Powell R, Haitchi HM, Holgate ST. The discovery and role of ADAM33, a new candidate gene for asthma. *Exp Rev Mol Med* 2004;6:1-12.
- Holgate ST, Davies DE, Rorke S, Cakebread J, Murphy G, Powell RM, et al. ADAM33 and its association with airway remodeling and hyperresponsiveness in asthma. *Clin Rev Allergy Immunol* 2004;27:23-34.
- Ober C, Cox NJ, Abney M, Di Rienzo A, Lander ES, Changyaleket B, et al. Collaborative Study on the Genetics of Asthma. Genome-wide search for asthma susceptibility loci in a founder population. *Hum Mol Genet* 1998;7:1393-8.
- Koppelman G, Stine C, Xu J, Howard T, Zheng S, Kauffman H, et al. Genome-wide search for atopy susceptibility genes in Dutch families with asthma. *J Allergy Clin Immunol* 2002;109:498-506.
- Ober C, Tsalenko A, Parry R, Cox NJ. A second-generation genomewide screen for asthma-susceptibility alleles in a founder population. *Am J Hum Genet* 2000;67:1154-64.
- Yokouchi Y, Nukaga Y, Shibasaki M, Noguchi E, Kimura K, Ito S, et al. Significant evidence for linkage of mite sensitive childhood asthma to chromosome 5131-q33 near the interleukin 12B locus by a genome wide screen in Japanese families. *Genomics* 2000;66:152-60.
- Kurz T, Strauch K, Heinzmann A, Braun S, Jung M, Ruschendorf F, et al. A European study on the genetics of mite sensitization. *J Allergy Clin Immunol* 2000;106:925-32.
- Xu X, Fang Z, Wang B, Chen C, Guang W, Jin JY, et al. A genome-wide search for quantitative-trait loci underlying asthma. *Am J Hum Genet* 2001;69:1271-7.
- Van Eerdewegh P, Little RD, Dupuis J, Del Mastro RG, Falls K, Simon J, et al. Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. *Nature* 2002;418:426-30.