

Genome-wide search for atopy susceptibility genes in Dutch families with asthma

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Background: Atopy is a phenotype associated with asthma that has a heritable component. However, the role of atopy-susceptibility genes in the development and expression of asthma and allergic disorders is not understood.

Objective: We sought to study the familial aggregation and co-occurrence of atopic phenotypes within family members of patients with asthma and to identify chromosomal regions that may contain genes that regulate different atopic phenotypes.

Methods: In 200 families (n = 1174) ascertained through a proband with asthma, genome-wide screen and linkage analysis was performed for the following atopic phenotypes: (1) specific IgE to common aeroallergens (Phadiatop assay); (2) specific IgE to Der p 1; (3) positive skin test responses to house dust mite; (4) positive skin test responses to 1 or more of 16 allergens; and (5) peripheral blood eosinophils. Results were compared with the linkage results for total serum IgE levels. **Results:** There was clear familial aggregation of atopy. A high total serum IgE level in combination with a positive Phadiatop result or a normal total IgE level in combination with a negative Phadiatop result was found in 56.1% of the probands and 66.9% of the offspring. Several chromosomal regions that showed evidence for linkage to an atopic phenotype (ie, 2q, 6p, 7q, and 13q) also showed evidence of linkage with total serum IgE (Xu et al. *Am J Hum Genet* 2000;67:1163-73). Specific regions of interest for atopic traits were also detected on chromosomes 11q, 17q, and 22q.

Conclusions: Atopic phenotypes show familial aggregation, although family members may differ in expression of atopy. Specific chromosomal regions appear to be important in susceptibility to different phenotypes of atopic responsiveness. (*J Allergy Clin Immunol* 2002;109:498-506.)

Key words: Genetics of atopy, asthma, specific IgE, eosinophils, genome-wide search

Abbreviations used

BHR: Bronchial hyperresponsiveness

LOD: Base 10 logarithm of the odds ratio favoring linkage

During the last 2 decades, there has been a worldwide increase in the prevalence of atopic diseases, including asthma and other allergic disorders.¹ Atopic diseases are caused by interactions between environmental factors and host or genetic susceptibility.² This recent increase in atopic diseases appears to be triggered by increases in environmental exposures that include indoor allergens and air pollutants, as well as changes in the causes of respiratory infections in early life, with an overall reduction in bacterial infections facilitating the development of allergic phenotypes (hygiene hypothesis).^{3,4} Allergic responsiveness is common in many populations, and genetic susceptibility is probably caused by a pattern of polymorphisms in multiple genes that regulate immunologic responses. Although the frequency of these polymorphisms may not be changing, alterations in environmental exposures could lead to expression of atopic phenotypes in previously unaffected but genetically susceptible individuals.⁵ Thus, it is important to identify chromosomal regions and the genes within these regions that regulate overall and specific allergic responsiveness. This will permit early identification of individuals at risk for allergic diseases and facilitate preventive public-health measures.

The close interrelation of asthma and atopy has enabled us to study atopic phenotypes in families ascertained through a proband with asthma. A genome-wide linkage analysis was then performed for 5 phenotypes related to atopy: (1) specific IgE to common aeroallergens (Phadiatop assay); (2) specific IgE to Der p 1; (3) positive skin test responses to house dust mite; (4) positive skin test responses to 1 or more of 16 allergens; and (5) peripheral blood eosinophils. These results are compared with linkage results for total serum IgE levels, which have been published previously.⁶

METHODS

Between 1962 and 1975, patients with newly diagnosed asthma from the northern part of The Netherlands were characterized at Beatrijcoord, a regional asthma referral center, by using a standard-

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TABLE I. Clinical characteristics of atopy in 200 Dutch families of probands with asthma

	Probands (1991-1999)	Spouses		Offspring	
		Of probands	Of children	Children	Grandchildren
No.	200	201*	68	531	136
Mean $\pm$ SD age, y	52.2 $\pm$ 8.5	51.0 $\pm$ 9.2	38.8 $\pm$ 6.2	24.0 $\pm$ 9.0	12.0 $\pm$ 5.3
Male, %	62.0	37.8	70.6	45.0	49.3
Mean total IgE† (range), kU/L	92.0 (1.0-2880)	26.2 (0.5-1940)	29.1 (3.0-1660)	62.8 (0-3360)	57.1 (0.5-2785)
Positive skin test, %					
$\geq$ 1 Allergen	81.8	31.0	42.6	53.5	31.3
House dust mite	71.4	14.9	19.1	37.6	17.9
Specific IgE, %					
Phadiatop	72.4	15.1	26.5	44.3	28.8
Der p 1	47.7	3.5	4.4	22.5	10.7
Mean eosinophils† (range), $10^7/L$	9.5 (0-126.5)	5.6 (0-63.8)	7.9 (2-31.9)	10.0 (0-294.8)	11.1 (0-109)

The following clinical data were not available for these reasons: not enough serum for Phadiatop measurement (n = 23) or Der p 1 measurement (n = 2); allergy skin test refused (n = 1); positive response to the negative control (n = 4); and no blood for total serum IgE analysis (n = 5) and eosinophil counts (n = 5).

*One proband married twice, and both spouses participated in the study.

†Mean values for IgE and eosinophils are geometric means.

ized clinical protocol.⁷ Two hundred probands were selected who met the following criteria at the time of initial evaluation: 45 years of age or younger, characteristic asthma symptoms, and bronchial hyperresponsiveness (BHR) to histamine. These 200 probands, together with their spouses, a minimum of 2 children, and, if available, their children's families, were restudied between 1990 and 1999.⁷ The study was approved by the Medical Ethics Committee of the University Hospital Groningen, as well as the institutional review boards of the University of Maryland and Wake Forest University. Written informed consent for all adults and written parental consent for children was obtained from all participants.

The following atopic phenotypes were studied: skin test responsiveness to common aeroallergens, total serum IgE, specific IgE to a group of common aeroallergens (Phadiatop assay) and to Der p 1, and peripheral blood eosinophils. Allergy skin testing was performed in adults by means of intracutaneous tests with 16 common aeroallergens, as well as positive and negative controls. Ten skin prick tests were performed in the children, with a positive and a negative control. The following allergens were tested in both adults and children: mixed grass and tree pollens, mixed weeds, house dust mite, dog and cat, a mixture of guinea pig and rabbit, horse, and the molds *Aspergillus fumigatus* and *Alternaria alternata*. In adults additional skin tests included a second mix of grass pollens and mixed tree pollens, storage mite, feather mix, and 3 molds (*Cladosporium herbarum*, *Penicillium notatum*, and *Botrytis cineria*; ALK). The maximum and perpendicular diameters of the wheal responses were recorded after 15 minutes. An intracutaneous skin test result was considered to be positive if the mean wheal diameter was 5 mm or larger; a skin prick test result was considered positive if the mean wheal diameter was 3 mm or greater. The skin test results were not used if the negative control caused a positive reaction.

Specific IgE was measured by using an in vitro test system (Pharmacia CAP system, Phadiatop FEIA; Pharmacia Diagnostics AB), according to the instructions of the manufacturer. This assay is composed of a mixture of inhalant allergens and is used as a general assessment of allergic responsiveness. It measures the presence of specific IgE to the following antigens: house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*); timothy grass (*Phleum pratense*), birch (*Betula verrucosa*), and olive (*Olea europaea*) pollen; 2 different weeds (*Artemisia vulgaris* and *Parietaria officinalis*); a mold (*Cladosporium herbarum*); and cat, dog, and horse dander. The test result was read as positive if the fluorescence score of the subject's serum was higher than that of the supplied reference. Specific IgE to Der p 1 (*D pteronyssinus*) was

measured by using the Cap method (Pharmacia & Upjohn). The lower level of detection was 0.35 kU/L. As a qualitative trait, specific IgE to Der p 1 was regarded to be present if the result of the Der p 1 assay was class 2 or higher, which is greater than 0.7 kU/L. Total peripheral blood eosinophils were counted in a counting chamber. Total IgE levels were measured by using standard methods.⁶ All participants were asked to discontinue asthma and allergy medication before the clinical testing, including oral antihistamines for 48 hours. Patients with asthma did not have a history of an exacerbation or require treatment with oral corticosteroids during the 6 weeks before the clinical evaluation.

DNA was isolated from peripheral blood by using standard methods (Puregene kit, Gentra Inc). For the genome-wide screen, the Weber version 8 set of markers (Marshfield Center for Medical Genetics) was used, which spans the human genome at an average interval of approximately 10 cM and consists of 366 polymorphic autosomal markers. Multiplex PCR was performed with fluorescence-labeled primers. PCR products were separated on denaturing polyacrylamide gels, and the fragments were detected with ABI 377 sequencers (Perkin Elmer Applied Biosystems). The fragments were scanned and scored with ABI software. A modified version of the program Linkage Designer was used to bin the alleles and check inheritance.⁸ Genotyping errors, double recombinants, and inheritance inconsistencies were detected with LINKAGE and CRIMAP software.

Nuclear families were stratified by 0, 1, and 2 parents with the trait to examine the familial aggregation of positive skin test responses and positive Phadiatop results and analyzed with SPSS 10.0 statistical software. Peripheral blood eosinophil count was log transformed to obtain a normal distribution and adjusted for age and sex in all analyses. Heritability was estimated from the variance components approach with the SOLAR program.⁹

Three-by-three contingency tables were generated and analyzed with SPSS software to study the overlap between phenotypes. High total serum IgE levels were defined as levels in the highest tertile for adults ($\geq$ 18 years) and children (6-17 years), giving IgE values for adults of 98 kU/L or greater and for children of 190 kU/L or greater. A positive skin test response was defined as the presence of one or more positive skin test results; specific IgE responsiveness was defined as the presence of a positive Phadiatop assay result. Linkage analysis for quantitative variables was performed with variance components analysis, as implemented in SOLAR.⁹ Linkage analyses for qualitative traits were performed with the nonparametric affected relative pairs method, as implemented in GENEHUNTER-PLUS.¹⁰ Allele-sharing base 10 logarithm of the odds ratio favoring

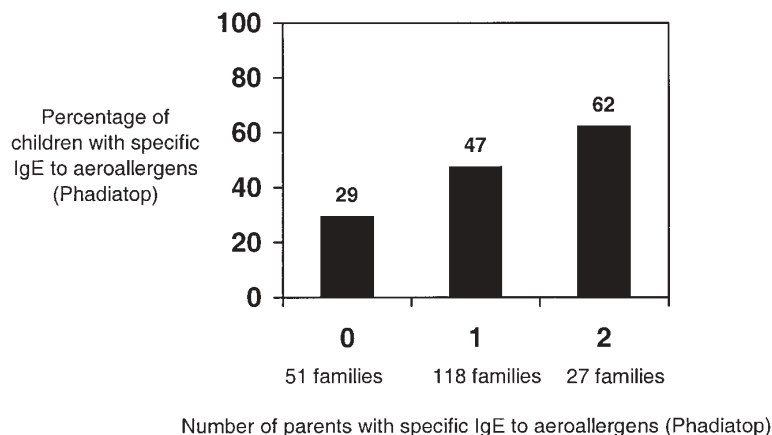


FIG 1. Familial aggregation of a specific IgE levels to common aeroallergens, as detected with the Phadiatop test, in nuclear families ascertained through a parent with asthma.

linkage (LOD) scores were calculated on the basis of the statistic *Z*-*all* and assigning equal weight to all families with the computer program ASM.¹¹ All linkage results with a minimal LOD score of 1.0 are reported.

RESULTS

The clinical characteristics of the probands and their family members included in the genome screen are reported in Table I. Eighty-two percent of the probands with asthma had one or more positive skin test responses compared with 31% of the spouses. Specific IgE, as detected with the Phadiatop assay, was present in 72% of the probands and in 15% of the spouses. The offspring showed frequencies intermediate between values of probands and spouses: 53% had a positive skin test response, and 44% had a positive Phadiatop result.

Heritability estimates were highest for specific IgE to Der p 1 (0.57); the heritability estimate was 0.41 for Phadiatop assay, 0.30 for log(eosinophil count), 0.29 for a positive skin test response to house dust mite, and 0.25 for any positive skin test response.

The prevalence of skin test positivity was 42.7% in children in the 26 families in which neither parent had a positive skin test response, 52.3% in the 119 families in which one parent had a positive skin test response, and 62.1% in the 50 families in which both parents had a positive skin test response. Similar results were observed for the familial aggregation of a positive Phadiatop test result (Fig 1).

Skin tests and Phadiatop assays gave consistent results (both positive or both negative) in 86.8% of the probands and in 78.6% of the offspring. High serum IgE levels were found in 60% of subjects who had a positive Phadiatop result and in 51% of subjects with one or more positive skin test responses. Forty-eight percent of the asthmatic probands with atopy had positive skin test responses and high total serum IgE and specific IgE levels. Thirty-seven percent had positive skin test responses and specific IgE levels but not high IgE levels. The remaining 15% of the atopic probands had various combinations of 1 or 2 of the 3 measurements of atopy. A

high total serum IgE level in combination with a positive Phadiatop result or a normal total IgE level in combination with a negative Phadiatop result was found in 66.9% of the offspring (Fig 2).

Fig 3, A to E, shows the results of the genome-wide linkage analysis for Phadiatop, specific IgE to Der p 1, positive skin test responses, positive skin test responses to house dust mite, and peripheral blood eosinophils, respectively. In Table II these linkage results are summarized together with the previously published linkage results for total serum IgE.⁶

Three chromosomal regions showed evidence of linkage for 2 or more of the 5 phenotypes in the current analysis: chromosome 11q (skin test to house dust mite, LOD = 1.90; Phadiatop, LOD = 1.27); 17q (Phadiatop, LOD = 1.38; eosinophils, LOD = 1.97; skin test, LOD = 1.55; skin test to house dust mite, LOD = 1.21), and 22q (Phadiatop, LOD = 1.08; skin test, LOD = 1.09; skin test to house dust mite, LOD = 1.02). Four chromosomal regions showed evidence of linkage to one of the atopic phenotypes in the current study and to total serum IgE levels in this population⁶: chromosome 2q (eosinophils, LOD = 1.49; total serum IgE, LOD = 1.96); 6p (eosinophils, LOD = 1.28; total serum IgE, LOD = 1.64); 7q (Phadiatop, LOD = 1.04; total serum IgE, LOD = 3.36); and 13q (Skin test, LOD = 1.27; total serum IgE, LOD = 2.28). Other chromosomal regions that showed evidence of linkage to a single atopic phenotype were chromosomes 3q (LOD = 1.53) and 18p (LOD = 1.25) for specific IgE to Der p 1; 10q (LOD = 1.04) for positive skin test responses; 5p (LOD = 1.09), 8p (LOD = 1.71), and 12p (LOD = 1.16) for positive skin test responses to house dust mite; and 15q (LOD = 2.19) for eosinophils.

DISCUSSION

This study shows evidence of familial aggregation for skin test reactivity, elevated specific IgE levels to aeroallergens, and eosinophil numbers. There are concordant results in the expression of high total IgE and specific

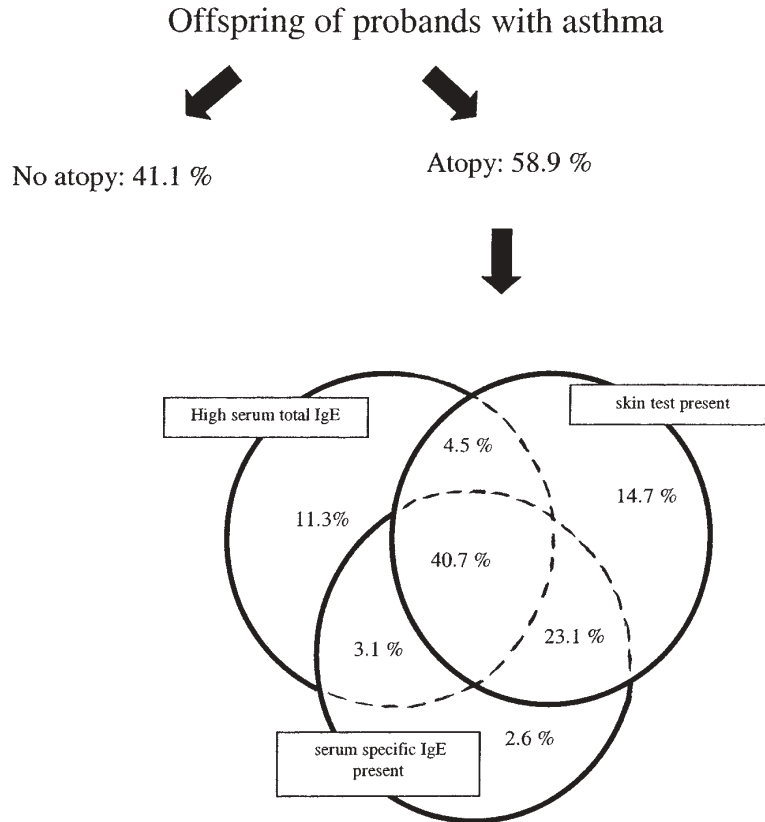


FIG 2. Offspring who had all tests successfully performed are included. High total serum IgE is defined as IgE levels in the highest tertile separately for adults and children. For adults (≥ 18 years of age), high IgE levels are 98 kU/L or greater, and for children under the age of 18 years, high IgE levels are defined as a total serum IgE level of 190 kU/L or greater. Skin test positivity is defined as the presence of one or more positive skin test responses; specific IgE positivity is defined as the presence of a positive Phadiatop assay result.

TABLE II. Linkage results for specific IgE levels to aeroallergens and to Der p 1, skin test positivity, and eosinophils compared with the linkage results of serum total IgE levels

Chromosome	Distance or interval	Flanking markers	LOD score	Phenotype
1p31	89.49-91.89	D1S3728-D1S2846	1.29	Total serum IgE
2q24-q32	173.00-186.21	D2S1776-D2S1391	1.49, 1.96	Eosinophils, total serum IgE
3q25-q26	181.87	D3S3053	1.53	Specific IgE to Der p 1
3q29	224.88-qter	D3S1311	2.11	Total serum IgE
5p15	7.77	D5S2849	1.09	Skin test to house dust mite
5q23-q31	129.83-139.33	D5S1505-D5S816	2.73	Total serum IgE
6p24-p22	25.08-34.23	D6S2434-D6S1959	1.28	Eosinophils
6p21	42.27-53.81	D6S2439-D6S2427	1.64	Total serum IgE
7q11-q22	98.44-109.12	D7S820-D7S821	3.36, 1.04	Total serum IgE, Phadiatop
8p23	8.34	D8S277	1.71	Skin test to house dust mite
10q21-22*	Est. 85*	D10S1*	1.04	Skin test
11q22	101.75	D11S2017	1.21, 1.55	Phadiatop, skin test to house dust mite
12p13	26.23	D12S391	1.16	Skin test to house dust mite
12q23-q24	109.47-125.31	PAH-D12S2070	2.46	Total serum IgE
13q12-q13	25.80-32.90	D13S1493-D13S218	2.28	Total serum IgE
13q14	45.55	D13S788	1.27	Skin test
15q11	12.30	D15S822	2.19	Eosinophils
17q21	82.00	D17S1290	1.21	Skin test to house dust mite
17q23	89.32-100.02	D17S2193-D17S1301	1.97	Eosinophils
17q25	116.86	D17S784	1.38, 1.55	Phadiatop, skin test
18p11	Est. 1.5†	D18S178†	1.25	Specific IgE to Der p 1
22q11	4.06	D22S420	1.09, 1.02	Sp IgE Der p 1, skin test to house mite
	19.32	D22S345	1.08, 1.09	Phadiatop, skin test

LOD scores greater than 1.0 are summarized for qualitative traits. The highest LOD scores for quantitative traits are shown. Map distance (in cM) are taken from the Marshfield map.

*D10S1 is not in the Marshfield map. Chromosomal distance was estimated relative to D10S1225 (80.77 cM) and D10S1432 (93.92 cM).

†D18S178 is not in the Marshfield map. Chromosomal distance was estimated relative to D18S976 (distance, 12.8 cM). The IgE results are from Xu et al.⁶

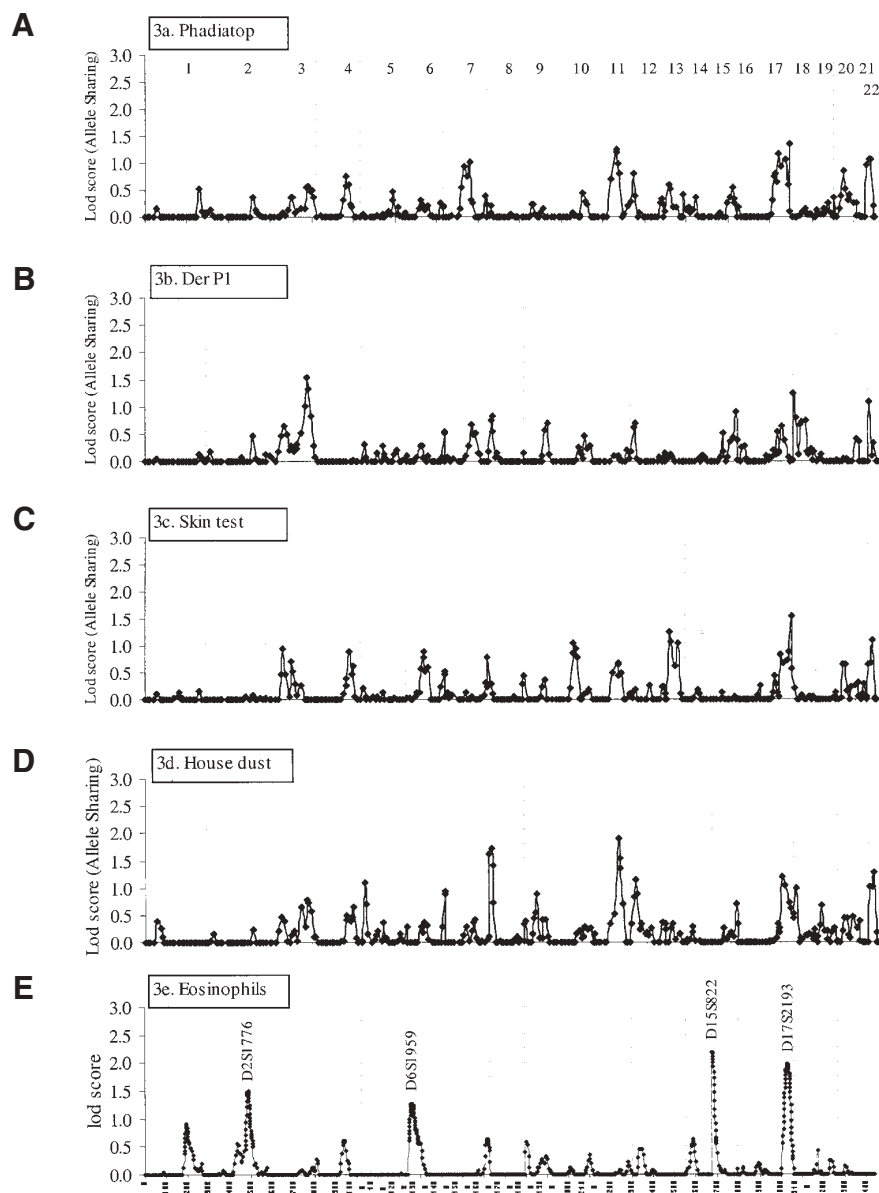


FIG 3. Genome-wide screen for atopy susceptibility genes in 200 Dutch families. Vertical dotted lines divide genomes into 22 chromosomes. **A**, Phadiatop; **B**, specific IgE to Der p 1; **C**, positive skin test responses; **D**, positive skin test responses to house dust mite; and **E**, peripheral blood eosinophils.

IgE, as detected by the Phadiatop assay, in 56% of the probands with asthma and 66.9% of the offspring. In these 200 families ascertained through a parent with asthma, there is evidence for linkage of atopic phenotypes to multiple chromosomal regions (Table II), providing an important basis for identifying specific genes that regulate host susceptibility for allergic responsiveness.

As has been reported in other studies, genome-wide criteria for a significant linkage are not always reached.¹² There may be several possibilities for this finding. First, although a genetic contribution to atopic traits has been well established, heritability estimates of various atopic phenotypes vary, ranging from 47% to 74% for total IgE, 34% for the RAST index, and 35% for positive skin test

responses.^{13,14} In these family data similar heritability estimates were observed: 55% for total IgE,⁶ 41% for Phadiatop assay, and 25% for positive skin test responses. Also, a quantitative trait (total serum IgE) may have better power to detect linkage than a qualitative trait (specific IgE).

Second, it is unknown how many genes contribute to atopy. If each of these genes confers a modest increased risk of atopy development, the trait may be heritable, but the power to find linkage produced by one gene in a specific chromosomal region may not be high. We have previously shown, using a 2-locus segregation analysis for total IgE levels, that 2 major genes account for 51.3% of the variance in total serum IgE levels in this population.⁶ No estimate of the number of (major) genes for other

TABLE III. Comparison of linkage results for atopic traits of the present study with published results*

Chromosome	Distance or interval (cM)	Phenotype					Candidate region studies (phenotype)
		Asthma and BHR	Total IgE	Specific IgE	Skin tests	Eosinophils	
1	89.49–91.89	French (asthma, BHR)	Dutch				
2	173.00–186.21		Dutch		US Hutterites (house dust mite)	Dutch	
5	129.83–139.33	US Hutterites (strict and symptom group)	Dutch	Japanese (1) (atopy)	Hutterites (pollen and cockroach)		US Amish (total IgE); Japanese (1) (asthma); Dutch (BHR); US Tucson (eosinophils)
6	25.08–34.23; 42.27–53.81	US Hutterites (loose); German; Japanese (2)	Dutch; German	German (≥1/13)	Hutterites (house dust mite and molds); Australian (atopy)	Dutch; German	
7	98.44–109.12	US Hutterites (BHR); Australian (BHR)	Dutch	Dutch (Phadiatop)	Hutterites (pollen and cockroach)		
8	0.73–22.41	US Hutterites (BHR)			Dutch (house dust mite)		English (house dust mite)
11	100.62–105.74			Dutch (Phadiatop)	US Hutterites (cockroach); Dutch (house dust mite)	Australian	European-HDM (house dust mite)
12	109.47–125.31	Japanese (2); Hutterites (loose)	Dutch		Hutterites (molds)	French	English (asthma and total IgE); Barbados (total IgE)
13	25.80–45.55	Japanese (2)	Dutch		US Hutterites (pollen); Dutch (1/16); Australian (atopy)	French	
17	82.00–116.86	US Hutterites (BHR)		Dutch (Phadiatop)	Dutch (house dust mite); Dutch (1/16); French (1/11); US Hutterites (house dust mite)	Dutch; French	

All studies with evidence for linkage ($P \leq .01$ for primary phenotype) in the same chromosomal region (spanning 20 cM from peak or interval markers) are included. If multiple markers showed evidence for linkage, the marker with the lowest P value was included. Map distance was taken from the Marshfield map. Data were taken from original papers and the Asthma Gene Database.

Sources and definitions of the phenotype:

- Australian¹⁸: Atopy was defined as one of 3 factors (ie, high serum total IgE level, one positive skin test response, or one positive specific IgE result).
- Barbados.¹⁹
- Dutch,^{6,20} present study: BHR to histamine.
- English¹³: Asthma was defined as an affirmative answer to the question, “Have you ever had asthma?”
- European-house dust mite:²¹ Families from England, Germany, Portugal, and Italy. Subpopulation: English families are part of this study.
- European-atopic dermatitis²²: Families were from Germany, Italy, Sweden, and Netherlands.
- French¹⁷-BHR to methacholine: Asthma is defined as the presence of asthma attacks or attacks of breathlessness at rest with wheezing combined with one of 4 factors (ie, BHR, reversibility of >12% after use of a bronchodilator, any hospitalization for asthma, or asthma therapy).
- German²³ Asthma: History of physician-diagnosed asthma.
- Japanese²⁴ (1): Asthma is defined as the presence of recurrent episodes of wheezing and shortness of breath in the preceding year that is reversible. Atopy was defined as the presence of one of 2 factors (ie, high total serum IgE levels or the presence of specific IgE to aeroallergens).
- Japanese²⁵(2): Asthma defined as the presence of 2 or more episodes of wheezing and shortness of breath, which was reversible and the presence of specific IgE to *D. farinae*.
- US-Hutterites¹⁶: BHR to methacholine. Strict asthma; BHR and symptoms; loose asthma: BHR or symptoms; symptom: asthma symptoms.
- US-CSGA²⁶: Asthma defined as presence of 2 of 3 symptoms (cough, wheeze, and dyspnea) and BHR to methacholine or reversible airways obstruction (≥15% increase from baseline).
- US-Arizona.²⁷
- US-Amish.²⁸

*Significance level: $P \leq .01$.

atopic phenotypes has been published.

Third, specific environmental factors may interact with a series of different genes, causing the expression of atopic responses. Thus although a large proportion of the

population may carry a susceptibility allele, only some individuals with a susceptibility allele will have atopic disease because of the requirement for exposure to specific environmental factors.

TABLE IV. Characteristics of published genome-wide studies compared with present study

Study	Study population	No. of families (No. of individuals)	Ascertainment strategy	Asthma or BHR	Atopic derma- titis	Total IgE	Specific IgE	Skin tests	Eosino- phils
Daniels et al ¹⁸	Australian	80 (n = 364)	Young families selected from population sample of 230 families	X		X		X	X
CSGA ²⁶	US-white, African American, and Hispanic subjects	Asthma: 199 nuclear and 67 extended families	Two siblings with asthma	X			X		
Wjst et al ²³	German	97 (n = 415)	Two siblings with asthma	X		X	X		X
Ober et al ¹⁶	US Hutterites	One extended pedigree (n = 693)	Population of 9 Hutterite colonies, all available individuals >5 years of age	X				X	
Yokouchi et al ²⁵	Japanese	47 (n = 197)	Two siblings with mite- sensitive asthma	X				X	
Dizier et al ¹⁷	French	107 (n = 493)	A proband with asthma or 2 siblings with asthma	X		X		X	X
Lee et al ²²	European (German, Italian, Swedish, and Dutch)	199 (n = 839)	Two siblings with atopic dermatitis		X	X	X		

Fourth, other effects, such as a parent-of-origin effect caused by imprinting or gene-gene interactions, have not been evaluated in most genetic studies.¹⁵ An important issue for the correct interpretation of linkage results in genome-wide screens is the level of significance. We have chosen to report the results of all results of an LOD of greater than 1 because a comprehensive approach showing all linkage results permits meaningful comparisons between different studies. Consistent replication of linkage results in different populations will provide important evidence for the locations of atopy-susceptibility genes (Table III).^{6,13,16-28} For example, the results of this study suggest a possible role of a gene on chromosome 17q for different phenotypes related to atopy (eosinophils, skin test response to house dust mite, and Phadiatop). Linkage of this region for the same phenotypes was also observed in the US Hutterite and French populations (Table III),^{16,17} making this an appropriate region for fine mapping to identify potential susceptibility genes for atopy.

Genome-wide screens of atopy-related phenotypes have been performed in several populations, although families were ascertained with different methods (Table IV).^{13,16,17,22,23,25,26} The type of ascertainment may affect the linkage results. For example, in the Japanese study 100% of the population with asthma were sensitive

to house dust mite, whereas only 52.1% of the Hutterites with an asthma phenotype had one or more positive skin prick test responses.¹⁶ Thus the linkage results reported in the Japanese study could be accounted for by genes important in asthma, atopy, or both. In addition to the genomic screens shown in Table IV, there has been a recent screen in the Finnish population, but the 2 regions with the strongest evidence for linkage (ie, 7p for asthma and total IgE on the basis of a cut-off point and chromosome 4 for asthma) were not seen in this study.²⁹ Replications of similar chromosomal regions with different phenotypes may identify a pleiotropic gene (ie, a gene causing different phenotypes, the expression of which is dependent on the interaction of other genetic and/or environmental factors). For atopy, evidence for shared and specific genetic factors in the regulation of serum total IgE, BHR, and specific IgE to aeroallergens have been suggested.¹⁴ Recently, evidence for shared genetic factors in skin test responsiveness and bronchial responsiveness to hypertonic saline has also been reported.³⁰ In addition, the combination of several genes in close proximity could account for the linkage signal observed in different chromosomal regions, such as 5q, 11q, and 12q. Different genes may regulate total and specific IgE responses, and different patterns of susceptibility genes may lead to the development atopy, asthma, or both.

On the basis of the analysis of 5 atopic phenotypes, evidence for linkage to atopy was observed in this study and at least one other study on chromosomes 2q, 6p, 7q, 11q, 13q, and 17q. Evidence for linkage to chromosome 8 was also observed, which is consistent with prior reports for linkage to asthma. Previous linkage results for total serum IgE levels in this population are consistent with evidence from other investigators demonstrating linkage to chromosomes 1p, 5q, and 12q for asthma- and atopy-related phenotypes (Table III). Linkage to chromosomes 5q, 6p, 7q, and 12q have been reported previously.^{6,20} Briefly, linkage to 5q was first seen for BHR in this population by using a candidate gene approach.²⁰ Segregation and linkage analysis for total serum IgE levels was performed by using log IgE as a quantitative trait. Evidence for linkage to several chromosomal regions was seen in the following order of significance: 7q, 5q, 12q, and 6p.⁶ We will now discuss the relevance of linkage on 8p, 11q, 13q, and 17q and relate them to positional candidate genes.

Linkage of asthma to chromosome 8p (marker D8S1130) was observed in the US-CSGA sample for the same marker that showed linkage to a positive skin test response to house dust mite in the present study.²⁶ The evidence for linkage for asthma increased after conditioning on the evidence for linkage to chromosome 11q21, indicating gene-gene interactions between loci at chromosomes 8p and 11q in asthma.²⁶ We observed evidence for linkage to both 8p (skin test to house dust mite) and 11q (Phadiatop and skin test to house dust mite). Chromosome 11q13 was one of the first regions identified in linkage studies of atopy and asthma.³¹ In the first 92 families in our population, we have previously reported that we did not find evidence for linkage on chromosome 11q to the number of positive skin test responses, total serum IgE levels, or BHR. This may seem contradictory; however, a positive skin test response to house dust mite and Phadiatop assay results were not analyzed in that study.³² The region on 11q (11q21-q24) that was linked to atopy in this study is approximately 35 to 45 cM telomeric to the FCeRIB region at chromosome 11q13. Linkage to this region (11q21) was also observed in the CSGA population for the asthma phenotype and to specific IgE to house dust mite in German families.^{21,26} Candidate genes in this region include matrix metalloproteinase genes 1, 3, and 8 and the δ , ϵ , and γ subunits of CD3. CD3 is found on T-cell surfaces associated with T-cell receptor α and β , binding antigen in association with the major histocompatibility complex proteins on host cell surfaces.

Evidence for chromosome 13q was observed in the Australian and French genome screen for atopy and eosinophils, respectively (Table III), and with total serum IgE and skin test reactivity in the current study. This region contains the candidate gene endothelin receptor B. A broad region on chromosome 17q has been implicated in 3 genome-wide searches, including this one, for phenotypes related to immune response, such as positive skin test responses or specific IgE. Several candidate genes have been proposed, including the transcription factor signal transducer and activator of transcription 5A, chemokine receptor 7, and the

small inducible cytokines subfamily A (RANTES eotaxin). RANTES is a chemokine responsible for recruitment of inflammatory cells, such as eosinophils and T lymphocytes, and promoter variants of RANTES have been associated with atopic dermatitis and skin prick test responses but not with total IgE levels.^{33,34} Eotaxin is a potent chemoattractant for eosinophils in inflamed tissues. We did not observe evidence for linkage of atopic traits to some chromosomal regions that have been in other genome-wide screens, such as chromosomes 4, 9, 11p, 16, 19, and 20 (Table III). This could reflect population heterogeneity or may be explained by a lack of power to identify all regions in each sample. In atopy different patterns of gene-gene and gene-environmental interaction may be important in different populations and for different allergic phenotypes.

In conclusion, this study shows familial aggregation of atopic phenotypes. High total serum IgE levels and the presence of specific IgE to common aeroallergens do not show complete overlap in family members of probands with asthma, and distinct regions on the genome are implicated for the different atopic phenotypes. Evidence was found for the presence of atopic susceptibility genes on chromosomes 2q, 5q, 6p, 7q, 8p, 11q, 12q, 13q, and 17q. Many of these regions have also shown evidence for linkage to atopic phenotypes in other genome-wide studies. On the basis of the clustering of linkage signals in genome-wide screens for atopic phenotypes, we propose that, in addition to shared genes, different genes may regulate total and specific serum IgE levels. Further replication of these findings and fine mapping studies will lead to identification of susceptibility genes for atopy and ultimately provide information on a basic understanding of the pathogenesis of atopic disease.

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APPENDIX

Electronic database information

Asthma Gene Database: <http://cooke.gsf.de/asthmagen>

Genemap 99: www.ncbi.nlm.nih.gov/genemap99

LDB (Genome Location Database): <http://cedar.genetics.soton.ac.uk>

LINKAGE and CRIMAP software: <http://linkage.rockefeller.edu/soft/linkage>

Marshfield Center for Medical Genetics: <http://research.marshfieldclinic.org/genetics>

OMIM (Online Mendelian Inheritance in Man): www.ncbi.nlm.nih.gov/omim

SAGE (Statistical Analysis for Genetic Epidemiology): <http://darwin.cwru.edu/pub/sage.html>

SOLAR (Sequential Oligogenic Linkage Analysis Routines): <http://www.sfbr.org/sfbr/public/software/solar/index.html>