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Genetics of asthma and atopy

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Chapter 6 | Genome-wide search for atopy susceptibility genes in Dutch families with asthma

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Abstract

Background

Atopy is a phenotype associated with asthma that has a heritable component. However, the exact role of atopy susceptibility genes is unclear.

Objective

To study the familial aggregation and association 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

Genome-wide screen and linkage analysis for atopic phenotypes were performed in 200 families (n=1174) ascertained through a proband with asthma. Specific IgE to common aeroallergens (Phadiatop assay) and Der P1, skin tests (positive to house dust mite or ≥ 1 out of 16 allergens) and peripheral blood eosinophils were evaluated and compared to the linkage results for total serum IgE.

Results

There was a clear familial aggregation of atopy. A high total serum IgE level in combination with a positive Phadiatop, or a normal total IgE level in combination with a negative Phadiatop was found in 56.1 % of the probands and 66.9 % of the offspring. Several chromosomal regions showed evidence for linkage to an atopic phenotype: chromosomes 2q, 6p, 7q, and 13q, and also showed evidence of linkage with total serum IgE (Xu et al., 2000). Specific regions of interest for atopic traits were also detected on chromosomes 11q, 17q, and 22q.

Conclusions

Atopic phenotypes show familial aggregation. Total IgE and specific IgE did not show complete overlap in members of families ascertained through a parent with asthma. Specific chromosomal regions appear to be important in susceptibility to different phenotypes of atopic responsiveness.

Introduction

The prevalence of atopic diseases that include asthma and other allergic disorders has been rising throughout the world during the last two decades.¹ Atopic diseases are caused by interactions between environmental factors and host or genetic susceptibility.² This worldwide increase in atopic diseases appears to be caused by recent changes in environmental exposures that include indoor allergens, air pollutants as well as alterations in the characteristics of respiratory infections in early life with a reduction in bacterial etiologies facilitating the development of allergic phenotypes (hygiene hypothesis).^{3,4} Allergic responsiveness is common in many populations, and genetic susceptibility is probably due to frequent polymorphisms in multiple genes. Although the frequency of these polymorphisms is probably not changing, an increase in environmental exposure would lead to expression of the phenotype in previously unaffected but genetically susceptible individuals.⁵ Thus, it is important to identify chromosomal regions and the genes that regulate overall (global) and specific allergic responsiveness. This will permit early identification of individuals at risk for allergic diseases and facilitate preventive public health measures by understanding gene-environment interactions.

Our primary approach to identify genes for atopy is through positional cloning, where regions of the human genome that may contain atopy susceptibility genes are identified by linkage analysis in families.⁶ Subsequent fine mapping and positional candidate gene studies will lead to the identification of atopy susceptibility genes. The close interrelation of asthma and atopy has enabled us to study atopic phenotypes in families ascertained through probands with asthma. A genome-wide linkage analysis was performed for five phenotypes related to atopy: specific serum IgE levels and allergy skin tests to common aeroallergens, specific IgE to Der P1, skin test to house dust mite and peripheral blood eosinophils. These results are compared to linkage results of total serum IgE levels, which have been published previously.⁷ The aims of the present study are first to study the familial aggregation of atopic phenotypes; second to examine the association of atopic phenotypes within family members of patients with asthma and third, to identify chromosomal regions that may contain genes that regulate different atopic phenotypes. Finally, we will compare these linkage results to previously published linkage studies in allergic disorders.

Methods

Study population

Between 1962 and 1975, patients with asthma from the northern part of the Netherlands were referred to Beatrixoord, a regional asthma center in Haren, the Netherlands. These newly diagnosed patients with symptomatic asthma who were not experiencing a current asthma exacerbation underwent a standardized, complete evaluation. For inclusion in the current study, at the time of initial testing all probands were younger than 45 years of age, displayed characteristic asthma symptoms, and had bronchial hyperresponsiveness (BHR) to histamine ($PC_{20} \leq 32$ mg/ml, 30 seconds inhalation protocol).⁸ Two hundred probands were restudied between 1990 and 1999 together with their spouses, a minimum of two children, and when available their children's spouses and grandchildren. 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. For adults, written informed consent and for children, written parental consent was obtained from all participants.

Clinical and laboratory evaluation

The following atopic phenotypes were studied: allergy skin tests, total serum IgE, specific IgE to a group of common aeroallergens (Phadiatop assay) and to Der P1 and peripheral blood eosinophils.

Allergy skin testing was performed in adults by intracutaneous tests with 16 common aeroallergens, as well as a positive and negative control. 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 moulds *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 three moulds (*Cladosporium herbarum*, *Penicillium notatum*, *Botrytis cineria*) (ALK, Nieuwegein, the Netherlands). The maximum diameter and the perpendicular diameter of the weal were recorded after 15 minutes. An intracutaneous skin test was considered to be positive if the mean weal diameter was ≥ 5 mm; a skin prick test was considered positive if the mean weal diameter was ≥ 3 mm. The skin tests were not used for further analysis if the negative control gave a positive reaction.

Total IgE was measured by solid phase immunoassay in the first 92 families. The mean of two duplicate tests of IgE was used, and measurements were repeated if the difference between duplicates was $> 5\%$. In the second set of 108 families, serum IgE levels were measured by an enzyme linked fluorescent assay (Mini Vidas, Biomerieux Inc., Marcy, France) The two methods showed high correlations in our laboratory ($r=0.95-0.99$, $p < 0.01$).

Specific IgE was measured by an in vitro test system (Pharmacia CAP system, Phadiatop FEIA) according to the instructions of the manufacturer

(Pharmacia Diagnostics AB, Uppsala, Sweden). This assay is composed of a mixture of inhalant-allergens and is used as a general assessment of allergic responsiveness. It determinates 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 pollen (*Olea europea*), two different weeds (*Artemisia vulgaris* and *Parietaria officinalis*), a mould (*Cladosporium herbarum*), and finally cat, dog and horse dander. The test was regarded positive if the fluorescence score of the subject's serum was higher than that of the reference as provided by the manufacturer. In the individuals who tested positive by the Phadiatop assay, specific IgE directed against the major allergen Der P 1 of the house dust mite *D. pteronyssinus* was measured by the Cap method (Pharmacia Diagnostics, Uppsala, Sweden). 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 P1 assay was class 2 or higher, which is > 0.7 kU/l. Finally, total peripheral blood eosinophils were counted in a counting chamber.

All participants were asked to discontinue asthma and allergy medication before the clinical testing if possible: specifically inhaled corticosteroids were stopped for 14 days, inhaled long acting beta-mimetics; and oral antihistamines for 48 hours, inhaled short acting beta-mimetics and anticholinergics for 8 hours. Asthma patients had not experienced an asthma exacerbation or require a course of oral prednisone during the 6 weeks prior to the study.

Molecular methods

DNA was isolated from peripheral blood by standard methods (Puregene kit, Gentra Inc., Minneapolis, USA).⁷ 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 ~10 cM, and consists of 366 polymorphic autosomal markers.⁷ Multiplex polymerase chain reaction (PCR) was performed using fluorescently labeled primers. PCR products were separated on denaturing polyacrylamide gels and the fragments were detected using ABI 377 sequencers (Perkin Elmer Applied Biosystems). The fragments were scanned and scored using ABI software. A modified version of the program Linkage Designer was used to bin the alleles and check inheritance.⁹ Genotyping errors, double recombinants, and inheritance inconsistencies were detected using the LINKAGE and CRIMAP software.

Statistical methods

To examine the familial aggregation of skin test positivity and Phadiatop positivity, nuclear families were stratified by 0, 1 and 2 parents with the trait. Three by two contingency tables were generated showing the percentage of first degree offspring with and without the trait using SPSS 10.0 statistical software. To examine the familial aggregation for eosinophil

levels, eosinophil number was log transformed and corrected for age and sex by multiple linear regression analysis. Correlation coefficients of log (eosinophil numbers) among various pairs of relatives were estimated from the sums of squares and from cross products from the pairs, using the computer program FCOR of S.A.G.E. (Statistical Analysis for Genetic Epidemiology). The option of equal weight to pairs is reported. Heritability was estimated from the variance components approach and implemented using the SOLAR (Sequential Oligogenic Linkage Analysis Routines) program.¹⁰

To study the overlap of high total serum IgE, positive allergy skin tests and the presence of serum specific IgE to common aeroallergens, three by three contingency tables were generated. High total serum IgE levels were defined as total serum IgE levels in the highest tertile for adults (≥ 18 years) and children (6 - 17 years). This resulted in cut-off values for adults of IgE ≥ 98 kU/l, and for children of IgE ≥ 190 kU/l. Skin test positivity was defined as the presence of one or more positive skin tests; specific IgE positivity was defined as the presence of a positive Phadiatop assay. These calculations were performed using SPSS 10.0 statistical software.

Linkage analysis for quantitative variables was performed using variance components analysis as implemented in the SOLAR package.¹⁰ First eosinophil number was log transformed to obtain a normal distribution and age and sex were included as covariates. The variance of a quantitative trait can be decomposed into residual genetic effects, random environmental effects, and quantitative trait loci (QTL). To perform a genome wide screen, the fit of the models with or without the effect of QTL in the observed phenotype and marker genotype data was compared. The difference between the \log_{10} likelihoods of the two models produces a LOD score that is equivalent to the classical LOD score of linkage.

Linkage analyses for qualitative traits were performed using the affected relative pairs method as implemented in GENEHUNTER-PLUS.^{11,12} The option of non-parametric methods was used, which compared the observed marker allele identical by descent (IBD) among the various affected relative pairs with its expected values under the null hypothesis of no linkage. Allele sharing LOD scores were calculated based on the statistic 'Z-all' and assigning equal weight to all families using the computer program ASM.¹² All linkage results with a minimal LOD score of 1.0. are reported. These linkage results were compared to the published literature and the Asthma Gene Database (<http://cooke.gsf.de/asthmagen>).¹³ A replication was defined as evidence for linkage ($p \leq 0.01$) of an asthma or atopy associated trait with the same or a closely linked marker (< 10 cM).

Results

Study population

The clinical characteristics of the probands and their family members included in the genome screen are illustrated in Table 1. Eighty-two percent of the probands with asthma had one or more positive skin tests compared to 31 % in the spouses. Specific IgE as detected by the Phadiatop assay was present in 72 % of the probands and 15 % of the spouses. First-degree offspring showed frequencies intermediate between values of probands and spouses: 53 % had a positive skin test and 44 % a positive Phadiatop.

Familial aggregation of skin test positivity, Phadiatop assay and eosinophil number

Heritability estimates (h^2) were highest for specific IgE to Der P1 (0.57); h^2 was 0.41 for Phadiatop; 0.30 for log(eosinophil count); 0.29 for skin test to house dust mite, and 0.25 for skin test positivity.

The prevalence of skin test positivity was 42.7% in children in the 26 families in which none of the parents had a positive skin test. This prevalence was 52.3 % in 119 families in which one of the parents had a positive skin test, and increased to 62.1 % in 50 families in which both of the parents had a positive skin test. Similar results were observed for the familial aggregation of a positive Phadiatop test (figure 1). The lowest prevalence of these traits was observed in families in which none of the parents had a positive Phadiatop test (29 %), which increased to 62 % in children of whom both of the parents expressed this phenotype.

Figure 1 Familial aggregation of specific IgE to aeroallergens

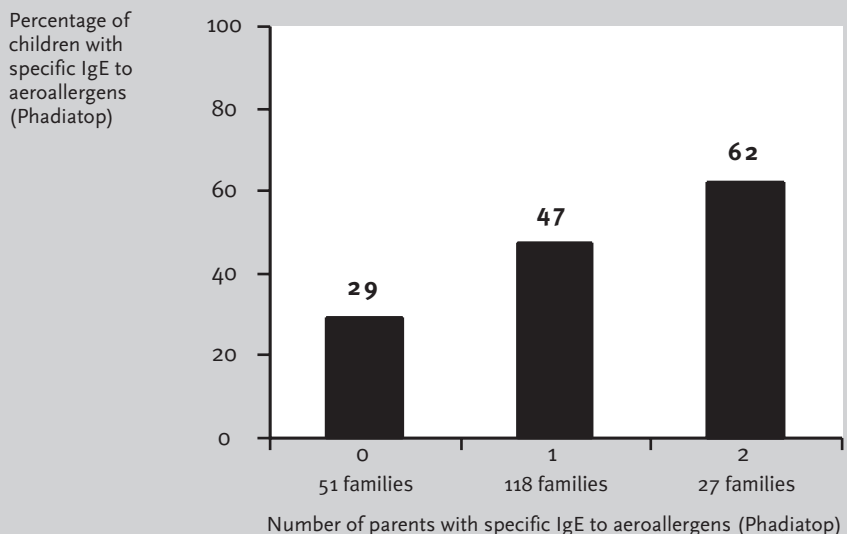
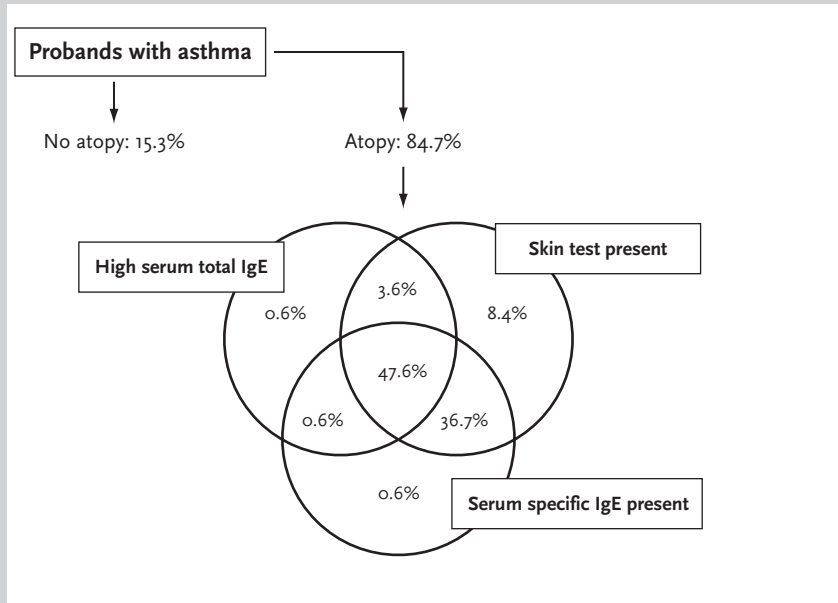


Table 1 Clinical characteristics of atopy in 200 Dutch families from probands with asthma

	Probands (1991-1999)	Spouses		Offspring	
		Of probands	Of 2nd and 3rd degree offspring	1st degree	2nd / 3rd degree
Number	200	201 #	68	531	136
Mean age (SD), years	52.2 (8.5)	51.0 (9.2)	38.8 (6.2)	24.0 (9.0)	12.0 (5.3)
Male, %	62.0	37.8	70.6	45.0	49.3
Total IgE, mean* (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, %					
≥ 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 P1	47.7	3.5	4.4	22.5	10.7
Eosinophils, mean* (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)

NOTE. The following clinical data was not present for these reasons: Not enough serum for Phadiatop measurement (23 individuals) or DerP1 measurement (additional 2); Allergy skin test refused by 1 individual; positive response to the negative control (n=4); no blood for total serum IgE analysis (5 individuals) and eosinophil counts (5 individuals). * Mean values for IgE and eosinophils are geometric means. # One proband married twice, both spouses participated in the study.

Figure 2a Overlap of atopic phenotypes in probands with asthma



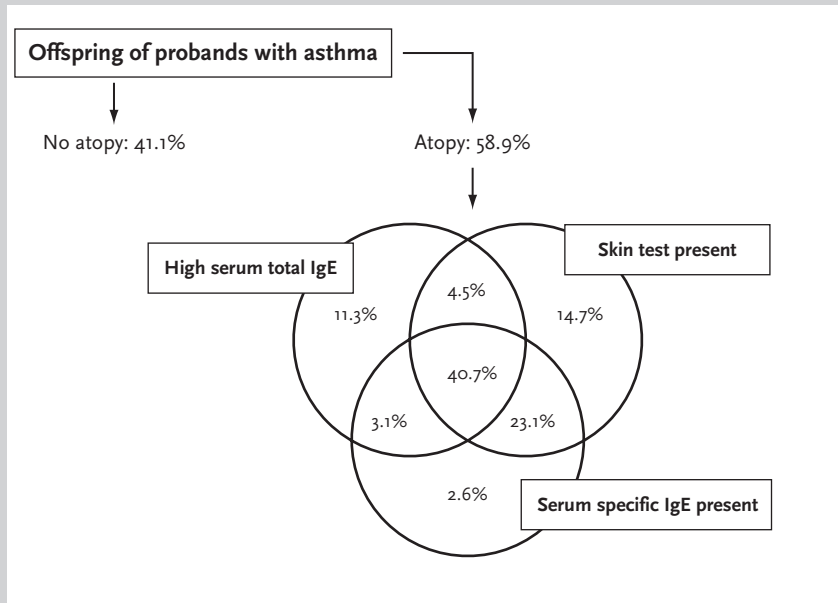
Panel A. Probands with asthma. (n=200) who participated in our genome screen. No atopy defined as normal serum IgE, no skin test positive, and absent serum specific IgE. Atopy defined as high total serum IgE or a positive skin test or specific IgE test. Percentages in Venn diagram is the percentage of all atopic probands with asthma. Missing data on one of three phenotypes in 4 individuals are not included in this figure.

The parent-offspring correlation coefficient of adjusted log(eosinophil) numbers was 0.12 in 1358 pairs (0.14 for 372 mother-daughter pairs, 0.14 for 317 mother-son pairs, 0.10 for 361 father-daughter pairs and 0.07 for 308 father-son pairs). The correlation between siblings was 0.18 in 684 sibling pairs (0.20 between 339 sister-brother pairs, 0.15 for 152 brother-pairs, and 0.16 for 193 sister-pairs). No correlation was observed between grandparents and children (-0.009 in 274 pairs), avuncular pairs (-0.07 in 559 pairs) and cousin pairs (0.002 in 395 cousin pairs).

Overlap of atopic phenotypes in probands with asthma and their offspring

Skin tests and Phadiatop gave consistent results (both positive or both negative) in 86.8 % of the probands and 78.6 % of the offspring. However, high serum IgE levels were found in 60 % of subjects who tested Phadiatop positive and in 51% of subjects with one or more positive skin tests. A high total serum IgE level in combination with a positive Phadiatop or a normal total IgE level in combination with a negative Phadiatop were found in 56.1 % of the probands and 66.9 % of the offspring (figure 2, panel A and B).

Figure 2b Overlap of atopic phenotypes in offspring of probands with asthma



Panel B. Offspring ($n=667$) of probands with asthma who participated in our genome screen. No atopy defined as normal serum IgE, no skin test positive, and absent serum specific IgE. Atopy defined as high total serum IgE or a positive skin test or specific IgE test. Percentages in Venn diagram is the percentage of all atopic offspring of the probands with asthma. Missing data on one of three phenotypes in 21 individuals are not included in this figure.

Genome-wide screen results

Figures 3a to 3e show the results of the genome-wide linkage analysis for Phadiatop, specific IgE to Der P1, skin test positivity, skin test to house dust mite and peripheral blood eosinophils, respectively. In table 2, these linkage results are summarized together with the previously published linkage results for total serum IgE.⁷

Three chromosomal regions showed evidence of linkage for two or more of the five phenotypes in the current analysis: chromosome 11q (skin test to house dust mite, LOD=1.90; Phadiatop, LOD=1.27); chromosome 17q (Phadiatop, LOD=1.38; eosinophils, LOD=1.97; skin test, LOD=1.55; skin test to house dust mite, LOD=1.21), and chromosome 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 our previous study: chromosome 2q (eosinophils, LOD=1.49; total serum IgE, LOD=1.96); chromosome 6p (eosinophils, LOD=1.28; total serum IgE, LOD=1.64); chromosome 7q (Phadiatop, LOD=1.04; total serum IgE, LOD=3.36); and chromosome 13q (Skin test, LOD=1.27; total serum IgE, LOD=2.28).

Figure 3a Genome wide search for major genes regulating specific IgE to aeroallergens (Phadiatop)

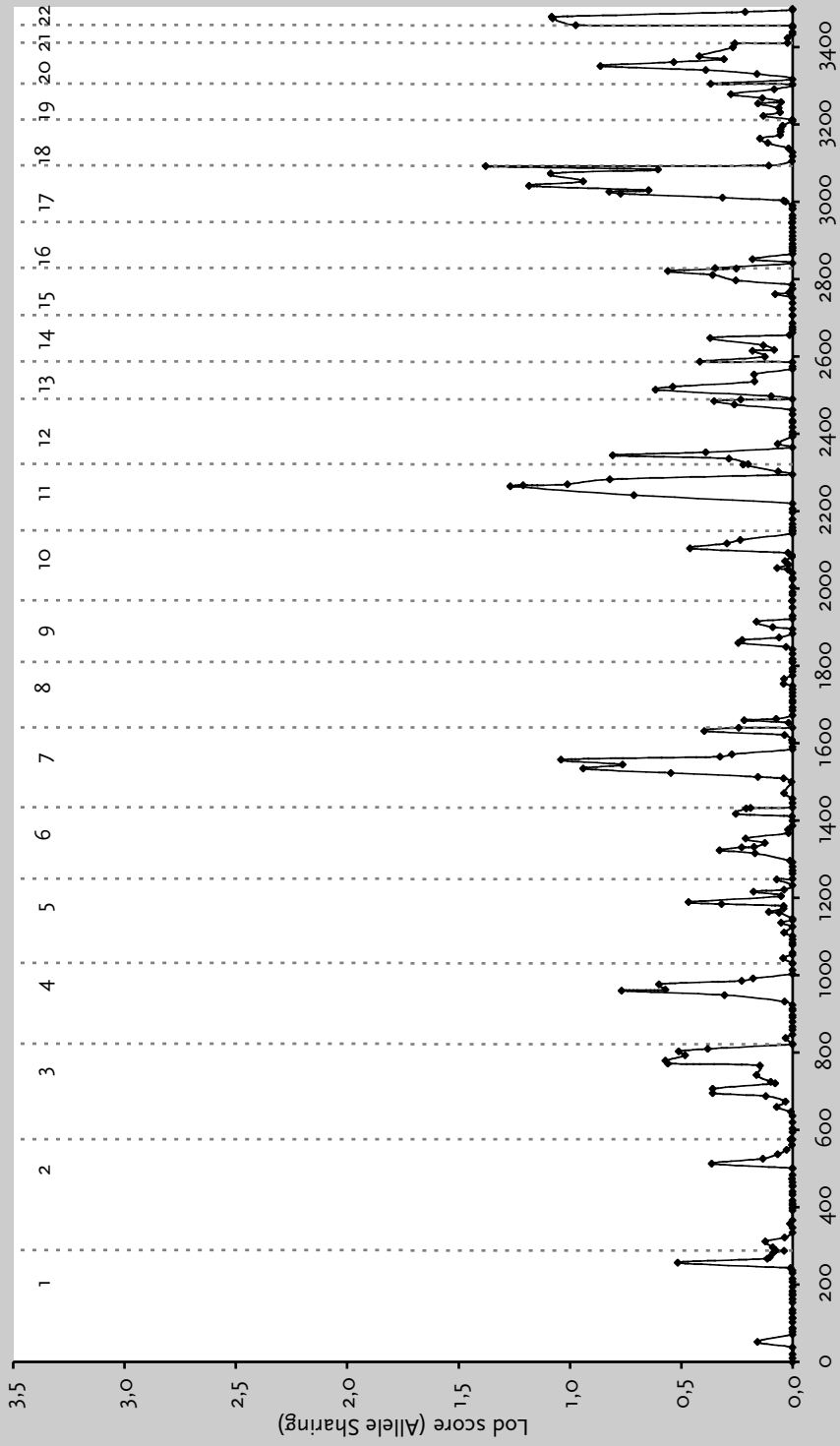


Figure 3b Genome-wide search for major genes regulating specific IgE to Der p 1

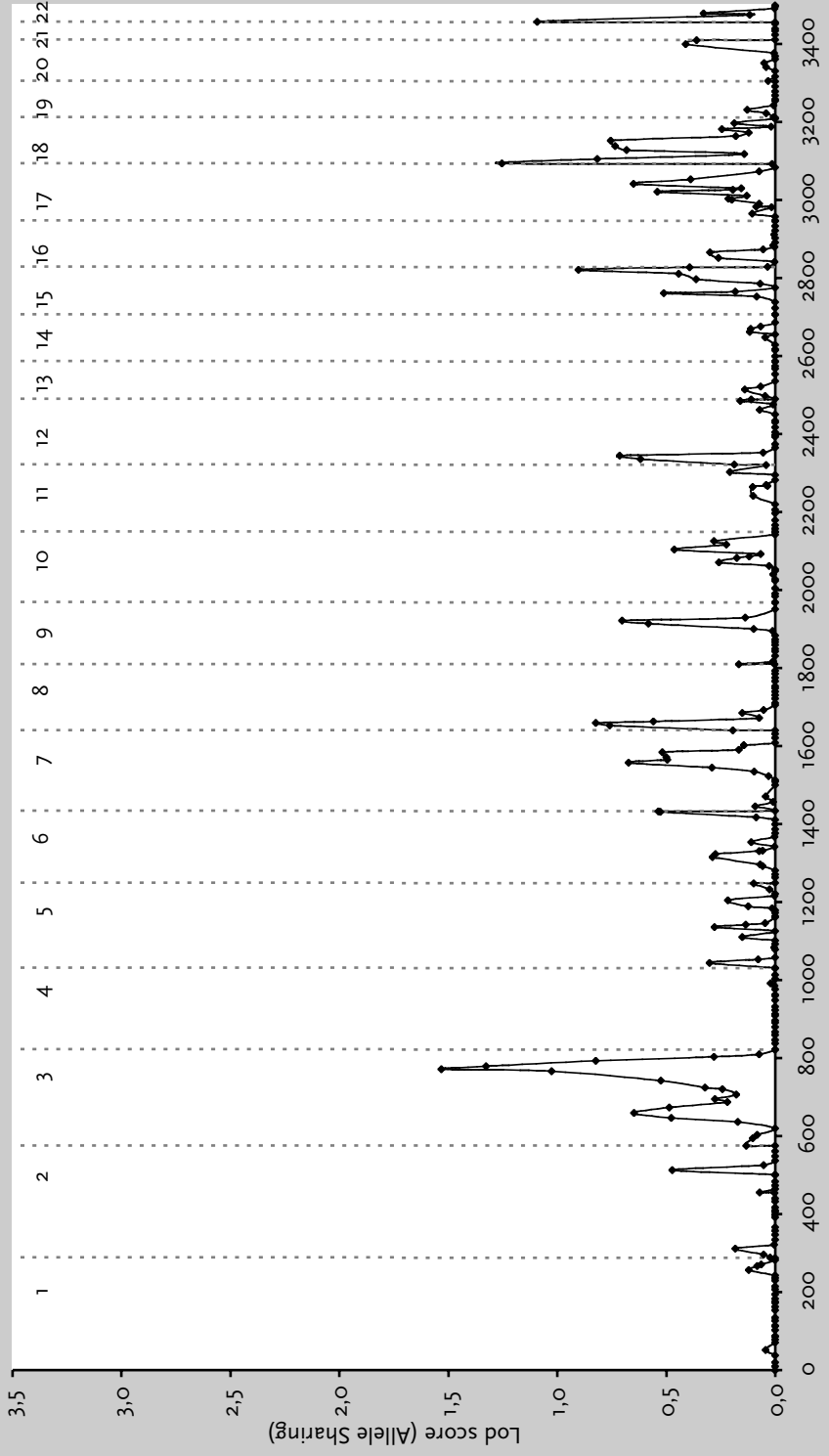


Figure 3c Genome wide search for major genes regulating skin test positivity

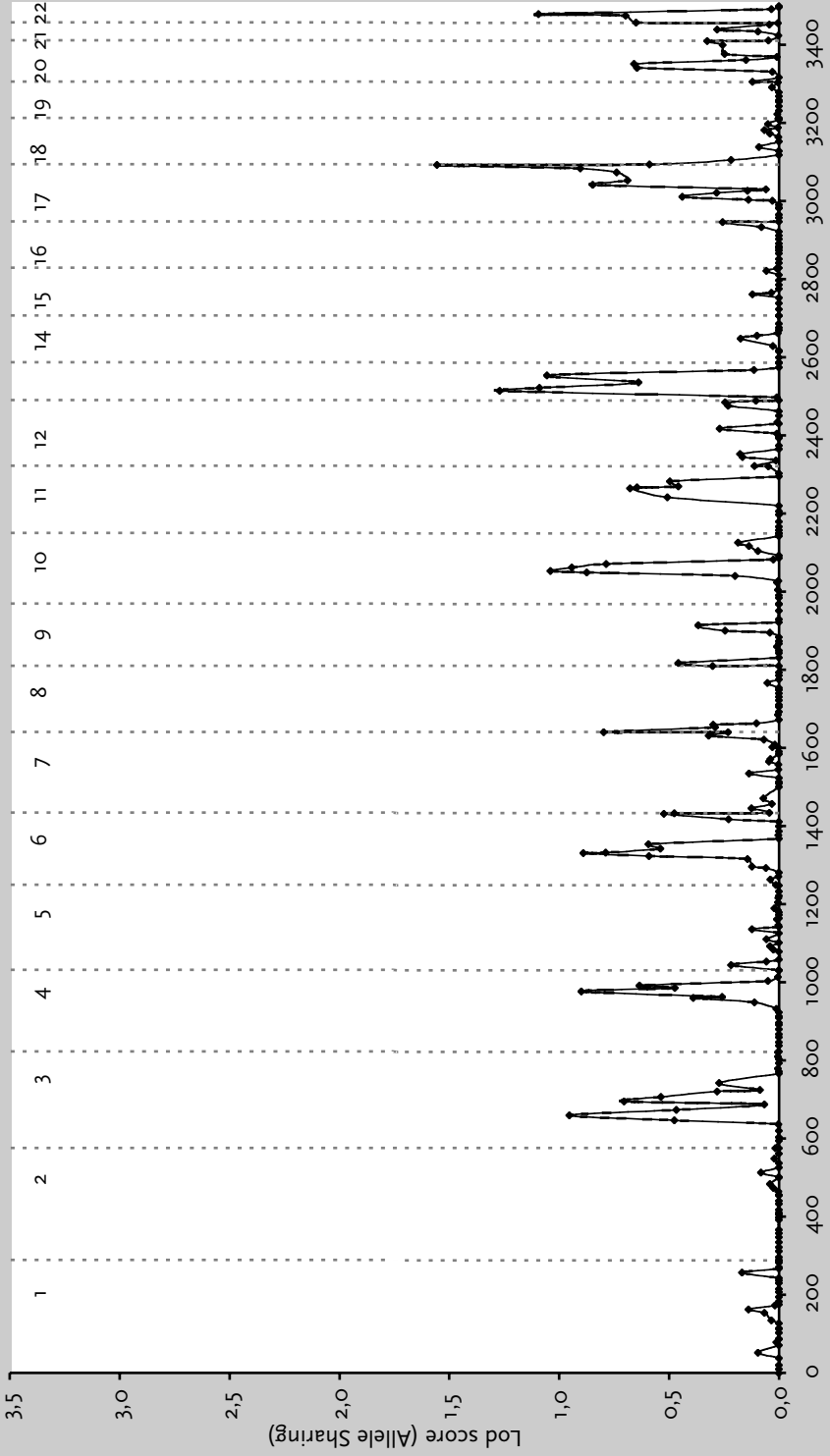


Figure 3d Genome wide search for major genes regulating skin test to house dust mite

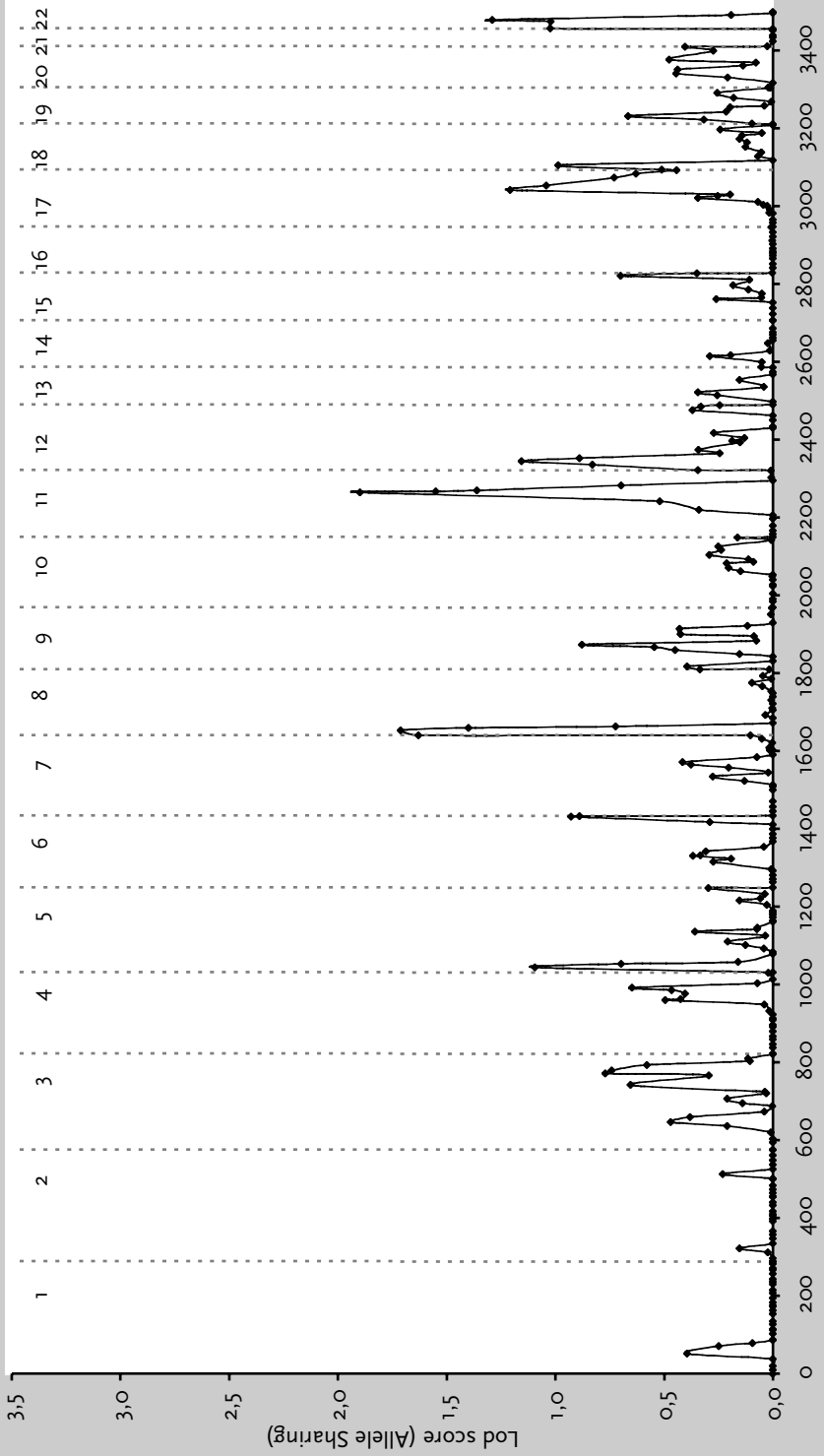


Figure 3e Genome-wide search for major genes regulating eosinophils

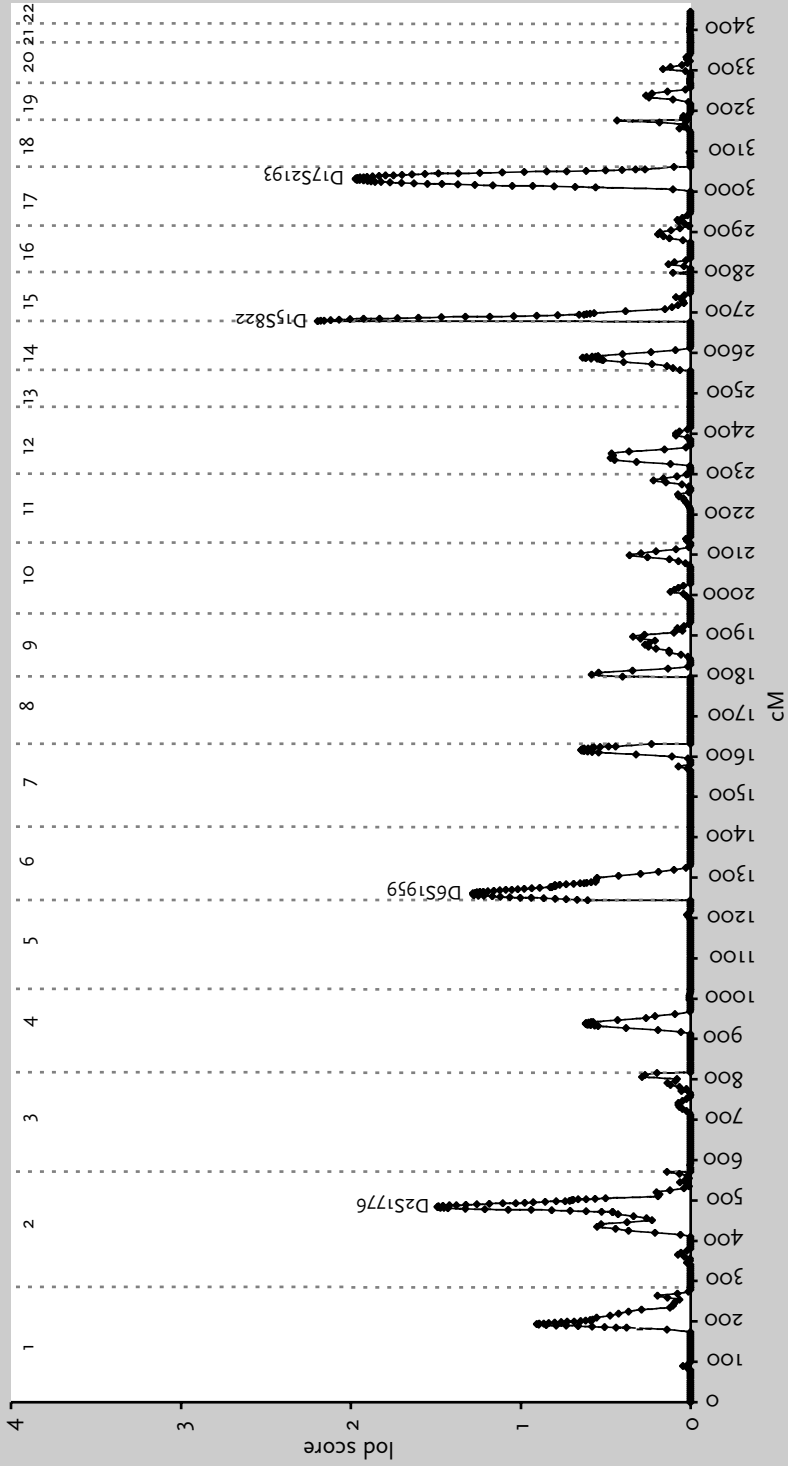


Table 2 Linkage results for specific IgE levels to aeroallergens and to Der P1, skin test positivity, eosinophils, compared to the linkage results of serum total IgE levels

Chromosome	Distance or interval* (sex-averaged in Kosambi cM)	(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	D3S3033	1.53	Specific IgE to Der P1
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 P1
22q11	41.06	D22S420	1.09, 1.02	Specific IgE Der P1, Skin test to house mite
	19.32	D22S345	1.08, 1.09	Phadiatop, skin test

LOD scores over 1.0 are summarized for qualitative traits. The highest LOD scores for quantitative traits are shown. * Map distance taken from Marshfield map. § D10S1 is a not in the Marshfield map. Chromosomal distance was estimated relative to D10S1225(80.77 cM) and D10S1432 (93.92 cM) # D18S178 not in Marshfield map. Chromosomal distance was estimated relative to D18S976 (distance 12.8 cM)

Other chromosomal regions showed evidence of linkage to a single atopic phenotype: chromosome 3q (LOD=1.53) and 18p (LOD=1.25) for specific IgE to Der P1; 10q (LOD=1.04) for skin tests; 5p (LOD=1.09), 8p (LOD=1.71), and 12p (LOD=1.16) for skin test to house dust mite; and 15q (LOD=2.19) for eosinophils.

Discussion

This study shows evidence of familial aggregation of skin test reactivity, elevated specific IgE to aeroallergens and eosinophil number. There are concordant results in the expression of high total IgE and specific IgE as detected by the Phadiatop assay in 56 % of the probands with asthma and 66.9 % of the offspring. In the 200 families ascertained through a proband with asthma, there is preliminary evidence for linkage of atopic phenotypes to multiple chromosomal regions (Table 2). These linkage results for several atopic phenotypes provide an important basis to identify specific genes that regulate host susceptibility for allergic responsiveness.

We have found several chromosomes to be implicated in the expression of atopy. As has been observed in other studies, genome-wide criteria for finding highly significant linkage are not always reached.¹⁴ There may be several explanations for this finding. First, although a genetic contribution to atopic traits has been well established, heritability estimates of various atopic phenotypes vary ranging for total IgE from 47 to 74 %.^{7,15,16} Estimates of heritability have been reported to be lower for other atopic phenotypes: 34 % for the the RAST index and 35 % for skin test positivity.^{15,16} In these family data, variance components analysis confirmed these findings: heritability estimates for total IgE were 55%, Phadiatop 41% , and skin test 25 %. Based on the genetic contribution to the trait, it is therefore plausible that the most significant results for linkage will be obtained for total serum IgE.^{7,17} In addition, the study of this 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 itself confers a modest increased risk to develop atopy, the trait may be heritable, but the power to find significant linkage produced by one gene in a specific chromosomal region will not be high.¹⁸ We have previously shown using a two-locus segregation analysis on total IgE that two major genes account for 51.3 % of the variance in total serum IgE levels in this population.⁷ No estimate of the number of (major) genes for other atopic phenotypes has been published. Third, specific environmental factors may interact with different genes to result in the expression of atopic responses. Thus, a large proportion of the population may carry a susceptibility allele, yet in only a part of these individuals the susceptibility allele is associated with the disease due to the exposure to a specific environmental factor. Fourth, other effects such as a parent of origin effects due to imprinting or gene-gene interactions have not been evaluated in most genet-

Table 3. Comparison of linkage results for atopic traits of the present study with published results (significance level: $p \leq 0.01$)

Chr.	Distance or interval (cM)	Phenotype						
		Asthma & BHR	Total IgE	Specific IgE	Skin tests	Eosinophils	Candidate region studies (phenotype)	
1	89.49 – 91.89	French (asthma, BHR)	Dutch		US - Hutterites (house dust mite)	Dutch		
2	173.00 - 186.21		Dutch		US - CSGA (dog and cockroach) Hutterites (pollen and cockroach)		US - Amish (total IgE) Japanese (1) Japanese (asthma) Dutch (BHR) US - Tucson (eosinophils)	
5	129.83 - 139.33	US - Hutterites (strict and symptom group)	Dutch	US - CSGA (Der P1, P2, and crude) Japanese (1) (atopy ^{*,**})				
6	25.08 - 34.23 42.27 - 53.81	US - Hutterites (loose) German Japanese (2)	Dutch German	German (≥ 1 of 13) US - CSGA (Der P1)	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 (one of 16) Australian (atopy ^{*,**})	French		
17	82.00 - 116.86	US - Hutterites (BHR)	Dutch (Phadiatop)		Dutch (house dust mite) Dutch (one of 16) French (one of 11) US - Hutterites (house dust mite)	Dutch French		

All studies with evidence for linkage ($p \leq 0.01$ for primary phenotype) in the same chromosomal region (spanning 20 cM from peak or interval markers) are included in the table. If multiple markers showed evidence for linkage, the marker with the lowest p-value was included in this table. BHR Bronchial hyperresponsiveness. Chr. Chromosome. Map distance taken from Marshfield map. Data were taken from original papers and the Asthma Gene Database. ⁽¹³⁾

Sources and definitions of the phenotype

- Australian. Atopy^{*} was defined as one of the three (high serum total IgE level, one skin test positive, or one specific IgE positive). Reference: Daniels *et al.* ⁽²²⁾
- Barbados. Reference Barnes *et al.* ⁽³⁸⁾
- Dutch: BHR to histamine. References: Xu *et al.* ⁽⁷⁾, Postma *et al.* ⁽³¹⁾, present study
- English. Asthma defined as positive answer to the question; have you ever had asthma? Reference Wilkinson *et al.* ⁽¹⁵⁾
- European - HDM. Families from England, Germany, Portugal, and Italy. Subpopulation English families is part of this study. Reference: Kurz *et al.* ⁽³⁴⁾
- European - AD (atopic dermatitis). Families from Germany, Italy, Sweden, and Netherlands. Reference Lee *et al.* ⁽²⁵⁾
- French - BHR to methacholine. Asthma defined as a the presence of asthma attacks or attacks of breathlessness at rest with wheezing, combined with one of the following four (BHR, reversibility >12 % after use of a bronchodilator, ever hospitalisation for asthma, or asthma therapy. Reference: Dizier *et al.* ⁽²¹⁾
- German. Asthma: History of physician diagnosed asthma. References: Wjst *et al.* ^(17;24)
- Japanese. (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 three (high total serum IgE levels or the presence of specific IgE to aeroallergens. Reference: Noguchi *et al.* ⁽³⁹⁾
- Japanese (2) Asthma defined as the presence of two or more episodes of wheezing and shortness of breath, which was reversible and the presence of specific IgE to D. farinae. Reference: Yokouchi *et al.* ⁽²⁶⁾
- US-Hutterites. BHR to methacholine. Strict asthma: BHR and symptoms. Loose asthma: BHR or symptoms. Symptom: asthma symptoms. Reference Ober *et al.* ⁽²⁰⁾
- US - CSGA. Asthma defined as presence of two of three symptoms (cough, wheeze, dyspnea), and BHR to methacholine or reversible airways obstruction ($\geq 15\%$ increase from baseline). References: CSGA ⁽³⁷⁾, Hizawa *et al.* ⁽²³⁾
- US - Tucson. Reference: Martinez *et al.* ⁽⁴⁰⁾
- US - Amish. Reference: Marsh *et al.* ⁽⁴¹⁾

ic studies.¹⁹ An important issue for the correct interpretation of linkage results in genome-wide screens is the level of significance. We reported all results of LOD > 1, since a comprehensive approach showing all linkage results permits meaningful comparisons between different studies. Consistent replication of linkage results in different populations may provide useful evidence for the locations of atopy susceptibility genes. For example, the results of this study suggests a possible role of chromosome 17q for different phenotypes related to atopy (eosinophils, skin test to house dust mite and Phadiatop). Linkage of this region for the same phenotypes was also observed in the US Hutterite and French population (table 3).^{20,21} Thus, this region of chromosome 17q represents a replicated regions that requires additional fine mapping to identify potential susceptibility genes for atopy.

Genome-wide screens of atopy related phenotypes have been performed in multiple populations^{17,20-26}, although families were ascertained using different methods, i.e. through two children with asthma^{21,24,26,27}, two children with atopic dermatitis²⁵, a random population sample, a single large pedigree from a genetically homogeneous population of the Hutterites²⁰, or a child or parent with asthma.^{7,22} The type of ascertainment may affect the linkage results (table 4). For example, in the Japanese study, 100 % of the population with asthma were house dust mite sensitive, whereas only 52.1 % of the Hutterites with asthma had one or more positive skin prick tests. Thus, the linkage results found in the Japanese study could be accounted for by genes important in asthma, atopy or both. Replications of similar chromosomal regions with different phenotypes may identify a pleiotropic gene (a gene causing different phenotypes depending on other genetic and or environmental factors). For atopy, evidence for shared and specific genetic factors in the regulation of serum total IgE, bronchial hyperresponsiveness to methacholine and specific IgE to aeroallergens has been suggested.¹⁶ Evidence for shared genetic factors in skin test positivity and bronchial hyperresponsiveness to hypertonic saline has also recently been reported.²⁸ In addition, the combination of different closely linked genes could account for the linkage signal observed in several chromosomal regions such as 5q, 11q, and 12q. When reviewing all published genome wide screens, a clustering of linkage signals for asthma and atopy can be observed. We speculate that different genes regulate total and specific serum IgE levels. In addition, the association of skin test positivity and the presence of specific IgE with asthma and allergic rhinitis may result from different combinations of 'atopy' and 'asthma' genes.^{29,30}

Based on the analysis of five atopic phenotypes in the current study, replicated evidence for linkage to atopy was observed on chromosomes 2q, 6p, 7q, 11q, 13q, and 17q. Evidence for linkage to chromosome 8 was also observed, consistent with previous reports for linkage to asthma. In addition, our previous linkage results for total serum IgE levels are consistent with

Table 4. Characteristics of published genome-wide studies compared to present study

First author	Study population	Number of families (number of individuals)	Ascertainment strategy	Average marker spacing	Asthma or BHR	Atopic dermatitis	Total IgE	Specific IgE	Skin tests	Eosinophils
Daniels ⁽²²⁾	Australian	80 (n=364)	Young families selected from population sample of 230 families	~11.5 cM	X		X		X	X
CSCA ^(23,37)	US - Caucasian, African American and Hispanics	Asthma: 199 nuclear and 67 extended families. (37) Specific IgE to Der P: 45 Caucasian and 53 Afr. Am. (n=580) (23)	Two siblings with asthma	~10 cM	X		X			
Wjst ^(17,24)	German	97 (n=415)	Two siblings with asthma	~10.4 cM ~10.7 cM	X		X	X		X
Ober ⁽²⁰⁾	US – Hutterites	1 extended pedigree (n=693)	Population of 9 Hutterite colonies. All available individuals > 5 years of age	~9.1 cM (genome screen)	X		X			
Yokouchi ⁽²⁶⁾	Japanese	47 (n=197)	Two siblings with mite sensitive asthma	376 markers (~8.2 cM)	X		X			
Dizier ⁽²¹⁾	French	107 (n=493)	A proband with asthma or two siblings with asthma	13 cM	X		X		X	X
Lee ⁽²⁵⁾	European (German, Italian, Swedish and Dutch)	199 (n=839)	Two siblings with atopic dermatitis	380 markers (~8.2 cM)		X	X	X		
Xu ⁽⁷⁾ , Present study	Dutch	200 (n=1174)	A proband with asthma (symptoms, BHR to histamine between 1962 and 1975)	~10 cM			X	X		X

BHR bronchial hyperresponsiveness

evidence from other investigators demonstrating linkage to chromosome 1p, 5q, and 12q for asthma and atopy related phenotypes (table 3). Because the results on 5q³¹, 6p, 7q and 12q⁷ have been discussed previously, we will now discuss the relevance of linkage on 8p, 11q, 13q and 17q and relate them to positional candidate genes based on Genemap '99 and the OMIM (Online Mendelian Inheritance in Man) database.

Linkage of asthma to chromosome 8p (marker D8S1130) was observed in a conditional analysis in the US-CSGA sample, the same marker that showed linkage to a positive skin test to house dust mite in the present study. Evidence for linkage for asthma to chromosome 8p in the CSGA study increased after conditioning on the evidence for linkage to chromosome 11q21, indicating gene-gene interactions between loci at chromosome 8p and 11q in asthma.²⁷ We observed evidence of linkage to chromosome 8p (skin test to house dust mite) and chromosome 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.^{32,22} In the first 92 families in this population, we have previously reported that we did not find evidence for linkage to the number of positive skin tests, total serum IgE levels or bronchial hyperresponsiveness. This may seem contradictory, however, a positive skin test to house dust mite and Phadiatop were not analyzed in that study.³³ The region on 11q (11q21-q24) that was linked to atopy in this study, is approximately 35-45 cM telomeric to the FCER1B region at chromosome 11q13. Linkage to 11q21 was also observed in the CSGA population for the asthma phenotype²⁷, and to specific IgE to house dust mite in German families.³⁴ 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 , that bind 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 3) and with total serum IgE and skin test positivity in the current study. It includes the candidate gene endothelin receptor B.²¹ A broad region on chromosome 17q has been implicated in three genome-wide searches, in particular for phenotypes related to the specific immune response. Skin test positivity and specific IgE as detected by the Phadiatop assay showed evidence for linkage in our study. Several candidate genes have been proposed.²¹ Examples are the transcription factor STAT5A (signal transducer and activator of transcription 5A), chemokine receptor 7 and members of the family small inducible cytokines subfamily A, such as RANTES (regulated upon activation, normally T-expressed, and presumably secreted) and eotaxin. RANTES is a chemokine responsible for recruitment of inflammatory cells such as eosinophils and T lymphocytes. Promoter variants of RANTES have been associated with atopic dermatitis and skin prick tests, but not total IgE levels.^{35,36} Eotaxin is a potent chemoattractant for eosinophils in inflamed tissue.

We did not observe evidence for linkage of atopic traits to some chromosomal regions that show evidence for linkage in other genome-wide screens, such as chromosome 4^{22,26}, 9^{20,24}, 11p^{17,21,26,37}, 16^{20,22}, 19^{20,21}, and 20^{20,22}. This could reflect population heterogeneity or may be explained by a lack of power to identify all regions in every population. In atopy, different patterns of gene-gene and gene-environmental interaction may be important in different populations and for different allergic phenotypes.²⁷

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 from probands with asthma. In addition, distinct regions on the genome are implicated for the different atopic phenotypes. Evidence was found for the presence of atopy susceptibility genes on chromosome 2q, 5q, 6p, 7q, 8p, 11q, 12q, 13q and 17q. Most of these regions have also shown evidence for linkage to atopy associated phenotypes in other genome-wide studies. Further replication and fine mapping studies will lead to identification of atopy susceptibility genes and ultimately provide information on the pathogenesis of atopic disease. Based on the clustering of linkage signals in genome wide screens on atopic phenotypes, we propose that in addition to shared genes, different genes may regulate total and specific serum IgE.

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Electronic Database information

Asthma Gene Database: <http://cooke.gsf.de/asthmagen>¹³
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
S.A.G.E. (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>

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