

Identifying genes predisposing to atopic eczema

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Background: Genetic and environmental factors are known to play a role in the development of atopic diseases, such as asthma, eczema, and rhinitis. However, the atopy gene (or genes) has yet to be defined. Studies of familial asthma have identified several regions that may contain genes predisposing to atopy, but the data for candidate regions do not show agreement, which may be due to heterogeneity, ascertainment bias, or stochastic factors. Factors such as an early age of onset, a positive family history, and a clearly defined phenotype favor a genetic origin and improve the chance of identifying genes that predispose to atopy.

Objective: We sought to define genes that predispose to the development of atopic eczema.

Methods: We have studied nuclear families with multiple cases of early-onset atopic eczema for involvement of the candidate regions on chromosomes 5q31 (*IL* gene cluster), 11q13 (high-affinity FC ϵ receptor), 14q11.2 (mast cell chymase), and 16p12 (*IL*-4 receptor α -chain, *IL4RA* gene).

Results: Using a recessive model, we find a maximum parametric log of the odds of linkage score of 2.25 and nonparametric score of 2.54 ($P = .006$) for a region on chromosome 5q31, which we postulate contains a gene predisposing to atopic eczema, but lack of support for linkage to 11q13. Transmission disequilibrium tests do not support an association with candidate polymorphisms in the mast cell chymase and *IL4RA* genes.

Conclusion: We have identified a clinically homogeneous cohort of patients with atopic eczema to identify genetic factors predisposing to the development of atopy. We postulate that there are certain loci that predispose to atopy in general and other loci that determine which of the atopic phenotypes is expressed. (*J Allergy Clin Immunol* 1999;104:1066-70.)

Key words: Eczema, candidate loci, mapping, 5q31, interleukin cluster, D5S404, atopy, linkage analysis, atopy, association studies

Abbreviations used

LOD: Log of the odds of linkage
MCC: Mast cell chymase
NPL: Nonparametric linkage
TDT: Transmission disequilibrium test

With the advent of new and rapid techniques for gene localization and cloning, over 200 single gene disorders have been mapped, and the genes have been identified. Researchers are now turning their attention toward complex traits and attempting to dissect the underlying biologic-genetic mechanisms that result in predispositions to particular disorders, such as atopy, heart disease, diabetes, and schizophrenia. Defining such genetic factors will shed light on pathophysiologic mechanisms, which in turn should result in better treatment strategies. However, the analysis of complex traits is complicated by the combined effects of genetic heterogeneity, phenocopies, gene interactions, and incomplete penetrance.

Atopy is a common, chronic debilitating condition in which treatment is often unsatisfactory. The atopic phenotypes include asthma, eczema, rhinitis, and food allergy. Most studies of the genetics of atopy have focused on individuals with respiratory symptoms (asthma) and an elevated serum IgE level.¹⁻⁹ They form a heterogeneous group.¹⁰

In looking for genetic factors that might predispose an individual to atopy, it is logical to examine the pathophysiologic pathways involved in allergic responses to focus the search initially on a few key genes. The key genomic regions of interest in the initial studies include chromosome 5q31 (in the region of the genes coding for *IL*-4, *IL*-5, *IL*-9, *IL*-13, interferon regulatory factor 1, GM-CSF-2, CSF-1 receptor, and platelet-derived growth factor receptor)^{1,2} and chromosome 11q13 (at the locus for the high-affinity FC ϵ receptor 1, FC ϵ R1).^{3-9,11,12} The locus on chromosome 11q13 has been associated with bronchial hyperreactivity, even in the absence of atopy.⁴ An alternative approach involves performing a genome-wide search to identify novel candidate regions.^{6,7}

The factors that favor a genetic origin and minimize genetic heterogeneity are an early age of onset, a positive family history, and a clearly defined phenotype.¹³ Atopic eczema has a peak prevalence in the first 5 years of life,¹⁴ and it is easy to diagnose and to gauge severity. The con-

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tribution of the genetic component to atopic eczema is evident in twin studies, which show concordance of disease in monozygotic twins (0.72) compared with dizygotic twins (0.21).¹⁵ Most atopic diseases have a heritability of between 0.60 and 0.72.¹⁶ The total serum IgE level is highest in patients with atopic eczema compared with patients with asthma, perennial rhinitis, and hay fever.¹⁶

There have been very few studies to date on eczema genetics, and discordant findings have been observed. One linkage study using markers on 11q13 flanking the *FCR1* locus gave negative data,¹² whereas a more recent study gave some evidence for support in a subgroup of families with atopic dermatitis.¹⁷ Studies in the Japanese population proposed an association between the presence of a polymorphic restriction site within the mast cell chymase (*MCC*) gene on chromosome 14q11.2 and atopic eczema¹⁸ and a second association with a polymorphism in the promoter of *IL-4* on chromosome 5q.¹⁹ Finally, Hershey et al²⁰ looked at the Arg576Gln variant of the IL-4R α chain, an excess of which was found in a study of people with hyper-IgE syndrome and severe eczema.

Using clearly defined clinical criteria, we have identified 50 families with sibships affected with severe eczema under the age of 1 year. We have performed linkage and association analyses for a number of candidate loci to clarify which genes are involved in the development of atopic eczema.

METHODS

Patients

Families with 2 or more children with atopic eczema attending the specialist allergy center at the Royal Children's Hospital were identified. This study was approved by the Human Ethics Review Board of the Royal Children's Hospital. The criteria for entry of the index case were as follows: severe generalized eczema in the first year of life diagnosed by using standard criteria^{21,22} by a pediatric allergy specialist (DH), an elevated serum IgE assay (>40 IU/mL for <2 years of age, >150 IU/mL for 2-7 years of age, and >200 IU/mL for >7 years of age; QuantiCLONE, Kallestad Diagnostics, Sanofi Diagnostics Pasteur, Inc), a positive skin prick test response to one or more dietary or inhalant allergens (wheal size greater than that of a skin prick test with 1 mg/mL histamine) and/or a positive RAST test response to common allergens (ELISA assay, Kallestad Allergo-coat EAST Conjugate Pack, Sanofi Diagnostics Pasteur, Inc; results expressed as Allergo-coat EAST units: negative at <0.35 AEU/mL and positive at >0.7 AEU/mL), and a sibling who had eczema diagnosed in the first year of life by one of us (DH). The clinical symptoms in the sibling may have been milder because of early dietary intervention. Parents were questioned about a history of atopy.

Microsatellite genotyping

Genomic DNA was isolated from mouthwash samples by standard methods.²³ In younger subjects mouth scrape samples were collected on a wooden spatula. Polymorphic markers close to the candidate loci on chromosomes 5q31 and 11q13 were selected. On chromosome 5q31, the markers D5S404, D5S642, D5S666, D5S471, and D5S659, spanning the IL-4 region, were analyzed. On chromosome 11q13, the CA microsatellite repeat in the fifth intron of the *FCR1* gene and the cIII-319ca marker adjacent to the gene were analyzed.⁵ The final concentrations of each 20- μ L PCR sample were as follows: 67 mmol/L Tris-HCl, pH 8.8; 16.6 mmol/L

(NH₄)₂SO₄; 0.45% Triton X-100; 0.02% gelatin; 1.5 to 4.0 mmol/L MgCl₂; 200 mmol/L deoxyribonucleoside triphosphate; and 500 nmol/L each of forward and reverse primers. Taq DNA polymerase (0.5 U, Boehringer Mannheim) was added. D5S404 was internally labeled by the addition of 1 μ Ci of α 32P-deoxycytidine triphosphate (3000 Ci/mmol). For D5S666, D5S642, D5S471, and D5S659, 1 primer was end labeled according to standard procedures by using 1 μ Ci of γ 32P-deoxyadenosine triphosphate and added to the PCR reaction, with the second primer unlabeled. PCR annealing temperatures were 50°C (D5S471), 57°C (D5S404 and D5S642), 59°C (D5S659), 60°C (D5S666), and 55°C (*FCR1* and MF). PCR products were resolved on 5% (D5S404, D5S642, D5S666, D5S659, and D5S471) and 8% (*FCR1* and MF) polyacrylamide gels. Genotypes were determined independently by 2 investigators.

MCC genotyping

Family members were analyzed for the BstX1 restriction enzyme site in the MCC region of chromosome 14q11.2. DNA was amplified by PCR with primers 5'-GGAAATGTGAGCAGATAGTCAGTC-3' and 5'-AATCCGGAGCTGGAGAACTCTTGTC-3'. The PCR products were digested with BstX1 (Boehringer Mannheim). Samples that did not amplify were subjected to a primary round of universal amplification by using degenerate oligonucleotide-primed PCR²⁴ before the specific PCR reaction for MCC alleles. Bands were separated by electrophoresis on 1.5% agarose TRIS borate EDTA gels.

IL4R genotyping

Family members were investigated for the polymorphic variant Arg576Gln caused by an A-to-G single-base substitution. The first-round PCR used primers 5'-AACCACTGTGCCCAACCTG-3' and 5'-GCTGCTTGGGAGATGTGAGC-3'. PCR was performed by using a touchdown protocol with annealing varying from 65°C to 55°C over 20 cycles and then 15 cycles at 58°C with 3 mmol/L MgCl₂. Two microliters of the first-round product was added to the second-round PCR in a final volume of 20 μ L. The primers used were 5'-CCGAAATGTCCTCCAGCATG-3' and 5'-CCAGTCAAAAGGTGAACAAGGGG-3'. The same touchdown protocol was used with 3 mmol/L MgCl₂. The polymorphism does not alter a restriction enzyme site, and therefore PCR products were sequenced by using the Thermo Sequenase radiolabeled terminator cycle sequencing kit (Amersham Pharmacia Biotech).

Linkage

For the regions on chromosome 5 and 11, where either an extended candidate region exists (Chr 5) or for which no candidate gene variants were known (Chr 11), the test for linkage was based on sib-pair allele-sharing methods by using GENEHUNTER, which permits combined multipoint parametric and nonparametric analyses.²⁵ Subjects were classified as *affected* if they had atopic eczema with an early childhood age of onset. Any parents with mild eczema were coded as *unknown*. Subjects without eczema were classified as unknown, irrespective of the presence or absence of other atopic conditions.

Population allele frequencies for the microsatellites in the linkage analysis were determined by using founder chromosomes in the families. The marker order and distance was obtained from the Genome Data Base (URL <http://gdbwww.gdb.org/>) and is as follows: D5S659 – 2.1 cM – D5S404 – 2.6 cM – D5S471 – 6.7 cM – D5S642 – 1.7 cM – D5S666.

By using existing epidemiologic and twin data as described below, 3 parametric models for disease segregation were considered. In the first 2 cases the disease gene was assumed to act recessively, the overall disease frequency to be between 5% and 10%, and the maximum penetrance to be 0.72 (based on twin studies²⁶).

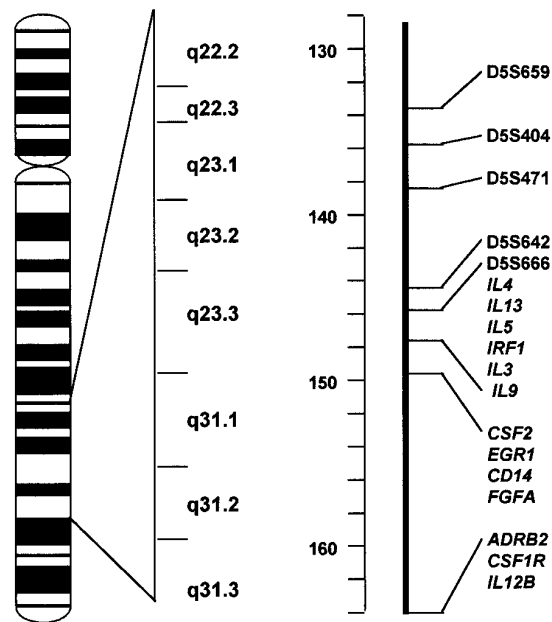


FIG 1. Chromosome 5q. Map of the relative position of markers and known genes (shown in italics).

The disease-associated allele frequency was set to be either 8% (model 1) or 25% (model 2), such that the proportion of affected cases in the population caused by homozygosity for the allele were 10% and 50%, respectively. In the third case the disease was assumed to act dominantly, with a disease-associated allele frequency of 3.8% (model 3). The proportion of affected cases having at least 1 copy of the affected allele was 54%. There were no age-related or sex-specific penetrance effects or liability class designations. To test the ability of the sample group to exclude a region of the genome, the EXCLUDE function of GENEHUNTER was used. The test parameter was λ s, and it was varied to enable tests to be performed for relative risks to siblings from 5 down to 2.

Transmission disequilibrium tests

In the case of the loci on chromosome 14 and 16, candidate variants of presumed functional importance have been identified. We sought to determine the relevance of these loci by using a family-based association method, the transmission disequilibrium test (TDT).²⁷ Standard TDT analysis is only a valid test of the null hypothesis of no linkage and no linkage disequilibrium when observations are independent; that is, only one trio is chosen per pedigree. A single offspring (the proband) was selected, and a standard TDT was performed.

Power calculations for association tests, such as the TDT, require substantial assumptions. We used genotype and allele frequency data from published case-control studies to infer dominance relationships and to estimate genotype penetrances consistent with known disease prevalence. We assumed that the variant tested was the disease locus itself (or in complete linkage disequilibrium with it). On the basis of these estimated parameters, power was calculated by using the methods of McGinnis.²⁸

RESULTS

Fifty pedigrees ($n = 231$ individuals) were analyzed. All families studied contained at least 2 children with atopic eczema assessed by a single pediatric allergist. All

TABLE I. Output file for chromosome 11

Position	LOD score (model 1)	LOD score (model 2)	LOD score (model 3)	NPL score	P value	Information
-5.00	-0.93	-1.19	-1.45	-0.021	.51	0.469
-4.00	-1.03	-1.30	-1.59	-0.023	.51	0.514
-3.00	-1.14	-1.42	-1.75	-0.025	.51	0.565
-2.00	-1.25	-1.56	-1.93	-0.026	.51	0.624
-1.00	-1.38	-1.70	-2.12	-0.028	.51	0.695
0.00	-1.51	-1.85	-2.34	-0.030	.51	0.793
1.20	-1.48	-1.77	-1.59	0.148	.44	0.714
2.40	-1.48	-1.72	-1.11	0.328	.37	0.681
3.60	-1.49	-1.69	-0.76	0.51	.30	0.675
4.80	-1.53	-1.69	-0.50	0.69	.24	0.693
6.00	-1.59	-1.71	-0.29	0.88	.19	0.753
7.00	-1.45	-1.57	-0.17	0.85	.20	0.663
8.00	-1.32	-1.44	-0.06	0.82	.20	0.598
9.00	-1.19	-1.32	0.02	0.78	.21	0.542
10.00	-1.08	-1.20	0.097	0.75	.22	0.495
11.00	-0.98	-1.10	0.164	0.73	.23	0.452

members of each nuclear family were genotyped. There were 131 children (77 boys). Sixty-eight boys and 43 girls had eczema ($\chi^2 = 1.85$, $P = .096$), and 20 sibs did not have eczema. The median age of onset of eczema in the index case was 2.0 months (range, 0.3-10 months). The median serum IgE level was 316 IU (range, 59-17200 IU). Of the 100 parents, 72 had an atopic disease; 43 had eczema (from mild to severe), and 29 had rhinitis or asthma without eczema.

Eczema was present in both parents of 3 families, in 1 parent in 28 families (mother in 16), and in neither parent in 19 families. There was no significant difference in the total number of mothers compared with fathers who reported a history of atopic eczema (19 vs 15, respectively; $\chi^2 = 0.47$, $P = .493$). In families in which only 1 parent had eczema, again there was no sex bias (16 vs 12; $\chi^2 = 0.57$, $P = .45$).

The pedigrees were analyzed for excess allele sharing in affected sib-pairs to loci on chromosomes 5q31 and 42 of the 50 pedigrees ($n = 194$) for 11q13 by using the GENEHUNTER multipoint linkage program.²⁵ Fig 1 indicates the position of the candidate genes on 5q31 relative to the markers used for analysis. Association with the MCC locus on 14q11.2 and the IL-4 receptor on 16p12 by TDT²⁷ was also examined.

Nonparametric analyses revealed evidence for excess allele sharing, which peaked at marker D5S404 (maximum nonparametric linkage [NPL] score = 2.54, $P = .006$; Fig 2, A) on chromosome 5q31. By using the parametric models, the multipoint log of the odds of linkage (LOD) scores at the same point were 1.83 (model 1), 2.25 (model 2), and -0.86 (model 3; Fig 2, B). No support for linkage was evident with the region on chromosome 11q13 by using any of the models (Table I). The EXCLUDE function of GENEHUNTER was performed; this sample group had the power to exclude linkage where the relative risk to siblings (λ s) is 2.9 but not for lower values.

We tested 2 genes known to harbor variants of possi-

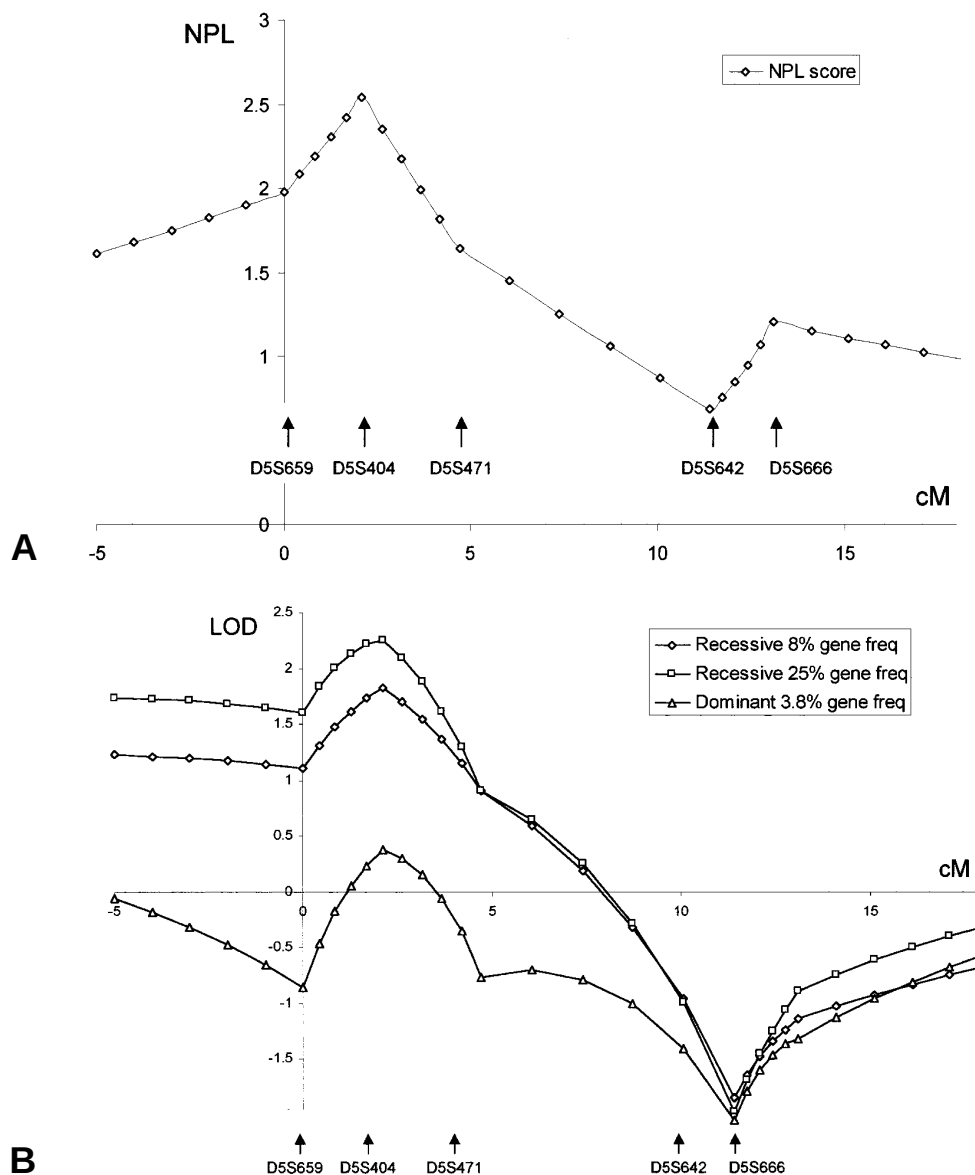


FIG 2. A, 5q output on a line plot; Y-axis gives NPL scores and X-axis gives marker position in centimorgans with order starting at 0 cM with D5S659 – 2.1 cM – D5S404 – 2.6 cM – D5S471 – 6.7 cM – D5S642 – 1.7cM – D5S666. B, 5q output for all models on a line plot; Y-axis gives LOD scores, and X-axis gives marker position and order as above.

ble functional significance for eczema by using family-based association methods. At the *MCC BstX1* polymorphism, there was no evidence for excess transmission to affected offspring when a single eczematous child (the proband) was analyzed ($\chi^2 = 1$, $P = .96$). There were 48 pedigrees, and assuming a recessive locus, there was 67% power to detect excess transmission at this locus at a 5% level of significance.²⁸ The second TDT analysis examined a variant of the *IL-4R α* chain. Again there was no evidence of disequilibrium in our sample of eczema patients by using a single affected child ($\chi^2 = 1.33$, $P = .25$). Here, 46 trios were analyzed, and assuming a dominant locus, this cohort had 94% power to detect excess transmission at this locus ($P = .001$).

DISCUSSION

Atopy is a common allergic condition affecting 10% to 20% of the population. We have identified a clinically homogeneous cohort of patients with atopic eczema to identify genetic factors predisposing to the development of atopy. The region of greatest interest is on chromosome 5q31, which contains the *IL4* cluster of genes. This area has been previously linked to the level of IgE production.¹ The maximum P value obtained in the Marsh study was .002 at D5S399.¹ The maximal score obtained in our study was with the D5S404 marker ($P = .006$). This marker is close to the gene cluster that includes the genes coding for IL-4, IL-5, IL-9, IL-13, interferon reg-

ulatory factor 1, GM-CSF-2, CSF-1 receptor, and platelet-derived growth factor receptor (Fig 1).

It has recently been shown that a gain-of-function mutation in the α -subunit of the IL-4 receptor confers a high relative risk of atopy on the basis of elevated IgE levels or specific IgE to one of a number of common allergens. Although the gene coding for this protein is located on chromosome 16p12.1, the finding further implicates the IL-4 pathway in susceptibility to atopy.²⁰ Our studies do not offer evidence of support for the involvement of the Q576R polymorphism in the IL-4 receptor, but the sample size was small. Twelve other polymorphic variants exist, 6 of which result in amino acid changes.^{29,30} None of these other variants have been tested in patients with atopic eczema to date.

The lack of evidence for linkage to the region on chromosome 11q13 is consistent with the suggestion that this region is involved in bronchial hyperreactivity rather than atopy per se.⁴ Although many of our subjects did not have eczema exclusively, the selection criteria meant that asthma was less common in our study compared with other studies.

Eczema has been previously associated with the BstXI restriction site in the gene coding for MCC in Japanese subjects.¹⁸ The data from our TDT analysis provides no evidence for disequilibrium. Alleles of this polymorphism in the gene for MCC do not predispose to eczema in the mixed, mainly white population studied here. This may be due to differences in predisposing genes between the Japanese and white populations or to population stratification in the Japanese study, which we avoid through the use of TDT.

Early-onset atopic eczema avoids some of the problems of phenotypic heterogeneity seen with adult asthma. Our data are consistent with the involvement of the IL cluster on chromosome 5q, which regulates IgE levels, in the development of atopy. We propose, first, that there is 1 major locus that causes a general predisposition to atopy at 5q31. This locus has been identified in our study as in many others. Second, there are different genes that combine with environmental factors to determine whether atopy manifests as eczema, asthma, or rhinitis.

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