Confirmation of genetic linkage between atopic IgE responses and chromosome 11q13

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Abstract
Genetic linkage between atopic IgE responses and chromosome 11q13 (D11S97) has been previously reported in a limited number of extended families. Difficulties of phenotyping in the older family members, poor family structure in some families, and genetic heterogeneity were proposed as possible explanations for the variability in lod scores. To test this finding a second linkage study of 64 young nuclear families was undertaken and gave a two point lod score of 3.8 at θ = 0.07 (assuming θm = 0θ). A test of genetic heterogeneity in the nuclear families shows that atopic IgE responses are linked to this locus in 60 to 100% of families (approximate 95% confidence limits).

Atopy is an allergic disorder that affects over 30% of some western populations1,2 and is the major (but not exclusive) cause of asthma, rhinitis, and eczema in children and young adults.3 Atopy is characterised by immunoglobulin E (IgE) responses to environmental proteins which are otherwise innocuous and predominantly found in house dust and plant pollens.4 Previous investigators have used clinical manifestations or raised total IgE or both as phenotype markers in genetic studies of atopy but have failed to agree on a consistent mode of inheritance.4,5 We have objectively assigned atopic IgE responsiveness (termed IGER and synonymous with atopy in this paper) to family members with either positive skin prick tests, raised specific IgE titres, or raised total IgE titre.6 We have used well established normal limits for these tests which have been determined by several population studies.7,10

Using these criteria we have described a dominant mode of inheritance of this immunological phenotype (IGER) although other factors, both genetic and environmental, are likely to influence the development of the clinical manifestations of atopy.8,14 Using this model, genetic linkage in seven extended families was found between IGER and a polymorphic marker (λMS.51) on chromosome 11q13 (lod score = 5.6).10 Lod score variation between families was attributed, in part, to the effects of age and smoking on reliably identifying the IGER phenotype. As a result, the possibility of genetic heterogeneity (more than one locus independently conferring disease phenotype) could not be resolved. We report the results of two point linkage and heterogeneity analysis in a large sample of atopic nuclear families suggesting the 11q13 region contains a major locus conferring atopic IgE responsiveness in these families.

Materials and methods
ASCERTAINMENT AND PHENOTYPING OF FAMILY MEMBERS
Families were recruited in which one or more children under 15 years of age reported symptoms consistent with eczema, asthma, or hay-fever, from an allergy clinic (n = 18), asthma clinic (n = 18), and after responses from a media appeal (n = 28). IGER phenotype was assigned, as previously described,10 to those with one or more of (1) a skin prick wheal of 2 mm or more than control to any one of a panel of common environmental antigens (Dome/Hollister-Steir, Spokane, Washington, USA), (2) a specific IGER RAST score of greater than 0.35 PRU/ml using solid phase immunoassay (Phadezyme RAST®, Pharmacia, Milton Keynes, UK) to the same antigens, or (3) a raised total serum IgE (Phadezyme IgE PRIST®, Pharmacia) corrected for age (>100 kU/l for persons over 10 years of age11 and children under 10 years as recommended10). Parents responded to a questionnaire modified from the American Thoracic Society respiratory questionnaire regarding lifetime symptoms suggestive of asthma, rhinitis, and eczema pertaining to themselves and their children. Phenotype status was assigned without previous knowledge of genotypes by two investigators independently.

DNA METHODS
Genomic DNA was extracted from whole blood and digested with TaqI, separated by gel electrophoresis in 0.8% agarose and 1 × TAE buffer, and transferred to nylon filters (Hybond N+, Amersham) by Southern blotting. The pAMS.51 probe (D11S97) was labelled by random hexanucleotide labelling (Amersham, hybridised with the transferred DNA overnight, and washed as previously described.10

LINKAGE AND HETEROGENEITY ANALYSIS
Two point linkage analysis was computed by the LINKAGE group of programs (version 5.0) using a one liability class model for phentoyping, assuming a gene frequency for atopy of 0.2 and 95% penetrance.9 A gene frequency of 0.2 is assumed to be consistent with a dominant disease affecting at least 30% of the
population with high penetrance. A penetrance of 95% is based on both previous studies of atopic families and from maximum likelihood estimation from previous data. The presence of genetic heterogeneity was tested by determining $\alpha$, the proportion of families in which atopy is linked to 11q. This was done by maximising the lod score over different values of $\theta$ and $\alpha$, under the hypothesis that atopy and the MS.51 locus show no linkage in a proportion $(1-\alpha)$ of families. The approximate 95% confidence limits for $\alpha$ were defined by subtracting one from the maximum lod score.

Results

Phenotyping
All families in which complete data on both parents and at least two children were obtained were used for study (63 Caucasian and one West Indian). Children's ages ranged from 1 to 18 years (mean age 8:0 years) with 65 girls and 88 boys. In 43 families one parent was atopic, in 16 both parents were atopic, and in five neither parent was demonstrably atopic. Of the designated atopic subjects (56% of the parents and 75% of children), 91% of parents and 74% of children were positive for skin prick testing or RAST or both, 77% of parents and 63% of children were positive for two or more criteria, 80% of parents and 78% of children reported symptoms, and 1% of parents and 4% of children were positive on skin testing only. Of the 79% of atopic subjects with symptoms during their life, 57% had asthma, 56% had rhinitis, and 39% had eczema.

Two Point Linkage Analysis
The maximum lod score was $3.80$ at $\theta=0.067$ (under the assumption $\theta_{\text{male}}=0/\theta_{\text{female}}$) from 32 informative families giving 46 meioses. However, a test of male/female difference in $\theta$ is significant with estimated $\theta_{\text{male}}=0.182$ and $\theta_{\text{female}}=0.0001 (x^2=6.44, 1$ df, $p<0.02$); the test statistic for linkage under this hypothesis is $5.21$ on the lod scale. The contributions from the families in which one, both, or neither parents were atopic were $3.59$, $6.2$, and $1.00$ respectively. The results were not sensitive to changes in penetrance: the maximum lod statistic was $5.50$ if penetrance was assumed to be $99$% and $4.87$ if it was assumed to be $90$%.

Heterogeneity Analysis
Using the two-point data between atopy and MS.51 in the nuclear families, under the assumption of equal male and female recombination frequencies, the maximum likelihood estimate of the proportion of linked families was $\alpha=1.0$ (no heterogeneity). The approximate 95% confidence region based on a $x^2$ distribution with one degree of freedom extends to $\alpha=0.6$ (that is, in 60 to 100% of families atopic IgE responsiveness appears to be linked to the 11q locus).

Discussion

Our results support the hypothesis that atopic IgE responsiveness (IGER) is linked to chromosome 11q13 (combined lod score in nuclear and extended families is in excess of 8). The testing of further families in the presence of incorrect assignment of phenotype, significant genetic heterogeneity, or phenocopies would have reduced rather than added to the lod score. In the present study of young families, phenotyping of atopy was considerably easier because the effects of smoking and old age were reduced. Of the designated atopic subjects, 74% of children and 91% of parents were positive for skin prick testing or RAST or both. A high total IgE was the sole criterion in 9% of parents and 26% of children, a high IgE being highly predictive for the development of atopic disorders (asthma, rhinitis, and eczema) in children. All parents were under the age of 52 years (mean 37.0 years, range 24 to 52) and all of the 9% of parents with a raised total IgE as their only criterion were non-smokers. Positive skin testing was the sole criterion in only 4% of the children and in only 1% of the parents.

In both the nuclear and extended family studies we have consistently found 5 to 10% of atopic subjects have apparently non-atopic parents. This could be the result of phenocopies, incomplete penetrance, or genetic heterogeneity. In the five nuclear families from this study in which neither parent was affected (8% of nuclear families), the lod score contribution was 1-0 (of the total score of 5.2), which supports our model and suggests that a non-penetrant heterozygote parent may be present in each of these families. The estimated difference in the male/female recombination frequencies is the subject of further analysis.

Though the precise assignment of individual nuclear families with small sibships to an unlinked group has limitations, the heterogeneity analysis has allowed us to define the proportion of families linked to 11q within confidence limits. The 95% confidence interval for $\alpha$ was 60 to 100% in the nuclear families in this study. We believe the recruitment of more families is necessary to test further for heterogeneity of this immunological phenotype at this locus. However, confirmation of linkage in this study of a larger sample of the atopic population, together with our heterogeneity analysis, suggests the 11q13 region contains a major locus conferring atopic IgE responsiveness, the underlying immunological abnormality that characterises the common allergic disorders of atopy.

We are happy to provide interested investigators with the phenotype and genotype data from this study. We thank ICI Ltd for the pMS.51 probe, the British Council for their support of RPY, the Wellcome Trust for their support of WOCMC and JMH, and the National Asthma Campaign and the Wellcome Trust for their support of this study.


