Atopy and bronchial hyperresponsiveness: exclusion of linkage to markers on chromosomes 11q and 6p

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Summary

Previous studies have reported a familial predisposition for the development of atopy, bronchial hyperresponsiveness and clinical asthma, and therefore have suggested the presence of a heritable component to these disorders. The specific contributions of genetic and environmental factors in the pathogenesis of allergic disease and asthma have not been determined although Cookson et al. [1] have postulated linkage between atopy and chromosome 11q. We have studied 20 families (two and three generations) ascertained through a proband identified as having asthma (90% were also allergic) during the period of time between 1962 and 1970. Of those who were originally skin test positive, 82% remained positive. All probands whose pulmonary function allowed retesting (FEV₁ > 1.2 L) remained hyperresponsive to histamine. The children of these probands are now in the same age range as their parents when they were originally evaluated; 66% are atopic using criteria described by Cookson et al. (one or more positive skin tests ≥ 2 mm, an elevated total serum IgE or a positive specific IgE) and 22% demonstrate bronchial hyperresponsiveness (PC20 FEV₁) to histamine. Using the highly polymorphic marker INT2 (which maps 2 cM from pMS.51 on chromosome 11q) and atopy, we obtained a lod score of -2.00 at a recombination fraction of 0.12. In addition, because many studies have suggested an association between atopy and certain HLA antigens, we investigated the possibility of linkage between atopy and bronchial hyperresponsiveness and D6S105, a polymorphic marker on chromosome 6p, located 7 cM from HLA-DR. For this marker and atopy, we observed a lod score of -2.00 with a recombination fraction of 0.07. Similar results were observed with both of these markers and bronchial hyperresponsiveness. By restudying these probands as well as their family members, we were unable to find evidence for linkage between atopy or bronchial hyperresponsiveness and these regions of chromosomes 11 and 6 in this population.

Introduction

Numerous family and twin studies have suggested the presence of a heritable component to atopy, bronchial hyperresponsiveness and asthma [2,3]. Obviously, exposures to inciting stimuli such as allergens, environmental pollutants and respiratory infections interact with a genetic predisposition in the development of allergic diseases and asthma. The number of genes involved in these disorders and their mode of inheritance have not been determined. Recently, Cookson et al. [4] suggested that there is dominant inheritance of a general immune response 'atopy gene' in subjects with atopic diseases and
allergic asthma. For a common trait such as atopy, it is difficult to distinguish between dominant inheritance of a common phenotype or clustering of the phenotype in families due to the high frequency of that trait in the general population. Studies which have utilized methods of complex segregation analysis have not found evidence for a dominantly inherited major gene for components of allergy and asthma, such as total serum IgE levels [5-8] or bronchial hyperresponsiveness to methacholine [9]. However, using their hypothesis of a dominantly inherited allergy and asthma, such as total serum IgE levels [5-8] or bronchial hyperresponsiveness to methacholine [9]. How- ever, using their hypothesis of a dominantly inherited ‘atopy gene', Cookson et al. [1] studied seven extended families (ascertained through a proband with a history of allergic asthma or allergic rhinitis), and by linkage analysis obtained a maximum lod score of 5.58 at a recombination fraction of 0.10 to the marker pMS.51 (D11S97), a minisatellite marker on chromosome 11q. With the inclusion of additional families, the maximum lod score increased to 10 at a recombination fraction of 0.08 [10]. Additional evidence for linkage was obtained from a study of four Japanese families [11]. However, more recently, these findings were not confirmed by Lympney et al. [12] who studied linkage to the same polymorphic marker on chromosome 11q in nine families who were ascertained through a family member with allergy or asthma. They reported a lod score of −0.30 at a recombination fraction of 0.30 for atopy and pMS.51, and a maximum lod of 0.80 at a recombination fraction of 0.30 for bronchial hyperresponsiveness to methacholine and pMS.51.

Because of these conflicting reports regarding linkage of the allergic phenotype to the region D11S97 on 11q, we have performed linkage analysis for atopy and bronchial hyperresponsiveness in 20 families from Northern Holland using a different polymorphic marker in this region, a PCR based marker INT2, which maps 2 cM from pMS.51 and is highly polymorphic (heterozygosity >0.70) [13,14]. In addition, a number of studies have demonstrated associations between certain HLA haplotype and response to specific allergens [3]. In 1978, Mendell et al. [15] postulated a dominant pattern of inheritance for allergic responses to ragweed allergen and linkage to the HLA loci. Thus, we have studied this candidate region on chromosome 6 in these same families using the highly polymorphic PCR marker, D6S105 [16].

These studies have been performed in a unique group of families from Northern Holland. We chose to study probands whose asthma and allergic status were originally evaluated between 1962 and 1970. Two and three generation families have been ascertained through the original patient with asthma. The children are now in the same age range as their affected parents when they were initially evaluated. The study of these families permitted us to overcome a major problem associated with the design of most families studies of the genetics of allergy and asthma. Specifically, we were able to control for age related changes that influence the expression of these diseases. Linkage studies for this analysis of candidate genes for atopy and bronchial hyperresponsiveness on chromosomes 11q and 6p are based on the detailed evaluation of 20 of these families.

Methods

Patient recruitment

Beatrixoord is a large hospital centre at Haren near Groningen, a city in Northern Holland, which since the early 1960s has served as a regional asthma and airways disease referral centre. Between 1962 and 1970, 1284 patients with obstructive airways disease entered the Beatrixoord Hospital Centre for a complete evaluation while in a stable clinical state. This patient population has been highly cooperative and has participated in previous studies on the natural history and progression of obstructive airways disease [17,18]. To date, 20 complete families have been studied. These families were selected through a proband with asthma who met the following criteria at the time of the first study: less than 45 years of age, bronchial hyperresponsiveness to histamine (provocation concentration causing a 20% fall in FEV₁ [PC20 FEV₁] <32 mg/ml), reversibility of FEV₁ (>15% above baseline) and no history of cigarette smoking (≤5 pack-years), i.e. a proband with typical asthma. The majority of the probands were also allergic (90%) based on skin testing to a variety of allergens.

Probands and their immediate family members agreed to participate in these studies and provided appropriate informed consent. The protocol was approved by the Ethics Committee at The University Hospital, Groningen and reviewed by the Institutional Review Boards at the University of Maryland and the Johns Hopkins Schools of Medicine. All available family members including the proband were evaluated. The same testing as originally performed on the probands was performed currently except that different preparations of the same or similar antigens were used. In each family, both parents and all available children were studied. Grandchildren under the age of 8 were excluded from testing.

Clinical evaluation

All family members completed an extended Dutch version of the British Medical Society Respiratory Questionnaire [19] which included additional questions that are pertinent to the diagnosis and assessment of asthma and allergic disease. Modifications were made using the

Adult subjects underwent intradermal skin testing to the following allergens (children ages 8–10 received skin prick tests to only those allergens identified with an asterisk): mixed grass pollens*, mixed trees, wheat*, feathers*, dog*, cat*, horse*, hair*, house dust mite*, mixed epithelium, moulds*: alternaria, aspergillus, cladosporium, penicillium, botrytis, negative* and positive* (histamine) controls. Between 1962 and 1970 skin tests were performed with mixed grasses, house dust mite, mixed trees, wheat, hay, mixed epithelium, moulds and spring pollens. Measurements of the largest diameter and the diameter perpendicular to the largest were taken of each resulting weal. In addition, measurements of total serum IgE (IU/l) were determined by enzyme allergosor-
from HLA-DR [16] was typed in these families. Both markers have a heterozygosity > 0.70 [13,16]. All laboratory personnel involved in the marker analysis were completely blinded to the clinical phenotypes of the subjects.

**Linkage analysis**

Linkage analysis was performed using the method of maximum likelihood as implemented in LINKAGE [24] and heterogeneity testing was performed using HOMOG [25]. An autosomal dominant model for atopy was used with a penetrance of 0.99 for affecteds and 0.01 for phenocopies. Basically, this penetrance value makes it unlikely that a non-allergic family member actually carries the postulated 'atopy' gene; likewise, atopic family members have a high probability of carrying the 'atopy' gene (i.e. are not phenocopies). The allergic phenotype was defined in a similar manner to Cookson et al. [1,4]. The presence of an allergic phenotype was based on the finding of one or more positive skin tests (≥ 2 mm), a positive RAST (≥ 35 RU/ml) or elevated total serum IgE levels [26].

Both an autosomal dominant and an autosomal recessive model were used for analysis of BHR. Family members with a PC_{20} FEV_{1} and ≤ 10 pack years of smoking were considered affected and those with a PC_{10} FEV_{1} with ≤ 10 pack years of smoking were considered ‘possible’. Any family member with a significant smoking history (> 10 pack years) was classified as phenotype unknown for the linkage analysis. BHR to histamine can be considered a primary marker of the asthma phenotype since virtually all subjects with asthma display BHR [27,28]. For the probands, the original data were used. One model was used with a penetrance of 0.99 for BHR, 0.80 for ‘possibles’. For all classes, the penetrance for phenocopies was set at 0.01. In addition, a recessive model for BHR was used with similar parameters since, as shown by Clerget-Darpoux et al. [29], model misspecification (dominant vs recessive) is a major problem in linkage analysis of complex diseases.

For both atopy and BHR, a test for genetic heterogeneity was performed on the resulting lod scores. Basically, this is a measure of the presence of genetic heterogeneity, i.e. are a portion of the families linked to the marker and the remaining families unlinked to this marker? A likelihood ratio for heterogeneity of 1 is seen when none of the families show evidence for linkage which represents a conservative approach to reporting exclusion mapping.

**Results**

In four probands, the FEV_{1} was too low to permit histamine challenge to be performed safely (< 1.21). The other 16 probands remained hyperresponsive to inhaled histamine. Of the 17 probands who were originally skin-test positive (two were skin-test negative and one had incomplete data on original testing), 14 are still skin-test positive. Allergy and asthma evaluations have been performed on 77 children and on all spouses. The children range in age from 8 to 44 years and 66% are atopic according to the criteria for atopy described by Cookson et al. [1]. Seventeen children (22%) are hyperresponsive to inhaled histamine, achieving a PC_{20} FEV_{1} during inhalation challenge to histamine. An additional 14 children (18%) develop a 10% fall in FEV_{1} (PC_{10} FEV_{1}).

A conservative approach to linkage analysis in complex traits is to determine whether affected members of a sib pair inherited the same allele from the affected parent. There were 54 possible combinations of sib pairs; 28 inherited the same INT2 allele from their affected parent and 26 did not. These results obviously do not differ from the 50% segregation ratio expected under the null hypothesis (absence of linkage).

The results of the maximum likelihood analysis give similar results (Table 1). Linkage was excluded to 12% recombination (based on a lod < -2.00). No evidence for genetic heterogeneity was observed; the likelihood ratio for heterogeneity was 1.0. An example of a pedigree from one of the families with the genotype results for INT2 is shown in Fig. 1. The father, an allergic asthmatic is the proband; his spouse is skin test negative and non-reactive to histamine. The two daughters with increased bronchial responsiveness inherited different alleles from their father (one inherited the ‘A’ allele, the other the ‘F’ allele). The skin test negative daughter and the skin test positive son inherited the same allele (‘A’) from their father. Clearly, no co-segregation of allergy or bronchial hyperresponsiveness with the marker allele is observed.

In all the families studied, there was only one family member who would have been reclassified if skin-test response is defined as ≥3 mm instead of ≥2 mm. Nine

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<th>Recombination fraction</th>
<th>( \theta ) when lod &lt; -2.00</th>
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<td>0.01</td>
<td>0.05</td>
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<tr>
<td>0.10</td>
<td>0.20</td>
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<td>0.30</td>
<td>0.40</td>
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<tr>
<td>Chromosome 11: INT2</td>
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<tr>
<td>-7.38</td>
<td>-4.28</td>
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<tr>
<td>-2.54</td>
<td>-0.93</td>
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<tr>
<td>-0.27</td>
<td>-0.04</td>
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<tr>
<td>0.12</td>
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<tr>
<td>Chromosome 6: D6S105</td>
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<tr>
<td>-6.45</td>
<td>-2.96</td>
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<td>-1.28</td>
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<td>0.18</td>
<td>0.11</td>
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family members were considered atopic because of an elevated total IgE level only; they had negative skin tests and negative RASTs. However, repeating the linkage analyses by reclassifying these individuals as non-atopic had little effect on the resulting lod scores.

Similar results were observed with the HLA linked marker on chromosome 6; the lod was $<-2.00$ at a recombination fraction of 0.07. For BHR, no evidence for linkage to chromosome 11 or chromosome 6 markers was found (Table 2) and no evidence for genetic heterogeneity was found (likelihood ratio for heterogeneity = 1). In addition, an autosomal recessive model was used for the inheritance of BHR with similar results.

### Discussion

Previous family studies have presented strong evidence for the role of genetic factors in the development of allergy, bronchial hyperresponsiveness (BHR), and clinical asthma although the number of major genes and the mode of inheritance remains to be clarified [2,3]. To determine the mode of inheritance of a disease or trait, it is necessary to perform family studies in a well-defined manner. For example, in this study, families are being ascertained through a single proband, one of the parents, and the spouse and all available children and grandchildren are studied. It is then possible to perform segregation analysis and test the fit of major gene models and a polygenic model; for example, is there evidence for a dominant gene that accounts for a significant component of the familial aggregation of allergy? Before performing such analyses, it is necessary to define the phenotype to be studied and determine important covariates such as age, sex and exposure.

There have been only a few studies where modern statistical genetic analysis techniques were applied. These included several studies of specific components of allergic disease such as total serum IgE levels, but no clear evidence for the presence of a major gene inherited either in a dominant or recessive manner has been found [5–8]. With regard to the asthma phenotype, Townley et al. [9] analysed their family data using complex segregation

<table>
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<th>Table 2. Results of linkage analysis for BHR</th>
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<td>Recombination fraction</td>
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<td>Model</td>
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<tr>
<td>Model 1: Dominant.</td>
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<td>Model 2: Recessive.</td>
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<td>Model used included weights for the possible classes of 0.80.</td>
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analysis and found evidence for a familial component for methacholine responsiveness that was probably not due to a major gene. Family studies have been difficult to perform because there are age and sex differences in the expression of allergy and asthma, and environmental exposure is difficult to quantify.

Cookson et al. [1,4] studied the genetics of clinical allergy, broadly defined to include any of the following: one or more positive skin-tests, one or more positive RAST tests and/or increased total serum IgE levels. Evidence for a dominant mode of inheritance was obtained although the findings are difficult to interpret because complex segregation analysis was not performed, and because of the high prevalence of allergy when it is defined in this manner. However, they did find evidence for linkage to a marker on chromosome 11q[1] suggesting the presence of at least one major gene for atopy. Because of the problems inherent in genetic studies of complex disorders, it is of major importance that a postulated linkage is replicated by additional studies. A study of Japanese families found evidence supporting this linkage in four families [11]. However, it is not clear why 134 families were excluded before the linkage analysis was performed and what effect this may have on the results reported in this abstract.

A recent study by Lymphe et al. [12] was unable to replicate the linkage to chromosome 11q. In our study, no evidence for linkage for atopy or bronchial hyperresponsiveness (a marker of the asthma phenotype) was found. In fact, linkage for atopy was excluded to at least 12 cM to INT2 (lod = -2.00) with no evidence for genetic heterogeneity (likelihood ratio for heterogeneity = 1). It is important to test for heterogeneity in any linkage study of a complex disorder since it is likely that there may be a subset of families showing linkage to a given marker and a subset of families not linked to that marker. Since the final lod score is calculated by summing the individual lods, it will probably be negative (or at least not significantly positive) due to the impact of the unlinked families. Therefore, the likelihood of heterogeneity versus homogeneity or no linkage is calculated, and if the resulting ratio is small as it is in our families, an exclusion interval may be reported.

The failure to replicate a postulated linkage has become a serious problem in studies on the genetics of complex disorders [30]. There are several possible reasons for this problem. First, the disease phenotype may be defined differently in different studies. In the current study, the allergic phenotype was defined in a similar manner to that described by Cookson et al., although there are still minor differences. While we performed a more extensive panel of skin tests, we only employed RAST testing to house dust mite and mixed grasses. However, as discussed by Cookson [1,4], the phenotype of only a few family members was dependent on the RAST measurement, i.e. those who are skin-test negative, have a low total IgE but a positive RAST. Although model misspecification is a problem in linkage studies of complex disorders, it should not be a reason for failure to replicate a linkage result. Also, if an incorrect model of inheritance was used, when affected sibs are analysed, they should share alleles in common which was not seen for atopic disease in our families.

Second, there may be a high degree of genetic heterogeneity present, i.e. genes which map in different regions of the genome may each separately be sufficient for disease expression. It is possible that either by chance or because of the type of families studied that different major genes are actually being mapped in the various studies. This does not seem to be a likely explanation for allergic disease. Although our families were ascertained through a parent with asthma, the type of allergy seen in these families reflects the allergic phenotype as found in the general population. As expected, the majority of allergic family members responded to house dust mite, and there were many allergic family members who did not demonstrate bronchial hyperresponsiveness to histamine.

A third reason may be that several genes that interact in disease expression are present (oligogenic inheritance). As shown by Suarez et al. [31], in these situations, it is often very difficult to replicate a 'true' linkage. In different sets of families, different proportions of the genes involved may be present and the 'linked' gene may not be detected in all studies. This differs from genetic heterogeneity as previously described and tested for in analysis of our families. In this case, there are several genes which interact to produce the phenotype, not that there are several major genes, any of which is sufficient to produce the phenotype (as in genetic heterogeneity). Obviously, for most complex disorders such as allergy and asthma, there may well be several major genes and a number of 'minor' genes which do interact in phenotype expression.

A fourth reason, as discussed by Risch [30] is the difficulty of interpreting a lod score calculated under an unlikely model. It is not clear from previous studies that an autosomal dominant model of inheritance as postulated by Cookson et al. [1,4] is appropriate for atopy. If it is not, the statistical interpretation of the resulting lod score is problematic.

One approach to the study of the genetics of complex disorders is to first perform segregation analyses, which are possible if the families are ascertained in a clearly defined manner and not for the presence of multiple allergic members. This approach will be used on data from the families that are currently being studied. Data from approximately 100 Dutch families ascertained.
through a parent with all family members carefully characterized with respect to allergy and asthma will be available. Then linkage analysis of markers throughout the genome can be performed using the genetic model obtained from the segregation analysis. Another approach is to perform linkage studies of candidate regions using either a non-parametric approach or a likelihood approach with a few well-defined models. This approach was used in these families for the candidate region of the HLA loci on chromosome 6. No evidence for linkage was found with either the allergic or the bronchial hyperresponsive/asthma phenotype. Although there are strong associations between the components of allergic disease and HLA haplotypes, linkage to a major gene for general atopy was not found. While this approach did not find evidence for linkage to markers on chromosomes 11q and 6p, it may be useful to study other candidate regions associated with findings related to atopic or asthmatic responses, e.g. cytokines, beta receptor function, etc.

Defining the role of genetic factors in the development of allergic disease and asthma will improve our understanding of the underlying mechanisms responsible for these disorders. We have reported our findings for polymorphic markers closely related to two candidate regions of the genome that have been reported to be associated with atopy and bronchial hyperresponsiveness. In this study we have attempted to control for age related changes in the phenotypic expression of allergy and bronchial responsiveness by restudying probands who were initially evaluated 20 to 30 years ago. Three of the 20 probands would have been incorrectly classified as non-allergic while four could not have had bronchial responsiveness categorized, if earlier data had not been available. Unfortunately, we were unable to find evidence for linkage for either atopy or bronchial hyperresponsiveness to these regions on chromosomes 11q and 6. Thus, further investigation is necessary to determine the relationship of atopy and bronchial hyperresponsiveness to chromosome 11q and the HLA locus on chromosome 6.

It would be preferable if these investigations included segregation analyses of families ascertained in a well-defined manner. Additional studies to define the allergic and asthmatic phenotype are necessary and should include studies of the effects of age, sex and allergen exposure on disease expression. Linkage studies should be undertaken using a few well-defined models for inheritance of the phenotype and, also, can be performed by analysing the proportions of alleles shared in common by affected members of the pedigrees. In addition, likelihood methods based on a dominant and a recessive model may be used especially when investigating candidate genes. Genome searches should use highly polymorphic PCR based markers. Marker technology and data management and analysis needs to be as automated as possible to avoid errors. Laboratory personnel should be ‘blinded’ to the phenotype of family members. All linkage analyses should include tests for heterogeneity since it is highly likely that multiple genes are involved in complex disorders such as asthma and allergy. The whole genome should be searched using highly polymorphic PCR based markers in a systematic manner to detect multiple genes. It is very appropriate to begin such studies of allergy and asthma given the availability of highly informative markers and well characterized families such as the ones that we are currently studying.

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