Genetic analysis using DNA polymorphism of the linkage between chromosome 11q13 and atopy and bronchial hyperresponsiveness to methacholine

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Previous studies have suggested that there is a genetic predisposition for the development of asthma and atopy. A recent study has also demonstrated that there is a striking link between chromosome 11q and the IgE response underlying asthma and rhinitis. To assess the linkage between chromosome 11q (region D11S97) and atopy or bronchial hyperresponsiveness (BH), we have studied nine families of two and, in many instances, three generations with the index case having asthma and/or atopy. With variable number of tandem repeat analysis with the probe, pλ-MS.51, we have been unable to confirm a significant link between region D11S97 of chromosome 11q and either atopy or BH to methacholine. We have demonstrated that atopy and BH produce similar log of odds scores with linkage analysis at each recombination fraction from 0.001 to 0.5 with both HinfI and TaqI restriction digests and that the use of either a positive skin prick test or positive RAST as a definition of atopy does not significantly alter the log of odds score. (J ALLERGY CLIN IMMUNOL 1992;89:619-28.)

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Asthma and hay fever are reported to occur in successive generations,[1, 2] suggesting that genetic factors may determine the development of atopic disorders. Van Arsdel and Motulsky[3] have demonstrated that the likelihood of an offspring of allergic parents developing allergy is dependent on parental phenotype with increasing probability of the offspring developing atopy or asthma if either or both parents are atopic. König and Godfrey[4] demonstrated that bronchial lability and atopy are more frequent in the families of infants with wheezy bronchitis than in families of normal children, supporting the hypothesis that there is a genetic basis for the tendency toward wheezing in infancy and asthma in childhood. Longo et al.[5] reported a bimodal distribution of bronchial hyperreactivity, suggesting that there may be two phenotypes for BH, thereby supporting the theory that this is controlled genetically to some extent.

Abbreviations used

VNTR:	Variable number of tandem repeats
SPT:	Skin prick test
BH:	Bronchial hyperresponsiveness
PBS:	Phosphate-buffered saline
SDS:	Sodium dodecyl sulfate
SSC:	Saline sodium citrate
LOD:	Log of odds

There is evidence for at least two types of genetic control of IgE responsiveness in man. In a genetic study of the IgE levels in nuclear families, Gerrard et al.[6] suggested the presence of a dominant major regulatory locus suppressing the production of persistent, high IgE levels. A study of the genetic control of total serum IgE has demonstrated a recessive mendelian model of inheritance of high total IgE levels.[7] Segregation analysis of the genetic basis of total IgE production[8] has suggested that 36% of total phenotypic variation in total IgE concentration is attributable to

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genetic factors and that this is comprised equally of a recessive Mendelian and a more general polygenic component. It has also previously been demonstrated that there is an HLA association between IgE responsiveness and specific allergens.[9] Additional investigations have demonstrated that there is a 90% association between IgE production to *Amb a* V and HLA-DR2, Dw2.[10] It has been suggested that this may involve a gene separate from HLA-D and near to the GLO locus.[11] A recent study[12] has demonstrated that immune responsiveness to *Lol p* III is associated with HLA-DR3 and that the presence of a specific amino acid sequence is important in the presentation of an epitope of the antigen. However, in a study of the familial occurrence of atopy, it was suggested that atopy is inherited as an autosomal dominant trait and that the clinical expression of the disease depends on other factors, such as environmental exposure to an allergen or developmental factors.[13] Cookson et al.[14] further demonstrated a linkage between IgE responses underlying asthma and rhinitis and chromosome 11q by studying seven families in which the index cases had histories of asthma or allergic rhinitis. The genomic DNA was analyzed with the single locus VNTR probe pλ-MS.51, and the linkage was analyzed with the linkage group of packages. The LOD scores for each family with respect to the marker locus and atopy were demonstrated to vary from -0.058 to 3.143 at a recombination fraction of 0.1, producing a maximum total LOD score of 5.58, assuming an autosomal dominant mode of inheritance.

The absence of a strict association between atopy and asthma[15] has been suggested to indicate that asthma and atopy are inherited separately. This suggestion prompted us to evaluate the linkage of chromosome 11 (region 11q13) with a VNTR probe pλ-MS.51 with BH and with the capacity of individuals to mount an IgE response.

VNTR probes are useful genetic markers because they are inherited in a stable manner and demonstrate polymorphism, and considerable experience has been gained from their use as DNA fingerprinting probes in pedigree and forensic analysis, as well as for diagnostic purposes. The VNTR probe, pλ-MS.51 (a single locus DNA fingerprint probe), is available for the region D11S97 on chromosome 11q (11q13).[16, 17] This probe is highly informative because most individuals are heterozygous at this locus (heterozygosity, 77%), and the alleles demonstrate codominant segregation.

MATERIAL AND METHODS

Subjects

Nine families with a history of asthma and/or atopy were studied. The index cases were selected from patients attending the allergy or chest clinics at Guy's Hospital on the basis of the size of the family and the number of generations willing to take part in the study. All subjects gave informed consent, both verbally and in writing, before taking part in the study. All individuals studied were white subjects of the United Kingdom. Forty-one male and 48 female subjects were studied. The ages of the subjects ranged from 2 to 84 years. All subjects were studied between January and April of the same year, and all members of each family were studied within 3 weeks of each other. BH in subjects older than the age of 2 years and younger than 8 years of age was investigated with an exercise test, and in subjects older than 8 years of age, BH was investigated with a methacholine inhalation test, as described below. Subjects were initially defined as atopic if they were positive to one or more of the following criteria: (1) a positive SPT, (2) a positive RAST score, and (3) a raised total IgE.

A questionnaire was administered to all subjects requesting information on smoking history, medication currently being taken, and history of asthmatic or allergic episodes.

Methacholine inhalation test

All subjects of 7 years of age or older were subjected to a methacholine (acetyl-β-methylcholine chloride, Sigma Ltd., Poole, Dorset, England) inhalation test administered by hand-held nebulizers according to the method previously described.[18] Subjects with BH were defined as subjects who had a fall of $\geq 20\%$ in their FEV_1 when a maximum cumulative dose of 8 μmol of methacholine was inhaled from a nebulizer, as previously described.[18] Subjects older than 2 years of age and younger than 7 years were required to undergo a 6-minute exercise test. The subjects ran on a treadmill at 4 miles per hour and at an angle of elevation of 25 degrees to raise the heart rate to 170 beats/min for 6 minutes. Peak flow rates were measured before exercise, then immediately after exercise, and then at 2.5-minute intervals up to 15 minutes. Subjects were defined as hyperresponsive if the peak flow fell by $\geq 12\%$ from the baseline value.

SPT

All subjects older than 2 years were assessed for SPT reactivity to 10 common allergens, as defined by induction of a wheal diameter of at least 2 mm larger than that elicited by a diluent control. The allergens used were mixed grass pollen (1/20 wt/vol), plane-tree pollen (1/20 wt/vol), silver-birch pollen (1/20 wt/vol), horse dander (1/10 wt/vol), house dust mite (30,000 Au/ml), cat dander (1/10 wt/vol), dog dander (1/10 wt/vol), *Alternaria* sp (1/10 wt/vol), *Aspergillus* sp, and milk (1/20 wt/vol) (Dome Hollister-Stier, Elkhart, Ind.). The diluent for all the allergens tested was used as a negative control and contained 50% vol/vol of glycerin in water (containing 0.5% phenol). The positive control was histamine (1 mg base in 1 ml of diluent). The skin prick sites were marked onto the inner forearm, and a small drop of each allergen or control was placed on the skin. A sterile lance was introduced through the drop to a depth of about 1 mm into the skin and pulled out, raising the skin slightly. The site was blotted, and the wheal diameter was measured in the two largest perpendicular di-

rections after 15 minutes. The mean diameter was calculated. A positive SPT was defined as a mean wheal diameter 2 mm or larger than that of the diluent control.

Total and specific IgE

Sera from all subjects were assayed for specific IgE to timothy-grass pollen, silver-birch pollen, plane-tree pollen, cat dander, dog dander, *Alternaria* sp, *Aspergillus* sp, horse dander, house dust mite, and milk allergens with RAST as previously described.[19, 20] Single estimations of each specific IgE level were made. RAST scores were measured with specific Phadebas solutions (Pharmacia, Ltd., Milton Keynes, England) corresponding to 17.5, 3.5, 0.7, and 0.35 Pharmacia RAST PRU/ml. The minimum level of detection of the assay was 0.35 PRU/ml. A score of 1+ or higher with the Pharmacia reference standards was accepted as being elevated. The standard solutions were appropriate for the measurement of specific IgE in subjects aged 1 year and older. The interassay coefficient of variation was 10.5% for RAST classes 3 and 4 and 21.3% for RAST classes 1 and 2. Total IgE antibodies were measured with an ELISA technique. Briefly, a purified antihuman IgE monoclonal antibody (712) (A. Sax, University of California-Los Angeles), diluted in coating buffer (0.1 mol/L of $NaHCO_3$ in PBS/azide), was added to each well of a microtiter plate (Nunclon, Gibco/Biocult, Hounslow, Middlesex, England) and incubated overnight at 4° C. The plate was then washed three times with PBS containing 0.5% polysorbate 20 (Tween 20) (Sigma, Ltd.) and dried. The control human serum, standard serum (from a pool of human serum with a known total IgE of 760 IU/ml), and the test sera were diluted in PBS buffer containing 0.5% horse serum (Sera Laboratories, Crawley, Sussex, England), and 0.5% Tween 20 (ELISA buffer) was added to each well. The plate was incubated for 3 hours at 4° C before being washed and dried three times as before. A rabbit antihuman IgE monoclonal antibody (prepared in our laboratory) was diluted in ELISA buffer and added to each well and incubated for 2 hours at 4° C. The plate was then washed as described previously. Then, a 1 mg/ml solution of *p*-nitrophenyl phosphate (Sigma, Ltd.) was added to each well. The plate was incubated at 37° C for 45 minutes. The absorbance of the individual wells was measured at 405 nm with a Titertek microtiter plate reader (Flow Laboratories, Rickmansworth, Hertfordshire, England). Each sample was assayed in duplicate. The concentration of the test sera was calculated from the standard curve, and the geometric mean of results for each sample was taken. The minimum level of detection was 4 IU/ml. Samples that were found to be on a nonlinear portion of the standard curve were diluted in ELISA buffer and reassayed. The coefficient of variation between assays was 14%. To account for total IgE variation with age, a raised total IgE was associated as 2 SD above the predicted mean for the age of the subject.

DNA extraction

Twenty-five milliliters of peripheral venous blood was withdrawn into 5% ethylenediaminetetraacetic acid (BDH chemicals, Ltd., Poole, Dorset, England) in PBS and stored at −20° C. Genomic DNA was extracted with a modified proteinase K, high-salt method.[21] Briefly, whole blood was thawed and then washed with 10 mmol/L of Tris (Sigma, Ltd.), and 1 mmol/L of ethylenediaminetetraacetic acid (BDH Chemicals) solution (TE). The mixture was centrifuged, and the cell pellet was resuspended in swelling buffer consisting of 10 mmol/L of Tris, 20 mmol/L of NaCl (BDH Chemicals), 5 mmol/L of $MgCl_2$ (BDH Chemicals), and 10% nonidet P-40 (NP40) (Sigma, Ltd.), left on ice for 15 minutes and then centrifuged as before. The pellet was resuspended in red cell-lysing buffer for 20 minutes before centrifugation as described above. The pellet was then resuspended in a white cell-lysing buffer, 0.07% SDS (BDH Chemicals) and 500 μg of proteinase K (Gibco/BRL, Uxbridge, Middlesex, England) at 37° C overnight; 5 mol/L of NaCl was then added to the solution before centrifugation. The supernatant was removed, and the DNA was precipitated with absolute ethanol. The DNA was washed with 70% ethanol before being resuspended in TE. The concentration of DNA was determined by measuring the ultraviolet absorption at 260 nm. An optical density reading of 1 in a cell of path length 1 cm corresponded to approximately 50 μg/ml of double-stranded DNA.

VNTR/restriction fragment-length polymorphism analysis

Eight to ten micrograms of prepared DNA was digested with TaqI or HinfI restriction endonuclease (Anglian Biotec, Ltd., Colchester, Essex, England). The resultant fragments were separated by size on 0.6% agarose gel (Gibco/BRL). DNA from the gel was denatured and transferred onto Hybond-N filters (Amersham, Buckinghamshire, England) according to the method described by Southern.[22] The filters were washed in SSC solution and cross-linked with an automatic cross linker (Hybaid, Teddington, Middlesex, England). The filters were prehybridized at 65° C for 2 hours in prehybridization buffer containing 1 mg of single-stranded sonicated salmon sperm DNA (Sigma, Ltd.).

The VNTR probes used were pλ-MS.51 (Cellmark Diagnostics, Abingdon, Oxfordshire, England) that is specific for chromosome 11q (region D11S97) and YNH24 (plasmid for which was donated by Nakamura et al.[23]) specific for chromosome 2p (region D2S44). Both probes demonstrate codominant segregation of restriction fragment-length polymorphism, that is, each individual demonstrates one allele from each patient. pλ-MS.51 hybridizes to nine alleles with an allelic range from 2 to 5 kb and has 77% heterozygosity,[16] and pYNH24 hybridizes to >30 alleles with an allelic range from 1 to 5 kb and with 97% heterozygosity.[23]

Each probe was labeled with ^{32}P-deoxycytidine triphosphate (Amersham) triethylammonium salt according to the method previously described.[24] A volume of labeled probe sufficient to produce 1×10^7 cpm was added to the prehybridized filters and buffer. The filters were hybridized to the ^{32}P-oligolabeled probe for 16 hours. The filters were then washed twice at 65° C in a solution of 0.3 mol/L NaCl and 0.04 mol/L of sodium citrate containing 0.2% SDS for 5 and 15 minutes, respectively, and then twice in a solution 0.03 mol/L NaCl and 0.004 mol/L of sodium citrate containing 0.2% SDS for 15 minutes. The filters were then

wrapped in Saran wrap (Dow Corning Genetic Research Instrumentation, Ltd., Dunmow, Essex, England) and exposed to Kodak XAR-5 film (Sigma, Ltd.) with intensifying screens at −70° C for 12 hours. All autoradiographs were analyzed according to band patterns and were confirmed after reanalysis by an independent investigator within our laboratories.

Definition of atopy and statistical analysis

The LOD scores were assessed with Liped 6 1987 (J. Ott, Columbia University, New York) within the Linksys shell package (v4.11, J. Attwood, Galton Laboratory, University College London, England). Individuals were assessed as having an atopic or nonatopic phenotype and, similarly, a phenotype for the presence or absence of BH. In an initial investigation with only seven families, the maximum frequency of atopy in the population over all age ranges was estimated to be between 10% and 35%, and the LOD scores were calculated with a range of frequencies of atopy in this range. The frequency producing the maximum LOD score was used in all subsequent analyses. The frequency of BH in the popultion was estimated at 7%.

The effect of variation in the initial definition of atopy (i.e., positive to one or more of the following criteria: (1) a positive skin prick test, (2) a positive RAST score, and (3) a raised total IgE on the LOD scores was also investigated. Atopy was defined in seven additional ways to include (1) positive SPT only, (2) raised specific IgE only, (3) raised total IgE only, (4) positive SPT and raised specific IgE only, (5) raised serum-specific IgE measured by RAST and raised total IgE only, (6) our original definition of atopy and/or previous history of asthma or rhinitis, and (7) our original definition of atopy and/or BH.

The mode of inheritance of atopy defined as a positive SPT and/or a raised specific IgE and/or a raised total IgE and of BH in the nine pedigrees studied was investigated with the statistical analysis for genetic epidemiology (S.A.G.E) (R.E.G.D version 3.0, Sorant et al., Louisiana State University Medical center, New Orleans, La.) package.

RESULTS

Atopic status

The family trees of all the pedigrees studied are illustrated in Fig. 1, *A* and *B*. Each individual was assessed for BH, SPT positivity, and serum-specific and total IgE concentration. The pedigrees studied had a maximum number of three generations, and the maximum number of siblings studied in any pedigree was seven.

The absolute numbers of atopic subjects and individuals with bronchial hyperreactivity within each pedigree and the number of current and exsmokers are presented in Table I.

VNTR analysis

Analysis of the alleles found by both Hinf1 and Taq1 digestion of genomic DNA and hybridization with the probe pλ-MS-51 demonstrated alleles ranging from size 1.8 to 5 kb for Hinf1 and 1.8 to 5.2 kb for Taq1 restriction digests. A representative example of each hybridization is illustrated in Fig. 2, *A* and *B*. The alleles obtained with the probe YNH24 varied in size from 1 to 5 kb. A representative example of VNTR analysis is illustrated in Fig. 2, *C*. Analysis of the alleles that occurred in each pedigree demonstrated that each allele could be traced from parents to offspring, indicating that the pedigree structure information that had been given by the family members was correct (data not presented).

The maximum LOD score obtained, when the linkage between D11S97 and atopy was calculated, occurred at an estimated population frequency of 35% for atopy with both Hinf1 and Taq1 restriction enzymes (Fig. 3, *A* and *B*). It was found that the maximum LOD score decreased as the estimated frequency of atopy was decreased from 35% to 10%. The maximum LOD score obtained was at 35% estimated frequency of atopy, and therefore the frequency of atopy in the general population used in all subsequent analyses was 35%.

The relative contribution of each family to the LOD score for atopy is presented in Table II. When the LOD scores for all pedigrees were calculated, the maximum LOD score for atopy compared to D11S97 was −0.3 at a recombination fraction of 0.3 with Hinf1 and −0.04 at a recombination fraction of 0.3 with Taq1 restriction enzyme (Fig. 3, *C*). Six of the nine families studied demonstrated a negative LOD score at the overall maximum recombination fraction with Hinf1 restriction digests and four of the nine families with Taq1 (Table II).

The relative contribution of each family to the LOD score for BH is presented in Table III. The maximum LOD score for BH was 0.80 for Hinf1 restriction digestion at a recombination fraction of 0.2, and for Taq1, the maximum LOD score was 0.1 at a recombination fraction of 0.3 (Fig. 3, *C*). Four of the nine families studied demonstrated a negative LOD score for BH at the overall maximum recombination fraction (Table III).

When a positive SPT only was used as the definition of atopy, the maximum LOD score changed to 0.17 or −0.07 at a recombination fraction of 0.3 with Hinf1 and Taq1 restriction enzymes, respectively (Table IV); when the definition of atopy included only a positive RAST score, the LOD score became −0.01 and 0.00 at a recombination fraction of 0.4 for Hinf1 and Taq1 restriction enzymes, respectively (Table IV). The effect of the use of raised total IgE only as a definition of atopy was to increase the LOD score to 0.21 or 0.14 for Hinf1 and Taq1 restriction enzymes, respectively (Table IV), at a recombination fraction

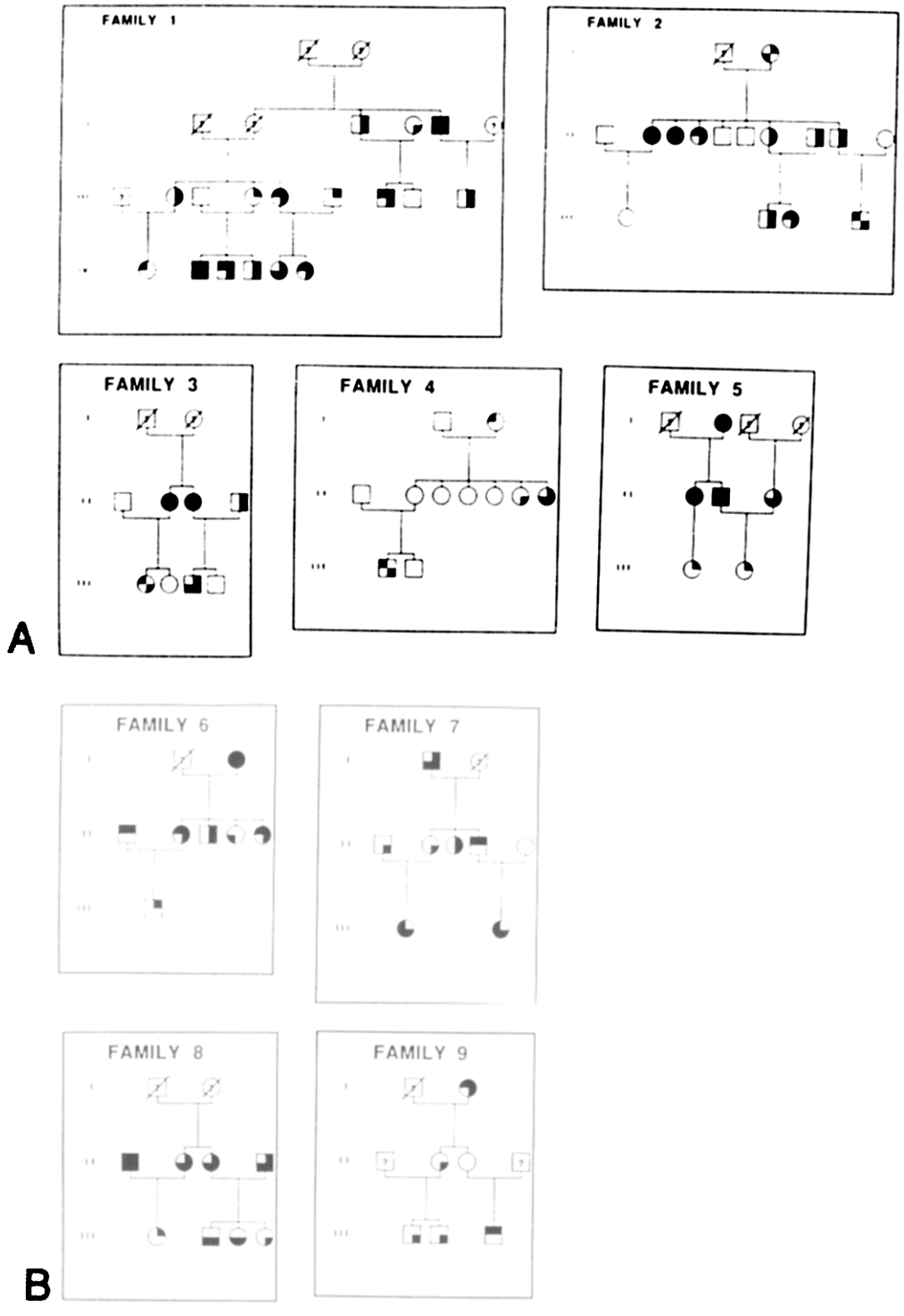

FIG. 1. A and **B,** Pedigree data. All subjects were assessed for BH (◔), positive SPT (◔), serum concentration of specific IgE (◔), and total IgE (◔); male (□), female (○), unknown status (◇), deceased (∅).

of 0.3. Variation in the definition of atopy to include a positive SPT and a positive RAST produced a LOD score −0.30 or −0.13 for HinfI and TaqI restriction enzymes, respectively (Table IV), at a recombination fraction of 0.3. The effect on the LOD score of variation in the definition of atopy to include only a positive RAST and a raised total IgE was to change the maximum LOD score to −0.11 or −0.03 at a recombination fraction of 0.3 with HinfI and TaqI restriction enzymes, respectively (Fig. 3, *D*). When a pre-

TABLE I. Assessment for atopic status and BH

Pedigree	No. of individuals	Smoking history		BH	Atopy
		Past	Current		
1	23	1	1	7	13
2	16	0	1	5	10
3	10	2	0	3	5
4	11	2	3	2	3
5	7	2	0	3	5
6	8	0	0	3	6
7	9	2	0	2	7
8	10	2	0	1	8
9	9	0	0	2	5

Atopy was defined as a positive SPT and/or a raised specific IgE and/or a raised total IgE. All subjects were asked details of their smoking history.

vious history of asthma or rhinitis was included in the original definition of atopy, no change resulted in the total LOD score obtained for atopy (Table IV). When the definition of atopy included BH, the maximum LOD score was found to be −0.23 at a recombination fraction of 0.3 for Hinf1 restriction enzyme-digested DNA and 0.19 at a recombination fraction of 0.3 for Taq1 restriction enzyme-digested DNA (Table IV).

With the S.A.G.E. program, it was found that atopy defined as a positive SPT and/or a raised specific IgE and/or a raised total IgE was not inherited in a simple mendelian fashion, either as an autosomal dominant or and autosomal recessive trait. (Maximum −2 log [likelihood] was −35.4.)

DISCUSSION

DNA polymorphisms are valuable tools in human genetic analysis and are used for the indirect localization of genetic disease by linkage analysis. With a similar study size to that previously published, we have been unable to confirm a strong positive LOD score between either atopy and D11S97 or between BH and the same chromosomal region. Our maximum LOD score for atopy and D11S97 was −0.29 or −0.04 with Hinf1 and Taq1 restriction digests, respectively, and occurred at a recombination fraction of 0.3. Similarly, the maximum LOD score for BH was 0.8 and occurred at a recombination fraction of 0.3 with Hinf1 restriction digests and 0.1 for Taq1 restriction digests at a recombination fraction of 0.3. In the previous study of genetic linkage and atopy,[13] which suggested a strong association with chromosome 11 (region 11q13), one family made a striking contribution to the total LOD score. Five of the remaining families produced small positive LOD scores, and one family produced only a small negative LOD score. Our criteria for a positive diagnosis of atopy do not differ from the latter study, but we have been unable to demonstrate a significant positive LOD score at the maximum recombination fraction, and six of the families studied demonstrated a negative LOD score at the maximum recombination fraction.

Hinf1-restriction enzyme digestion was used in this study because the allelic range and heterozygosity are known for this enzyme with the probe pλ-MS.51, and Taq1 restriction was used so that data could be directly compared to previously published results. We were unable to reproduce the 10.8 kb allele associated with atopy in the previous study[13] and suggest that this band size reported previously may be the result of either a point mutation or a chromosomal deletion, resulting in the site for the restriction enzyme being removed.

A potential problem in a study of this type is the ascertainment of the correct familial relationships in each pedigree. YNH24 is a highly polymorphic chromosome 2-specific VNTR probe (approximately 30 alleles) that is useful in establishing pedigree information. These data from analysis of band patterns obtained from each pedigree with this probe demonstrated that the familial relationships within each pedigree in this study were compatible with relationships stated when the families were recruited.

The use of specific IgE and/or total IgE instead of SPT positivity and/or specific IgE and/or total IgE as a definition of atopy produced a small increase in LOD score compared to the use of all three criteria. Although this increase in LOD score was not sufficient to demonstrate significant linkage, it indicates that the criteria for the definition of atopy are particularly important and that a broad definition of atopy may be responsible for a change in the significance of the LOD scores. This view is supported by analyzing these data when the criteria for a definition of atopy were broadened to include BH. The LOD score produced by

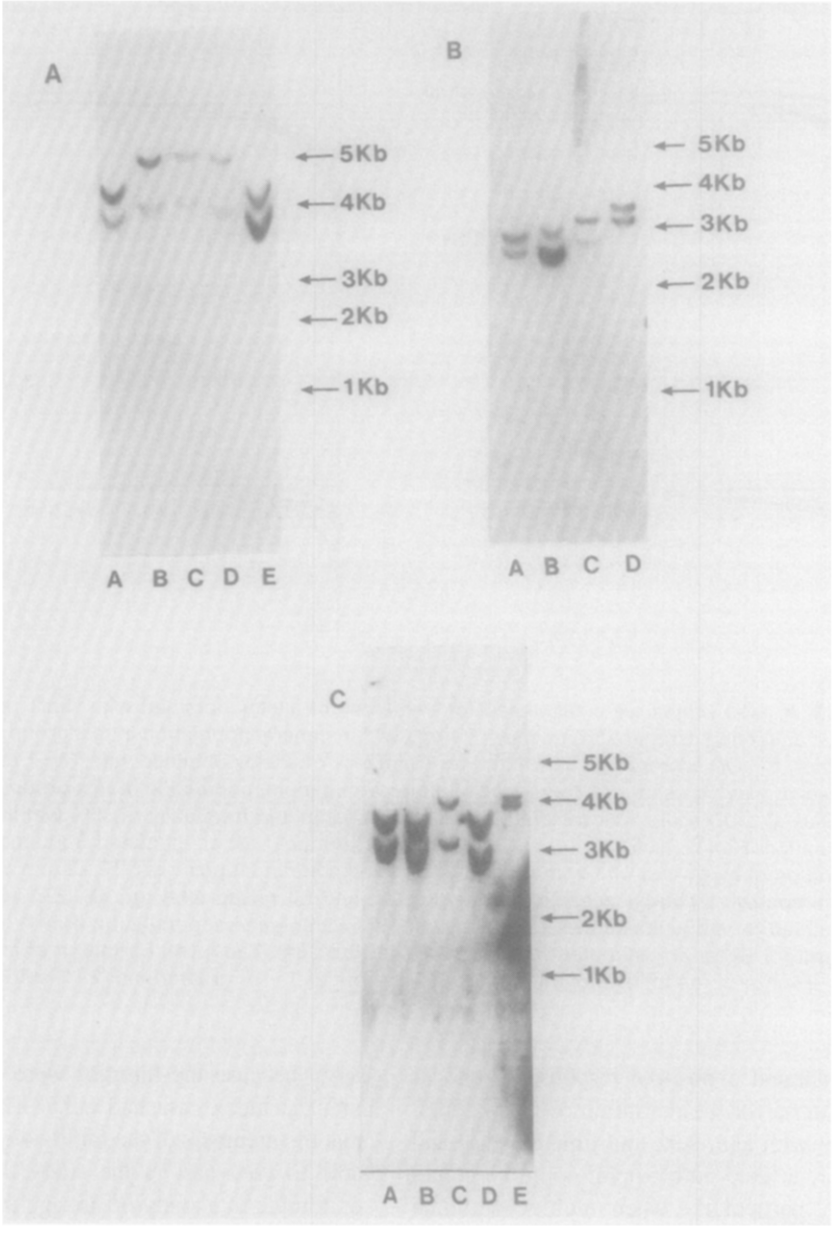

FIG. 2. A, Representative example of hybridization of genomic DNA digested to completion with Hinf 1 restriction enzyme with Pλ-MS.51. Tracks *A, B, C, D,* and *E* illustrate alleles present in members of one family. Subjects *B* and *D* are offspring of subjects *A* and *C.* Subject *E* is a sibling of subject *A.* Kilobase markers *(kb)* are indicated by *arrows* and were obtained from DNA 1 kb ladder. **B,** Representative example of hybridization of genomic DNA digested to completion with Taq 1 restriction enzyme, blotted onto nylon membrane, and hybridized with radiolabeled pλ-MS.51. Tracks *A, B, C,* and *D* illustrate alleles present in members of one family. Subjects *A* and *D* are parents of subjects *B* and *C.* Kilobase markers *(kb)* are indicated by *arrows* and were obtained from DNA 1 kb ladder. **C,** Representative example of hybridization of genomic DNA digested to completion with Taq 1 restriction enzyme, blotted onto nylon membrane, hybridized with radiolabeled pYNH24. Tracks A, B, C, and D illustrate alleles present in members of one family. Subjects *A, B,* and *C* are offspring of subjects *D* and *E.* Kilobase markers *(kb)* are indicated by *arrows* and were obtained from DNA 1 kb ladder.

analyzing these data in this way changed slightly from -0.29 to -0.23 at a recombination fraction of 0.3 for Hinf1 restriction digests and from -0.04 to 0.19 for Taq1 restriction digests at a recombination fraction of 0.3. The LOD score was unchanged from our original definition when the definition of atopy was broadened to include subjects who had recorded a previous history of asthma or rhinitis, since all of these subjects

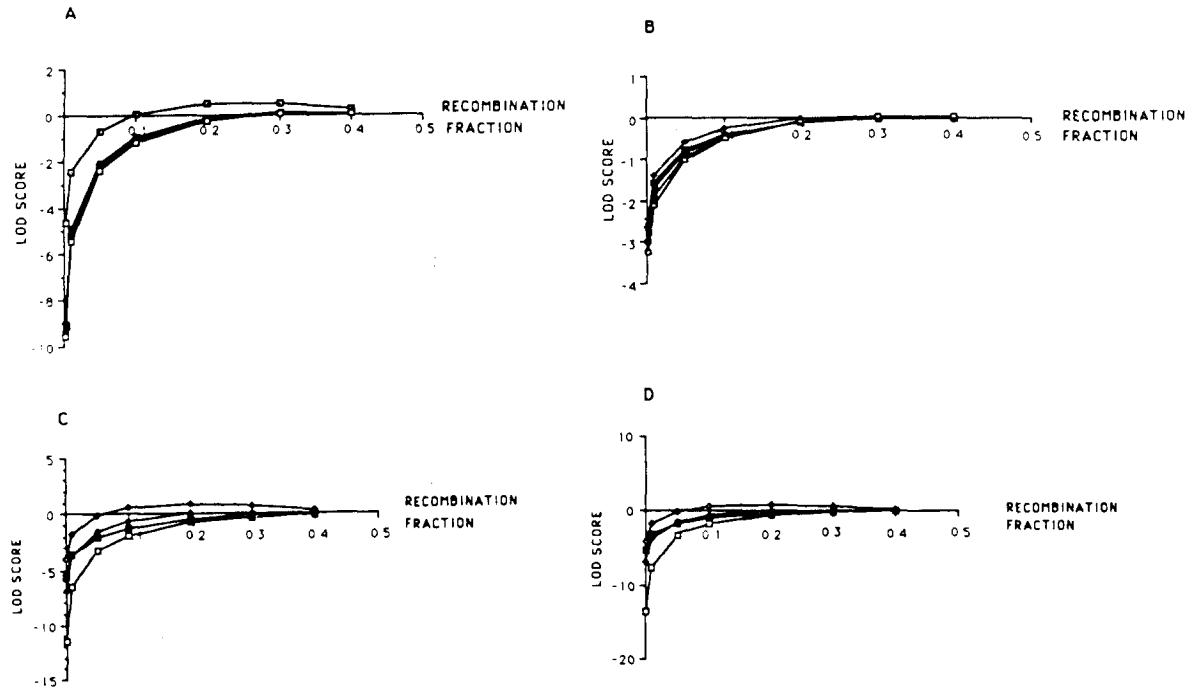

FIG. 3. A, LOD scores were calculated for frequency of alleles obtained with Hinf1 restriction digest at varying estimated frequency of atopy within general population in test group of seven families. **B,** LOD scores were calculated for frequency of alleles obtained with Taq 1 restriction digests at varying estimated frequency of atopy with general population in test group of seven families. **C,** LOD score was calculated for atopy at estimated frequency of 35% within general population (Hinf1) (□) (Taq1) (△) and for BH (Hinf1) (■) (aq1) (▲) at estimated frequency of 7%. Definition of atopy was one or more of the following criteria: (1) positive SPT ≥2 mm of normal saline control, (2) RAST score of 1+ or greater, and (3) raised total IgE. **D,** LOD score was calculated for atopy at estimated frequency of 35% within general population (Hinf1) (□) (Taq1) (△) and for BH at an estimated frequency of 7% (Hinf1) (■) (Taq1) (▲). Definition of atopy was one or more of the following criteria: (1) RAST score of 1+ or greater and (2) raised total IgE.

already demonstrated a positive response to one or more of the criteria for the definition of atopy. Total IgE levels vary with age, sex, and time of year, and in consequence, a fixed cutoff point could be somewhat arbitrary, particularly when multigenerational families are studied.

A study of this nature is strongly dependent on both the size and composition of the pedigrees studied. Within our families there were three individuals unavailable to take part in our study. However, Liped 6 calculates the conditional probabilities for the phenotype of such individuals and calculates the weighted LOD scores accordingly. In addition, the selection of the original pedigrees may be biased by selecting pedigrees with a high incidence of the disease of interest. For a disease of high incidence within the general population, it may be possible to select families at random via electoral role or from schools. In this study, the pedigrees were not specifically selected to include families with multiple cases of asthma or allergy because the families were chosen on the basis that the index case had either asthma or allergy and that the members of the family were willing and available to take part in the study. Large pedigrees are preferable in a study of linkage in which the mode of inheritance and the penetrance are not known.

Pedigrees with three generations and multiple sibships will add power to the study. Power is lost to some degree when individuals with atopy intermarry and produce atopic offspring, a fact that has been predicted in previous publications.[8] It is therefore possible that a larger study would add weight to the statistical analysis and produce a significant LOD score ($> +3$ or < -2). However, it should be emphasized that six of the nine pedigrees we have studied demonstrated negative LOD scores for atopy and four for BH, indicating no linkage with chromosome 11q. We suggest that our data confirm the data of previous studies that atopy may be a polygenic disease.

We thank David Briggs for reanalyzing our autoradi-

TABLE II. Relative contribution to LOD score for atopy

Pedigree	No. of individuals	Recombination fraction (θ)						
		0.001	0.01	0.05	0.1	0.2	0.3	0.4
A								
1	23	−0.34	−0.32	−0.24	−0.18	−0.09	−0.04	−0.01
2	16	0.22	0.22	0.21	0.17	0.13	0.08	0.04
3	10	−4.82	−2.83	−1.45	−0.88	−0.37	−0.14	−0.03
4	11	−6.90	−3.92	−1.91	−1.12	−0.45	−0.16	−0.04
5	7	−0.05	−0.05	−0.04	−0.03	−0.02	−0.01	−0.00
6	8	0.01	0.01	0.01	0.00	−0.01	−0.01	−0.01
7	9	0.10	0.08	0.06	0.03	−0.01	−0.03	−0.02
8	10	0.11	0.10	0.09	0.07	0.04	0.02	0.01
9	0.31	0.14	0.14	0.13	0.11	0.06	0.03	0.01
B								
1	23	−0.11	−0.11	−0.08	−0.05	−0.02	−0.01	0.00
2	16	−0.83	−0.81	−0.64	−0.44	−0.18	−0.06	−0.01
3	10	−2.67	−1.66	−0.92	−0.59	−0.27	−0.11	−0.03
4	11	0.24	0.23	0.20	0.17	0.11	0.05	0.02
5	7	0.04	0.04	0.04	0.03	0.03	0.02	0.02
6	8	−0.10	−0.10	−0.09	−0.07	0.04	−0.02	−0.01
7	9	0.10	0.10	0.09	0.08	0.06	0.04	0.02
8	10	0.11	0.10	0.09	0.07	0.04	0.02	0.01
9	9	0.11	0.11	0.11	0.10	0.08	0.05	0.03

The relative contribution of each of the nine families studied to the total LOD score for atopy at an estimated frequency of atopy within the general population of 35% was assessed with the restriction enzymes HinfI *(A)* and TaqI *(B)*.

TABLE III. Relative contribution to LOD score for BH

Pedigree	No. of individuals	Recombination fraction (θ)						
		0.001	0.01	0.05	0.1	0.2	0.3	0.4
A								
1	23	−3.97	−2.75	−1.62	−1.08	−0.53	−0.23	−0.06
2	16	−0.12	−0.11	−0.09	−0.08	−0.05	−0.02	−0.01
3	10	−1.97	−0.98	−0.35	−0.14	−0.01	0.00	0.00
4	11	1.50	1.47	1.35	1.19	0.86	0.50	0.17
5	7	−0.37	−0.35	−0.27	−0.19	−0.10	−0.04	−0.01
6	8	0.30	0.29	0.27	0.25	0.20	0.14	0.08
7	9	−0.05	−0.04	0.02	0.06	0.11	0.11	0.07
8	10	0.62	0.61	0.55	0.48	0.32	0.17	0.05
9	9	−0.01	−0.01	0.00	0.00	0.00	0.00	0.00
B								
1	23	−1.91	−0.09	−0.29	−0.07	0.07	0.08	0.05
2	16	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	10	0.49	0.47	0.42	0.34	0.20	0.09	0.02
4	11	0.30	0.30	0.26	0.22	0.14	0.07	0.02
5	7	−0.90	−0.85	−0.68	−0.53	−0.33	−0.19	−0.08
6	8	1.18	1.16	1.07	0.96	0.70	0.43	0.17
7	9	−5.31	−3.10	−1.72	−1.14	−0.59	−0.30	−0.12
8	10	0.02	0.02	0.02	0.01	0.01	0.00	0.00
9	9	−0.91	−0.85	−0.64	−0.45	−0.22	−0.09	−0.02

The relative contribution of each of the nine families studied to the total LOD score for BH at an estimated frequency within the general population of 7% was assessed with the restriction enzymes HinfI *(A)* and TaqI *(B)*.

TABLE IV. Effect of variation of the definition of atopy on the LOD score

Definition of atopy	LOD score		Recombination fraction (θ)	
	Hinf1	Taq1	Hinf1	Taq1
SPT positive only	0.17	−0.07	0.3	0.3
Raised specific IgE only	−0.01	0.00	0.4	0.4
Raised total IgE only	0.21	0.14	0.3	0.2
SPT positive and/or raised specific IgE	−0.30	−0.13	0.3	0.3
Raised specific IgE and/or raised total IgE	−0.11	−0.03	0.3	0.3
SPT positive and/or raised specific IgE and/or raised total IgE and/or asthma and rhinitis	−0.29	−0.04	0.3	0.3
SPT positive and/or raised specific IgE and/or raised total IgE and/or BH	−0.23	0.19	0.3	0.3
SPT positive and/or raised specific IgE and/or raised total IgE[13, 14]	−0.29	−0.04	0.3	0.3

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