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Review Article

# Genetics, atopy and asthma

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## ABSTRACT

Asthma is a complex disease which is due to the interaction of an unknown number of genes with strong environmental factors. Segregation analysis suggests the presence of major genes underlying asthma and atopy. Different genetic effects have been recognized which predispose to generalized atopy, or modify the atopic response to particular allergens, or enhance bronchial inflammation, or modify bronchial tone. These known genes or genetic loci do not account for all of the familial clustering of asthma and atopy. Many studies are now under way to identify the remaining genes.

**Key words**: asthma, atopy, genetics, immunoglobulin E.

## INTRODUCTION

The familial nature of atopy and asthma implies that they are due, at least in part, to inherited genetic factors. Study of their genetics will first increase understanding of the aetiology and pathophysiology of these diseases. Second, it may be hoped that the early identification of children at genetic risk of asthma may open new approaches to the prevention of illness. Third, the involvement of particular genes may identify a particular clinical course and response to therapy. Fourth and perhaps most distantly, genetics may lead to new pharmacological treatments for asthma.

Asthma is a complex disease that is likely to be due to the interaction of several genes with important environmental factors. In contrast to single gene disorders, such as cystic fibrosis or muscular dystrophy, genes predisposing to asthma will not contain mutations. Rather, as for genes influencing lipid metabolism, they will be variants of normal genes, whose evolutionary advantage has been lost in the current Western environment.

Asthma is almost certainly not one disease but many. In children, 95% of asthma is allergic, also known as atopic. The term atopy, meaning 'strange disease', describes a familial syndrome of asthma, seasonal rhinitis (hay fever) and infantile eczema.

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Of the various types of asthma, atopic asthma is clinically most easily recognized and defined and has the most obvious familial clustering. For this reason, most effort towards elucidating the genetic causes of asthma have been directed at asthma in children and in young adults and at the underlying condition of atopy.

The identification of genes causing disease is through two processes: the study of candidate genes, or by genetic linkage and positional cloning. These two approaches, which will be described in more detail later, both begin with a definition of phenotype, which may be the disease itself or the choice of disease-related parameter (known as an intermediate phenotype). The choice of phenotype may be critical in the eventual ability to discover the genetic lesions or variations that predispose to disease.

## ASTHMA AND ATOPY PHENOTYPES

Asthma may be recognized by questionnaire, physical examination or the demonstration of a variable reduction of airflow. In the absence of an attack of asthma, airflow limitation can be demonstrated by challenge tests. Challenges in common use include exercise, cold air and inhaled bronchial spasmogens, such as histamine or methacholine. Of these challenges, spasmogen inhalation gives the most reliable measure of underlying airway lability. Challenge tests have been widely used in the investigation of asthma, both in the clinical setting and in large epidemiological surveys.

Atopy is distinguished by immunoglobulin E (IgE) responses to inhaled proteins, known as allergens. Typical allergen sources include house dust mite (HDM), grass pollens and animal danders. The total annual exposure to allergens is small, often in the order of micrograms. IgE binds by its high affinity receptor (FcεRI) most notably to mast cells in the skin and in mucosal surfaces of the lung and intestines. Mast cells contain dense granules, which contain histamine and other inflammatory mediators, in addition to pro-inflammatory cytokines. In sensitized individuals, exposure to an allergen produces cross-linking of IgE, the triggering of high affinity receptors and the release of mast cell granules. The subsequent inflammation is in two waves; the first is immediate and the second occurs some hours later. Inflammation produces airway narrowing with wheeze

when occurring in the lung and sneezing and obstruction when it occurs in the nose. The regulation of IgE and of some components of early and late inflammation are under the control of antigen-specific T cells.

The atopic state is detected most easily by skin prick tests. In these, the allergen, in a dilute solution, is placed on the skin and a superficial prick is made to introduce minute amounts of allergen below the dermis. Sensitization and mast cell degranulation is detected by a weal, which is maximal after 10–15 min. A significant weal is judged to be between 2 and 4 mm greater in size than a negative control.

Atopy may also be detected by elevation of the total serum IgE, or by elevation of serum IgE titres against common allergens. Elevation of antigen-specific IgE is detected by RAST or ELISA techniques. In Western populations, there is a close correlation between prick skin tests, specific IgE titres (RAST), the total serum IgE and symptoms of wheeze or rhinitis. Despite these close correlations, the relationship between the variables is complex.

Atopy, defined by skin tests, is very common and has been shown in several large Western population samples to affect between 40 and 50% of young adults.[1–3] The prevalence of asthma has risen steadily through this century.[4] The prevalence of seasonal rhinitis also appears to have risen, although the evidence for this is less clear. The reasons for these increases, which cannot be due to changes in the gene pool, must be environmental.

Any trait as common as atopy cannot be considered abnormal and it is obvious that the atopic state gives some advantage to those who carry it. The most likely evolutionary reason for atopy to exist is that IgE is particularly important in handling parasite infestations.[5,6] In our society, we are now largely free of parasite infection, the implications of which are also discussed in the later section on environment.

## Factors confounding genetic studies of atopy

The high poulation prevalence of atopy may seriously confound genetic studies.[7] If atopy is present in 40–50% of the population, then one-fifth of marriages may be between two atopics. Any large pedigree is therefore likely to contain several atopy genes introduced through different individuals, instead of a single abnormal gene introduced through one progenitor. If atopy was due to a single gene disorder, then many of the population would be homozygous. If, as is likely, more than one gene predisposes to the syndrome, then many individuals will carry two or more of these genes.

The substantial prevalence of atopy means that great care has to be taken in the recruitment of families for genetic studies. Ascertainment by public appeals for families with asthma[8] or with eczema[9] produced samples in which 70 and 80%, respectively, were atopic, with considerable loss of power to detect linkage.[8] For this reason, in Oxford we now recruit families either from population samples (i.e. complete ascertainment), or through a defined proband with atopic disease.

For the purposes of genetic investigations, it is necessary to decide which measures of the atopy or asthma phenotype should be studied. The total serum IgE is an attractive parameter for genetic study, as it has well established normal values and, in large population surveys, correlates well with the presence of asthma. However, approximately 45% of the variation in the total serum IgE is attributable to the specific IgE (RAST) to HDM or grass pollen.[10] When multiple regressions are carried out on population data, asthma and bronchial hyper-responsiveness are found to relate to variation in the specific IgE, most notably to HDM.[10,11] Once specific IgE is taken into account, the residual total IgE does not correlate with the presence of asthma. The specific IgE, either detected indirectly by skin tests or directly in the serum by RAST or ELISA techniques, may therefore be suitable for genetic analysis. Bronchial hyperresponsiveness is a further intermediate phenotype that is currently being investigated.

It is also possible to study asthma as the principal phenotype. If this is done, care needs to be taken that the asthma being investigated is as clinically homogeneous as possible, which will usually mean the asthma of children and young adults. As there is no clear-cut division between normal and abnormal individuals, studies of asthma should exclude marginal phenotypes and should concentrate on 'barn door' affected and unaffected subjects.

Selection will affect the type of genetic effects found in particular samples of subjects or families. Even if total or specific IgE is used as a phenotype, the factors influencing the IgE in asthma patients may be different from those affecting the IgE in children with eczema, or in subjects selected for the presence of positive skin tests rather than for symptoms.

The behaviour of atopy with age presents a particular problem for geneticists. While many diseases have increasing penetrance throughout life, atopy has a low penetrance in infancy, which rises to a maximum from 15 to 25 years of age. Thereafter serum IgE and skin prick test responses decline steadily until, at the age of 45, the serum IgE may be half of its value at the age of 15.[2]

## Inheritance of atopy

Geneticists have traditionally carried out segregation analysis as a first step in the study of a genetic disorder. Segregation analysis is intended to detect the presence of major genes predisposing to the disease, or to suggest that the illness is polygenetic, or environmental, or a mixture of environmental and genetic factors. Segregation analysis may be used to estimate the values of parameters related to the mode of inheritance of a trait, its penetrance and its prevalence. Taken together, the estimates from segregation analysis make up a model that can be used to test for genetic linkage of a trait to particular chromosomal regions.

The pattern of inheritance of atopy has been the subject of much debate. In 1916, Cooke and Van der Veer, in a study of 1000 patients, found that if one parent was allergic then 50% of children were similarly affected and that if both parents were allergic then, so too, were 75% of their children.[12] This neat Mendelian finding was disputed by Schwartz, who found families in which atopic children had normal parents. He proposed a dominant model of inheritance tempered with incomplete penetrance.[13] Weiner *et al.* observed a similar pattern in 1936.[14]

These early authors studied the inheritance of the whole syndrome without reducing it to its component parts. Later studies concentrated on the inheritance of specific illnesses, such as asthma or hay fever. In these circumstances a pattern of inheritance was much harder to define. Sibbald and Turner-Warwick[15] studied the first degree relatives of atopic and non-atopic asthmatics, mostly by questionnaire, and found some evidence of familial aggregation of atopy without any clear cut pattern of inheritance.[15] Edfors-Lubs studied 7000 twin pairs for asthma and atopy, relying primarily on responses to a questionnaire. She concluded that asthma was polygenic.[16]

After atopy was shown to be mediated by IgE, workers then concentrated on the genetics of this parameter, as it was now quantifiable in a way that was not possible with symptoms alone. Bazaral *et al.* studied IgE levels in infants and mothers, concluding that there was simple Mendelian inheritance of basal IgE levels.[17] A subsequent study by the same investigators showed identical twins to be highly concordant for total serum IgE and that this effect was not linked to HLA haplotypes.[18] The result indicated an important genetic component to the control of total serum IgE. Hanson *et al.* studied pulmonary function, total serum IgE and specific IgE responses (RAST) in mono-zygotic and dizygotic twins reared together and apart.[19] He too found that monozygotic twins reared apart or together were concordant for total IgE, but that they differed in specific IgE responses.

Marsh and colleagues studied many families for the inheritance of total IgE.[20] They found that there was no simple pattern of Mendelian inheritance of the high IgE trait, but that a model in which high IgE was recessive best fitted the data. Gerrard *et al.* also studied many families with complex segregation analysis, and concluded that a major locus controlled IgE levels with a recessive allele determining high IgE levels, but that other genes influenced the trait.[21] Blumenthal *et al.*, however, considered that a dominant allele coded for high IgE levels in some families while a recessive allele coded for the trait in others.[22]

Borecki *et al.* studied a Canadian population for the inheritance of atopy. They found that if the total serum IgE was the only measure of atopy, then an autosomal recessive pattern of inheritance best fitted the data.[23] When they included symptoms in their definition, they then found that a dominant pattern of inheritance best explained their findings.

Using a definition of atopy that included responses to skin prick tests and serum specific IgE estimation in addition to total IgE (which they termed IgE responsiveness), Cookson and Hopkin examined the genetics of atopy in a limited number of nuclear and extended families.[24] Their findings suggested that there was a major genetic component to atopy. As with the kindreds studied by Marsh, at least 10% of atopic subjects did not have atopic parents, which the authors attributed to dominant inheritance with incomplete penetrance.[24]

Thus, diverse models have been proposed for the inheritance of atopy at different times, with varying definitions of atopy and different methods of analysis. It is perhaps as a result of the diversity of approaches that dominant, dominant with incomplete penetrance, recessive and polygenic modes of inheritance have all been suggested. None of these hypotheses explain the results of many studies that have shown the risk of atopy is much higher in children of atopic mothers than in children of atopic fathers. That asthmatic mothers had more asthmatic children than asthmatic fathers was reported 60 years ago[25] and large studies have shown a similar maternal pattern for the inheritance of elevations of the cord blood IgE,[26,27] atopic symptoms[28,29] and skin prick test responses to common allergens.[30] This finding may be due to interactions between the mother and child *in utero*, through the placenta, or *post partum*, through the breast milk. Genomic imprinting, in which a paternal 'atopy gene' may be suppressed during spermatogenesis, is also possible.[31]

One new approach to the problem of a complex phenotype has been the application of regressive models to segregation analysis. Despite close correlations between symptoms, total serum IgE and specific IgE, genetic effects independently modifying these different variables can be dissected out with these models. This type of segregation analysis has been applied to total serum IgE. The results demonstrate that, at least in the population studied, total IgE is influenced by at least one gene that is independent of genes affecting skin tests or positive RAST tests.[32]

The failure to show a simple consistent model of inheritance is most simply explained by accepting that the most likely possibility is several genes interacting with a strong environmental component. For the purpose of identifying genes causing atopy, an eclectic approach to phenotype definition is necessary, allowing for potential differences in the genes influencing skin tests and RAST, total serum IgE or disease states, such as asthma.

## Finding genes

As with other complex diseases, genes contributing to atopy may be found either by examining candidate genes or by genetic linkage. The enormous increase in the understanding of complex cytokine networks that influence atopy has meant that a plausible case could be put for as many as 20 different candidates. The role of candidate genes may be assessed by defining polymorphisms within the respective genes and by testing for associations with disease. At the moment, a systematic search through the various candidates has not been carried out. Two candidates, *IL-4* and the beta chain of the high affinity receptor of IgE (*FcεRIβ*), have been implicated by genetic linkage studies.

Genetic linkage relies on the demonstration of co-inheritance of disease and genetic markers of known chromosomal localization. This approach has the advantage of not requiring any pre-existing knowledge of the pathophysiology of the disease. However, the power to detect linkage in multigenic diseases is very limited (Table 1), so that several hundred families may be necessary to detect linkage to a gene affecting one-third of subjects with the disease. A further problem with complex diseases is that of replication of linkage.[33] Linkage to a heterogeneous trait will normally only be found fortuitously in samples which contain an exceptional proportion of individuals or families influenced by that particular gene. Simulation experiments have shown that, in these circumstances, many studies may be necessary before replication occurs.

**Table 1.** The power to detect linkage

| Fraction linked | Recessive inheritance | Dominant inheritance | Imprinted inheritance |
|---|---|---|---|
| 0.80 | 22 | 69 | 29 |
| 0.50 | 62 | 181 | 87 |
| 0.30 | 181 | 508 | 253 |
| 0.20 | 412 | 1152 | 571 |
| 0.10 | 1662 | 4637 | 2310 |

The table shows the number of affected sibling pairs required to detect loci at P=0.05 with 90% power at u=0.005, with different proportions of families linked to the putative locus. The required numbers of sib-pairs are estimated for three modes of inheritance. The table assumes 70% marker informativeness. (Table courtesy of Dr Alan Young, Statistical Genetics Group, Wellcome Centre for Human Genetic Disease, Oxford, UK.)

## GENES INFLUENCING ASTHMA AND ATOPY

Many different kinds of genes may be involved in atopy and asthma. These genes can be divided into four classes:
Class I: Genes predisposing in general to IgE-mediated inflammation.
Class II: Genes influencing the specific IgE response.
Class III: Genes influencing bronchial hyper-responsiveness independently of atopy.
Class IV: Genes influencing non-IgE mediated inflammation.

### Class I asthma genes

Genes predisposing to generalized atopy have been identified on chromosomes 11 and 5 by a combination of genetic linkage and candidate gene approaches.

#### *Chromosome 11q12–13*

The first suggested linkage of atopy was to the marker d11s97 on chromosome 11q13.[34,35] Following some controversy,[36] this linkage has been replicated by another two research groups.[37,38] This linkage was confounded by the high prevalence of atopy and because the linkage was predominantly seen in maternal meioses.[37,39] In the largest study described, linkage was exclusively maternal.[39] The reasons for the maternal linkage are not known and it is not clear that this maternal phenomenon corresponds to the phenotypic maternal inheritance of atopy that has been previously noted.

Recognition of the maternal linkage allowed better localization of the atopy locus, to within a 7 cM one lod unit support interval.[40,41] This interval was centromeric to and excluded the original d11s97 marker to which linkage was first observed. A lymphocyte surface marker, *CD20*, was noted to be within the interval. *CD20* shows sequence homology to the beta chain of the high affinity receptor for IgE (*FcεRIβ*) and has been localized close to that gene on mouse chromosome 19.[42] The human *FcεRIβ* was subsequently found to be on chromosome 11q13, in close genetic linkage to atopy.[40] Two coding polymorphisms have now been identified within the gene, *FcεRIβ Leu181* and *FcεRIβ Leu181/Leu183*.[43] These both show strong associations with atopy when maternally inherited. The population prevalence of *FcεRIβ Leu181/Leu183* is approximately 4%[44] and *FcεRIβ Leu181* has been reported in 15% of asthmatic patients.[43]

These results with *FcεRIβ* variants have not been replicated outside of the Oxford group and a reliable assay system for the variants has not yet been established. *FcεRIβ Leu181* does not show functional differences from the wild-type receptor (JP Kinet, pers. comm. 1995). A further complication is the detection of a third homologous gene, *Htm4*, in close proximity to *FcεRIβ* and *CD20*,[45] so that it is not clear how many members of the gene family are present. As it stands, it is therefore not yet established whether the chromosome 11q atopy gene is *FcεRIβ* or whether it is some other gene in linkage disequilibrium with the *FcεRIβ* variants.

#### *Chromosome 5*

Linkage of total serum IgE to markers near the cytokine cluster on chromosome 5q31–33 has been demonstrated by Marsh *et al*.[46] Marsh and colleagues studied Amish pedigrees selected to contain members with positive skin prick tests. Linkage was, however, strongest in families with the lowest serum IgE. A linkage to the same general region was found by Myers *et al*.[47] in Dutch asthmatic families. The regions of linkage in these two studies may not coincide. Linkage has not been found in other studies of extended families (S Rich, pers. comm. 1995). My group have tested 1500 individuals from 300 nuclear families and have not found any evidence for linkage either by sib-pair or by lod score methods. However, in order to test the claim that linkage is predominantly seen with the low IgE phenotype, we have used class D regressive models to account for specific IgE response. Residual IgE shows evidence of linkage to a microsatellite repeat found in IL4, but not to other polymorphic markers studied by Marsh or Myers (Dizier *et al*. unpubl. data, 1996). We found no linkage to asthma or bronchial responsiveness at this locus.

The region contains a number of cytokines, the most important of which, from the point of view of atopy, are *IL-4*, *IL-13*, the

p40 subunit of *IL-12* and *IL-5*. Other cytokines include *IL-9* and granulocyte-colony stimulating factor (*G-CSF*). A substantial amount of work is now required to establish which of these various candidates accounts for the linkage.

## Class II asthma genes

Atopic individuals differ in the particular allergens to which they react. This difference is of clinical significance, as asthma and bronchial hyper-responsiveness are associated with allergy to HDM but not to grass pollens.[10,11] It is therefore of interest to examine whether particular genes influence the IgE response to specific allergens. In addition, study of these genes may give an insight into the inheritance of normal variation within the immune system and the functional consequences of such variation.

There are two classes of genes that are likely candidates for constraining specific IgE reactions. These are the genes encoding the human leucocyte antigen (HLA) proteins and the genes for the T-cell receptor (TCR). These modules are central to the handling and recognition of foreign antigens.

Inhaled allergen sources, such as HDM, are complex mixtures of many proteins. A number of 'major allergens' to which IgE responses are consistently found in most individuals have been identified from each allergen source. It is likely that genetic associations will be better detected with reactions to purified major allergens rather than with complex allergen sources. Major allergens include *Der p* I (25.4 kDa) and *Der p* II (14.1 kDa) from the HDM *Dermatophagoides pteronyssinus*, *Alt a*l (28 kDa) from the mould *Alternaria alternata*, *Can f* I (25 kDa) from the dog *Canis familiaris*, *Fel d* I (18 kDa) from the cat *Felis domesticus* and *Phl p* V (30 kDa) from Timothy grass (*Phleum pratense*).

### *Human leukocyte antigen*

The human major histocompatibility complex (MHC) includes genes coding for HLA class II molecules (HLA-DR, DQ and DP), which are involved in the recognition and presentation of exogenous peptides.

An HLA influence on the IgE response was first noted by Levine *et al.*[48] who found an association between HLA class I haplotypes and IgE responses to antigen E derived from ragweed (*Ambrosia artemisifolia*) allergen. This association has been subsequently found to be due to the restriction of the response to a minor component of ragweed antigen (*Amb a* V) by HLA-DR2.[49] To date, the association of *Amb a* V (molecular weight 5000) and HLA-DR2, is the only HLA association to have been consistently confirmed.[48–50] Other suggested associations are of the rye grass antigens *Lol p* I, *Lol p* II and *Lol p* III with HLA-DR3 (in the same 53 allergic subjects),[51,52] American feverfew (*Parthenium hysterophorus*) and HLA-DR3 in 22 subjects from the Indian sub-continent,[53] the IgE response to *Bet v* I (the major allergen of birch pollen) and HLA-DR3 in 37 European subjects[54] and an HLA-DR5 association with another ragweed antigen *Amb a* VI in 38 subjects.[55]

Other authors have reported negative associations with particular allergens. These include HLA-DR4 and IgE responses to mountain cedar pollen (37 subjects)[56] and HLA-DR4 and melittin (from bee venom; 22 subjects).[57] Non-responsivenes to Japanese cedar pollen may be associated with HLA-DQw8.[58]

There is, to date, no confirmation of many of these results and the number of subjects has generally not approached that required to establish an unequivocal HLA association. In addition, there has not been recognition of the problems of reactivity to multiple allergens: significant relationships between HLA-DR alleles and five antigens (*Amb a* V, *Lol p* I, *Lol p* II, *Lol p* III and *Amb a* V) have been claimed from the same pool of approximately 200 subjects.[49,51,52,55]

In order to test more definitively if HLA class II gene products have a general influence on the ability to react to common allergens, we have genotyped for HLA-DR and HLA-DP in a large sample of atopic subjects from the British population.[59] Subjects were tested for IgE responses to the most common British major allergens.

Four hundred and thirty-one subjects from 83 families were genotyped at the HLA-DR and HLA-DP loci and serotyped for IgE responses to six major allergens from common aeroallergen sources. Three hundred subjects were used as controls. The subjects and controls have come from the same relatively homogeneous population. In the United Kingdom and Europe, allergens other than *Bet v* I and those tested for in our study are uncommon causes of sensitization and IgE-mediated allergy.

The results showed only weak associations between HLA-DR allele frequencies and IgE responses to common allergens. A possible excess of HLA-DR1 was found in subjects who were responsive to *Fel d* I compared with those who were not [Odds Ratio (OR)=2; $P$=0.002]) and a possible excess of HLA-DR4 was found in subjects responsive to *Alt a* I (OR=1.9; $P$=0.006). Increased sharing of HLA-DR/DP haplotypes was seen in sibling pairs responding to both allergens. *Der p* I, *Der p* II, *Phl p* V and *Can f* I were not associated with any definite excess of HLA-DR alleles. No significant correlations were seen with HLA-DP genotype and reactivity to any of the allergens.

Of all possible associations, that of *Alt a* I with HLA-DR4 and of *Fel d* I with HLA-DR1 were supported by a finding of excess sharing of a HLA haplotype in affected sibling pairs. Regression analysis showed that the apparent association of *Phl p* V with HLA-DR4 was due to the presence of many individuals who reacted with an IgE response to both *Alt a* I and *Phl p* V. The association of HLA-DR1 and *Fel d* I is the strongest statistically and is significant even taking the multiple comparisons into account.

The study was the first to investigate HLA-DP alleles and reactivity to common allergens. As no definite correlation was found between any antigen response and HLA-DP genotypes with substantial numbers of subjects, HLA-DP genes are unlikely to have a major role in restricting IgE responses to these allergens.

The results suggest that HLA-DR alleles do modify the ability to mount an IgE response to particular antigens. However, the

OR for the association of *Alt a* I with HLA-DR4 was only 1.9 while that of *Fel d* I with HLA-DR1 was 2.0. Thus, class II HLA restriction seems insufficient to account for individual differences in reactivity to common allergens. It is therefore likely that environmental factors or other loci, such as T-cell receptor genes, may be of greater relevance in determining an individual's susceptibility to specific allergens.

### *T-cell receptor*

The T-cell receptor (TCR) is usually made up of α and β chains, although 5% of receptors consist of γ and δ chains. The β chain locus is on chromosome 7 and the α chain locus is on chromosome 14. The δ chain genes are found within the α chain locus.

An enormous potential for TCR variety follows from the presence of many variable (V) and junctional (J) segments within the TCR loci. However, the usage of the TCR Vα and Vβ segments by lymphocytes is not random and may be under genetic control.[60–63]

In order to examine if the TCR genes influence susceptibility to particular allergens, we have tested for genetic linkage between IgE responses and microsatellites from the TCR-α/δ and TCR-β regions.[64] Two independent sets of families, one British and one Australian, were investigated. Because the mode of inheritance was unknown and because of interactions from the environment and other loci, affected sibling pair methods were used to test for linkage.

Linkage of IgE serotypes to TCR-β was not detected, but significant linkage of IgE responses to the HDM allergens *Der p* I and *Der p* II, the cat allergen *Fel d* I and total serum IgE to TCR-α was seen in both family groups. The results show that a locus in the TCR-α/δ region is modulating IgE responses. The close correlation between total and specific IgE makes it difficult to determine if the locus controls specific IgE reactions to particular allergens or confers generalized IgE responsiveness. Nevertheless, linkage was strongest with highly purified allergens, suggesting that the locus primarily influences specific responses. The pattern of allele sharing seen with some serotypes suggests a recessive genetic effect, making it possible that this linkage corresponds to the recessive atopy locus implied by previous segregation analyses.[32,65]

Replication of positive results of linkage in a second set of subjects is important in interpreting this study. Differences between the populations for the serotypes showing TCR-α allele sharing may be due to different allergen exposures, as grass pollen responses were much more common in Australian subjects. In addition, British subjects were recruited through clinics, whereas Australian subjects were not selected by symptoms.

No association was seen between particular IgE responses and specific TCR-α microsatellite alleles, implying that the microsatellite is not in immediate proximity to the IgE modulating elements. The degree of linkage disequilibrium across the TCR-α/δ locus seems low[66] and the microsatellite has only been localized within a 900 kb yeast artificial chromosome.[67] The observed linkage may, therefore, be with any elements of TCR-α or TCR-δ, or with other genes in the locality.

Several Vα genes have been recognized to be polymorphic[68] and limitation of the response to an allergen may correspond to these polymorphisms. Particular TCR-Vα usage may induce IL-4 dominant (Th2) helper T cells, which enhance IgE production.[69] A reported non-random usage of Vα13 in *Lol p* I specific T-cell clones supports independently the possibility of Vα genes controlling IgE responses.[70]

The TCR-δ locus is also a candidate for this linkage. The function of TCR-γ/δ cells is not known, but their location on mucosal surfaces, where allergens initiate IgE responses, could suggest a role in IgE regulation.[71]

This study has, therefore, identified a further genetic locus affecting atopy. The genetic restriction of specific IgE responses may be of clinical significance and may be of general interest in understanding the control of humoral immunity. Further localization of this genetic effect requires the identification of TCR-α/δ elements showing allelic associations with specific IgE responses. Studies are also needed to investigate the interactions between this chromosome 14 linkage and the HLA class II genes.

## Class III asthma genes

No genes have yet been identified that predispose to bronchial hyper-responsiveness independently of atopy. Variants in the beta adrenergic receptor have been identified and it has been suggested that these may be associated with nocturnal asthma or other subdivisions of the asthma phenotype.[72]

## Class IV asthma genes

Airway inflammation is a characteristic of asthma that may be independent of mechanisms controlling atopy. Tumour necrosis factor alpha (TNF-α) is a potent pro-inflammatory cytokine that shows constitutional variations in the level of secretion which are linked to polymorphisms in the TNF gene complex.[73–75] We have, therefore, investigated TNF polymorphisms for association with asthma in 800 normal and abnormal subjects from the general population and from asthma clinic samples. We have found that asthma was significantly increased in subjects with alleles associated with increased secretion of TNF-α, most notably the TNF-α promoter polymorphism TNFα-308. Considering unrelated subjects only (the parents) from both populations, the OR for asthma in individuals homozygous for the high secretor allele was 3.9 compared with homozygotes for the low secretor alleles (95% CI 1.4–11.0; $P=0.007$).[76]

In the Japanese population, it is possible that a common mutation in platelet activating factor acetylhydrolase predisposes to asthma,[77] but this has not yet been confirmed in large datasets nor has it been linked to a structural change in the gene. This gene is, however, an excellent candidate for predis-

posing to asthmatic airway inflammation in both Japanese and Western populations.

## WHOLE GENOME SCREENS FOR ATOPY AND ASTHMA

The four loci described above do not account for all atopy. The chromosome 5 gene does not seem to have major effects on the population as a whole and HLA and TCR-α loci modify the specific response rather than endowing any general predisposition to atopy. Segregation analysis is unable to predict with any accuracy the number and nature of genes contributing to atopy and asthma. In order to discover if atopy is a genuine polygenic disorder, my group have performed a complete genome screen in 80 nuclear families, with 300 markers spaced at approximately 10% recombination. Using sib-pair analysis we have discovered potential new linkages ($P<0.001$) to serum IgE or other asthma-associated phenotypes. These results are currently being evaluated for their significance in further sets of subjects. Similar large scale genome scans are to be carried out in the United States and Canada, so that it is likely general agreement will soon be reached on the number and nature of the most important loci causing atopy.

## GENES AND ENVIRONMENT

No description of the genetics of asthma would be complete without some consideration of the effects of environment. It is self-evident that in the absence of environmental precipitants allergic asthma and hay fever would not exist. Such conditions are found on mountains, where there is little pollen and where the low humidity prevents HDM growth. School children raised at high altitudes develop fewer allergies than those raised at sea level.[78] Similarly, children living in the dry interior of Australia develop fewer allergies than those living in more humid conditions near the coast.[4]

Data from a number of sources indicate that events in early infancy are critical in determining the subsequent course of allergic disease. In Scandinavian countries, a short intense spring flowering of birch trees is accompanied by symptoms in many individuals. Children born in the 3 months around the pollen season carry an increased risk of allergy to birch pollen for the rest of their life.[79] In English children, the level of HDM in infants' bedding during the first year of life correlates with the subsequent risk of childhood asthma.[80]

The enormous increase in the prevalence of asthma in the past two decades cannot be attributed to changes in gene frequencies in affected populations and must be due to an environmental factor or factors. Air pollution has been suggested as a cause of this increase, although atmospheric pollution has declined steadily since the 1950s in England and Western Europe and ozone levels remain stable despite an increase in the number of cars. Comparative studies of the prevalence of asthma have been carried out between East and West Germany, two regions with genetically similar populations, but with far higher levels of atmospheric pollution in the East.[81] Surprisingly, the prevalence of asthma is lower in the East than it is in the West. This decrease is matched by a lower prevalence of atopy, as detected by skin tests to common allergens.[82] Similar results are seen when the prevalence of asthma in the Baltic States is compared to that of Sweden.[83] This difference may be attributable to childhood respiratory infections, as pollution and overcrowding, both of which are more common in the East, predispose to infantile infection. Support for this hypothesis is given by the finding that the youngest children in large sibships have significantly less asthma than their older siblings.[84] At the cellular level it is suggested that early infections program the immature immune system towards a Th-1 rather than a Th-2 helper cell profile, thereafter favouring a cellular rather than humoral immune response.

Thus, even in genetically similar people, the dose and timing of allergen exposure will have important effects on subsequent manifestations of the atopy phenotype. This places an additional requirement for careful study design and interpretation in attempts to unravel the genetics of atopic disease.

Another environmental factor to consider is that of parasitism. Slum-dwelling Venezuelan children have higher levels of serum IgE and lower levels of asthma than their more affluent compatriots.[85] In endemically parasitized Australian Abori-gines, the presence of a positive RAST to HDM correlates poorly both with skin test responses to the same allergen and the presence of asthma.[86] Multiple regression shows that the discrepancy between RAST and skin tests is accountable by the elevation of total serum IgE. The results fit the hypothesis that parasitism, by causing an increase in polyclonal IgE, is protective against atopy.

## SCREENING

The increase in the prevalence of asthma in recent decades has an important corollary: asthma is preventable. Recognition of children or infants genetically predisposed to asthma is likely to be the first step in strategies for prevention by environmental manipulation or vaccination in the first year of life. It is likely that many genetic variants that predispose to asthma will be identified in the near future and that a comprehensive estimation of genetic risk to a particular infant will be feasible by the end of the decade.

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