

# Atopy, airway responsiveness, and genes

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## Introductory articles

### Linkage of high-affinity IgE receptor gene with bronchial hyperreactivity, even in absence of atopy

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*Asthma is a manifestation of bronchial hyperreactivity (BHR) and forms part of the spectrum of atopic disease. Some pedigree studies of atopy have suggested linkage with the high-affinity IgE receptor ( $Fc\epsilon R1\beta$ ) gene on chromosome 11q13, but others find no linkage. The molecular genetics of asthma and BHR have not been studied in the general population. We examined the genetic linkage of the  $Fc\epsilon R1\beta$  gene with clinical asthma and the underlying phenotypes of BHR (to methacholine) and atopy (defined by skinprick testing) in 123 affected sibling-pairs recruited from the general population. We found evidence of significant linkage of a highly polymorphic microsatellite marker in the fifth intron of the  $Fc\epsilon R1\beta$  gene to a diagnosis of asthma (18.0% excess of shared alleles,  $P=0.002$ ) and to BHR (21.7% excess of shared alleles,  $P=0.001$ ). Significant linkage was also observed in siblings sharing BHR when those with atopy were excluded (32.8% excess of shared alleles,  $P=0.004$ ). Atopy in the absence of BHR did not show significant linkage to the  $Fc\epsilon R1\beta$  gene (7.2% excess of shared alleles,  $P=0.124$ ). These findings suggest that mutations in the  $Fc\epsilon R1\beta$  gene or a closely linked gene influence the BHR underlying asthma, even in the absence of atopy. (Lancet 1995;346:1262-65)*

### Genetic susceptibility to asthma – bronchial hyperresponsiveness coinherited with a major gene for atopy

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**Background.** Bronchial hyperresponsiveness, a risk factor for asthma, consists of a heightened bronchoconstrictor response to a variety of stimuli. The condition has a heritable component and is closely related to serum IgE levels and airway inflammation. The basis for these relations is unknown, as is the mechanism of genetic susceptibility to bronchial hyperresponsiveness. We attempted to define the interrelation between atopy and bronchial hyperresponsiveness and to investigate the chromosomal location of this component of asthma. **Methods.** We studied 303 children and grandchildren of 84 probands with asthma selected from a homogeneous population in the Netherlands. Ventilatory function, bronchial responsiveness to histamine, and serum total IgE were measured. The association between the last two variables was evaluated. Using analyses involving pairs of siblings, we tested for linkage between bronchial hyperresponsiveness and genetic markers on chromosome 5q31-q33, previously shown to be linked to a genetic locus regulating serum total IgE levels. **Results.** Serum total IgE levels were strongly correlated ( $r=0.65$ ,  $P<0.01$ ) in pairs of siblings concordant for bronchial hyperresponsiveness (defined as a  $\geq 20$  percent decrease in the forced expiratory volume in one second produced by histamine [threshold dose,  $\leq 16$  mg per milliliter]), suggesting that these traits are coinherited. However, bronchial hyperresponsiveness was not correlated with serum IgE levels ( $r=0.04$ ,  $P>0.10$ ). Analyses of pairs of siblings showed linkage of bronchial hyperresponsiveness with several genetic markers on chromosome 5q, including D5S436 ( $P<0.001$  for a histamine threshold value of  $\leq 16$  mg per milliliter). **Conclusions.** The study demonstrates that a trait for an elevated level of serum

*total IgE is coinherited with a trait for bronchial hyperresponsiveness and that a gene governing bronchial hyperresponsiveness is located near a major locus that regulates serum IgE levels on chromosome 5q. These findings are consistent with the existence of one or more genes on chromosome 5q31-q33 causing susceptibility to asthma. (N Engl J Med 1995;333:894-900)*

### Markers and linkage

Perhaps the central issue in the aetiology of asthma is the relationship between bronchial hyperreactivity (BHR) measured by methacholine or histamine challenge, atopy defined as the propensity to make an exuberant IgE antibody response to antigen, and T cell cytokines. Family studies of BHR point to a strong genetic influence in man, with one or more genes controlling the response.<sup>1</sup> Unfortunately, human studies give an imperfect approach to such questions because the breeding choices of the participants are not open to experiment. Mice, on the other hand, can be bred for differences in response to methacholine or their propensity to make IgE antibodies. Researchers can knock out individual murine genes to assess their contribution to a disease phenotype. In contrast, human aetiological studies require a very large number of subjects to be studied to control for the variables introduced by the genetic heterogeneity of an individual.

The identification of genes responsible for human disease has been fastest for disorders with a readily identifiable phenotype determined by only a single genetic locus – the rapid progress made in identifying the genes and aetiology of X-linked immunodeficiencies is a case in point.<sup>2</sup> On the other hand, when genes on different chromosomes act together to produce a disorder, large numbers of subjects have to be studied to identify links between the inherited DNA (genotype) and the disorder (phenotype).

Linkage studies<sup>3</sup> have become possible only in the past decade as microsatellite markers have been mapped to individual chromosomes. Microsatellites are short tandem repeats of two or more nucleotides that vary between individuals. Only small amounts of blood are required and the polymerase chain reactions used to identify microsatellites can be semi-automated.<sup>4</sup> The human genetic map based entirely on microsatellites has just been updated<sup>5</sup> and now comprises 5264 repeat polymorphisms distributed in 2335 positions with a total length of 3699 cM and mean interval of 1.6 cM. A centimorgan (cM) refers to one genetic map unit which is the distance between gene pairs for which one product of meiosis out of 100 is recombinant – that is, a recombinant frequency of 1%. The density of this map should make it easier to localise candidate genes linked to markers.

Linkage studies of complex multigene disorders, such as type 1 diabetes, first proved of value when they were “focused” on a specific group of genes such as the major histocompatibility complex (MHC) locus.<sup>6</sup> However, the MHC gene is not the only one which contributes susceptibility to diabetes and a genome-wide search found that there were linked loci on chromosomes 6, 11, and 2 (the loci on 6 and 11 are close to the major histocompatibility complex and the insulin gene<sup>7</sup>). This knowledge is a stimulus to new approaches to prevention (for diabetes) and the analysis of genes contributing to asthma will be most valuable if it points to new aetiologies and new approaches to treatment.

The introductory articles by van Herwerden *et al*<sup>8</sup> and Postma *et al*<sup>9</sup> illustrate the “focused” approach to

linkage studies in which siblings, parents, and children who share asthma are tested for shared DNA markers around specific chromosomal sites of interest. Sharing in excess of what would be predicted to occur by chance then implicates one or more genes close to the marker in the pathogenesis of the asthma. At first sight the reports appear contradictory with their fingers pointing to chromosome 11 and chromosome 5, respectively. Studies in mice point to one resolution for this dilemma and may help to guide further human studies.

### Genetic control of bronchial hyperreactivity in mice

Inbred strains of mice differ in their bronchial reactivity to methacholine.<sup>10</sup> Crosses between high and low responder strains yielded distinct high and low responders<sup>11</sup> with an inheritance pattern which suggested that BHR could be inherited as an autosomal recessive trait. Markers for all mouse chromosomes have subsequently become available and De Sanctis and colleagues<sup>12</sup> have now used these to localise genes contributing to BHR in mice. Figure 3 from their paper (reproduced here as fig 1) shows how the LOD score, the geneticist’s measurement of linkage, rises to a peak as markers up and down the chromosome are examined. They found that about 50% of the variability in murine response to methacholine was genetically determined with the remaining 50% assumed to reflect environmental factors. When A/J mice (which have the highest response) and C57BL/6J (the lowest methacholine responders) were mated, the BHR of the offspring was normally distributed with a wide standard deviation, a result suggestive of a polygenic trait. The BHR of individual mice was determined by the origin of their chromosomes 2, 15, and 17, with the candidate genes on these being named BHR1, BHR2, and BHR3. The genes tended to interact in pairs, BHR1 with BHR3, and BHR2 with BHR3. These interactions make it difficult to estimate the contribution of individual loci to BHR but, taken together, the three loci account for about 26% of the genetic variance in BHR.

C57BL/6 mice sensitised to egg protein (ovalbumin) by injection and then challenged with an aerosol of the same protein make IgE antibodies and acquire BHR with increased numbers of circulating and bronchial lavage fluid eosinophils.<sup>13</sup> The lungs of the sensitised animals show lymphocyte and neutrophil infiltrates with microvascular leakage, mucosal oedema and, in some animals, thickening of the epithelial basement membrane. Interleukin (IL)-5-deficient mice of the same strain also make IgE antibodies to injected and aerosolised ovalbumin, but they do not develop eosinophilia, lung damage, or BHR. While these results demonstrate that IL-5 is necessary for the eosinophilic response and that eosinophilia is accompanied by BHR, they should not be interpreted as implying that this is the sole pathway to developing BHR. In this respect, BALB/c mice which, like A/J mice, are high responders to methacholine, are not protected from aeroallergen induced BHR by blocking IL-5 even though their eos-

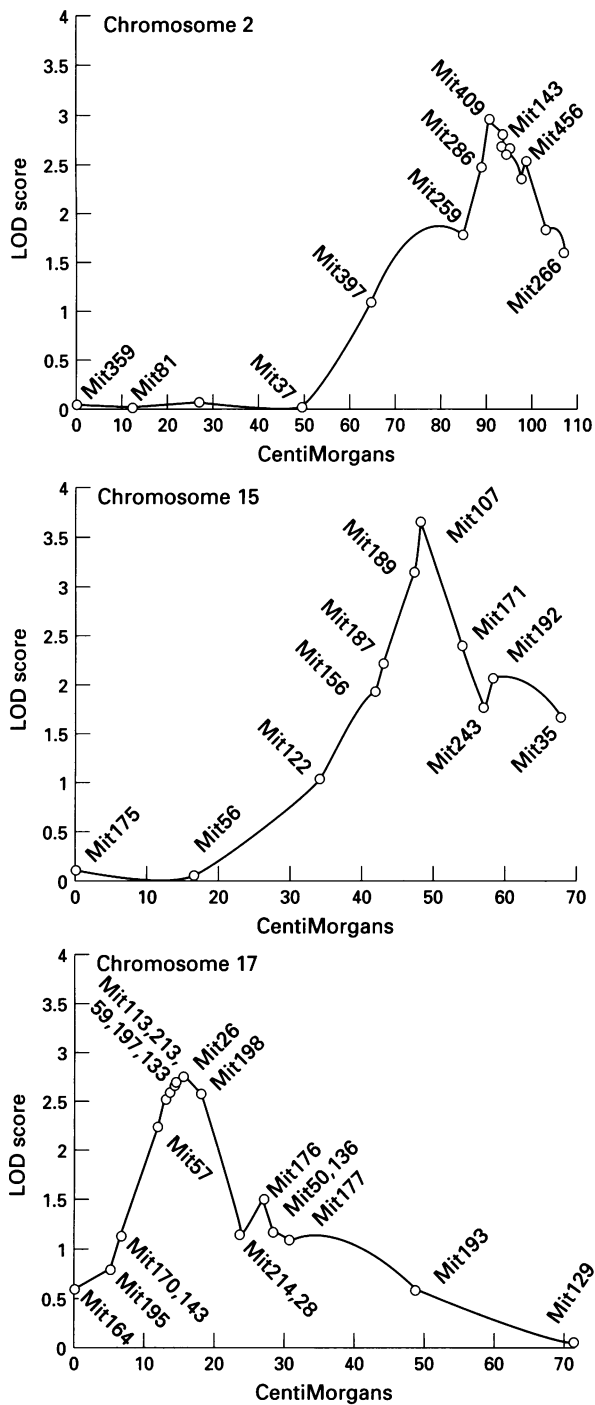


Figure 1 Calculation of LOD scores from genotypes of murine SSLP markers on 321 backcross progeny [(C57BL/6J × A/J) × C57BL/6J] on chromosomes 2, 15, and 17. Reproduced from De Sanctis et al<sup>12</sup> with permission.

inophilia is abolished.<sup>14</sup> BALB/c mice were protected from BHR by neutralising their IL-4 even though this did not prevent the appearance of eosinophils in the lung. The differences between the responses of the C57BL/6 and the BALB/c mice may at least in part reflect differences in their BHR genotypes. There are likely to be at least two independent pathways leading to the development of airway inflammation and BHR in mice. One would be mediated through IL-4, a high IgE and independent of eosinophils, the other mediated by eosinophils and requiring IL-5.<sup>15</sup>

#### Human studies

Although the molecular strategies for identifying genes

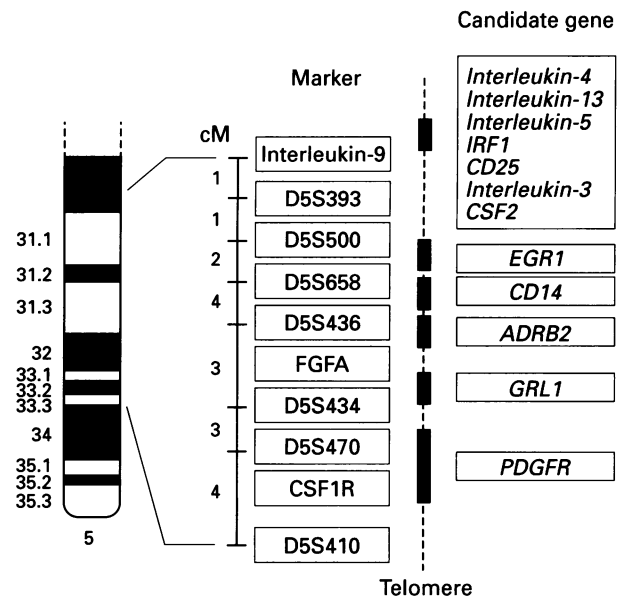


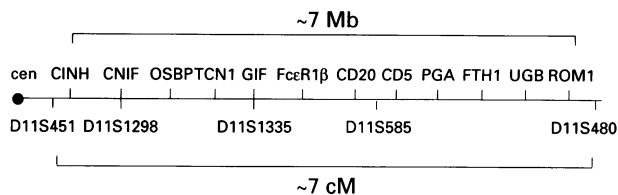
Figure 2 Map showing the relative order of and distance between the polymorphic genetic markers used and the approximate location of the gene candidates for asthma, bronchial hyperresponsiveness, and atopy relative to the markers studied. The map includes the following genes: interleukin-4, 13, 5, and 3; immune regulatory factor 1 (IRF1); cell division cycle 25 (CDC25); granulocyte-macrophage colony-stimulating factor (CSF2); early growth response gene 1 (EGR1); CD14;  $\beta_2$  adrenergic receptor (ADRB2); lymphocyte-specific glucocorticoid receptor (GRL1); and platelet-derived growth factor receptor (PDGFR). Reproduced from Postma et al<sup>9</sup> with permission.

which contribute to asthma are now very sophisticated,<sup>2</sup> the two studies which form the basis for this review illustrate the potentially conflicting conclusions which could be drawn. Meyers<sup>16</sup> gives an outstanding account of the pitfalls in genetic studies of asthma, along with a crisp explanation of the jargon of linkage. Reports in 1995 continued to focus on linkage of asthma to genes on chromosome 11 in the area of the high affinity IgE receptor<sup>17</sup> and genes on chromosome 5 in the area of a cluster of cytokines.<sup>18</sup> The focus itself is a potential pitfall because, without a genome-wide search, we cannot estimate the number of genes which may contribute to the clinical phenotype of asthma, nor can we estimate their relative importance. The hot spots of asthma research have arisen partly from serendipity – for example, the availability of a polymorphic marker – and partly from the likely involvement of the eosinophil and/or IgE systems in the pathogenesis of this disease.

#### HUMAN CHROMOSOME 5

The balance between interferon- $\gamma$  and IL-4 responses in mice<sup>15</sup> reflects the participation of separate populations of T cells, described as Th1 and Th2 cells, respectively. IL-4 is required for IgE antibody responses and the mice separate into high and low IgE responders on the basis of their cytokine phenotype. The human chromosome 5 has several cytokine genes of which IL-3, IL-4, IL-5, IL-9, and IL-13 are close to band 5q31.1. Serum IgE levels show a very close linkage to a polymorphism in the second intron (that is, the non-coding segment) of the IL-4 gene in the Pennsylvania Old Order Amish.<sup>19</sup>

The new sibling pair study of 303 children and grandchildren of 84 Dutch asthmatics by Postma and colleagues<sup>9</sup> showed that the inheritance of BHR was linked



**Figure 3** Map showing the order of some genes of interest in the human c11q12–13 region. The entire region encompasses only about 7 Mb of DNA and the CC10 gene (shown as UGB) is separated by only 3 Mb of DNA from the FCε RIβ gene. ROM1 = retinal outer membrane protein; FTH1 = ferritin heavy polypeptide 1; PGA = pepsinogen; GIF = gastric intrinsic factor; TCN1 = transcobalamin 1. Figure contributed by Dr Pamela Fain and compiled from Single Chromosome Workshop on Chromosome 11, 1994. Genome database.

to the inheritance of a marker in the D5S436 region of 5q31–q33 (fig 2). The subjects with BHR tended to be concordant with respect to their serum IgE levels with the correlation increasing as BHR was more stringently defined. The serum IgE levels did not correlate with the degree of histamine sensitivity in the BHR tests. The authors interpret their findings as showing the presence of one or more loci in the 5q31–q33 regions which contributes to BHR and to regulating IgE levels, while acknowledging that they cannot incriminate any particular gene in the region, nor exclude the presence of other BHR associated loci elsewhere in the genome. In light of the results in the murine model reviewed above, one must wonder whether polymorphisms affecting IL-5 availability may contribute to BHR through an eosinophilia mechanism.

#### HUMAN CHROMOSOME 11

A microsatellite polymorphism at c11q led Cookson and coworkers to a linkage with asthma in 1989.<sup>20</sup> Subsequent studies have narrowed the linkage to a stretch of DNA of about 7 cM located between D11S480 and D11S451 (fig 3).<sup>17,21</sup> The interval contains the important candidate gene for the high affinity IgE receptor beta subunit (FCε RIβ). However, the FCε RIβ gene is not the only potential culprit on chromosome 11 and Hay and coworkers have recently pointed out that the CC10 gene product is expressed in non-ciliated airway epithelial cells.<sup>22</sup> Production of the CC10 protein in the lung is increased by corticosteroids and the protein

itself is known to inhibit phospholipase A2, which is the rate limiting step in the synthesis of prostaglandin and leukotrienes. This suggests that the CC10 gene product may be important in modulating inflammation within the airways. However, the FCε RIβ and CC10 genes are both close to the centromere and are separated by about 3 megabases (Mb, one million base pairs) of DNA, too close for linkage mapping to tell us whether either of these genes, or other genes to which each may be linked, is directly involved in the pathogenesis of asthma. The studies focusing on chromosome 11 do not exclude the possibility of a linkage between c5q31 and asthma (and BHR). The entire human genome is thought to comprise about 3000 Mb and to encode perhaps 100 000 genes.

#### Clinical relevance

Do genetic linkage studies only obscure the management of chronic and sometimes dangerous disease with detail, inaccessible to therapeutic manipulation? The studies summarised above are hampered by the absence of a unique biochemical defect to characterise the disease. Furthermore, common diseases may have more than one underlying cause, genetic or otherwise. Not only are there differences based on the definition of the disease and the population being studied, but clearly the environment shapes the phenotypes encountered by clinicians in their daily practice. In this respect, whether asthma develops in the genetically susceptible individual may depend on exposure to maternal smoking, allergen load and duration of exposure, as well as other unrecognised stimuli that could influence the balance between Th1 and Th2 cells.

New phenotypes of asthma continue to be identified: nocturnal asthma has been linked to a Gly16 polymorphism of the β<sub>2</sub> adrenergic receptor<sup>23</sup> while patients with steroid resistant asthma have a distinct pattern of cytokine gene expression compared with patients with steroid sensitive asthma.<sup>24</sup> Given the growing numbers of phenotypes, the number of genes contributing to asthma may be large. Separate contributions by genes on different chromosomes are likely to result in a rather broad distribution for BHR in a population. The existence of intermediate phenotypes, and the potential for interactions between genes, could complicate the problem. For example, with prolonged exposure in certain occupational settings, a high proportion of

## LEARNING POINTS

- \* For diseases where there is a strong familial trend, but no simple inheritance, a minimum estimate for the number of genes which contribute susceptibility can be obtained by linkage analysis.
- \* Genome-wide linkage studies are the ideal way to start, but are so costly that they have been undertaken only for a small number of diseases, such as type 1 diabetes and essential hypertension.
- \* Linkage studies which focus on individual genes do not usually exclude a role for other genes, so apparently conflicting conclusions may be drawn.
- \* The results of linkage studies are also greatly influenced by the populations in which they are carried out.
- \* In the context of asthma, there is hope that linkage studies will point the way to identifying important pathogenic mechanisms and better directed treatments.

people may develop asthma which may continue even after the exposure is terminated.<sup>25</sup>

Expectations for a quick and dramatic transition from "bench to bedside" of genetic research have met with a number of obstacles. The fact that the well designed studies of van Herwerden *et al*<sup>8</sup> and Postma *et al*<sup>9</sup> reach different conclusions regarding the genetic linkage of asthma underscores the complexity of the disease that clinicians struggle with in their daily practice. Nevertheless, the recent identification of major loci associated with diabetes,<sup>26</sup> breast cancer,<sup>27</sup> and essential hypertension<sup>28</sup> confirms the usefulness of these methods for investigating the pathogenesis of complex genetic disorders. Thus, genetic studies of asthma and allergic diseases may help to identify the underlying immunopathological mechanisms. These studies could eventually lead to diagnosis prior to onset of clinical disease and help in the development of prevention strategies. If a genetic cause for asthma can be identified, then gene transfer therapy might be a feasible treatment option in the future.

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