Wheeze, sneeze and genes

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Recurrent wheeze and sneeze are becoming increasingly prevalent symptoms in developed countries. The principal cause is atopy or an allergic response to otherwise innocuous inhaled antigens such as the house dust mite, grass pollens and mould spores. The allergic response is mediated by exuberant production of *immunoglobulin* E (IgE) to these antigens [1]. In the mucosa of the lung and nose, IgE is bound to mast cells by the high affinity IgE receptor (FcεRI). Inhaled allergen reacts with mast cell bound IgE to trigger, via FcεRI, a cascade of transduction events leading to release or cell surface expression of a range of pro-inflammatory mediators [2]. They cause intense local mucosal inflammation and thereby the characteristic symptoms of allergic asthma and rhinitis including wheeze, cough, chest tightness, sneeze, nasal discharge and blockage.

Available indicators suggest that atopy results from the interaction of genetic and environmental factors, each of which may have multiple origins. The recent increase in the prevalence of atopic symptoms must be attributable to some form of 'environmental' changes but there is no clear indication what this or these might be. Most atopic individuals respond to multiple allergens and that suggests idiosyncratic or innate responsiveness. There has long been a strong clinical impression of familial aggregation of atopy but no simple mode of inheritance has been agreed. Twin studies, including those of twins reared together and apart, have consistently suggested a heritability (that fraction of phenotype attributable to genetic factors) of approximately 50% for total serum IgE levels [3].

In Oxford, we have attempted to re-investigate the genetics of atopy—applying broad ranging measurements of IgE responses allied to a positional cloning approach using molecular genetic methods. IgE responses were defined by skin prick testing to the common allergens noted above, plus solid phase immunoassay for circulating IgE to the same allergens, and total serum IgE levels. These assays are all related, but it is generally acknowledged that the factors governing generalised IgE responsiveness (response to *any* one or more common allergens) are different from those determining allergen specific response [4]. Positional cloning describes an approach to the identification of a disease-conferring gene by increasingly refined regional chromosomal assignment. This approach is premised on searching for genetic linkage between the disease, however it may be inherited, and a range of highly polymorphic DNA marker loci. Many such markers are now available and are amenable to streamlined application.

In our early studies on families with atopic asthmatic probands, we observed vertical transmission of atopy from an affected parent in 90% of instances when atopy was assigned by a raised total serum IgE or a positive specific IgE response to *any* one or more common allergens. We then suspected predominantly dominant inheritance which now proves to be an over-simplification of the heterogeneous genetics of atopy [6]. However, our observation that atopy was often vertically transmitted encouraged us to proceed to molecular genetic linkage studies based on a random chromosome search.

Fairly quickly, genetic linkage was detected between atopy and a marker (D11S97) on the long arm of chromosome 11 [7] and this was robust to phenotype assignment. Linkage was detected in approximately 50–60% of the families recruited through a young atopic asthmatic proband [8,9]; evidently other genetic loci at other chromosomal regions should contribute to the atopy in the remaining families. This genetic heterogeneity is likely to account for the various other reports of positive, null or negative linkage between atopy and chromosome 11q13 [10]. There are now also reports of linkage between low total serum IgE levels and the interleukin cluster on chromosome 5 [11] and atopic IgE responses and the α/δ T cell receptor gene-complex region on chromosome 14 [12].

Further analysis of our extensive family data using affected sib pair methods unearthed a curiosity in the linkage of atopy to chromsome 11q13; the linkage was confined entirely to alleles from maternal chromosomes [13]. This observation was independent of the atopic status of the mother and suggests the possibility of genomic imprinting [14]. In this process, which is becoming increasingly recognised in both human and murine genetics, there is parent-specific silencing or imprinting of certain autosomal genes for one generation. Therefore there may be paternal imprinting, that is silencing of alleles transmitted through spermatogenesis, at the chromosome 11 atopy locus.

The observation that linkage was confined to maternal chromosomes was crucial for linkage mapping of the atopy locus on chromosome 11. In this exercise,

atopy was assigned to a confidence interval that included the gene for CD20, a differentiation antigen for B lymphocytes [9]. The co-location of CD20 and the partially homologous beta subunit of the high affinity IgE receptor (FcεRI-β) on mouse chromosome 19 suggested the latter might also be co-located with CD20 in man and therefore be a strong candidate for the atopy locus on chromosome 11. We isolated human FcεRI-β using a DNA amplification strategy based on a highly conserved FcεRI-β sequence in mice and rats. Human FcεRI-β sequences so obtained allowed the identification of a highly polymorphic micro-satellite repeat in the 5th intron of the gene; this proved valuable in assigning the gene securely to human chromosome 11q13 by genetic linkage.

These data and the central role of FcεRI in triggering allergic mast cell events made the beta subunit gene an excellent candidate gene for the chromosome 11 atopy locus.

We proceeded to conduct comparative sequence analysis on the whole of the coding sequence and splice site regions of FcεRI-β in atopics and controls. We found an atopic subject's chromosome with three nucleotide substitutions in the 6th exon. These mutations predicted the substitution of leucine for isoleucine (position 181) and leucine for valine (position 183) within the 4th transmembrane (TM) domain of FcεRI-β (Fig 1).

To test the prevalence of leucine residues at 181 and 183 of FcεRI-β, and their relationship to atopy, we used allele-specific DNA amplification (ARMS) and studied two subject groups: a random sample of patients (unselected for atopy) and 60 nuclear families ascertained through an atopic asthmatic proband with at least one other sibling and both parents (regardless of atopy status) available for study.

In the random patient sample, Leu 181 was found in 25 of 163 individuals (17%)—one of whom was homozygous, but none showed a Leu 183 substitution. The presence of Leu 181 was associated with high total serum IgE (odds ratio 3.07 (95% confidence interval 1.25–7.55)) and with positive IgE tests to grass pollen antigen (odds ratio 2.61 (95% CI 1.07–6.4)). Thirteen (56%) of the Leu 181 positive subjects were designated atopic (12 by positive RAST tests) and had a mean total serum IgE of 300 kU/L. Although total serum IgE varies with age, race and other variables, the upper limit of normal, by association with allergen sensitisation and allergic symptoms, is about 100 kU/L in non-smoking adults in Western populations.

Any association of Leu 181 with atopy in a random patient sample was likely to be weakened by the documented maternal inheritance of atopy on chromsome 11q. This predicted that half the individuals inheriting a chromosome 11q atopy mutation, ie those receiving the allele paternally, would be non-atopic; indeed, in our random patient sample, 11 of 24 (46%) Leu 181 heterozygotes were non-atopic. Family studies to identify the parental origin of Leu 181 were therefore necessary to clarify the relationship between genotype and phenotype. Ten (17%) of the 60 unrelated nuclear families (with origins scattered through eight counties in England and Wales) were found to have the Leu 181 variant segregating (Fig 2); this was confirmed by DNA sequencing. In each family the Leu 181 was maternally inherited ($p < 0.0001$). Amongst their children, Leu 181 was strongly associated with atopy (all 12 children with Leu 181 were atopic, ten of 12 Leu 181 negative children were not atopic, $p < 0.0001$). Atopy was present in a child without Leu 181 in two

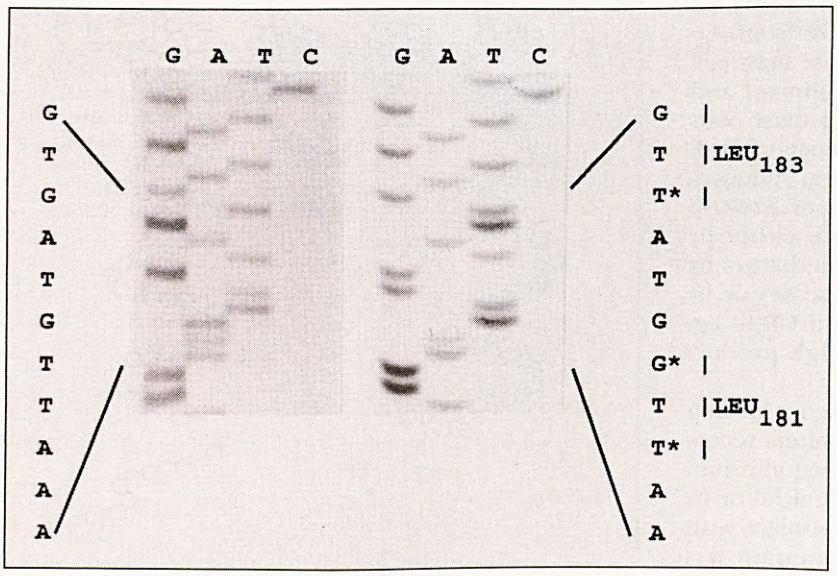


Fig 1. *DNA sequence at the junction of the 5th intron and 6th exon of the β subunit of FcεRI showing wild type sequence and sequence from an atopic individual with three base substitutions converting isoleucine to leucine 181 and valine to leucine 183.*

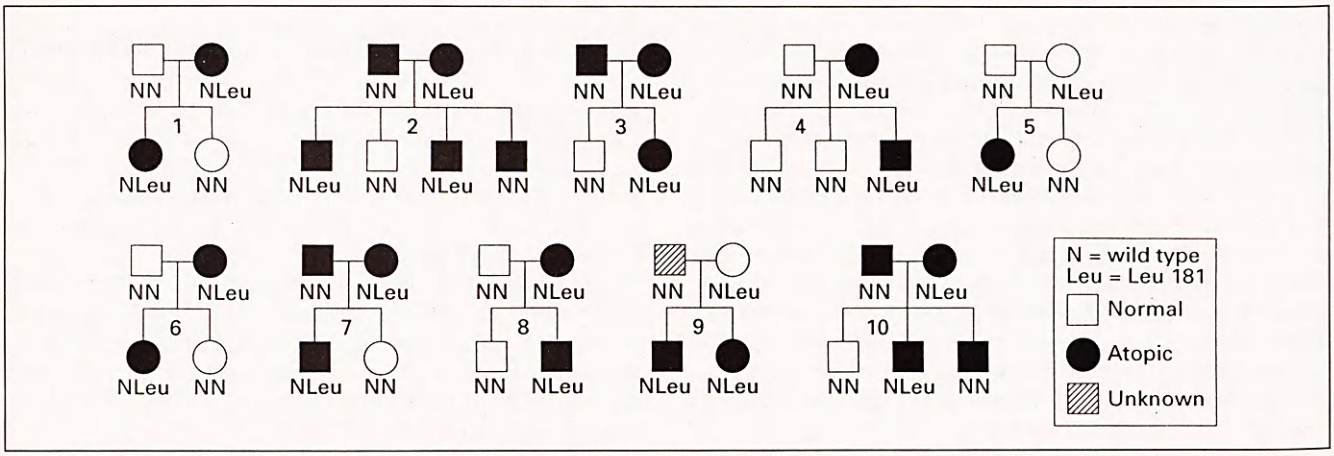


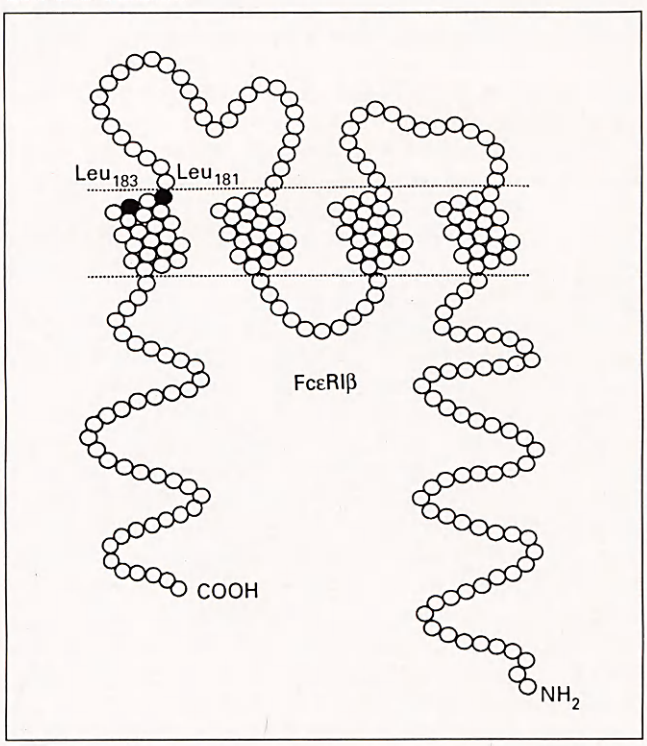
Fig 2. *Schematic model of the β-subunit of FcεRI demonstrating four transmembrane domains and the position of the leucine substitutions (181 and 183 as solid symbols) within the 4th transmembrane domain.*

families, and in each instance the father also had atopy without Leu 181. Eight of the ten Leu 181 heterozygous mothers were themselves atopic. DNA was available from both maternal grandparents in two families; Leu 181 was of grandmaternal origin where the Leu 181 mother was atopic, and of grandparental origin where the Leu 181 mother was non-atopic. The results strongly supported a maternal effect on phenotype development at the chromosome 11 atopy locus.

The strong association between maternally inherited Leu 181 and atopy in a set of unrelated families suggests that variants of FcεRI-β are one cause of atopic IgE responsiveness; this possibility is consistent with the known biological functions of the high affinity IgE receptor [2, 15]. FcεRI is comprised of three subunits α,β and γ_2 and is expressed on mast cells, basophils, monocytes and Langerhans cells [16]. The receptor plays a central role in the mediation of IgE dependent allergic inflammation and also in IgE metabolism and mast cell and B-lymphocyte differentiation and growth. Stimulation of FcεRI causes release from the mast cell of cytokines [2,12] which promote mast cell and helper T cell subtype 2 (TH2) development and IgE production by B-lymphocytes. Lung mast cells expressing CD40 ligand may, in the presence of IL-4, regulate local B lymphocyte IgE production independently of T lymphocytes [18]. Variants of FcεRI-β might therefore promote the atopic state either by enhanced release of pro-inflammatory mediators by mast cells (to cause more symptomatic disease) or by enhanced mast cell expression of IL-4 and CD40 ligand (to cause more local B lymphocyte IgE production).

We do not now know whether the presence of Leu 181 within the 4th TM of FcεRI-β (Fig 3) alters receptor function or whether it is in linkage disequilibrium with undiscovered functional variants of FceRI-ß or its controlling elements. But in the atopic subject with both Leu 181 and Leu 183, no other mutation was detected in full coding and splice site sequences of FcεRI-β. Alpha-helical TMs do play a central role in the function of FcεRI and similar receptors in which non-ionic interactions between non-polar amino acids regulate the relationship of the helices and influence signal transduction [19]. Single amino acid changes within TMs of other seven-helix bundle receptors have major functional effects; these include 10–20 fold

Fig 3. *ARMS testing for Leu 181 in 60 nuclear families identified through an asthmatic proband. The ten families with the variant are shown. No family was found with the Leu 183 variant.*



changes in ligand binding in the 5-hydroxytryptamine receptor [20], and causation of familial male precocious puberty in the luteinising hormone receptor [21]. The exchanges of aliphatic amino acids (Ise-Val-Leu) within a TM of FcεRI-β parallel species-specific variants of the brain cholecystokinin-B/gastrin receptor which result in 20-fold altered affinity for benzodiazepine-based antagonists [22].

If FcεRI-β variants are a cause of atopy, their relatively high population frequency suggests that they may also confer some evolutionary advantages. Exuberant IgE responses and inflammatory cascades might enhance resistance to certain infections, in particular the intestinal helminths [23].

In summary, atopy is a heterogeneous disorder, determined by genetic and environmental interactions; it is characterised by enhanced IgE responses and underlies the common diseases of allergic asthma and rhinitis. We have observed maternal genetic linkage of atopy to chromosome 11q13 in some 50–60% of families with an atopic proband suffering from asthma and rhinitis. We have found variants of the β subunit of the high affinity IgE receptor (FcεRI-β), encoded on chromosome 11q13, in which there are substitutions of leucine for isoleucine and valine at positions 181 (Leu 181) and 183 (Leu 183) within the 4th transmembrane domain of the molecule. Leu 181 is strongly associated with atopy by maternal descent.

Functional studies on the cellular and molecular consequences of the FcεRI-β variants are in progress.

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