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

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Summary

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 (FcεRIβ) 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 FcεRIβ gene with clinical asthma and the underlying phenotypes of BHR (to methacholine) and atopy (defined by skin prick 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 FcεRIβ 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 FcεRIβ gene (7.2% excess of shared alleles, \(p=0.124\)).

These findings suggest that mutations in the FcεRIβ gene or a closely linked gene influence the BHR underlying asthma, even in the absence of atopy.

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Introduction

Asthma results when genetically predisposed individuals are exposed to certain environmental risk factors. Much effort has been directed towards the identification of an asthma gene, although most studies have examined the phenotype of atopy,1–8 which is a predisposition to produce excess amounts of IgE. Atopy commonly exists without overt clinical manifestation but is associated with a heterogeneous group of clinical disorders including eczema, hay fever, and asthma. In the general population, the majority of atopic subjects do not have asthma. Furthermore, asthma may occur in the absence of other obvious allergic features, although most young asthmatic patients are atopic, and allergy to common inhaled environmental proteins is the commonest aetiologic stimulus to the disease process.

Some1,2 but not all14 studies have reported linkage of atopy to chromosome 11q13, and specifically to the gene encoding the β subunit of the high-affinity IgE receptor (FcεRIβ) in this chromosomal region. The conflicting findings may reflect clinical as well as genetic heterogeneity in atopic subjects. Insufficient sample size, ascertainment bias, and inadequate phenotypic classification may also contribute to the discrepant results.

Our aim was to examine linkage of the FcεRIβ gene to asthma defined in a clinically relevant way and to the underlying phenotypes of bronchial hyperreactivity (BHR) and atopy. A sample of suitable size was drawn from the general Melbourne population, and the affected sibling-pair method was used.

Methods

These studies were approved by the Alfred Hospital Ethics Review Committee. Recruitment for screening was part of the European Community Respiratory Health Survey (ECRHS).9 Our centre was the only Australian centre in this international study of lung health in young adults. 4500 individuals aged 20–45 years selected randomly from the electoral roll were invited to participate. 3200 individuals completed a screening questionnaire, which provided demographic information and details of respiratory and allergic symptoms. A subgroup of 757 attended our respiratory function laboratory for more detailed characterisation of atopy and BHR. The validity of the questionnaire has been documented elsewhere.10,11 We identified individuals who satisfied the criteria for BHR, atopy, or both, and reported that they had a natural sibling living in Melbourne. Siblings were invited to participate, complete the questionnaire, and have the same phenotypic tests. We identified 123 affected sibling-pairs (114 men, 132 women) who shared at least one of the phenotypes.

The asthma phenotype was defined according to the criteria adopted for follow-up in the ECRHS study10—ie, an episode of asthma in the previous 12 months, or the use of oral or inhaled asthma medication, or nocturnal shortness of breath.

Skin sensitivity to specific allergens was used to define atopic status. All antigens were standard extracts and from the same batch. They included cat, housedust-mite, rye grass, timothy grass, plantain, birch, olive, ragweed, parietaria, alternaria, and cladosporium. Individual antigen-coated lancets (Phazets, Kabi- Pharmacia, Uppsala, Sweden) and two control lancets (histamine
were affected and concordant for at least one phenotype. The resolution of this system was able to detect differences in DNA by standard techniques. Primers flanking the variable nucleotide repeat sequence in intron 5 of the FcE Rip gene\(^3\) were used to amplify the marker by PCR. PCR reactions were carried out in 15 \(\mu\)L mixtures of 100–200 ng genomic DNA, 2 \(\mu\)mol/L of each primer, 200 \(\mu\)mol/L of each deoxynucleotide, 2 mmol/L magnesium chloride, and 0.5 units Taq polymerase (Boehringer Mannheim). Amplification conditions were as described previously.\(^3\) Alleles were visualised by laser scanning (Molecular Dynamics FluorImager, California, USA) after separation on 8% polyacrylamide gels and 20 min after staining with SYBR green II dye (Molecular Probes Inc, California, USA). The largest diameter and that perpendicular to it were measured.\(^*\) We defined atopy as a wheal of diameter 3 mm or greater to one or more antigens in the presence of a positive histamine control and a negative uncoated control.

A methacholine challenge was used for bronchial provocation to assess BHR. Methacholine was administered in a standard reproducible way by dosimeter (Mefar, Borezzi, Italy). We used methacholine rather than histamine because it produced fewer side-effects and a higher effective dose could be administered. Lung function (forced expiratory volume in 1 s: FEV\(_1\)) was measured with a computerised rolling-scan spirometer (Sensor Medics, California, USA). BHR was defined as a reduction in FEV\(_1\) of 20% or more (PD\(_{20}\)) at a cumulative dose of 2 mg (10-2 \(\mu\)moles) or less of methacholine.

Probands and siblings were questioned about parental history of asthma. Further phenotypic characterisation or blood sampling of parents was not part of this study.

10 mL samples of blood in EDTA were taken for extraction of DNA by standard techniques. Primers flanking the variable nucleotide repeat sequence in intron 5 of the FcE Rip gene\(^3\) were used to amplify the marker by PCR. PCR reactions were carried out in 15 \(\mu\)L mixtures of 100–200 ng genomic DNA, 2 \(\mu\)mol/L of each primer, 200 \(\mu\)mol/L of each deoxynucleotide, 2 mmol/L magnesium chloride, and 0.5 units Taq polymerase (Boehringer Mannheim). Amplification conditions were as described previously. Alleles were visualised by laser scanning (Molecular Dynamics FluorImager, California, USA) after separation on 8% polyacrylamide gels and 20 min after staining with SYBR green II dye (Molecular Probes Inc, California, USA). The resolution of this system was able to detect differences in allele size of 1 base-pair.

Genotyping was undertaken in 137 sibling-pairs, 123 of whom were affected and concordant for at least one phenotype. The remaining 14 pairs did not share an affected phenotype (discordant). These pairs were included randomly in PCR experiments as controls. Allele sizes were evaluated independently by two investigators (LvH, SBH) who were unaware of the phenotypic characteristics or concordance of the subjects. When the investigators disagreed about allele sharing by siblings or about allele sizes, they reviewed the gels together and reached consensus. Occasionally genotyping had to be repeated before consensus was reached.

Linkage was defined as sharing of marker alleles by affected siblings concordant for a phenotype greater than that expected by chance alone from the identity-by-state methods of Lange.\(^*\) The population marker allele frequencies were estimated from the sample data.\(^*\) Linkage analysis was based on the clinical and underlying phenotypes. Therefore, sibling-pairs were grouped according to concordance for asthma or atopy or BHR. To separate the relation between the Fce RI\(\beta\) receptor gene and atopy or BHR alone, we also analysed sibling-pairs selected on the basis of two phenotypes—ie, concordance for atopy without BHR, or concordance for BHR without atopy, or concordance for both atopy and BHR.

### Results

We identified 11 different alleles in the 274 individuals genotyped (table 1). The findings were similar whether all siblings or only the 137 unrelated probands were analysed. The calculated heterozygosity index in our population was 82% with a normal distribution of allele sizes.

67 sibling-pairs were concordant for clinical asthma and shared 18-0% more alleles than would be expected in the absence of linkage (table 2, \(p=0.002\)). Concordance for BHR was found in 53 sibling-pairs, all of whom reported clinical asthma, and alleles of the Fce RI\(\beta\) gene marker were shared 21.7% more frequently than expected (\(p=0.001\)). When 36 sibling-pairs concordant for BHR with atopy were excluded from the analysis, the remaining 17 sibling-pairs concordant for BHR but not atopy showed a significant 32.8% excess of shared alleles (\(p=0.004\)).

106 sibling-pairs were concordant for atopy (with or without BHR) and showed linkage to the Fce RI\(\beta\) locus with a 10.3% excess of shared alleles (\(p=0.02\)). The 70 sibling-pairs concordant for atopy without BHR did not show significant evidence of genetic linkage to the Fce RI\(\beta\) gene (7.2% excess, \(p=0.124\)). The pairs concordant for both BHR and atopy showed an intermediate (16.5%, \(p=0.028\)) excess of shared alleles.

Our conclusions did not depend on a single arbitrary definition of BHR (ie, a reduction of 20% at least in FEV\(_1\) in response to methacholine). When we examined sibling-pairs (\(n=18\)) who were concordant for a reduction in FEV\(_1\) of between 10% and 20% we also found significant linkage to the Fce RI\(\beta\) locus (data not shown, \(p=0.007\)). Furthermore, we could find no significant evidence of linkage in atopic siblings (\(p=0.268\)) when we excluded those with BHR defined as a 10% or greater fall in FEV\(_1\).

42 of 123 probands reported a parental history of asthma (22 fathers, 20 mothers). The prevalence of maternal and paternal history of asthma was indistinguishable statistically (McNemar's test, \(\chi^2=0.21, p=0.65\)). Of the 70 sibling-pairs concordant for atopy without BHR, 12 reported a maternal history and nine a paternal history of asthma. Of the 17 sibling-pairs with BHR in the absence of atopy, none reported a maternal history of asthma, whereas five reported a paternal history.

### Discussion

In this study we found genetic linkage between the Fce RI\(\beta\) locus on chromosome 11q13 and clinical asthma. This is the first study of the genetics of asthma in the general population. Previous studies have been based on a general allergic tendency as characterised by atopy or have involved families recruited through clinic-based ascertainment.

Our study also gave some genetic insight into the phenotypes underlying asthma—BHR and atopy.
Although previous studies have reported linkage between atopy and the FcεRIβ locus, this linkage did not seem to explain the association between this locus and asthma in our population. There was evidence of significant linkage between BHR and the FcεRIβ locus, but we could find no evidence of significant linkage between atopy and the locus. The link with BHR independent of atopy was emphasised by the analysis of non-atopic sibling-pairs with BHR, who showed the strongest evidence of linkage (as measured by the excess of shared alleles) with the 11q1 locus. These findings suggest that the genetics of clinical asthma in the general population depend on a mutation or mutations in or near the FcεRIβ locus that affects the reactivity of the airways.

The nature of the gene on chromosome 11q13 that causes BHR is not yet clear. The FcεRIβ locus is simply a marker in these analyses. It is possible that mutation in the IgE receptor itself might alter the interaction with ligands resulting in BHR by enhancement of the inflammatory response of mast cells, even in the absence of atopy. However, it is also conceivable that other closely linked genes in this region may cause BHR through effects on airway mechanics, structure and function of bronchial smooth muscle, or autonomic neural control.

Not all asthma is associated with allergy, and most atopic individuals are not asthmatic. BHR may occur in atopic individuals as a result of sensitisation—i.e. IgE antibody production after allergen exposure. However, non-atopic BHR may be produced by other environmental factors such as viral infection, occupational chemical exposure, and air pollution in the absence of specific IgE. Genetic predisposition to atopy and BHR may be determined separately; there is evidence from biometric family studies to support this possibility. We believe that an underlying tendency to BHR is determined by a gene in close relation to the IgE receptor on chromosome 11q13. Other studies have suggested that susceptibility to atopy may be the result of another gene on chromosome 5q31. The isolated actions of a BHR gene might explain intrinsic or cryptogenic asthma, whereas an atopic gene might lead to typical allergic asthma associated with hay fever or eczema. The inheritance of both BHR and atopy genes would result in greater susceptibility to BHR and increase the likelihood of asthma in an atopic individual.

Our observations are relevant to the disagreement about linkage of atopy to chromosome 11q. Previous discrepant findings have been explained in terms of genetic heterogeneity, ascertainment bias, insufficient statistical power, environmental differences, and maternal inheritance. However, overlap between the subsets of atopic and BHR in the asthmatic population might confound genetic analyses. Our findings suggest that detection of linkage with the 11q13 locus in unselected atopic subjects might depend on the underlying proportion with BHR. This proportion depends partly on the method of ascertainment. For instance, Sandford et al. found positive linkage in subjects recruited largely through a respiratory clinic. Although BHR was not measured, this group of atopic individuals might be expected to have particular predisposition to BHR. Studies that did not detect linkage between chromosome 11q and BHR had low proportions of BHR or did not assess BHR. In many studies, subject numbers and statistical power were unsatisfactorily low.

Our analysis was based on the affected sibling-pair method. We chose this technique because it avoids the confounding introduced when genetically predisposed individuals do not express a phenotype (false negatives) because of age-related or seasonal variation. It also offers the advantage that a large number of small families is likely to be more representative than a small number of large families. However, the identity-by-state method used in this study relies on an estimate of the frequency distribution of alleles and is sensitive to the polymorphism of the markers used. It appears that the phenotype expression of an 11q13 locus depends on maternal inheritance. In general, we found linkage for BHR and atopy in groups that reported similar distribution of asthma in mothers and fathers. In particular, in the group with apparently strongest linkage (BHR without atopy), no subject reported maternal asthma, whereas five reported paternal asthma. A more formal study of this feature would require phenotypic characterisation and DNA analysis of all parents.

We have shown that the gene encoding the β chain of the high-affinity IgE receptor is linked to asthma, predominantly through BHR. This finding may lead to important insights into the relation between atopy and asthma and the induction of BHR in non-atopic individuals.

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Gut inflammation in children with cystic fibrosis on high-dose enzyme supplements

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We used a whole-gut perfusion technique to study subclinical gut inflammation in children with cystic fibrosis (18 elective tests, three lavages to treat distal intestinal obstruction syndrome); and in 12 control children with constipation or pre-colonoscopy. We assayed for haemoglobin, IgG, albumin, alpha-1-antitrypsin, granulocyte elastase, interleukin-1β (IL-1β) and IL-8 concentrations in whole-gut lavage fluid. Results for two children with distal intestinal obstruction syndrome, the only children in the series taking Nutrizym 22, were strikingly abnormal. This new test has revealed subclinical gut mucosal inflammation in a minority of CF children, for which distal intestinal obstruction syndrome, Nutrizym 22 treatment, or both, may be risk factors.

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The development of fibrotic strictures and ulceration of the ascending colon in some children with cystic fibrosis (CF), is known as fibrosing colonopathy (FC), and was first reported in January, 1994.1 The pathogenesis of this disorder is unknown, but there is a possible association with use of new formulations of high-dose pancreatic enzyme supplements.2

Whole-gut lavage is used in children and adults for bowel cleansing, in the management of severe constipation, and to treat distal intestinal obstruction syndrome in CF. Tests on the resulting clear rectal fluid, essentially a gut perfusate, can provide information on gut immunity and inflammation.2 It seemed likely that if subclinical gut inflammation were present in CF children, it could be detected by this method.

Whole-gut lavage was performed electively, in 12 CF children (11 boys, one girl, age 3-14 years) taking high-dose enzyme supplements. In five, a repeat lavage was performed after they changed to low-dose enzymes. Three CF children had lavage performed as treatment for distal intestinal obstruction syndrome; all three had abdominal pain, constipation, and a palpable right iliac fossa mass. In two children, barium enema examinations 10 and 2 months later (after they had changed to low-dose pancreatic enzymes) were normal. One child had a repeat lavage test after changing to low-dose pancreatic enzyme supplements. In the case of albumin, concentrations were higher than the adult reference range in nine specimens from CF children: four electively studied while on high-dose pancreatic enzyme supplements for alpha-1-antitrypsin, marginally high values were found in only two children, both on high-dose pancreatic enzyme supplements. In the case of albumin, concentrations were higher than the adult reference range in nine specimens from CF children: four electively studied while on high-dose pancreatic enzyme supplements two who had repeat tests on low-dose pancreatic enzyme supplements, and all three with distal intestinal obstruction syndrome. As a group, the children with CF had significantly higher concentrations than control children, probably because albumin is sensitive to proteolysis and the CF children had pancreatic insufficiency.

We found high values for whole-gut lavage fluid haemoglobin were found in three CF children on high-dose pancreatic enzyme supplements.

Abnormally high concentrations of IgG were present in whole-gut lavage fluid from two CF children. Both had lavage treatment for distal intestinal obstructive syndrome and were taking high-dose pancreatic enzyme supplements. For alpha-1-antitrypsin, marginally high values were found in only two children, both on high-dose pancreatic enzyme supplements. In the case of albumin, concentrations were higher than the adult reference range in nine specimens from CF children: four electively studied while on high-dose pancreatic enzyme supplements two who had repeat tests on low-dose pancreatic enzyme supplements, and all three with distal intestinal obstruction syndrome. As a group, the children with CF had significantly higher concentrations than control children, probably because albumin is sensitive to proteolysis and the CF children had pancreatic insufficiency.

We found high values for whole-gut lavage fluid granulocyte elastase in six of 21 tests in CF children; one child on low-dose pancreatic enzyme supplements and five taking high-dose pancreatic enzyme supplements, including extremely high concentrations (725, 1943 nKat/L), of the same order of magnitude as we find in active Crohn’s disease, in specimens from the two children with distal intestinal obstruction syndrome taking high-dose pancreatic enzyme supplements.