Mutational analysis of the high affinity immunoglobulin E receptor β subunit gene in asthma

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Abstract

***Background*—The gene for the β subunit of the high affinity receptor for immunoglobulin E (FcεRI-β) on chromosome 11q13 is linked with clinical asthma and certain mutations have been identified. A study was undertaken to identify DNA variation in the FcεRI-β gene in a population sample in which linkage between 11q13 and asthma was explained by bronchial hyperreactivity (BHR) but not atopy.**

***Methods*—DNA samples from 71 subjects with asthma, atopy, or BHR were analysed. The complete coding region, some of the introns, and some of the 5' untranscribed region of the FcεRI-β gene were sequenced.**

***Results*—In the subjects studied there were no deviations from the published sequence in any of the seven coding exons of the FcεRI-β gene. In particular, the three previously reported mutations (Ile181, Leu183, Glu237) were not detected. Two new polymorphisms were discovered, one at position 243 in the 5' untranscribed region and one at position 4390 in intron III. Neither of these variants showed significant association with asthma, atopy, or BHR.**

***Conclusions*—These results suggest that, in the population studied, linkage of asthma and BHR to 11q13 is not explained by mutations in the FcεRI-β gene. Other mutations in the non-coding region of this gene or in adjacent genes must explain the linkage findings in this study.**

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Asthma results when genetically predisposed individuals are exposed to certain environmental risk factors. Much effort has been directed towards the definition of the "asthma gene" although asthma is likely to be genetically and phenotypically complex. Allergic tendency, manifest as atopy and non-specific bronchial hyperreactivity (BHR), are considered underlying phenotypes.

Atopy, as defined by a skin prick sensitivity test to common allergens, frequently exists without overt clinical manifestation, but is associated with a heterogeneous group of clinical conditions including eczema, hayfever, or asthma. In the general population most atopic subjects do not have asthma. Furthermore, asthma may occur in the absence of other obvious allergic features, although most young asthmatics are atopic. BHR to non-specific stimuli is a common feature in asthma, to such an extent that it is used as a diagnostic test. There is some overlap, but also independence of BHR and atopy, and these two phenotypes may have separate genetic causes.

Some[1–3] but not all[4–10] studies have reported linkage of atopy to chromosome 11q13, and specifically to a candidate gene in this chromosomal region: the gene for the β subunit of the high affinity immunoglobulin E receptor (FcεRI-β). The coincidence or independence of atopy and BHR was not analysed in these studies. However, we have reported linkage between the FcεRI-β gene and asthma.[11] This linkage was explained by BHR rather than atopy. It has been suggested that particular mutations in the FcεRI-β gene may alter the function of the receptor and predispose to asthma through atopy.[2 12] The aim of this study was to determine whether we could detect mutations in the FcεRI-β gene that could explain the observed linkage with BHR and asthma in our population.

Methods

SUBJECTS AND CLINICAL PROTOCOL

These studies were approved by the Alfred Hospital ethics review committee. The selection of subjects and phenotypic determination were as described previously.[11] In brief, recruitment for screening was part of the European Community Respiratory Health Survey (ECRHS) which has been described elsewhere.[13] Four thousand five hundred adults aged 20–44 years were randomly selected from the electoral roll. Postal questionnaires were returned by 3200 (72%) subjects in the first phase of the ECRHS. A total of 757 subjects attended the laboratory for testing. They comprised a random sample of 553 young adults and an additional 204 subjects with symptoms of asthma. The latter group were included according to the ECRHS protocol to enrich samples for asthma,[13] although the prevalence of asthma in our random sample was already relatively high (17.4%).[14] Clinical asthma was defined using a validated questionnaire,[13] as wheeze or the use of asthma medications in the previous 12 months. Skin sensitivity (a weal of more than 3 mm diameter) to common aeroallergens was used to define atopic status.[11] A methacholine challenge was used for bronchial provocation to determine BHR which was

Table 1 PCR primers for amplification of the coding region, the intron-exon boundaries, and some 5' untranscribed region of the β subunit of the high affinity immunoglobulin E receptor gene

Primer	*Primer sequence*	*Nucleotide position (5'-3')*	*PCR product (bp)*
Exons 1, 2 and 3			
IGER135	5'-GGG ACA ATT CCA GAA GAA GGG-3'	65–86	2303
IGER133	5'-GGG CTA AAT GTT TAA CCC ACC G-3'	2368–2347	
Exons 4, 5 and 6			
IGER465	5'-CCA CAC CCG CCT TAT TCG TA-3'	4270–4289	1534
IGER463	5'-TTT TAA GGA ATA AAG CTC CAA TTC G-3'	5804–5780	
Exon 7			
IGER75	5'-GAG TTT AAT GAC AGA GAG CGT-3'	7100–7120	406
IGER73	5'-AAA TAG AAG GAG CAT ATT AAG GT-3'	7506–7484	

defined as a reduction in forced expiratory volume in one second (FEV_1) of 20% or more at a cumulative dose of ≤2 mg (10.2 μmol) methacholine.[11]

For our original linkage analysis we obtained DNA from sibling pairs (n = 123 pairs) who shared at least one of the relevant phenotypes.[11] Because of the genetic link between the FcεRI-β gene and BHR in this population,[11] we searched initially for evidence of DNA mutations in subjects expressing the BHR phenotype alone. There were 34 individuals who expressed BHR without atopy[11] and we sequenced successfully DNA in 32 of these subjects. In addition, we sequenced the DNA from eight subjects chosen at random from the 72 individuals with BHR and atopy for whom DNA was available. Observed sequences were compared against published data. However, for additional control comparison we also sequenced DNA from a random sample of 20 subjects from our population without BHR (12 subjects with atopy in the absence of BHR and eight with neither BHR nor atopy). In a small number of individuals, complete unequivocal sequence was not obtained for some gene regions. Exact numbers are reported in the results section. For the case-control comparison of the new mutations (see below) we selected at random further individuals expressing particular phenotypes until the phenotype distribution was balanced and provided suitable statistical power. This resulted in the study of 71 subjects with phenotypes distributed as follows: asthma, 44 affected, 27 unaffected; BHR, 48 affected, 25 unaffected; and atopy, 44 affected, 27 unaffected.

GENOMIC AMPLIFICATION AND SEQUENCING

A 10 ml sample of blood in EDTA was drawn to extract DNA by standard techniques.[15]

Three sets of primers were chosen to produce three fragments encompassing the whole protein coding region and a section of the 5' untranscribed region of the FcεRI-β gene (table 1). Primer sequences were derived from the human FcεRI-β gene sequence.[16] The fragment sizes ranged from 406 bp to 2303 bp. Standard polymerase chain reaction (PCR) was carried out in a 50 μl volume containing 100 ng genomic DNA, 10 pmol of each primer, 67 mM Tris/HCl (pH 8.8), 16 mM $(NH_4)_2SO_4$, 0.2 mg/ml gelatin, 0.45% Triton X-100, 1.5 mM $MgCl_2$, 200 μM each dNTP and 0.5 U *Taq* polymerase (Bresatec). Samples were processed in a Corbett Research thermal cycler. After initial denaturation at 95°C for five minutes, 35 temperature cycles were carried out consisting of 1 min at 94°C, 1.5 min at 60°C, and 2.5 min at 72°C, followed by a final extension at 72°C for 5 min. For the exon 7 amplification the annealing and extension times were reduced to 1 min each. All amplifications produced a single band, with no bands detected in the no DNA or single oligonucleotide controls. After amplification, PCR products were purified using the QIAquick purification kit (QIAGEN).

PCR fragments were sequenced manually using the CircumVent thermal cycle sequencing kit (New England Biolabs) and ^{32}P-labelled sequencing oligonucleotides according to the manufacturer's instructions. The following sequencing primers were used (location of the 3' end of the primer given in parentheses): 5' untranscribed region, 5'-CTT ACT GCA TGC TCT GAA TAG GC-3' (368); exon 1, 5'-AAA GTT TCA TCT CCT AAG CAC CG-3' (574); exon 2, 5'-CTC CCC TTT CTG TCT GTC GAG-3' (1321); exon 3, 5'-AAA CAA CTG GTT AGA TCT GAG-3' (2218); exon 4, 5'-CTT CTT ATC TTT TCA AGG ATG GAC-3' (4555); exon 5, 5'-CCA GCC CTG AAA TGA AGA TAG G-3' (5020); exon 6, 5'-CTT TTG GGG CGA ATA CCA ATG TG-3' (5604); exon 7, 5'-CTT GAG CGA GAC TTC TAG GGA T-3' (7178). In view of some discrepancies in published results using different methods[17] for the detection of the Leu181 and Leu183 variants, we performed controls to determine whether we could detect the Ile181Leu variant. We generated the Leu181 variant in vitro by site directed mutagenesis.[18] Wild type and mutant PCR fragments were then mixed 1:1 and 3:1 to model a heterozygote and a weak heterozygote, respectively. The fragments were sequenced and the Leu181 variant was clearly seen even when the mutant fragment constituted only 25% of the template for sequencing. We were therefore able to detect the in vitro generated variant without difficulty.

STATISTICAL ANALYSIS

The proportional distribution of genotypes in affected and unaffected individuals for each phenotype was compared using contingency tables and χ^2 analysis or Fisher's exact test. Statistical significance was accepted as $p<0.05$.

Results

We initially screened by sequencing for the previously described Leu181 and Leu183 variants in exon 6 of the FcεRI-β gene.[2] We

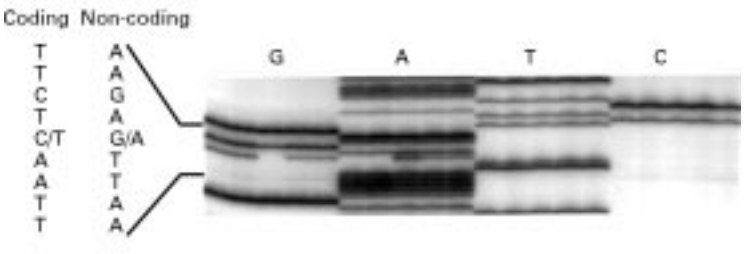

Figure 1 Direct sequencing of genomic DNA. The nucleotide sequence from five subjects around the polymorphism at position 243 in the FcεRI-β gene is presented. The nucleotide sequence of the coding and non-coding strands of a section of the sequence is shown.

analysed DNA from 32 subjects with BHR alone, eight subjects with atopy and BHR, 12 subjects with atopy but not BHR, and eight unaffected subjects. However, in none of the subjects tested did we find the Leu181 or Leu183 variants.

We then sequenced the other six exons and the intron-exon boundaries of the FcεRI-β gene in the same group of subjects (32 subjects with BHR alone, four with atopy and BHR, four with atopy but not BHR, and four unaffected controls). In none of the subjects did we detect any deviation from the published sequence. In particular, we did not detect the recently reported Gly237 variant in exon 7.[12]

In the course of sequencing the FcεRI-β gene we discovered two previously undescribed polymorphisms. The first was a C/T polymorphism at position 243 in the 5' untranscribed region and the second was a G/T polymorphism at position 4390 in intron III. The sequencing of the C/T polymorphism at position 243 in the 5' untranscribed region is shown in fig 1.

We analysed each mutation for association with asthma, BHR, and atopy. For the C/T polymorphism at position 243 in the 5' untranscribed region we sequenced 32 subjects with BHR alone, 10 with BHR and atopy, 24 with atopy alone, and five unaffected subjects. For the G/T polymorphism at position 4390 in intron III we sequenced 32 subjects with BHR alone, 23 with atopy alone, two with atopy and BHR, and four unaffected individuals. The results in table 2 indicate that neither polymorphism showed significant association with asthma, atopy or BHR.

Table 2 Characterisation of DNA sequence variants in the FcεRI-β gene

Genotype	*C/C*	*C/T*	*T/T*	*Phenotype p*
5' untranscribed region polymorphism (position 243)				
Asthma				
No	6	15	5	
Yes	11	17	16	
				0.232
Atopy				
No	5	10	7	
Yes	12	22	14	
				0.967
BHR				
No	6	11	7	
Yes	11	20	13	
				0.999
Intron III polymorphism (position 4390)				
Asthma				
No	6	7	6	
Yes	9	18	13	
				0.732
Atopy				
No	4	7	8	
Yes	11	18	11	
				0.530
BHR				
No	6	10	5	
Yes	9	12	14	
				0.438

Discussion

It has been suggested that mutations in the FcεRI-β gene may alter the function of the receptor and predispose towards asthma.[2 12] In this study we used a subject group drawn from the general population in Melbourne which was carefully phenotyped for asthma, atopy, and BHR. Linkage analysis of this group showed significant association between the FcεRI-β gene and asthma.[11] When the underlying phenotypes of atopy and BHR were examined it was found that the linkage could be explained by BHR alone. We could not find evidence of significant linkage between the FcεRI-β gene and atopy alone. The aim of this study was to determine whether mutations in the FcεRI-β gene were associated with BHR or asthma in our population.

We found no mutations in the coding region of the FcεRI-β gene in the subjects studied. Other investigators have reported particular mutations of the FcεRI-β gene in asthmatic or atopic populations. These mutations have been found in the coding region of the gene and include substitution of Leu for Ile at position 181, Leu for Val at position 183, and Gly for Glu at position 237. Where such mutations have been found, they have been linked to atopy.[2 12 19]

Both the Ile181Leu and the Val183Leu are very conservative substitutions and retain the non-polar character expected of residues predicted to be in a membrane spanning region of FcεRI-β protein. At this stage there is no direct evidence that these mutations can alter the function of the receptor. The population frequency of these mutations is variable. The Ile181Leu mutation was found in 15% of individuals in Britain but could not be detected in another Australian population.[17]

The Glu237Gly mutation has the potential to affect receptor function as it is located in the predicted cytoplasmic tail of the FcεRI-β protein. The cytoplasmic tail of FcεRI-β protein could be involved in signalling as it contains a conserved motif that has been shown in other receptors to be sufficient for coupling to signalling systems.[20] However, there is no direct evidence that the Glu237Gly mutation can alter receptor function. The population frequency of this mutation in other studies was 5.3%.[12] If the E237G mutation was present at the same prevalence in our population we would have had a 96% chance of detecting it in at least one of our subjects.

We identified two new polymorphisms in the FcεRI-β gene. The mutation in intron III would be unlikely to be involved in receptor function. The guanine substitution in this region was common, being present in 53% of

alleles in our subject group. It showed no association with any of the phenotypes related to asthma in our study population.

The polymorphism in the 5' untranscribed region was associated with a cytosine to thymine transition. The thymine substitution is present in 53% of alleles in our subject group. This polymorphism is likely to be in the promoter region of the FcεRI-β gene. It therefore could alter the rate of transcription of the FcεRI-β gene and so change the level of expression of the FcεRI-β protein. However, our data indicate that the 5' untranscribed region polymorphism is not associated with asthma, BHR, or atopy, which suggests that, if there are changes in the rate of FcεRI-β gene transcription, it is not of pathophysiological importance.

A recent genome-wide screen underscored the complexity of the genetic basis of asthma with a number of regions being linked to asthma, atopy, and BHR.[21] This raises the possibility that more than one gene in the 11q13 region may have a role in asthma. We have clearly shown that a gene in this region is linked to BHR. The results in this paper suggest that mutations in the FcεRI-β gene are not associated with asthmatic phenotypes. This raises the possibility that other genes on 11q13 may explain linkage with BHR.

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