Linkage and association studies of atopy and the chromosome 11q13 region

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Abstract

The clinical syndrome atopy is largely determined by genetic factors. In 1989, the first linkage of markers within and flanking the chromosomal region 11q13 and atopy was reported. In the following years, the gene coding for the β chain of the high affinity IgE receptor was localised to this region and two polymorphisms in this gene have been shown to be associated with the atopic phenotype.

We investigated two independent populations (population based and outpatient department) with different degrees of clinical symptoms. Using highly polymorphic markers we could find no evidence for linkage or allelic association of this particular genomic region to the atopic phenotype defined by enhanced IgE responsiveness ($p>0.05$). Neither did we succeed in finding either of the two polymorphisms described, nor could we identify any other polymorphisms within the gene. However, we found weak evidence for linkage in asthmatic sib pairs regarding maternal alleles ($p=0.03$).

We conclude from our data that in our populations the gene for the β chain of the high affinity IgE receptor is of minor importance for enhanced IgE responsiveness, and that it might influence atopy with clinical signs like asthma through maternally derived alleles.

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Atopy is the familial syndrome clinically characterised by bronchial asthma, atopic eczema, and allergic rhinitis. It is characterised immunologically by enhanced IgE responsiveness.[1] This syndrome is largely determined by genetic factors besides environmental influences.[2 3] The observed high prevalence in the population, age dependent penetrance, and assumed heterogeneity make it difficult to characterise the mode of inheritance and the genes involved.[4] The presence of a major gene for atopy has been discussed controversially.[5] Several genes and genetic regions have been named which affect the atopic phenotype.[6] In 1989, Cookson *et al*[7] first reported close linkage of enhanced IgE responsiveness underlying the clinical symptoms of allergic asthma and rhinoconjunctivitis and a locus on chromosome 11q13. In 1994, the β chain of the high affinitiy IgE receptor (FcεRI-β) was localised to the same chromosomal region and close genetic linkage to the atopic syndrome could be shown.[8] The same group described two distinct mutations in the coding gene leading to amino acid changes Ile181Leu and Glu237Gly. Both of them were shown to be associated with specific sensitisation to common inhalant allergens,[9–11] which was confirmed by Shirakawa *et al*.[12] So far, there is no proof of altered receptor functioning caused by the two mutations.

Linkage of atopy to chromosome 11q13 and the frequency and value of the published mutations have been discussed controversially. For example, Amelung *et al*[13] were unable to confirm linkage of 11q13 to asthma and allergy, nor could they find in their population the coding variants described. Collée *et al*[14] found linkage of 11q markers, but not of the FcεRI-β gene with atopy, whereas Shirakawa *et al*[15] confirmed the initial results in a Japanese population.

Two observations are common to most of the positive linkage studies. First, transmission of atopy at the 11q13 locus was only found through alleles derived from the mother.[7 9] Second, linkage was only seen in populations with clinical signs of atopy or severe manifestations.[8 14–17]

We have tested for linkage and association of enhanced IgE responsiveness as well as clinical manifestations of atopy to the chromosomal region 11q13 and the presence of polymorphisms in the gene. We looked at independent populations from the south western part of Germany.

Subjects and methods

FAMILIES

Two different sets of nuclear families are presented in this study. Population 1 was recruited through a population based study; 463 randomly contacted families with children of school age from the urban area of Freiburg and one community in the Black Forest participated in this study.[18] The sensitisation of all family members was verified by three consecutive skin prick tests using standardised procedures and standardised aeroallergens (mixtures of grass pollens, birch pollens, house dust mites, and cat dander; ALK, Horsholm, Denmark).[19] A total of 302 families underwent all three SPTs on all family members. Families showing at least one sensitised member were asked to participate in our recent study; 64 (52%) of the target population of 123 families participated. Population 2 was recruited through the outpatient department of the University Children's Hospital, Freiburg. All fami-

lies with more than one affected child (criteria shown below) who presented to the outpatient department were asked to participate in this study and 45 families participated. In both populations, a medical history was taken, skin prick tests were performed using the same aeroallergens, and a blood sample was drawn for IgE measurements and genotyping.

PHENOTYPE ANALYSIS

Specific IgE was detected by enzyme linked immunosorbent assays against two mixtures of grass pollens, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat dander, and birch pollens (Magic Lite, ALK, Denmark). The cut off point for a positive titre was 1.4 mU/l.[20] Detectable IgE to one of the four allergens or a positive skin prick test response to one of them were the criteria for "any specific sensitisation". Measurement of total serum IgE was carried out by an enzyme allergosorbent test (Phadezym, Pharmacia, Uppsala, Sweden). The cut off point for a raised titre was 100 kU/l.[21] The diagnostic criterion "atopy" required any specific sensitisation or a raised total serum IgE or both and corresponds to the "combined definition". Asthma was diagnosed on the basis of the patient's history of doctor's diagnosis or recurrent wheezing or both.

GENOTYPE ANALYSIS

DNA was extracted from peripheral blood leucocytes following standard procedures and column purified (DNA midi kit, Qiagen, Germany). Genotypes were determined by polymerase chain reaction for two microsatellites within and flanking the FcεRI-β gene. These were (1) FcεRI-β[22] in the fifth intron of the gene. Primer sequences were (5'→3') ATCTACTGCAAGTGACGATC and CATCTCCCTACCATCTGACC. The amplified alleles were 114 to 130 bp in length. (2) cCI11-319ca,[23] 5.9 Mb from the FcεRI-β gene. Primer sequences were (5'→3') CCCTCTGCCTCTCTTCAAAT and TTTGAGGTAGGCTTCGTATA. The amplified alleles were 189 to 203 bp in length. As internal reference one subject was included in all reactions yielding alleles of 116 and 120 bp for FcεRI-β and 189 and 191 bp for cCI11-319ca.

PCR was carried out in a volume of 10 µl containing 30 ng genomic DNA, 2.5 pmol of each primer, 0.5 U *Taq* polymerase (Pharmacia, Uppsala, Sweden), and 200 µmol/l of each dNTP, but 2.5 µmol/l dCTP, with the buffer recommended by the supplier. (^{32}P)dCTP (200 µmol/l) was incorporated into the PCR reaction. After denaturation for five minutes at 94°C, 35 cycles were carried out, each cycle consisting of 60 seconds at 94°C, 90 seconds at 62°C, and 120 seconds at 72°C. The last synthesis step was extended to seven minutes. The amplified products were resolved on 8% denaturing polyacrylamide gels and detected by autoradiography (Kodak X-omat XAR, 1.5-3 hours). Allele sizes were determined using known standards. The genotyping was performed by two investigators unaware of the phenotypes.

SEQUENCING

Sequencing by the dideoxy chain termination method[24] was done using DIG labelled primers at a distance of 50 to 60 bp from the exons and immunological detection (Boehringer, Mannheim, Germany). Sequencing of exon 6 was performed using the primer 5'-AAGGAATAAAGCTCCAATTCGTTAA-3'.

SSCP ANALYSIS[25]

SSCP analysis was performed using amplified DNA fragments of all exons and splice sites (about 200 bp each). After denaturation for five minutes at 94°C in a solution of 95% deionised formamide, the DNA fragments were analysed by electrophoresis through a non-denaturing 10% polyacrylamide gel containing 10% glycerol at 15°C and 370 V for two hours. The gels were silver stained as described elsewhere.[26]

STATISTICAL ANALYSIS

All informative genotypes of affected sib pairs were included in this study. Sib pair analysis was performed on the basis of identity by descent (IBD).[27] The observed distribution of shared and non-shared alleles in affected sibs was compared to the distribution expected under the hypothesis of non-linkage (50%/50%). Testing for significance was performed using χ^2 goodness of fit testing.

Regarding the fact that the same set of data was interpreted under three different phenotype definitions in the total population as well as the separate populations, the level of significance was adjusted to a p value of 0.008.

Linkage disequilibrium analysis was performed using the transmission/disequilibrium test (TDT).[28] The number of transmissions of the two most common alleles of each probe in heterozygous parents was compared to the expected 1 to 1 ratio by χ^2 goodness of fit testing.

Allelic association was tested regarding the parents of both populations as independent subjects. Testing was done for the allelic distribution in parents showing no criterion for atopy towards those groups showing a single or both criteria. The frequency of each of the alleles in the group of unaffected parents was compared to the observed frequency in the respective affected population and differences were tested using χ^2 goodness of fit testing.

Results

Seventy-one families with more than one child affected by atopy according to the above criteria were studied (table 1). Population 1 was

Table 1 Statistical features of the two populations of atopic subjects presented in this study

	No of nuclear families	*Average age of children (y)*	*No of affected children*	*No of affected children per family*	*No of sib pairs (IgE >100 kU/l)*	*No of sib pairs (specific sensitisation)*	*No of sib pairs (combined definition)*	*No of sib pairs (asthmatic)*
Population 1	35	16 11/12	82	2.3	48	50	67	0
Population 2	36	12 1/12	88	2.4	57	73	77	15
Pooled	71		170	2.4	105	123	144	15

Table 2 Number of parental alleles shared in affected sib pairs in population 2. The data are based on three phenotype criteria. Results are also given for maternal alleles only. The p values of 0.03 and 0.07 did not reach significance in respect of multiple tests being performed

		Both parents				Mothers		
		Alleles shared				Alleles shared		
Microsatellite	Phenotype	0	1	2	p	0	1	p
FcεRI-βca	Specific sens	10	15	13	NS	28	29	NS
Pop 2	IgE >100	9	12	11	NS	19	25	NS
	Combined	10	15	13	NS	31	30	NS
	Asthma	2	6	4	NS	4	11	0.07
cCI11-319ca	Specific sens	7	18	8	NS	24	24	NS
Pop 2	IgE >100	4	13	8	NS	16	20	NS
	Combined	8	20	9	NS	27	25	NS
	Asthma	0	4	4	NS	3	11	0.03

recruited through a population based study and population 2 through an outpatient department. Thus, 5.4% of the children of population 1 had asthma and 45.8% of population 2.

Genotyping with the two markers FcεRI-βca and cCI11-319ca showed no significant sharing of alleles in affected sib pairs with enhanced IgE responsiveness. Furthermore, no maternal effect was seen regarding parental alleles separately.

For both markers, sharing of maternal but not paternal alleles was seen in the 15 affected sib pairs of population 2 presenting with asthma (table 2). However, the results were not shown to be statistically significant (p=0.03 and 0.07 respectively) under the correction for multiple testing.

Transmission disequilibrium testing of the two most frequent alleles of each marker was performed without yielding significant results (data not shown).

The mutation Ile181Leu of FcεRI-β in the fourth transmembrane region corresponds to a G/T exchange in exon 6 of the coding gene. We tested for its presence in all children involved in the original genotyping. Neither ARMS testing using the same primers and test conditions as those described by Shirakawa *et al*,[9] nor direct genomic sequencing showed polymorphisms in the region described.

To find further mutations in the gene, we looked for polymorphisms by mobility shift assays (SSCP) of all exons in all children and we performed direct genomic sequencing of all exons of the oldest affected child in each family. We could not find evidence for any other polymorphism in this gene.

Discussion

The aim of this study was to investigate the influence of the β chain of the high affinity IgE receptor on the development of the atopic phenotype, as postulated by Cookson *et al*.[8 9 11] In order to make the comparison more valuable, we have chosen the same phenotypes for the study as the group from Oxford, we used the same microsatellite probes for linkage analysis, and checked for the same polymorphisms. We studied two populations, one recruited through a population based study and the other through our outpatient department. Thus, the much higher number of clinically affected children in the second population (45.8% versus 5.4%) was expected. Assuming that atopy represents a genetically heterogeneous disease, it might well be associated with a different set of genes involved or a different degree of involvement of each gene in our populations.

We could not find definite evidence for involvement of the the β chain of the high affinity IgE receptor on enhanced IgE responsiveness in a cognate (specific sensitisation) or non-cognate (total IgE) fashion, neither by affected sib pair analysis nor by allelic association and transmission disequilibrium testing. However, the significance of linkage results largely depends on the number of affected sib pairs recruited.[29] As there were 123 sib pairs in our study, a minor effect of FcεRI-β polymorphisms on IgE responsivenesss cannot be excluded.

Strikingly, the only sharing of alleles was seen in the 15 asthmatic sib pairs and only for maternal alleles, although the level of significance was not reached (table 2). Linkage of atopy to the 11q13 locus has mainly been seen in asthmatic or symptomatic sib pairs.[8 14–17] This might be congruent with the fact that linkage of the 11q13 locus with bronchial hyperreactivity has been seen even in the absence of atopy,[16] indicating that FcεRI-β may mainly affect asthmatic symptoms rather than IgE responsiveness. Furthermore, allele sharing was only observed in maternal alleles, as reported previously.[7 9 30] The basis for the observed maternal effect remains unclear. The genetic mechanism of imprinting as well as effects of the maternal immune status on the fetus and nursed child and a possible selection bias have been discussed.[9 11]

Screening for the mutation Ile181Leu by the ARMS method followed the same procedures as those described by Shirakawa *et al*,[9] who confirmed association with the atopic phenotype. Our negative findings were supported by sequencing results showing homozygous genotypes according to the DNA sequence published by Kuester *et al*.[31]

A search for further polymorphisms in the atopic children of our families on the basis of electrophoretic mobility shifts (SSCP) was negative. SSCP analysis in all children of population 2 included exon 7 as well, where a further polymorphism associated with atopy and bronchial hyperresponsiveness has been postulated.[11]

Although the Glu237Gly polymorphism had been reported at a frequency of 5%[11] and the Ile181Leu polymorphism at a frequency of about 15-17%[9] in presumably genetically similiar populations, we could not find any subject bearing either of the two polymorphisms. This might be the result of recent admixture of different populations with different allele frequencies, genetic drift, population bottlenecks, and new mutations in linkage disequilibrium with those already described.[32]

All techniques are based on an initial PCR reaction. It has been proposed by Hill and Cookson[33] that negative findings are at least partially based on differential amplification of mutation bearing versus wild type alleles. The microsatellite FCE5CA is situated in intron 5

of the gene next to the mutation and was included in the sequencing reactions. Differential amplification should also lead to a high degree of false homozygous genotypes or at least one faint band on microsatellite typing. This was not seen in our studies. On the contrary, the allelic distribution resembled the one published by the Oxford group.[22] A further explanation could be that the observed association could well result from different mutations in linkage disequilibrium with the two mutations examined. Thus, absence of the two mutations would not exclude linkage effects.

We conclude from our data that in our populations the β chain of the high affinity IgE receptor is of minor importance for enhanced IgE responsiveness. However, because of the low power of detection using sib pair methods and a relatively small sample size, we cannot exclude a minor influence of this gene on the development of the atopic phenotype. Sharing of maternal alleles in asthmatic sib pairs might indicate an influence of the genetic region on atopy with clinical signs, although the level of significance was not reached.

The knowledge of further mutations in the FcεRI-β gene associated with altered receptor functioning and of other genes involved in the development of the atopic phenotype should define the contribution of single genes to the whole process.

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