

Short communication

Lack of association between atopy and RsaI polymorphism within intron 2 of the Fc_εRI-β gene in a Spanish population sample

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Background The B genotype of RasI polymorphism located within intron 2 of the Fc-IgE receptor I (Fc_εRI) gene was previously found to be increased in atopic patients from a Japanese population sample.

Methods We studied these A/B genotypes in 70 Spanish atopic patients, and the results were compared to those of 51 nonatopic controls. RasI polymorphisms were studied by specific digestion of polymerase chain reaction fragments followed by polyacrylamide gel electrophoresis.

Results The polymorphism frequency (A/A: 25/70, A/B: 28/70, B/B: 17/70) found in patients did not differ from the frequency in nonatopic control subjects.

Conclusions We did not find RasI polymorphisms associated with atopic disease. The genetic findings in atopy and asthma may be very different according to ethnic and local characteristics, and they must be carefully verified in different population samples.

Key words: genetics; IgE receptor; polymorphism.

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Atopy is a common immunologic disorder characterized by increased IgE antibody response to common allergens (1). The atopy reaction occurs through the interaction of allergens and IgE molecules bound to the high-affinity receptor (Fc_εRI). The Oxford group presented evidence of a linkage between atopic disease and a locus on chromosome 11 (C11q13), the "atopy locus" (2), although their results were later disputed. The coding gene of the beta chain of Fc_εRI is also located in this atopy locus, and, after extensive study, it was found to contain seven exons (Fig. 1).

van Herwerden et al. (3) found asthma to be significantly linked to a highly polymorphic microsatellite marker (CA repeat) placed on the fifth intron of the Fc_εRI-β gene. The linkage was observed in siblings sharing bronchial hyperreactivity (BHR), but atopy in the absence of BHR did not show linkage. This finding is apparently surprising, but asthma is a complex disease and is

probably due to the interaction of several genes with environmental factors, and the relationship among asthma, atopy, and BHR remains unclear. In children, the pathogenesis of asthma frequently has an atopic background, although asthma and atopy are independent disorders.

In recent years, many authors have sought chromosome regions and candidate genes in asthma and atopy. The choice of C11q13 for atopy linkage studies is based not only on the presence of the Fc_εRI-β gene, but also on the availability of many polymorphic markers (4). In a Japanese population sample, Shirakawa et al. (5) developed a simple polymerase chain reaction (PCR)-based technique for RasI RFLP within intron 2 of the Fc_εRI-β gene and reported a significant association between atopic asthma and a B allele of the RasI polymorphism placed in intron 2 of the Fc_εRI-β gene (Fig. 1).

This study aimed to determine whether the

Chromosome 11q13 map

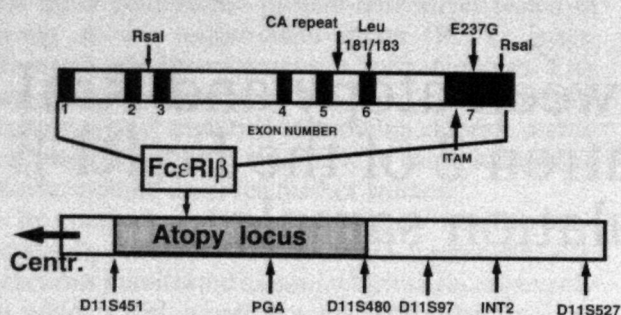


Fig. 1. Schematic representation of long arm of chromosome 11q13 showing relevant atopy markers. RsaI polymorphism is located within intron 2 of Fc ϵ RI- β gene.

frequency of this polymorphism located on the Fc ϵ RI- β gene is also increased among atopic patients in a Spanish population sample.

Material and methods

Subjects

Our study included 70 patients, 43 males and 27 females, recruited from the pediatric allergology unit. The mean age was 17 years (range 3–42), because some adults were also included. The atopy diagnosis was based on clinical symptoms and positive skin prick test in agreement with the clinical history. The total serum IgE level was determined in all patients, and it was generally increased with the exception of the youngest patients. The specific serum IgE-mediated antibodies (FAST, Pharmacia) were determined when necessary for resolving diagnostic doubts. The clinical atopy picture was asthma and/or rhinoconjunctivitis. Fifty-one nonatopic children were used

as controls. These children did not show atopic dermatitis, food intolerance, or respiratory symptoms suggesting IgE-mediated disease.

The genotype and phenotype results were independently assessed by two different researchers and matched at the end of the study.

DNA methods

DNA was extracted from peripheral blood leukocytes by the phenol-chloroform method (6). The primers used were as follows:

sense:

5'-CAG AAT GTT CTC ATG ACT GAA TTG-3'

antisense:

5'-CAA GTA CAG AGC AGA CAA CTG-3'

They were placed on intron 1 and exon 3, respectively, and the RsaI polymorphic site was located in the middle of intron 2. The RsaI site is created by the substitution of C for A at nucleotide position 1660 of the Fc ϵ RI- β gene (Gen EMBL file M89796), as follows: 1651 TAA CTG GGT C(A)CT TTT 1665. The restriction enzyme RsaI was used. PCR conditions were the same as previously described elsewhere (7); 749-, 428-, and 321-bp fragments were obtained after digestion of 5 μ l of PCR product with 5 U of RsaI enzyme at 37°C for 1 h in a 10- μ l final volume of appropriate buffer. The digested products were identified by electrophoresis on a 6% polyacrylamide gel and stained with ethidium bromide.

The A/A homozygote cases showed bands with the smallest molecular weight (321 and 428 bp), whereas the band for the B/B genotype was heavier (749 bp). The heterozygote subjects showed both band patterns (Fig. 2).

Statistical analysis

The difference of genotype frequency between cases and controls was assessed by the chi-square test.

Results

The A/A genotype was found in 25/70 (36%) atopic patients, the A/B genotype in 28/70 (40%), and the B/B genotype in 17/70 (24%). The observed distribution was very similar to expected values, and it did not differ from nonatopic subject data (Table 1). A comparative diagram with percentages published by other authors is shown in Fig. 3.



Fig. 2. Polyacrylamide gel (6%) electrophoresis stained with ethidium bromide, showing genotypes observed by restriction enzyme analysis after digestion with RsaI. A/A: lanes 2, 3, 5, and 6; A/B: lanes 1, 8, and 10; B/B: lanes 7 and 9.

Table 1. Association between RsaI polymorphism (intron 2) of Fc ϵ RI- β gene and atopy

	Atopic patients	Nonatopic controls
A/A genotype^a		
Observed frequency	25	20
Expected values	26	19
A/B genotype^a		
Observed frequency	28	19
Expected values	27	20
B/B genotype^a		
Observed frequency	17	12
Expected values	17	12
P value	NS ^b	

^a Explained in text.

^b NS: no significant difference.

Discussion

Many studies have suggested a probable relationship between asthma or atopy and variations in the Fc ϵ RI- β gene; nevertheless, none of them have been definitely accepted. In fact, genetic studies are strongly dependent on ethnic and environmental conditions, and these need to be determined in each region showing particular conditions. The linkage analysis studies focusing on the "atopy locus" of C11q13 have derived from Cookson et al.'s original paper (2). It was at first assumed that about 60% of Caucasian atopic asthmatic families are linked to chromosome 11q13 (5), although the exact genes remain unknown. Several polymorphisms located on the Fc ϵ RI- β gene have been described in asthmatic patients. They are exactly placed in exon 6 (I181L/I183V and 181L) and exon 7 (Fc ϵ RI- β E237G), adjacent to the immunoreceptor tyrosine activation motif (ITAM) (7).

In 1996, Shirakawa et al. (8) found a strong linkage between atopy, in a Japanese population

sample, and a different genotype in the second intron of the Fc ϵ RI- β gene, with the RasI restriction enzyme. They reported that the A/B and B/B genotypes are predominately detected in atopic subjects. This means not only that the RasI polymorphism is linked to one of the loci determining atopy, but also that the substitution causing genotype B shows linkage disequilibrium with a causative mutation for atopy.

In our population, the A/A, A/B, and B/B genotypes were distributed in a similar percentage in atopic and nonatopic subjects, and the distribution found was similar to the expected frequencies (Table 1). The difference between both groups was not significant ($P=0.9$). These findings clearly disagree with data published by Shirakawa et al. (7), who reported a decreased frequency of A/A genotype in atopic patients in relationship to data from nonatopic controls. The results from Spanish nonatopic controls were also different from Japanese controls (Fig. 3). Special attention must be paid to racial variabilities in genetic studies, although in this case Fukao et al. (9) did not find statistical differences either, in studying another Japanese population sample. A polymorphism linked to a genetic disease, like atopy, may arise in a patient, and then all descendants will inherit this marker, which will be detected only in a local atopic population.

Having ruled out intron 2 as a causative mutation for atopy, several authors have focused their attention on the study of intron 5. The analysis of its polymorphisms and highly polymorphic microsatellite markers will help clarify the genetics of atopy.

In summary, many papers proposing atopy-linked genes on the 11q13 chromosome have been published in recent years. Unfortunately, their conclusions could not be confirmed. A great deal of research work must be developed in the next few years for a better understanding of the genetics of atopy, and the findings need to be carefully verified since they are strongly influenced by ethnic variabilities.

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GENOTYPE PERCENTAGE

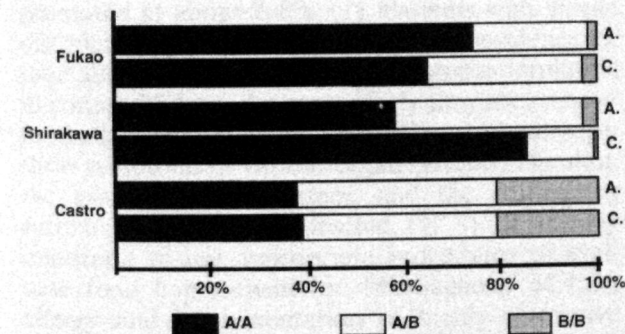


Fig. 3. Comparative diagram of allele frequency percentages reported by Fukao et al. (9), Shirakawa et al. (7), and Castro et al. (this study). (A) atopic population; (C) control population.

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