

Original article

Molecular analysis of sequence variants in the Fcε receptor I β gene and IL-4 gene promoter in Italian atopic families

Background: The genetic variants in the Fcε receptor I β gene (Glu237Gly) and the T allele of the (C590T) polymorphism of interleukin (IL)-4 gene promoter were reported to be associated with atopy. But the data of the studies in different populations are contrasting with one another.

Methods: A group of 25 Italian nuclear families were studied. In each family at least two allergic subjects were present. The allergic children were 65 and the allergic relatives were 35. One hundred and three nonallergic unrelated controls included outpatients with no history of atopy. The (C590T) promoter polymorphism of the IL-4 and the genetic variant Glu237Gly of Fcε RI β genes were analysed by the polymerase chain reaction-restriction fragment length polymorphism method.

Results: A significant difference was observed in the genotype frequency at codon 237 of the Fcε RI β gene between allergic children and nonatopic control ($P < 0.01$) and in the allergic relatives ($P < 0.001$). In the children, the Glu237Gly polymorphism was also associated with elevated circulating levels of immunoglobulin E. The -590C/T allele of IL-4 promoter gene showed no association with atopy.

Conclusions: In our study, the Glu237Gly polymorphism of the Fcε RI β gene was associated with atopy. Our results have not pointed out an association between the (C590T) promoter polymorphism of the IL-4 gene and atopy. These data suggest the potential role of the Fc RI β gene in the development of the allergy.

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Atopy, expressed clinically as asthma, atopic dermatitis and/or rhinoconjunctivitis, is characterized by a genetic predisposition for generating immunoglobulin (Ig) E antibodies against common environmental allergens. Several family and twin studies have demonstrated an involvement of genetic components in the development of allergy of asthma (1). Several loci linked to allergy and asthma have been suggested through genome-wide linkage studies (2, 3).

Among the genes predisposing individuals to atopy, several variants of the Fcε receptor I β gene (Fcε RI β) were reported to be associated with allergic asthma (4–6). Indeed, IgE-dependent activation of mast cells and basophils through Fcε RI is involved in the pathogenesis of allergen-induced immune response in allergic diseases (7). Several studies have reported a linkage or association between the measurements of allergy and chromosome 11q13 (8). The gene encoding the β subunit of the high-affinity IgE receptor in this chromosomal region is considered to be a most likely candidate from these findings (9). Recently, animal models have provided unequivocal genetic evidence that Fcε RI-β functions are

an amplifier of immune responses, especially those induced by mast cells and basophils.

As regards the genetics of allergic diseases, Marsh et al. (10) showed a linkage between total serum IgE and markers on chromosome 5q31–33, which contain the interleukin (IL)-3, IL-4, IL-5 and IL-13 genes and the granulocyte-macrophage colony stimulating factor. Interleukin-4 is a pleiotropic cytokine, which plays a crucial role in IgE-dependent disorders. It is central to B cells switching to IgE antibody production and to the maturation of T-helper cells to the Th2 phenotype (type 2 T-helper lymphocytes). Therefore, IL-4 is thought to be a direct participant in the generation of high serum IgE levels observed in allergic subjects (11).

In our study, the prevalence of Fcε RI β gene Glu237Gly and the IL-4 promoter C-590T polymorphisms have been investigated in nuclear allergic families of Southern Italy (Sicily) and in nonallergic control subjects in order to determine their possible role in modulating the allergic response and to find a correlation between allergic sensitization, elevated total IgE, specific IgE, positive skin prick tests.

Subjects and methods

Patients

One hundred patients (58 females and 42 males) suffering from allergic diseases (i.e. asthma, atopic dermatitis and rhinoconjunctivitis) were selected. This group consisted of 25 nuclear families from the region of Sicily (Southern Italy) recruited from among allergic children attending the Department of Paediatrics of the University of Messina, Italy.

In each family at least two allergic subjects were present. The total number of children suffering from allergy, asthma and/or rhinitis consisted of 65 (30 females and 35 males). The median age of the allergic children was 10 years (range 5–13 years) (Table 1).

The total number of the relative allergic patients was 35 and their median age was 42 years (range 29–48 years) (Table 2).

One hundred and three (50 females and 53 males) nonallergic unrelated controls included outpatients with no history of allergic diseases, such as bronchial asthma, atopic dermatitis and allergic rhinitis. They had negative skin test responses to common aeroallergens (from cats, dogs, house dust mites, grass, pollen, moulds and *Parietaria*).

In this group, 63 children (25 females and 38 males) were studied and their median age was 11 years (range 6–14 years) (Table 1).

The median age of the remaining 40 healthy control subjects (23 females and 17 males) was 47 years (range 33–49 years) (Table 2).

The study was approved by the Committee for ethics in medical research and was, therefore, carried out in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All the subjects gave their informed consent prior to their inclusion in the study.

Assessment

The families of the allergic members were subdivided into six groups. Moreover, the total number of the parents, the children and their relatives was calculated (Table 3).

In group I, five families were identified with both parents affected by atopy and at least two children. The total number of the allergic subjects was 26.

Seven families with two affected parents and one child were assigned to group II. The total number of the allergic subjects was 23.

Group III consisted of four families with an affected mother and at least two children. The affected patients were 15.

Table 1. Demographic characteristics of children with atopy and healthy controls

	Healthy controls	Children
Age (years)	10 (range 5–13)	11 (range 6–14)
Sex, F/M	30/35	25/38
Allergy	Yes	No

Table 2. Demographic characteristics of relatives with atopy and healthy controls

	Healthy controls	Relatives
Age (years)	42 (range 29–48)	47 (range 33–49)
Sex, F/M	20/15	23/17
Allergy	Yes	No

Table 3. Distribution of atopic members in the families studied

	Members affected	Families
Mother, father, at least two children	5	26
Mother, father, one child	7	23
Mother, at least two children	4	15
Father, at least two children	3	10
Mother, one child	4	10
Two or more children, no parents affected	2	16
Total	25	100

In group IV, three families were identified with an affected father and at least two children. The total number of patients was 10.

Group V consisted of four families with an affected mother and one child. The total number of allergic patients was 10.

Two families with two or more affected children, without affected parents, but only some relatives were assigned to group VI. The total number of patients was 16.

Phenotype analysis

Atopy was defined by the presence of at least two of the following criteria:

1. A positive skin prick test (wheal diameter ≥ 5 mm) to one or more common inhalant allergens.
2. Specific IgE at least class 2 (>0.35 kU/l) to one or more inhalant allergens.
3. Elevated circulating total IgE: cut-off > 100 kU/l.

Skin prick test

Skin tests were carried out on the forearm with inhalant allergen panel of Southern Italy: *Dermatophagoides pteronyssinus* and *D. farinae*, *Parietaria judaica*, *Phleum pratense* and grass mix, *Artemisia*, *Olea*, dog, cat, *Cladosporium* and *Alternaria* (Soluprick ALK Denmark).

The measurement of the immediate-phase cutaneous responses was assessed 15 min later, in accordance with the recommendations of the European Academy of Allergology and Clinical Immunology (12). Wheal size was expressed by the formula $(D + d)/2$, where D is the maximum diameter and d the perpendicular diameter at the midpoint. A mean diameter of ≥ 5 mm, was considered a positive result.

Total and specific IgE determination

Total IgE and specific IgE concentration in sera were determined with CAP system – Pharmacia and Upjion Diagnostics, Uppsala Sweden.

Specific IgE concentration for inhalant allergen was detected according to the supplier's instructions.

Molecular methods

Genotype analysis

Genotype analysis was performed on genomic DNA extracted from whole blood (13).

Polymorphisms of the Fcε receptor I β gene and IL-4 gene promoter

Screening for polymorphisms of the Fcε receptor I β gene (Glu237Gly)

Screening for Glu237Gly variant of the Fcε receptor I β gene was performed by allele-specific polymerase chain reaction (PCR) and PCR-single strand confirmational polymorphism (SSCP). The allele-specific PCR primers to detect Glu and Gly alleles were 5'-GACAGCAGATATACTAAGGAATAACC-3' and 5'-CTTA TAAATCAATGGGAGGAGACAATT-3' for the Glu allele, 5'-ACAGTGAGTTGGAAGACCCAGGCGG-3' and 5'-GGAGC ATATTAAGGTGGACAGAAGC-3' for the Gly allele, respectively.

Allele-specific PCR was carried out on Ready-To-Go PCR Beads (Amersham Pharmacia Biotech, Uppsala, Sweden) in a total volume of 25 μl containing 20 ng of genomic DNA. The PCR profile used was as follows: initial denaturation at 94°C for 5 min, followed by 40 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 15 s. The final extension was at 72°C for 7 min. PCR products for Glu and Gly alleles gave 342- and 228-bp bands, respectively. The PCR-SSCP was performed to confirm the results of allele-specific PCR, using 30 randomly chosen samples. The PCR amplification was performed under the same conditions as allele-specific PCR with primers of 5'-GACAGCAGATATACTAAGGAAT AACC-3' and 5'-GGAG CATATTAAGGTGGACAGAAGC-3'. The PCR product was 517 bp, including codon 237 of the Fcε receptor I β gene and digested with restriction enzyme MboI (Takara Biomedical, Shiga, Japan) to shorten the fragment optimally for SSCP analysis. Glu and Gly alleles were clearly separated, and the results confirmed the data by means of allele-specific PCR.

Screening for polymorphisms of IL-4 -590C/T promoter

We performed -590C/T allotyping by PCR-restriction fragment length polymorphism (RFLP) as previously described (14). Briefly, PCR was performed with the primer pair (5'-TAAACTTGGG AGAACATGGT-3' and 5'-TGGGGAAAGATAGAGTAATA-3') and the products were digested with AvaII followed by the separation in a 4% agarose gel.

Statistical analysis

Statistical analysis was performed through use of the STATA program (15). To assess the Fcε receptor I β gene Glu237Gly and IL-4 -590C/T promoter polymorphisms as a risk factor for allergy, 95% CIs and *P* were calculated for allergy through use of Fisher's test.

We then compared total serum IgE levels to assess correlation with genotypes of the Fcε receptor I β gene Glu237Gly and IL-4 -590C/T promoter polymorphisms (separate comparisons for allergic patients and healthy control subjects). Total serum IgE levels were log-transformed to normalize the distribution.

Results

The allergic sensitization of children is shown in Table 4.

Polymorphism of the Fcε receptor I β gene (Glu237Gly)

Allergic patients were different in the genotype frequency at codon 237 of the Fcε receptor I β gene in respect to the 105 nonatopic subjects (*P* = 0.044).

Table 4. Sensitization to inhalant allergens assessed by prick and radioallergo-sorbent tests of allergic children

Common allergens	No. of children
<i>Dermatophagoides pteronyssinus</i> and <i>D. farinae</i>	13
<i>Parietaria judaica</i>	12
<i>Phleum pratense</i>	9
Grass mix	5
<i>Artemisia</i>	3
<i>Olea</i>	4
Dog	6
Cat	6
<i>Cladosporium</i>	4
<i>Alternaria</i>	3

Results in 65 children affected by atopic asthma, rhinoconjunctivitis and dermatitis.

Table 5. Genotype frequency of Fcε receptor I β gene (Glu237gly)

	Glu/Glu	Glu/Gly	Gly/Gly	95% CI*	Risk ratio	<i>P</i> -value
Children (128)						
Affected children (65)	53	9	3			
Healthy children (63)	62	1	0	0.38–0.64	0.49	<0.01
Relatives (75)						
Affected relatives (35)	26	7	2			
Healthy relatives (40)	40	0	0	0.29–0.53	0.39	<0.001

* Glu/Glu vs Glu/Gly+Gly/Gly between controls and each group of patients.

Table 6. Genotype frequency of IL-4 promoter (-590C/T)

	C/C	C/T	T/T	95% CI*	Risk ratio	<i>P</i> -value
Children (103)						
Affected children (65)	61	4	0			
Healthy children (63)	60	3	0	0.44–1.68	0.86	ns
Relatives (75)						
Affected relatives (35)	35	0	0			
Healthy relatives (40)	40	0	0	0.35–0.58	0.45	ns

* C/C vs C/T +T/T between controls and each group of patients. ns, not significant.

A significant difference was observed in the genotype frequency at codon 237 of the Fcε receptor I β gene between allergic children and nonatopic control (*P* < 0.01) (Table 5). This association has also been found in the allergic relatives (*P* < 0.001).

Polymorphism of IL-4 promoter

The -590C/T allele of IL-4 promoter showed no association with allergic diseases between atopic children and controls. Moreover, there was no difference in allelic frequency between atopic and nonatopic relatives (Table 6).

Association with the IgE levels and skin prick tests

A significant difference was observed in the genotype frequency at codon 237 of the Fcε receptor I β gene

Table 7. Demographic characteristics of children with atopy and healthy controls according to Glu237Gly genotype of FCER1B gene

	Children with Atopy			Healthy controls		
	Glu/Glu	Glu/Gly	Gly/Gly	Glu/Glu	Gly/Glu	Gly/Gly
No. of subjects	53	9	3	62	1	0
Sex, F/M	25/28	5/4	1/2	29/33	1 M	0
Prick test	+	+	+	–	–	–
Total IgE · log (IU/ml)	2.63 ± 0.56	2.37 ± 0.56	2.44 ± 0.56	1.73 ± 0.57	1.73 ± 0.58	1.75 ± 0.57

Data are mean ± SD.

$P = 0.0044$ among genotypes in subjects with atopy by multivariate analysis using sex and age as covariates.

IgE, immunoglobulin E.

Table 8. Demographic characteristics of relatives with atopy and healthy controls according to Glu237Gly genotype of FCER1B gene

	Relatives with atopy			Healthy controls		
	Glu/Glu	Glu/Gly	Gly/Gly	Glu/Glu	Gly/Glu	Gly/Gly
No. of subjects	26	7	2	40	0	0
Sex, F/M	14/12	3/4	2 F	21/19	0	0
Prick test	+	+	+	–	–	–
Total IgE · log (IU/ml)	2.98 ± 0.43	2.76 ± 0.43	2.54 ± 0.48	1.67 ± 0.50	1.65 ± 0.58	1.64 ± 0.45

Data are mean ± SD.

$P < 0.001$ among genotypes in the relatives with atopy by multivariate analysis using sex and age as covariates.

IgE, immunoglobulin E.

between children with elevated circulating levels of IgE ≥ 100 kU/l and nonatopic controls (Table 7).

A significant association was also observed between the allergic children with positive skin prick test and nonatopic subjects. These significant differences were also observed between the allergic and nonallergic relatives (Table 8).

These associations were not observed between the polymorphism -590C/T of IL-4 promoter and the elevated levels of IgE and skin prick test.

Discussion

Rosenwasser et al. (16) identified a C \rightarrow T exchange at -590 base pair (bp) from the open reading frame within the IL-4 gene promoter. The T allele of -590C/T was associated with allergic asthma. Their findings suggest that the C-590T polymorphism may be a true nuclear variant that explains some of the linkages found in 5q31–q33. Nevertheless, it has been also reported that there is no statistically significant association between total serum IgE levels and this variation (17, 18).

Atopy, which predisposes individuals to develop asthma, hay fever and atopic dermatitis, is usually associated with dramatically elevated total serum IgE levels and is thought to be controlled by a major susceptibility gene and multiple minor susceptibility genes (2). Candidate atopy genes include those encoding the β -chain of high-affinity receptor for IgE (Fce receptor I) on chromosome 11q (4–6), chromosome 5q cytokines (IL-4, IL-13, etc.) (9), IL-4 receptor α (2),

human leucocyte antigens (2), and the T cell receptor (2).

Among genes predisposing individuals to atopy, two coding polymorphisms in the Fce receptor I β gene (Ile181Leu and Ile181Leu/Ile183Val) were initially identified to be associated with atopy (19). Another variant of the FCER1B gene (Glu237Gly) was reported to be associated with Japanese atopic asthma (20). An amino acid substitution (glutamic acid \rightarrow glycine) at position 237 in the FCER1B gene was associated with total and specific IgE levels and with atopic asthma. The 237 glycine allele was present in approximately 5% of Japanese and white populations (2, 20). A study of 1004 white Australian subjects found out that the patients with the Glu237Gly allele had a relative risk for asthma compared with nonasthmatic nonatopic control subjects (9). This is the first study that shows the frequency of Glu237Gly polymorphism of the FCER1B gene in a population of Southern Italy.

A marginally significant difference was observed in the genotype frequency at codon 237 of the FCER1B gene between 100 atopic patients and 105 nonatopic controls ($P = 0.044$). However, a significant difference has been observed in the genotype frequency at codon 237 of the Fce receptor I β gene between allergic children and nonatopic controls ($P < 0.01$). This association has also been found in the allergic relatives ($P < 0.001$). Moreover, this polymorphism was associated with elevated levels of IgE, both in children ($P = 0.0044$) and in adults ($P < 0.001$).

The correlation between the FCER1B gene and the IgE levels is well known. Indeed, the β -chain of the high-

affinity receptor for IgE is found on mast cells and basophils. Cross-linking of this receptor leads to increased IL-4 production by these cells. Polymorphisms in the FCER1B gene could alter IL-4 production and thus modify IgE levels. Interleukin-4 is a pleiotropic cytokine, which plays a crucial role in IgE-dependent disorders (9). It is central to B cells switching to IgE antibody production and to maturation of T-helper cells to the Th2 phenotype (type 2 T-helper lymphocytes).

In our study we found that the FCER1B gene *237Gly allele was associated with a relative risk of atopy. However, these data need further studies in a larger series of cases to confirm these observations.

Finally, in the present work there was a lack of association of the polymorphism of IL-4 promoter -590C/T with the atopy. Rosenwasser et al. (14) found a polymorphism with a C → T exchange at position 590 upstream from the open reading frame of the IL-4 gene (C590T) and showed that it was associated with elevated levels of IgE phenotype in asthmatic families. However, in a later report, no association was found between this polymorphism and atopy or the total serum IgE levels in a Caucasian population from Britain and in Australian families (21, 22). These data could reflect a genetic heterogeneity in the pathogenesis of atopy. Indeed,

different risk alleles may be important in different populations. Alternatively, the lack of association may be due to differences between populations in the amount or the type of exposure to environmental factors. Moreover, among the patients, the definition of severity of allergic diseases (i.e. asthma, rhinitis and atopic dermatitis) is complicated 'per se' and is the expression of the 'polygenic background' of these kinds of diseases.

Nevertheless, the results of our study suggest that genetic markers of atopy can be identified. The understanding of the genes involved in allergic diseases may identify new molecular targets and may also predict the response to different forms of therapy (pharmacogenetics). One suitable target may be IL-4. If the practical problems encountered by gene therapy are taken into consideration, this approach seems unlikely in the foreseeable future, except for the proof of concept studies. The possibility of developing a cure for atopy is remote because of the complex interactions between atopic status and other related airways and skin related alterations in determining allergic phenotypes (i.e. asthma, rhinitis and atopic dermatitis). However, strategies to inhibit the development of sensitization in early childhood (23, 24) offer such a prospect in the future.

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