

Short communication

The α -chain of high-affinity receptor for IgE (Fc ϵ RI α) gene polymorphisms and serum IgE levels

Background: The high-affinity receptor for immunoglobulin-E (IgE) (Fc ϵ RI) plays a major role in the pathogenesis of allergy, but there are only two published studies on its α subunit (Fc ϵ RI α) genetic variability in allergic diseases.

Aims of the study: Mutational screening in the region of the Fc ϵ RI α gene promoter and the first exon with subsequent genetic variability assessment in allergic patients and a random population sample.

Methods: Allergic subjects were individuals with asthma or urticaria. Age- and sex-matched controls were randomly selected from a large population sample. Mutational screening was performed using a single-stranded conformational polymorphism and subsequent sequencing. Detected polymorphisms were genotyped by restriction fragment length polymorphism. Total serum IgE was measured in allergic subjects and controls. Skin prick tests, blood eosinophil count and aspirin challenge test were performed only in the subjects. A subgroup of the subjects was further characterized by autologous serum skin test, histamine release test, Phadiatop and IgE antibodies against staphylococcal enterotoxins.

Results: Two linked polymorphisms –344 C > T and –95 T > C were found within the Fc ϵ RI α gene. The allele –344 T frequency was 0.45 vs 0.37 ($P = 0.33$), and the allele –95 C frequency was 0.26 in subjects vs 0.30 in controls ($P = 0.62$). Serum IgE was significantly higher in subjects homozygous for the –344T allele (TT genotype) than in those carrying the –344 C allele (CT or CC genotype; $P = 0.003$), but this association was not detectable in controls.

Conclusions: Our findings of genotype-related differences in IgE levels in allergic patients suggest an impact of –344 C > T but not –95 T > C gene polymorphism of Fc ϵ RI α on total levels of IgE. The genetic variability in Fc ϵ RI α at the –344 nucleotide of its regulatory sequence, though not related to atopy, predicts higher levels of the immunoglobulin.

**D. P. Potaczek¹, M. Sanak¹,
L. Mastalerz¹, M. Setkowicz²,
M. Kaczor¹, E. Nizankowska¹,
A. Szczeklik¹**

Departments of ¹Medicine and ²Dermatology,
Jagiellonian University School of Medicine, Cracow,
Poland

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Dr A. Szczeklik
Department of Medicine
Jagiellonian University School of Medicine
8 Skawinska Str.
31-066 Cracow
Poland

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The high-affinity receptor for immunoglobulin-E (IgE) (Fc ϵ RI)-mediated activation of basophils and mast cells by IgE is crucial in the pathogenesis of allergic diseases, such as asthma and urticaria. Genetic variability in the β subunit of this receptor (Fc ϵ RI β) and its association with atopic disorders has been extensively studied recently (1, 2). The α -chain of Fc ϵ RI receptor (Fc ϵ RI α) was characterized in 1993 (3); however, to our knowledge, there were only two reports on a relationship between Fc ϵ RI α genetic variability and allergic diseases, of which only one was related to Caucasians (4, 5).

We therefore performed a mutational screening within the Fc ϵ RI α gene promoter region and the first 32 bp of the coding sequence in patients with asthma or urticaria.

We studied 101 patients with asthma ($n = 52$) or urticaria ($n = 49$) (aged 45.0 ± 1.3 years; male : female, 29 : 72) (Table 1). All the patients were in apparent clinical remission and denied taking systemic steroids, antihistaminics or leukotriene receptor antagonists. Only

inhaled steroids and long-acting β_2 -agonists were allowed. The control group consisted of 101 age- and sex-matched individuals (aged 44.7 ± 1.3 years; male : female, 29 : 72) selected from a random population sample of Cracow City, Poland ($n = 550$) (Table 1). The study was approved by the Jagiellonian University Ethics Committee for Human Subjects Studies, and all the patients gave their written informed consent.

Total serum IgE levels were determined by a latex immunonephelometry (Dade Behring, Marburg, Germany) in all studied individuals. Peripheral blood eosinophil count, skin prick test (Allergopharma, Reinbek, Germany) and aspirin challenge tests (6, 7) were performed in the allergic subjects only. Autologous serum skin test (ASST) (8), a modified histamine release test (HRT) (9, 10), Phadiatop screening test for atopy, serum specific IgE against staphylococcal enterotoxins (IgE-SEs) A (IgE-SEA), B (IgE-SEB), C (IgE-SEC), D (IgE-SED) and toxic shock syndrome toxin-1 (IgE-TSST-1) were

Table 1. Subject characteristics

	Allergic subjects (<i>n</i> = 101*; <i>n</i> = 99†)	Controls (<i>n</i> = 101)
Age (years)‡	45.0 ± 1.3; 44.9 ± 1.3	44.7 ± 1.3
Sex (m : f)	29 : 72; 29 : 70	29 : 72
Asthma	52; 50	ND
Urticaria	49; 49	ND
Aspirin sensitivity	49; 47	ND
Duration of disease (years)‡	8.4 ± 0.9; 8.3 ± 0.9	ND
Total IgE (IU/ml)§	92.0 ± 208.4; 92.0 ± 206.8	26.6 ± 55.7
Positive skin prick tests	46; 45	ND
Total IgE >100 IU/ml and/or positive skin prick tests	70; 69	ND
Eos (/ μ l)†	238.9 ± 27.5; 239.0 ± 27.8	ND

ND, not determined; Eos, peripheral blood eosinophil count.

*344 C>T polymorphism

†95 T>C polymorphism.

‡Mean ± SEM.

§Median ± IQR.

carried out by fluoroenzymeimmunoassay (CAP II System; Pharmacia & Upjohn Diagnostics, Uppsala, Sweden) in a subgroup of allergic subjects, but not in controls.

Genomic DNA was isolated from peripheral blood leukocytes. DNA fragment encompassing promoter and the 32 bp of the first exon of FcεRIα gene was amplified by polymerase chain reaction (PCR) in standard conditions (primer set 1: ACC TGG CAT ATG TTT GGT ATT CAG and TCA TGG ACT CCT GGT GCT TAC T). Thereafter, a single-stranded conformational polymorphism was used for screening of genetic variability (DNA Pointer System; Kucharczyk TE, Warsaw, Poland) in DNA samples from a randomly selected 24 allergic individuals. PCR products of altered electrophoretic mobility were cloned using TOPO TA-Cloning kit (Invitrogen, Carlsbad, CA) and sequenced (BigDye Terminator v3.1 Cycle Sequencing kit and ABI Prism 377; Applied Biosystems, Foster City, CA). Subsequently, genotyping of detected FcεRIα polymorphisms was completed in all individuals studied by a digestion of PCR product and restriction fragment length polymorphism (PCR-RFLP). The amplification product obtained with primers set 1 was digested with *MnII* endonuclease (Fermentas, Vilnius, Lithuania) for -344 C>T polymorphism. Genotyping of -95 T>C polymorphism was completed by PCR with primer set 2: GCT GAT GGG TTA ACC AGA TGT G and CAC AGT AAA GGT TCC TAC CC, in which an underlined nucleotide introduced a restriction site for *NmuCI* (Fermentas). In two allergic subjects -95 T>C genotype was undetermined due to degeneration of DNA.

Clinical data were expressed as mean ± standard error of mean; serum IgE levels were reported as median ± interquartile range. Comparison of total serum IgE levels required logarithmic transformation of the data. Phadiatop ≥0.35 KU/l was considered as positive (+), similarly IgE antibodies against staphylo-

coccal enterotoxins >0.35 KU/l were considered positive (+). Haplotype frequencies were reconstructed using Arlequin software version 2.0 available at <http://lgb.unige.ch/arlequin>. Genotype or haplotype groups were compared by using unpaired Student's *t*-test, chi-squared test or Fisher's exact test as appropriate. The most prevalent IgE against enterotoxin B was correlated with ordered -344 C>T FcεRIα promoter genotypes using a logistic regression. *P* < 0.05 was considered significant.

The mutational screening of the FcεRIα gene promoter and the 32 bp of the first exon in allergic subjects revealed two common single nucleotide C>T transitions located in position -344 C>T and -95 T>C, which were formerly referred to as -335 T>C by Shikani et al. (4) and -66 C>T by Hasegawa et al. (5). The T allele frequency of -344 single nucleotide polymorphism (SNP) was 0.45 in allergic subjects and 0.37 in control group. The minor variant at -95 SNP, C allele had frequency of 0.26 in patients vs 0.3 in controls. The allelic variants were in Hardy-Weinberg equilibrium in both groups. The genotyping data are summarized in Table 2. The frequency of the -344 C>T and -95 T>C polymorphisms did not differ significantly between allergic subjects and controls (*P* = 0.33 and 0.62). There was no significant difference in the prevalence of polymorphisms between males and females in either group. The two SNPs were in a complete linkage disequilibrium, and only three haplotypes were reconstructed in allergic subjects and controls. The most common one (-344T|-95T) had a frequency of 0.45 in the subjects and 0.40 in controls. Two other haplotype variants (-344C|-95T) and (-344C|-95C) also did not differ in their frequency between the subjects and the controls (0.29 vs 0.31 and 0.26 vs 0.29). Haplotypes frequencies conformed to the genetic equilibrium. The polymorphisms were not related to aspirin sensitivity in allergic subjects (Table 2) or peripheral blood eosinophil count, which was 238.7 ± 32.5 μ l in allergic subjects carrying allele C at nucleotide -344 and 239.4 ± 51.2 μ l in -344 TT homozygotes. Allergic subjects homozygous for the -344 T allele had significantly higher (*P* = 0.003) total serum IgE levels (TT: 162.0 ± 317.4 IU/ml) than these carrying at least one -344 C allele (CT or CC: 72.8 ± 163.5 IU/ml). No similar association was detectable in the control group (18.2 ± 42.7 vs 30.5 ± 76.0; *P* = 0.44) (Table 3). The two genotype classes of allergic subjects did not differ in ASST and histamine released in HRT (TT: 75.4 ± 7.0 vs CT or CC: 68.0 ± 4.6 ng/ml) (Table 4). The ratio of positive Phadiatop, IgE-SEA, IgE-SEB, IgE-SEC, IgE-SED, and IgE-TSST-1 were similar in TT and CT or CC allergic subjects (Table 4). There was a significant correlation between IgE-SEB positivity and ordered (from CC by CT to TT) FcεRIα genotype (*P* = 0.03) in a logistic regression model; however, the odds ratio (4.67) of prediction for SEB antibodies on -344 C>T polymorphism had only a borderline significance (95% confidence interval 0.94 -25.45). The -95 T>C polymorphism did not correlate with clinical data

Table 2. Genotype frequencies of the –344 C>T and –95 T>C FcεRIα polymorphisms

	–344 C>T polymorphism				–95 T>C polymorphism			
	Genotype frequencies			T allele frequency	Genotype frequencies			C allele frequency
	CC	CT	TT		TT	TC	CC	
Allergic subjects, <i>n</i> (%)	33 (32.7)	46 (45.5)	22 (21.8)	0.45	55 (55.5)	37 (37.4)	7 (7.1)	0.26
Male, <i>n</i> (%)	9 (31.0)	15 (51.7)	5 (17.3)	0.43	12 (41.4)	15 (51.7)	2 (6.9)	0.33
Female, <i>n</i> (%)	24 (33.3)	31 (43.1)	17 (23.6)	0.45	43 (61.5)	22 (31.4)	5 (7.1)	0.23
Aspirin-sensitive, <i>n</i> (%)	15 (30.6)	24 (49.0)	10 (20.4)	0.45	29 (61.7)	16 (34.0)	2 (4.3)	0.21
Aspirin-tolerant, <i>n</i> (%)	18 (34.6)	22 (42.3)	12 (23.1)	0.44	26 (50.0)	21 (40.4)	5 (9.6)	0.30
Controls, <i>n</i> (%)	41 (40.6)	45 (44.6)	15 (14.8)	0.37	52 (51.5)	37 (36.6)	12 (11.9)	0.30
Male, <i>n</i> (%)	8 (27.6)	18 (62.1)	3 (10.3)	0.41	15 (51.7)	10 (34.5)	4 (13.8)	0.31
Female, <i>n</i> (%)	33 (45.8)	27 (37.5)	12 (16.7)	0.35	37 (51.4)	27 (37.5)	8 (11.1)	0.30

Table 3. Genotype groups of the –344 C>T and –95 T>C FcεRIα polymorphisms and selected quantitative parameters

	–344 C>T polymorphism			–95 T>C polymorphism		
	Genotype class		<i>P</i> -value	Genotype class		<i>P</i> -value
	CC or CT	TT		TT	TC or CC	
Patients (<i>n</i> = 101†; <i>n</i> = 99‡)						
Total IgE (IU/ml)§	72.8 ± 163.5	162.0 ± 317.4	0.003*	89.1 ± 222.3	103.2 ± 160.4	0.98*
Eos (/μl)	238.7 ± 32.5	239.4 ± 51.2	0.99	215.4 ± 33.4	267.3 ± 46.4	0.36
Hist (ng/ml)	68.0 ± 4.6	75.4 ± 7.0	0.43	74.1 ± 5.1	64.1 ± 6.2	0.21
Controls (<i>n</i> = 101)						
Total IgE (IU/ml)§	30.5 ± 76.0	18.2 ± 42.7	0.44*	22.6 ± 49.7	36.4 ± 72.5	0.67*

Eos, peripheral blood eosinophil count; Hist, histamine released in the histamine release test.

**P*-value calculated for log-transformed values.

†344 C>T polymorphism.

‡95 T>C polymorphism.

§Mean ± SEM, otherwise as median ± IQR.

Table 4. Prevalence of positive test for allergy and genotype classes of the –344 C>T and –95 T>C FcεRIα polymorphisms

	Allergic subjects (<i>n</i>)	–344 C>T polymorphism			–95 T>C polymorphism		
		Genotype class		<i>P</i> -value	Genotype class		<i>P</i> -value
		CC or CT	TT		TT	TC or CC	
ASST (±)	90†; 88‡	33/39	7/11	0.6	22/26	18/22	0.89
HRT (±)	99†; 97‡	41/36	13/9	0.63	27/28	27/15	0.14
Phadiatop (±)	55	12/29	5/9	0.91	10/18	7/20	0.62
IgE-SEA (±)	45	2/32	0/11	0.57*	1/22	1/21	1.0*
IgE-SEB (±)	45	2/32	3/8	0.09*	4/19	1/21	0.37
IgE-SEC (±)	45	3/31	0/11	0.42*	1/22	2/20	0.97
IgE-SED (±)	45	2/32	1/10	0.75	2/21	1/21	0.97
IgE-TSST-1 (±)	45	2/32	0/11	0.57*	0/23	2/20	0.23*
IgE-any-SE (±)	45	8/26	3/8	0.88	7/16	4/18	0.54

ASST, autologous serum skin test; HRT, histamine release test; IgE-SEA, IgE antibodies against staphylococcal enterotoxin A; IgE-SEB, IgE antibodies against staphylococcal enterotoxin B; IgE-SEC, IgE antibodies against staphylococcal enterotoxin C; IgE-SED, IgE antibodies against staphylococcal enterotoxin D; IgE-TSST-1, IgE antibodies against toxic shock syndrome toxin-1; IgE-any-SE, IgE antibodies against any staphylococcal enterotoxin.

P-value calculated by chi-squared test, otherwise by *Fisher exact test.

†344 C>T polymorphism.

‡95 T>C polymorphism.

(Tables 3 and 4) and haplotype analysis did not reveal additional differences related to the variance at the –95 nucleotide of the FcεRIα gene promoter.

The –344 C>T FcεRIα promoter polymorphism has been previously described by Shikanai et al. in asthmatics and healthy controls (4). The allelic frequencies in our

study were similar to those determined by Shikanai et al. in Caucasians, and likewise we did not find any differences in genotype frequencies between allergic subjects and controls. However, our patient group included both asthma and urticaria. Moreover, our controls were age and sex matched from a random population sample rather than healthy individuals. Thus, our finding of higher IgE levels in allergic subjects homozygous for allele T indirectly corresponds to the result of Shikanai et al. (4) who described a more frequent CC genotype in asthmatic patients with lower serum IgE levels.

The -95T > C FcεRIα polymorphism has been previously described by Hasegawa et al. in Japanese subjects (5) as -66 C > T. This inconsistency reflected indexing of the variable nucleotide from the translation start rather than transcription start. The allelic frequency of -95 T > C polymorphism in Caucasians has not been published previously.

Autologous serum skin test and HRT were developed as a scientific tool to investigate the autoimmune background of allergic diseases, mostly chronic idiopathic urticaria (8–10). We found no genotype-related differences in the results of ASST or histamine released in HRT, ascertained in most of the allergic subjects. Similarly, no differences were found within Phadiatop and IgE-SE results between genotype groups. The latter

was recently associated with a pathogenesis of various allergic conditions, for example atopic eczema/dermatitis syndrome, nasal polyposis, and allergic rhinitis (11–13). Taken together, none of the additional tests added to the interaction between total serum IgE levels and the -344 C > T FcεRIα polymorphism in the allergic subjects.

Our finding of higher IgE levels in patients with asthma or urticaria characterized by TT genotype of -344 C > T FcεRIα polymorphism supports the role of FcεRIα and its genetic variability in the pathogenesis of allergy. A recent introduction of a novel therapy based on recombinant anti-IgE antibody, omalizumab, pointed to the pivotal role of serum IgE level and downregulation of FcεRI subsequent to this treatment (14). The second promoter polymorphism, -95 T > C, although frequent, linked to the previous one and located closer to the coding sequence, in our data did not seem to explain this association additionally. Moreover, there was a strong statistical trend toward more frequent IgE-SEB positivity in relation to the -344 T allele, probably reflecting propensity to produce more of the specific IgE in atopic patients exposed to this common antigen. One might suggest that genotyping for FcεRIα polymorphisms could prove useful for prediction of anti-IgE efficacy, especially in patients suffering from severe asthma (15).

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