

Sequence variants in the FcεRI alpha chain gene

TOSHIKI SHIKANAI,¹ ERIC S. SILVERMAN,¹ BRIAN W. MORSE,¹
CRAIG M. LILLY,¹ HIROSHI INOUE,² AND JEFFREY M. DRAZEN¹

¹*Division of Pulmonary and Critical Care Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115; and* ²*Iwate Medical University, Morioka, Iwate 020-8505, Japan*

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Shikanai, Toshiki, Eric S. Silverman, Brian W. Morse, Craig M. Lilly, Hiroshi Inoue, and Jeffrey M. Drazen. Sequence variants in the FcεRI alpha chain gene. *J Appl Physiol* 93: 37–41, 2002. First published February 15, 2002; 10.1152/japplphysiol.00993.2001.—There is a relationship between IgE levels and expression of high-affinity IgE receptors (FcεRI). Because the alpha chain is the only portion of the receptor that binds directly to IgE, we reasoned that sequence variants in the FcεRI alpha gene may exist that alter these binding events. We screened all of the exons and the promoter region of the FcεRI alpha chain gene with genomic DNA from 389 asthmatic and 341 normal control subjects for mutations by using single-stranded conformational polymorphism analysis. No nonsynonymous single nucleotide polymorphisms (SNPs) were identified in the coding region. Three SNPs were found in the promoter region: an A/C transversion at –770 from the translation start site; a G/A transition at –664; and a T/C transition at –335. No differences in allele frequencies were detected between asthmatic subjects and controls. Homozygosity for the C variant at locus –335 was more common in Caucasian asthmatic patients with IgE levels in the lower quartile than in the upper quartile ($P = 0.032$). An analysis of highly polymorphic SNPs indicated that this association is unlikely to be due to population substructure. We conclude that homozygosity for the C allele of FcεRI alpha chain variant is associated with lower IgE levels.

asthma; atopy; immunoglobulin E; polymorphism; association study; genetics; allergy

CROSS-LINKING OF HIGH-AFFINITY IgE receptors (FcεRIs) by IgE and multivalent antigens on the surface of mast cells and eosinophils is an important early step in the sequence of atopic reactions. FcεRI is a heterotetrameric protein consisting of one alpha, one beta, and two gamma chains found exclusively on mast cells, eosinophils, basophils, monocytes, and Langerhans' cells (5, 8, 9, 11). The binding of IgE via the alpha chain of the receptor is an important factor regulating the number of FcεRIs on the cell surface (4, 17).

The FcεRI alpha chain gene is located on chromosome 1q23 in humans and consists of five exons and a 5'-untranslated region with known promoter activity. It is known that there is a direct relationship between

the level of expression of FcεRI and IgE levels in the blood of both humans and mice (4, 15, 17). We reasoned that naturally occurring DNA sequence variants in the FcεRI alpha chain gene might be associated with IgE levels; thus factors that could modify the expression of FcεRI could be associated with circulating IgE levels. To test this hypothesis, we used single-stranded conformational polymorphism (SSCP) analysis to screen genomic DNA derived from asthmatic subjects for sequence variation in the core promoter and all exons (including exon-intron boundaries) of the FcεRI alpha chain gene. No coding region single nucleotide polymorphisms (SNPs) were identified. A total of three polymorphisms were identified, one of which showed an association with IgE levels in an asthmatic population.

MATERIALS AND METHODS

Subjects. DNA was extracted by standard techniques from peripheral blood drawn from 389 patients with asthma diagnosed according to American Thoracic Society criteria (1). Patients consisted of 305 Caucasians and 84 African-Americans, whose only asthma medication was inhaled albuterol used on an as-needed basis. These patients were enrolled at over 20 centers in the United States as part of a drug treatment trial. They had disease of moderate severity, as indicated by an average forced expiratory volume in 1 s of 62.3% of predicted when tested at least 8 h after inhaled albuterol. Control DNA from 341 normal individuals (201 Caucasians and 140 African-Americans) without a history of asthma or atopy (as determined by responses to a questionnaire) was also obtained; these subjects were all enrolled in Boston.

PCR SSCP analysis. Genomic DNA was screened for mutations by SSCP analysis according to the method of Orita et al. (12) with minor modifications. Oligonucleotide primers (listed in Table 1) were designed from the published sequence (GenBank accession number L14075) (13). Forward and reverse primers were end labeled with [γ -³²P]ATP (DuPont NEN, Boston, MA) by using polynucleotide kinase (Boehringer Mannheim, Mannheim, Germany). PCR reactions were performed according to the methods previously described by our laboratory (2) with minor modifications. The radioactive amplified products were electrophoresed, transferred to chromatography paper (Whatmann, Maidstone, UK), and exposed to X-ray film after drying.

Address for reprint requests and other correspondence: J. M. Drazen, Division of Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, Harvard Medical School, 75 Francis St., Boston, MA 02115 (E-mail: jdrazen@nejm.org).

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Table 1. PCR primers for genotyping the *FceRI* alpha chain gene

Region	Primer	Sequence (5'–3')
Promoter	1	TTC ATG TGG AAT GAC TGG TTT C TCA AGG CTC AAC CTT CTT CC
Promoter	2	TTT GCA AAT GAC AAG CCA CT TCA GAA CAC AGG AAG GCA GA
Promoter	3	AGGGAGATCATGTTGGGAAA TTC ACT GAT GAT CCG CTG TC
Promoter	4	GCACTAACTGATCAGCAGGAA TGT TTT GGG GTG CCA ACT AT
Promoter	5	ATA GTT GGC ACC CCA AAA CA CTT AGA AAA GTG GGA TGC AAG G
Promoter	6	CAT ATG ACT AAG AGT TTG ACT TAG G GGC ATA GGT CTA GCA CAA TC
Promoter	7	TAT ATG TGG AGG TGG GAT AGG G CTG GTG CTT ACT GTG CTG GA
Exon 1	8	CTC CAG CAC AGT AAG CAC CA CCT GGG AGG GGT AAT TGA AT
Exon 2	9	TCC TGA AAA GAC GGT TGG TC AAG CAG AAG TGA TGG GAC GA
Exon 3	10	TTT TCG AAG TCC CTC AGA AAC C GTG GAA CCA TTT GGT GGA AC
	11	CAC CAA ATG GTT CCA CAA TG CCA CTG AAG ACT TCC AGG TAC A
Exon 4	12	CTT CAG ACT GGC TGC TCC TT CCA GTA CTT GAG AGC TTC ACC A
	13	TCC TCA GGT GCC ATG GTT CCT GCT ATG GAT GCT TTT CC
Exon 5	14	TGT TCC ACT ACA GCT CCG C TTG CTG ATG CTG GAA AAA AA
	15	AAC CCA CAT CCT AAG CCA AA CAT GCC ACT TAA CCA GTT TCA
	16	GAA ACG TCT GTG CTC AAG GA AAC TTC ATG GTC CTT GCC TTT

Restriction fragment length polymorphism analysis. Restriction fragment length polymorphism analysis was used to genotype at the T/C-335 locus. PCR was performed with the following primers: forward 5'-CATATGACTAAGAGTTTG-CTTAGG-3' and reverse 5'-GGCATAGGCTAGCACAA-TC-3'. PCR products were digested with *Mnl* I (New England Biolabs, Beverly, MA), according to the manufacturer's instructions and resolved by electrophoresis. DNA with the homozygous T genotype produced a digest pattern consisting of 106 and 232 bp; the heterozygous T/C genotype produced a digest pattern consisting of 73, 106, 159, and 232 bp, and the homozygous C genotype produced a digest pattern consisting of 73, 106, and 159 bp. Selected samples (5 samples per each genotype) were confirmed by direct sequencing with complete concordance at each locus.

IgE levels. Total plasma IgE levels of asthmatic patients and controls were measured by using the UniCAP system (Pharmacia and Upjohn, Uppsala, Sweden), according to the manufacturer's instructions.

Stratification analysis. Evidence of population stratification between the high and low IgE groups was sought by the method of Pritchard and Rosenberg (14). Participants were genotyped at 40 candidate SNPs selected from the SNP consortium project website (<http://snp.cshl.org/genome.shtml>). SNPs were selected to be at least 20 Mb apart, and thus are unlinked. Under the null hypothesis of genotype frequencies in the high and low IgE groups at each marker locus, contingency tables were constructed for each homozygous-wild type vs. (heterozygous + homozygous) mutant genotype (recessive model), and a χ^2 statistic was calculated. Because our interest is in whether loci show genotype-fre-

quency differences as a group, the test statistics from each locus were summed as $X_s^2 = \sum_{i=1}^L X_i^2$, where X_i^2 is the χ^2 statistic computed at the i th marker locus and L is the set of unlinked marker loci typed in all individuals. Under the null hypothesis, H_0 , X_s^2 is χ^2 distributed, with degrees of freedom equal to the sum of the degrees of freedom of individual loci. In the RESULTS section, the mean number ($\pm$ SD) of genotypes at each locus is provided along with a distribution of χ^2 values.

Statistical analysis other than for population stratification. Associations between allele frequency and asthma phenotype, IgE level, or race were determined by χ^2 analysis. Consistency of genotype frequencies with Hardy-Weinberg equilibrium was tested with goodness of fit for a χ^2 test on a contingency table of observed vs. predicted genotype frequencies. Results are expressed as fractions and considered statistically significant at the $P < 0.05$ level. Computations were performed with StatView 4.0 software (Abacus Concepts, Berkeley, CA).

RESULTS

We screened 1.3 kb of the 5' flanking region and all five exons (1.4 kb), including exon-intron junctions, of *FceRI* alpha chain gene in 389 asthmatic patients for DNA sequence variants by PCR SSCP analysis. Three SNPs (Fig. 1) were detected; each was located in the 5' flanking region. No sequence variants were detected in

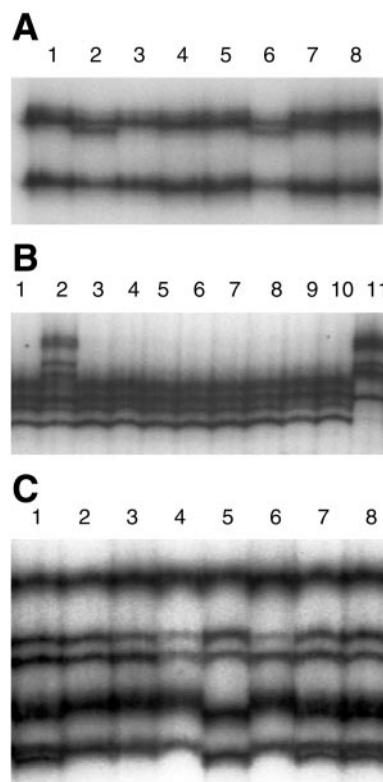


Fig. 1. Three sequence variants were identified by single-stranded conformational polymorphism (SSCP) analysis in the *FceRI* alpha chain gene. A: detection of A/C-770 variant in 5' flanking region. Lanes 2 and 6 are heterozygous, whereas lanes 1, 3, 4, 5, 7, and 8 are wild type. B: detection of G/A-664 variant. Lane 2 is heterozygous, and lane 10 shows no wild-type allele; all other lanes are wild type. C: detection of T/C-335 variant in 5' flanking region. Lanes 1, 7, and 8 are T/C. Lanes 2, 3, 4, and 6 are TT. Lane 5 is CC.

the coding region of the F ϵ cRI alpha chain gene. Once variants were detected by SSCP and confirmed by direct sequencing, the 389 asthmatic patients and 341 normal controls were genotyped by restriction fragment length polymorphism (T/C-335) or SSCP (A/C-770 and G/A-664). Genotype data are summarized in Table 2. The most common polymorphism consisted of a T/C transition at -335 bp from the translation start site and had a minor allele (T) frequency of 0.36 for all genotyped individuals. The A/C transversion at -770 bp from the ATG start site had a minor allele (C) frequency of 0.045, and the G/A transition at -664 had a minor allele (A) frequency of 0.030.

All sequence variants were in Hardy-Weinberg equilibrium, except those at G/A-664 in both Caucasian and African-American asthmatic patients when stratified by race; there were no significant differences in allele frequencies or genotypes between asthmatic patients and nonasthmatic patients. The frequency of the T/C-335 polymorphism differed significantly between Caucasians and African-Americans in both the asthmatic population (0.423 vs. 0.146, respectively; $P < 0.0001$) and the normal population (0.445 vs. 0.161, respectively; $P < 0.0001$). The allele frequencies of A/C-770 were marginally different between Caucasian asthmatic patients and African-American asthmatic patients ($P = 0.039$).

The total average IgE levels of the Caucasian and African-American asthmatic patients were 307.46 ± 441.83 and 401.02 ± 445.03 kU/l, respectively. The total IgE levels of Caucasian and African-American controls were 49.38 ± 100.75 and 145.64 ± 376.31 kU/l, respectively. There was substantial variance in the IgE levels (Fig. 2). To determine whether there was a relationship between genotype and IgE level, we examined the distribution of genotypes in the upper and lower quartiles of the IgE distribution. There was a greater proportion of genotype CC (T/C-335) than of

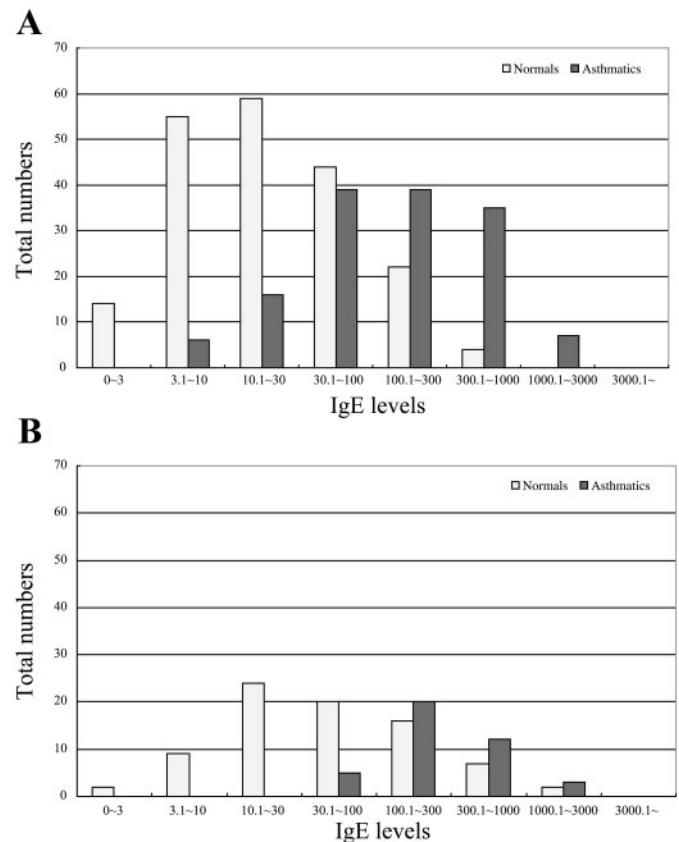


Fig. 2. Total IgE levels in asthmatic patients and controls. A: total IgE levels of Caucasians. B: total IgE levels of African-Americans. There were significant differences between patients with asthma and normal controls ($P < 0.0001$), between Caucasian asthmatic patients and African-American asthmatic patients ($P = 0.006$), and between Caucasian controls and African-American controls ($P < 0.0001$).

TT or TC in the Caucasian patients in the lowest quartile of IgE level compared with Caucasian patients in the highest quartile ($P = 0.025$; Table 3). A similar difference was not observed in African-American asthmatic patients. There were no significant genotype-stratified differences in forced expiratory volume in 1 s, total eosinophil counts, or total IgE levels.

Stratification analysis. The total number of genotypes for the stratification analysis of high and low IgE groups was 1,644, with an average of 41.1 ± 6.4 genotypes per locus. No evidence of stratification was detected within the high and low IgE groups ($\chi^2_{40} = 30.6$, $P = 0.86$). The distribution of each genotype as a function of χ^2 value is provided (Fig. 3). Only 1 of 40

Table 2. Genotype frequencies of sequence variants in F ϵ cRI alpha chain gene

Genotype	Caucasian		African-American	
	Patients, %	Controls, %	Patients, %	Controls, %
A/C-770				
AA	94.6(140)	88.3(106)	87.8(72)	91.9(79)
AC	5.4(8)	11.7(14)	11.0(9)	8.1(7)
CC	0(0)	0(0)	1.2(1)	0(0)
H-W-P	0.740	0.497	0.263	0.094
G/A-664				
GG	94.7(197)	95.0(172)	92.9(78)	93.6(131)
GA	4.8(10)	0.5(9)	6.0(5)	6.4(9)
AA	0.5(1)	0(0)	1.2(1)	0(0)
H-W-P	0.040	0.732	0.019	0.693
T/C-335				
TT	16.7(51)	19.4(39)	2.4(2)	3.4(3)
TC	51.1(156)	50.2(101)	24.4(20)	25.3(22)
CC	32.1(98)	30.3(61)	73.2(60)	71.3(62)
H-W-P	0.040	0.808	0.829	0.333

Genotype distributions of Caucasian asthmatic patients and controls and African-American asthmatic patients and controls are shown. Values in parentheses denote the n value. H-W-P, P value for the Hardy-Weinberg equilibrium patients in question.

Table 3. Analysis of total IgE levels and genotype results of T/C-335 in F ϵ cRI alpha chain gene

Genotype	Caucasian		African-American	
	Upper Quartile IgE	Lower Quartile IgE	Upper Quartile IgE	Lower Quartile IgE
T/C-335				
TT + TC	27	19	2	2
CC	8	17	8	8
P value	0.032		>0.999	

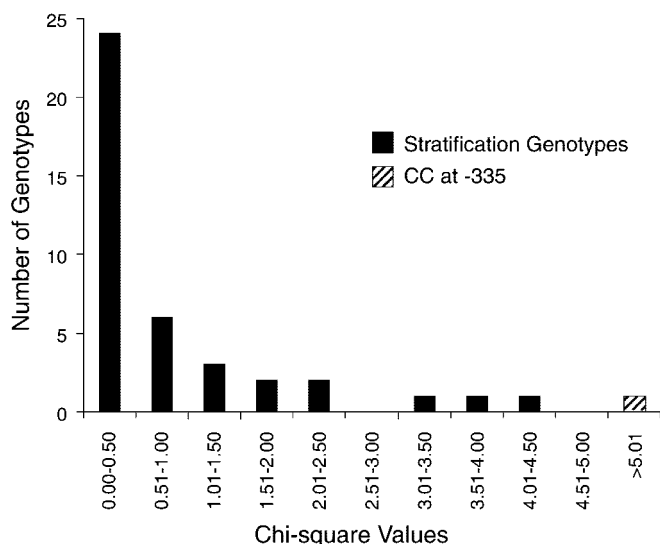


Fig. 3. Histogram of χ^2 values from contingency tables comparing the number of subjects having two polymorphic alleles at a given locus with those having no or one polymorphic allele. Among the 40 test loci, only 1 had a χ^2 value exceeding 4, whereas the locus of interest in the alpha chain in the FcεRI had the highest χ^2 value.

loci, other than -335, was significantly different between high and low IgE groups, and the strength of the association was greatest for the -335 locus; locus -335 had the highest χ^2 value of all genotypes ($\chi^2 = 5.04$, $P = 0.025$).

DISCUSSION

The alpha chain of the FcεRI demonstrates a remarkable consistency of its DNA sequence in the coding region. Because on average at least 1 SNP per 1,000 of coding sequence is expected (3), this observation suggests that there have been evolutionary pressures to retain the intact sequence. Although the coding region was not polymorphic, we found three SNPs in the 5' flanking region of the FcεRI alpha chain. The most common SNP, with an allele frequency of 0.47 in Caucasian asthmatic patients, demonstrated an association with IgE levels.

We used SSCP analysis, which has about a 70% efficiency in identifying variants (10), to screen for DNA sequence variants in the 5' untranslated region and coding exons of the gene for the alpha chain of the FcεRI. We identified no sequence variants in the coding region of the gene. Because we screened 542 chromosomes, we had theoretical power of >99% to identify alleles with a frequency of ≥ 0.04 (7). Even if the <70% efficiency of SSCP reduced the effective number of chromosomes screened to 389, we still had 98% power to identify sequence variants at this minor allele frequency. We acknowledge, however, that there may be variants that are not resolved by SSCP and that may have escaped detection by this method.

The lack of DNA sequence variants in the coding region of the alpha chain of FcεRI suggests that maintaining fidelity in this region of the genome is important to homeostasis. Sequence variants in the FcεRI

beta chain gene have been reported (6, 16) that may be associated with atopy or bronchial hyperresponsiveness. There are no data available on sequence variants in the gamma chain. An interpretation of these data is that binding of IgE by the alpha chain of the FcεRI is an important event in atopic diathesis.

Although we found no coding region variants in the alpha chain, we found variants in the 5' untranslated region of the gene. Two of the variants were uncommon, with minor allele frequencies of <0.05, whereas the T/C transition at -335 was relatively common, with a minor T allele frequency of 0.47 in Caucasian asthmatic patients. The frequency of the T allele in African-American asthmatic patients was much lower (0.15). Thus this allele is one with marked differences in frequency among individuals of varying ethnicity.

In the entire cohort of Caucasian asthmatic patients, the T/C transition was not associated with IgE levels. That is, patients with a TT genotype did not have a different mean IgE level than patients with a CC genotype. We reasoned, however, that there may be multiple pathways leading to altered IgE levels and thus examined the population extremes to determine whether there was a significant difference in the proportion of genotypic assignments between Caucasian asthmatic patients with high and low IgE levels (Table 3). Our data show that there were nearly equal proportions of TT + TC and CC genotype among the individuals with the lowest IgE levels, whereas the proportion of patients with the CC genotype was lower among Caucasian asthmatic patients with the highest IgE levels. We speculate that, in Caucasian asthmatic patients, the CC genotype skews toward a lower IgE response. The same trend was not observed in African-American asthmatic patients, thus suggesting that the panel of genes controlling IgE levels differs in different ethnic groups.

Because case-control association studies may be confounded by population substructure, we subjected the DNA from patients in the upper and lower quartiles of IgE levels to an analysis to see whether there were underlying identifiable genetic differences between the two groups unassociated with the loci identified at the alpha chain of the FcεRI. Our analysis shows that, among 40 polymorphic loci scored, only one, as would be expected, had a $\chi^2 P$ value of <0.05 (Fig. 3). On the basis of this analysis, it is unlikely that our finding is spurious. Although a functional effect of the C/T transition has not been identified, our observation opens this region of the alpha chain of the FcεRI to such study.

It is known that circulating IgE levels are closely related to the levels of expression of FcεRI. Because the alpha chain is the binding unit for IgE, we speculate that the T/C transition at position -335 of the alpha chain of FcεRI leads to lower IgE binding and represents a potential mechanism responsible for our finding. In following this line of reasoning, it seems reasonable to speculate that the microenvironmental availability of the alpha chain of the FcεRI may participate in the regulation of IgE levels.

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