

Letter to the Editor

Correlation of *FCER1B* gene haplotypes with histamine release from basophils in atopic asthma

To the Editor:

The pathophysiologic features of atopic disorders, including atopic asthma, consist of enhanced IgE production, increased mediator release from inflammatory cells, and hypersensitivity of target organs.¹ The interaction between allergen-specific IgE and its high-affinity receptor, FcεRI, which is present on the surface of human mast cells and basophils, is the key to the onset and propagation of allergic diseases.²

Allergic individuals have higher serum IgE levels and higher numbers of IgE receptors (FcεRI) per basophil than healthy subjects.³ Also, FcεRI-mediated histamine release (HR) is significantly greater in asthmatic patients than control subjects, indicating that FcεRI-mediated HR plays an important role in the pathogenesis of asthma.² In addition, a significant intrapair correlation coefficient of anti-IgE-induced and calcium ionophore A23187-induced HR was found in monozygotic twins but not in dizygotic twins,⁴ suggesting that both FcεRI-mediated and non-FcεRI-mediated HRs are determined in part by genetic factors.

Previous linkage studies showed that HR from basophils to anti-IgE stimulus was linked to the genetic marker of chromosome 11q13, where the gene for the β-subunit of the high-affinity IgE receptor (*FCER1B*) is located.³ This subunit enhances the surface expression of FcεRI by association with the α-subunit and functions as an amplifier of signal transduction mediated by the γ-subunit.^{2,5} Several polymorphisms have been identified in the *FCER1B* gene,^{2,5-7} a few of which alter/regulate the function of the receptor by changing the expression of the β-chain. Particularly, the E237G polymorphism has

been associated with increased HR from basophils in response to anti-IgE stimuli¹ and with increased FcεRI expression on human basophils.⁵ In our earlier study, using a G/A polymorphism in intron 2, a C/T polymorphism in the 3'-untranslated region (3'-UTR), and a dinucleotide (CA)_n repeat in intron 5, we identified 2 major haplotypes, A₁₆C and G₁₈T, to be positively and negatively associated with asthma, respectively. However, the E237G variant was noninformative in our population (minor allele frequency <3%).⁶ Because *FCER1B* has numerous polymorphisms,^{2,6,7} we wondered whether the identified risk and protective haplotypes of this gene could correlate with IgE receptor-mediated (anti-human IgE) HR, non-IgE receptor-mediated (calcium ionophore A23187) HR, or both.

We recruited 36 unrelated atopic asthmatic patients (mean age, 25.39 ± 10.76 years; male/female ratio, 0.55:0.45) having specific haplotypes from northern India. Ethical approval was obtained from the institutional review board. Written informed consent was obtained from all participants. Patients were diagnosed for asthma as detailed earlier.⁶ Briefly, asthma in the recruited study population was defined by clinical history and validated later by interview questions. Fifteen common environmental allergens were used for the skin prick test, and all subjects had positive results to at least one allergen. Total serum IgE levels were estimated by using ELISA (2.76 ± 0.66 IU/mL).⁶

Leukocyte suspensions from peripheral blood were isolated as previously described.¹ HR to 2 different stimuli, goat anti-human IgE (Bethyl Laboratories, Montgomery, Tex) and calcium ionophore-A23187 (Sigma, St Louis, Mo), at 3 different concentrations (anti-IgE: 1:1000, 1:100, 1:10; calcium ionophore: 1, 3, and 10 mM) was measured as described previously with a spectrofluorometer (Molecular Devices, Sunnyvale, Calif).¹ HR was calculated as follows:

TABLE 1. HR induced from basophils in patients with asthma according to *FCER1B* gene polymorphisms and haplotypes

Genotypes/haplotypes	No.	HR-anti-IgE (%)	HR-A23187 (%)	HR ratio
Intron 2_3'-UTR				
a. AA_CC*	8	9.94 ± 5.88	10.37 ± 9.62	1.87 ± 1.49
Non-AA_CC	28	4.93 ± 2.71	13.29 ± 13.89	0.67 ± 0.57
b. GG_TT†	12	2.92 ± 2.03	14.01 ± 10.71	0.25 ± 0.16
Non-GG_TT	24	9.67 ± 6.98	12.80 ± 11.07	1.16 ± 0.95
Three-locus haplotype				
a. A ₁₆ C/A ₁₆ C‡	6	11.01 ± 5.69	13.30 ± 9.37	1.21 ± 0.92
Non-A ₁₆ C/A ₁₆ C	30	4.87 ± 3.74	14.75 ± 15.03	0.52 ± 0.49
b. G ₁₈ T/G ₁₈ T§	10	3.52 ± 2.22	20.33 ± 20.26	0.29 ± 0.21
Non-G ₁₈ T/G ₁₈ T	26	9.48 ± 6.70	12.81 ± 10.85	1.17 ± 0.94

HR-anti-IgE, Maximal HR from basophils at 3 different concentrations of anti-IgE (% [mean ± SD]); HR-A23187, maximal HR from basophils at 3 different concentrations of calcium ionophore A23187 (% [mean ± SD]); HR ratio, maximal HR from basophils to anti-IgE divided by maximal HR from basophils to calcium ionophore A23187 (mean ± SD).

*F ratio = 12.07, *df* = 1, *P* = .001 and F ratio = 12.57, *df* = 1, *P* = .001 for HR-anti-IgE and HR ratio, respectively.

†F ratio = 10.62, *df* = 1, *P* = .0025 and F ratio = 10.54, *df* = 1, *P* = .003 for HR-anti-IgE and HR ratio, respectively.

‡F ratio = 11.25, *df* = 1, *P* = .002 and F ratio = 7.26, *df* = 1, *P* = .011 for HR-anti-IgE and HR-ratios, respectively.

§F ratio = 7.51, *df* = 1, *P* = .01 and F ratio = 8.29, *df* = 1, *P* = .007 for HR-anti-IgE and HR-ratios, respectively.

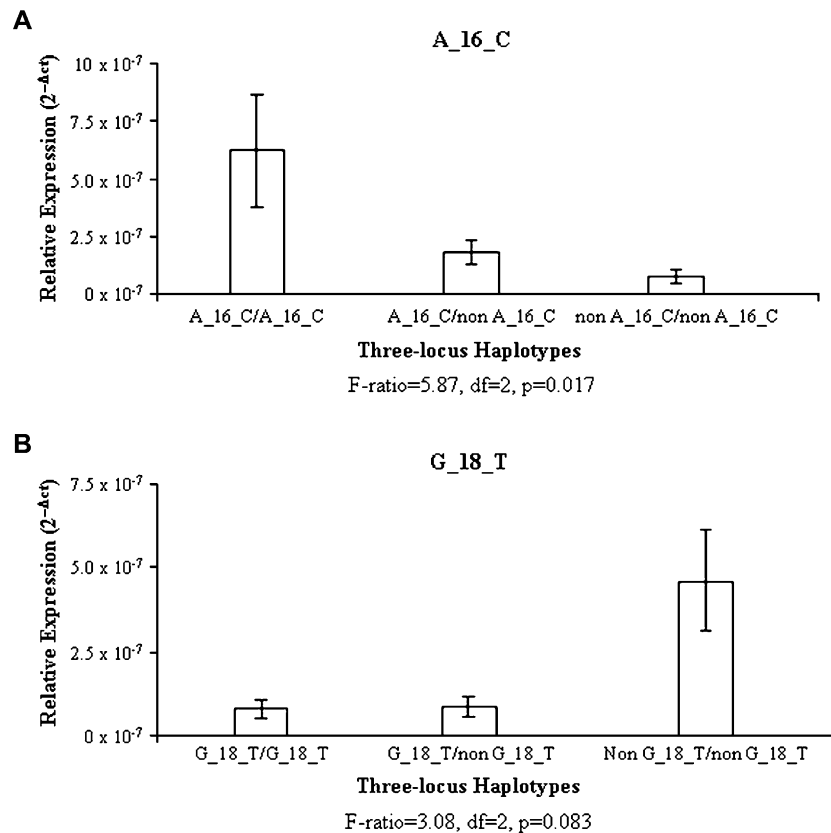


FIG 1. TaqMan real-time quantitative RT-PCR analysis for *FCER1B* transcripts in individuals with specific haplotypes: **A**, A_16_C; **B**, G_18_T. Each bar represents the mean $\pm$ SEM. There were more than 3 cases for each haplotype. ΔCt values ($Ct_{FCER1B} - Ct_{18S}$) were calculated from duplicate samples normalized to human 18S rRNA and were converted to linear scale by using the formula $2^{-\Delta Ct}$. The y-axis represents the $2^{-\Delta Ct}$ values.

$$HR(\%) = \frac{([Induced\ HR - Spontaneous\ HR])}{[Total\ histamine\ content]} \times 100.$$

Total RNA was extracted from the leukocyte suspensions ($n = 15$) and converted to cDNA (cDNA archive kit; Applied Biosystems, Foster City, Calif). The amount of *FCER1B* mRNA was quantified by using a 7900 Real-Time PCR System (Applied Biosystems) with an Assays-on-Demand gene expression product (no. Hs00175091_m1# for human *FCER1B*) and TaqMan universal PCR mastermix (Applied Biosystems).

Genomic DNA was extracted and genotyped for polymorphisms in the *FCER1B* gene, as described earlier.⁶ Haplotypes were generated with PHASE version 2.0.2.⁶ ANOVA was used to assess the significance of association between *FCER1B* haplotypes and HR from basophils.

Our earlier results suggested that the CC genotype at the 3'-UTR and the AA genotype at intron 2 together conferred higher risk.⁶ Interestingly, when we compared individuals for HR induced by anti-IgE (HR-anti-IgE) and for the HR ratios (HR-anti-IgE/HR-A23187) between the AA_CC and non-AA_CC genotypes (intron 2_3'-UTR), significant differences were obtained (F ratio = 12.07, $df = 1$, $P = .001$ and F ratio = 12.57, $df = 1$, $P = .001$ for HR-anti-IgE and HR ratio, respectively),

with a higher HR associated with individuals having the AA_CC combination of genotypes (Table I). On the other hand, the GG_TT genotype combination (associated with lower risk in the earlier study)⁶ was associated with lower HR (F ratio = 10.62, $df = 1$, $P = .0025$ and F ratio = 10.54, $df = 1$, $P = .003$ for HR-anti-IgE and HR ratio, respectively; Table I). However, no difference was observed when spontaneous HR, total histamine content, and maximal HR to A23187 were compared for these groups ($P > .05$).

Also, when we correlated the haplotypes A_16_C and G_18_T with the HR from basophils, significant differences were obtained ($P < .05$). As evident from Table I, individuals with the protective haplotype G_18_T had lower HR profiles (F ratio = 7.51, $df = 1$, $P = .01$ and F ratio = 8.29, $df = 1$, $P = 0.007$ for HR-anti-IgE and HR ratios, respectively) compared with individuals with the risk haplotype A_16_C (F ratio = 11.25, $df = 1$, $P = .002$ and F ratio = 7.26, $df = 1$, $P = .011$ for HR-anti-IgE and HR ratios, respectively).

Because the FcεRI function is dependent on the expression of the β -chain, we measured its transcript levels. Interestingly, we found that the individuals homozygous for the risk haplotype A_16_C had significantly higher levels of the transcripts (Fig 1, A) compared with the A_16_C heterozygous and the non-A_16_C

homozygous individuals ($P = .017$), whereas individuals homozygous for the protective haplotype G_18_T (Fig 1, B) had lower levels of the transcripts compared with the G_18_T heterozygous and the non-G_18_T individuals ($P = .083$), thus further corroborating the results in Table I.

Therefore, these results indicate that individuals homozygous for the A_16_C haplotype are at increased risk, whereas individuals homozygous for G_18_T are at lower risk because of the differences in their HR profiles. Because the non-IgE receptor (A23187)-mediated HR is unaffected by specific haplotypes/genotypes, the difference in HR could be due to FcεRI-mediated signaling differences in specific haplotypes/genotypes. Although no causal role has been attributed to these polymorphisms, our results indicate that the presence of these haplotypes in an asthmatic individual modulate FcεRI-mediated signaling in response to receptor cross-linking, presumably by altering the expression of *FCER1B*. Alternatively, these haplotypes might be present in linkage disequilibrium with any other causal mutation in the gene⁵ that confers higher risk for asthma and related atopic disorders.

In summary, this study correlates *FCER1B* haplotypes with HR and *FCER1B* expression and thus could be useful for identifying individuals predisposed to atopic asthma.

We thank our collaborating physicians for helping us in sample collection. We also thank all patients for participating in this study. The financial support from the Task Force Project SMM-0006 of the Council of Scientific and Industrial Research (CSIR), India, is greatly appreciated. Shilpy Sharma and Amrendra Kumar acknowledge CSIR for their fellowship.

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Disclosure of potential conflict of interest: The authors have all received grant support from the Council of Scientific and Industrial Research and are all employed by the Institute of Genomics and Integrative Biology.

Supported by the Council for Scientific and Industrial Research (CSIR), India.

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doi:10.1016/j.jaci.2006.06.039