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A Common *FCER1B* Gene Promoter Polymorphism* Influences Total Serum IgE Levels in a Japanese Population

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Genetic factors are important in defining total serum IgE levels. Linkage analyses have localized a gene or genes that influence atopic phenotype at chromosome 11q13. Variants of the *FCER1B* gene have been identified, which are associated with an increased risk of developing atopy and bronchial asthma. Given uncertain functional consequences and low frequencies of these coding variants of *FCER1B*, we screened for new mutations using 24 subjects with atopic asthma. A common −109C/T polymorphism at the promoter region of *FCER1B* was identified, although no variant was found in the entire coding region. We genotyped this promoter polymorphism in 226 healthy control subjects and 226 asthmatic subjects using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis. Allele frequencies were 0.697 for −109T and 0.303 for −109C in 226 healthy control subjects. No significant difference in the distribution of −109C/T polymorphism was found between asthmatic subjects and healthy control subjects. A homozygosity for the −109T allele, however, was associated with increased total serum IgE levels in 226 subjects with asthma ($p = 0.0015$). The strongest evidence for an association between total serum IgE levels and −109C/T polymorphism ($p = 0.0004$) was obtained when age at onset of asthma was incorporated into the analysis. Our findings may represent genetic heterogeneity and complex interactions between genetic and environmental components involved in the regulation of total IgE levels, providing evidence that the −109C/T polymorphism of the *FCER1B* promoter region is one of the genetic factors identified thus far, which affects total serum IgE levels in a Japanese population. Hizawa N, Yamaguchi E, Jinushi E, Kawakami Y. A common ***FCER1B*** gene promoter polymorphism influences total serum IgE levels in a Japanese population. AM J RESPIR CRIT CARE MED 2000;161:906–909.

Total serum IgE levels are a quantitative trait associated with atopic diseases including bronchial asthma, allergic rhinitis, and atopic dermatitis. Immunoglobulin E-dependent activation of mast cells and basophils through the high-affinity IgE receptor (FcεRI) is involved in the pathogenesis of allergen-induced immune responses in atopic diseases. Several studies have reported linkage or association between the measures of atopy and chromosome 11q13 (1–3). The gene encoding the β subunit of the high-affinity IgE receptor in this chromosomal region is considered to be the most likely candidate for these findings (4). Recently, animal models have provided unequivocal genetic evidence that FcεRI-β functions as an amplifier of immune responses, especially those induced by mast cells and basophils (5).

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Three coding variants, I181L/V183L, I181L, and E237G in the *FCER1B* gene have previously been described. An isoleucine to leucine substitution at position 181 (I181L) of FcεRI-β is associated with atopy through maternal descent in a British population (6). In addition, a glutamic acid-to-glycine substitution (E237G) occurs in approximately 5% of Australian and British populations and is associated with higher prevalence of atopy and bronchial hyperresponsiveness (7). It is, however, apparent that these variants are neither necessary nor sufficient to cause atopy, because these variants are comparatively rare (7, 8) and the functional roles of these *FCER1B* gene variants in atopy and asthma are still obscure.

We previously failed to identify I181L variants in 120 unrelated Japanese subjects (3). In the present study, therefore, we searched for a mutation in the promoter region, and examined its relationship to the development of bronchial asthma, atopy, or total serum IgE levels using 226 unrelated asthmatic subjects and 226 unrelated healthy control subjects.

METHODS

Screening for Mutations

Twenty-four unrelated patients with atopic asthma, who had both elevated total serum IgE levels (≥ 300 international units [IU]/ml) and elevated specific IgE antibody (Ab) levels (≥ 0.35 UA/ml) toward at least one common inhaled allergen, were selected for mutation analysis in the *FCER1B* promoter region. Genomic DNA was amplified

under standard polymerase chain reaction (PCR) conditions with a primer pair of 5′-GTG GGG ACA ATT CCA GAA GA-3′ (sense) and 5′-CCG AGC TGT CCA GGA ATA AA-3′ (antisense). The PCR product was 382 base pairs in length, corresponding to nucleotides −291 to +91 of the human *FCER1B* sequence. Sequence analysis was performed using the Perkin-Elmer dye terminator cycle sequencing kit on an ABI PRISM 310 Genetic Analyzer (PE Applied Biosystems, CA). A C to T substitution at −109 bp (from the transcription initiation site) in the *FCER1B* promoter region generated a *Tru9I* restriction site, and we used a restriction enzyme based assay to study more subjects and determine the prevalence of this −109C/T polymorphism. Briefly, *Tru9I* (New England Biolabs) digested the PCR products, and digestions were analyzed by electrophoresis on a 3% agarose gel. The PCR products corresponding to the −109C allele produced fragments of 221 and 161 bp, whereas the PCR products corresponding to the −109T allele produced fragments of 182, 161, and 39 bp (a fragment of 39 bp was not visible on the gel).

We also searched for mutations in seven exons of *FCER1B*, including I181L, I183V, and E237G in the same 24 subjects with atopic asthma.

Subjects

Subjects with bronchial asthma (n = 226) were recruited from the pulmonary clinic at the First Department of Medicine, Hokkaido University Hospital. Subjects with bronchial asthma were included on the basis of history (the presence of cough, wheeze, or dyspnea) and a demonstrated reversible airflow limitation (15% variability in forced expiratory volume in one second [FEV_1] or in peak expiratory flow rate either spontaneously or with an inhaled short-acting β_2-agonist), increased airway responsiveness to methacholine, or both. Healthy control subjects (n = 226) who had requested annual physical examinations were included only if they had no history of allergic diseases, including bronchial asthma, allergic rhinitis, and atopic dermatitis.

Total serum IgE levels (IU/ml) and specific IgE responses to 10 common inhaled allergens, including *Dermatophagoides farinae* (*Der f*), grass pollens, animal danders, and molds, were determined. A specific IgE Ab level (IgE CAP RAST) equal to or more than 0.35 UA/ml was considered positive. We defined atopy as having positive RAST scores ($\geq$ 0.35 UA/ml) to at least one of 10 common allergens. All subjects in the study (n = 552) were Japanese, and all gave informed consent for enrollment in the study and for blood drawing.

Statistical Analysis

The allelic frequency distribution was tested for Hardy-Weinberg equilibrium by the chi-square test. To test whether the −109C/T polymorphism is an independent contributor to the development of bronchial asthma or atopy, we performed a logistic regression analysis using sex, age, smoking status, and −109C/T polymorphism as independent variables. Differences in serum total IgE levels between C/C homozygotes, C/T heterozygotes, and T/T homozygotes were then examined using multiple linear regression models adjusted for age, sex, and smoking status in healthy control subjects and in asthmatic subjects separately. Because it appeared that elevated total IgE levels were confined to the T/T homozygotes, C/T and C/C genotypes were combined into a single category for subsequent analyses.

Given the evidence for genetic heterogeneity of bronchial asthma according to age at onset of the disease (9), we studied the genetic association between the −109C/T promoter polymorphism and total IgE levels, while incorporating age at onset of asthma as a covariate in a multiple linear regression analysis. Logistic regression and multiple linear regression analyses were completed using the program Prophet 5.0 (BBN Systems and Technologies).

We also examined the interaction between genetic effects of the −109C/T polymorphism and disease status in the regulation of total IgE levels using a factorial ANOVA on a model which included disease status (asthmatic or healthy) and disease status −109C/T polymorphism in addition to age, sex, smoking status, and −109C/T polymorphism (SYSTAT; SYSTAT Inc., Evanston, IL).

For these analyses, total IgE levels (IU/ml) were log transformed to normalize the distribution.

RESULTS

Allelic Variation in the *FCER1B* Promoter Region

The −109C/T biallelic polymorphism was identified at 109 bp upstream from the transcription initiating site in the *FCER1B* promoter region (Figure 1). The 24 subjects with atopic asthma exhibited three homozygotes for −109C allele, 14 homozygotes for −109T allele, and seven heterozygotes. Sequencing of seven exons did not reveal any additional variants such as I181L, V183L at exon 6, or E237G at exon 7 in 24 subjects with atopic asthma (data not shown).

Study Subjects

Detailed characteristics of study subjects are described in Table 1. Subjects with bronchial asthma had higher total IgE levels than healthy control subjects (2.48 ± 0.58 [mean ± SD] log IU/ml versus 1.74 ± 0.59, $p < 0.0001$, *t* test). A total of 172 (76.1%) subjects with asthma and 65 healthy control subjects (28.8%) were atopic.

Mean age at onset of asthma in the 54 subjects who had no positive RAST scores toward any of the allergens was 40.6 ± 18.4 y, whereas it was 28.6 ± 19.8 yr in the 172 subjects who had positive RAST scores toward at least one allergen. One hundred (83.3%) of 120 subjects who had developed asthma at 40 yr or younger, and 43 (40.6%) of 106 subjects who had developed asthma at an age older than 40 yr were atopic.

Association Analysis

The −109T allele occurred in 315 of 452 chromosomes (69.7%) in unrelated healthy control subjects, and 273 of 452 chromosomes (64.8%) in unrelated asthmatic subjects. The −109C/T genotypes were distributed according to Hardy-Weinberg equilibrium ($p > 0.05$) in the whole population, indicating asthmatic subjects and healthy control subjects were recruited from the same population. Logistic regression analysis was used to adjust for age, sex, and smoking status and provided no evidence for a significant difference in genotype distribution between healthy control subjects and 226 asthmatic subjects or between 237 atopic and 215 nonatopic subjects. The odds ratios were 1.31 (95% CI 0.95 to 1.79) for asthma, and 112 (0.79 to 1.59) for atopy when subject had a −109T allele. However,

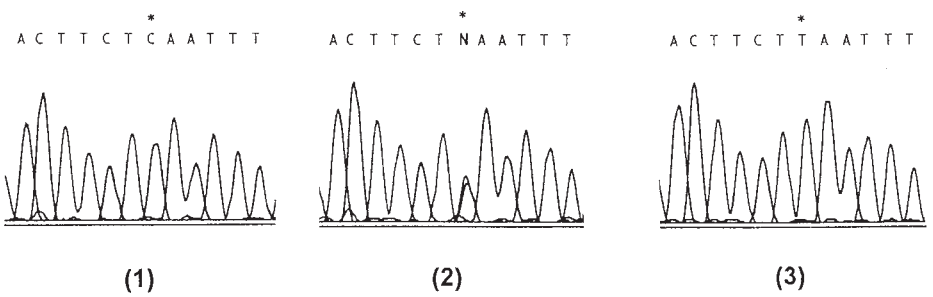

Figure 1. Identification of −109C/T polymorphism. Representative sequencing results of the promoter region of *FCER1B* showing −109C/C homozygous (1), −109C/T heterozygous (2), and −109T/T homozygous sequence (3). *Asterisks* correspond to −109 bp from transcription initiation site of *FCER1B*.

TABLE 1

DEMOGRAPHIC CHARACTERISTICS OF SUBJECTS WITH ASTHMA AND HEALTHY CONTROLS ACCORDING TO THE −109C/T GENOTYPE*

	Subjects with Asthma			Healthy Controls		
	T/T	C/T	C/C	T/T	C/T	C/C
No. of subjects	85	123	18	108	99	19
Age, yr	45.8 ± 16.5	44.3 ± 16.5	42.8 ± 16.5	41.6 ± 11.5	42.8 ± 11.5	39.4 ± 11.5
Sex, F/M	45/40	64/59	10/08	53/55	39/60	10/09
Current smoker	17 (20.0%)	28 (22.8%)	5 (27.8%)	30 (27.8%)	33 (33.3%)	7 (36.8%)
Positive RAST (≥ 0.35 UA/ml)	68 (80.0%)	87 (70.7%)	17 (94.4%)	30 (27.8%)	31 (31.3%)	4 (21.1%)
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 means ± SD.
† $p = 0.0060$ among genotypes in subjects with asthma by multivariate analysis using sex, age, and smoking status as covariates.

we found a significant difference in total serum IgE levels among genotypes in subjects with asthma ($p = 0.0060$) (Table 1). Using the T/T homozygotes as a reference, we observed an association between the T/T genotype and elevated serum IgE levels in subjects with asthma ($p = 0.0015$) (Table 2b). The association of total IgE levels with the *FCER1B* promoter polymorphism was found to be highly significant ($p = 0.0004$) when age at onset of asthma was controlled for in the model (Table 2c). Conversely, there was no significant difference in total serum IgE levels between T/T and C/T or C/C genotypes ($p > 0.05$) in healthy control subjects (Table 2a).

Finally, we identified a significant interaction between genetic effects of −109C/T polymorphism and asthma status in defining total serum IgE levels (F statistics = 4.98, $p = 0.026$ for −109C/T polymorphism disease status) using a whole population (226 asthmatic subjects and 226 healthy control subjects) (*see* METHODS).

DISCUSSION

We have identified a common −109C/T polymorphism in the transcriptional control region upstream from the *FCER1B* coding sequence. Although the functional consequences are still unknown at the present time, the location within the proximal 5′-flanking region suggested that this polymorphism had a direct role in the regulation of transcription. According to the analysis with the MatInspector program (10), the sequence between −94 bp and −107 bp from the transcription initiation site corresponds to the consensus sequence of NFAT binding site. Additionally, transition from C to T at −109 bp in the promoter region produced new transcription factor binding sites for HFH-3, Nkx-2.5, and S8. Luciferase assays revealed that Nkx-2.5 served as a modest transcription activator (11). Furthermore, a high prevalence of each −109C/T allele may provide a strong biological rationale for the involvement of this promoter polymorphism in the regulation of serum total IgE levels, although the high prevalence of the allele could be attributable to random genetic drift.

The distribution of −109C/T polymorphism was similar between asthmatic subjects and healthy control subjects and between atopic and nonatopic subjects, suggesting that this promoter polymorphism was unlikely to be a significant genetic factor contributing to the development of bronchial asthma or atopy. Homozygosity for the −109T allele, however, remained an independent risk factor for elevated total IgE levels especially in subjects with bronchial asthma. A significant interaction was also identified between −109C/T polymorphism and disease status, and it appears that additional genetic and environmental factors are required for the −109C/T polymorphism to manifest genetic influences on total serum IgE levels. Furthermore, these factors may include higher exposure levels to inhaled allergens or elevated interleukin-4 (IL-4) or specific IgE Ab levels in asthmatic subjects (12, 13). Similar interactions between gene and environment have recently been described in population-based association studies between total serum IgE levels and *CD14* promoter polymorphism (14).

There is considerable variation in age at onset of bronchial asthma. Interestingly, we found the strongest association when the analysis was confined to asthmatic subjects and age at onset of asthma was controlled for in the model. A previous study also noted that genetic effects of *FCER1B* were much more prominent in childhood asthma (15). Taken together,

TABLE 2

REGRESSION MODELS FOR TOTAL IgE LEVELS*

	(a) 226 Healthy Controls			(b) 226 Subjects with Asthma			(c) 226 Subjects with Asthma¶		
Variables	Coefficient (SD)	t	p Value	Coefficient (SD)	t	p Value	Coefficient (SD)	t	p Value
Age	0.004 (0.0036)	1.102	0.27	−0.0071 (0.0023)	−3.02	0.0028	−0.0084 (0.003)	−2.73	0.0069
Sex†	0.088 (0.0865)	1.013	0.31	0.2792 (0.0775)	3.6	0.0004	0.26 (0.081)	3.19	0.0017
Smoking status‡	0.122 (0.0908)	1.341	0.18	0.0174 (0.0932)	0.187	0.85	0.133 (0.099)	1.33	0.183
FCER1B promoter polymorphism§	0.0053 (0.0826)	0.064	0.95	−0.2485 (0.0774)	−3.21	0.0015	−0.287 (0.079)	−3.59	0.00041
Age at onset of asthma	—	—	—	—	—	—	2.61 (0.105)	1.2	0.23

* The contribution of genotype to prediction of total IgE levels was modeled using multivariate regression analysis.
† Male = 2, female = 1.
‡ Current smoker = 2, Ex. or Never smoker = 1.
§ Log [total IgE] (log IU/ml) were 1.725 ± 0.059 for T/T and 1.731 ± 0.057 for C/T + C/C in the model (a) (226 healthy controls). Log [total IgE] (log IU/ml) were 2.63 ± 0.55, or 2.65 ± 0.57 for T/T and 2.38 ± 0.51, or 2.36 ± 0.53 for C/T + C/C in the model (b) or (c), respectively.
¶ In the model (c), age at onset of asthma was also controlled for in addition to age, sex, smoking status, and −109C/T genotype. In the models (b) and (c), the regression was highly significant ($p < 0.0001$), and about 14% or 23% of the variability in total IgE levels in asthmatic subjects is accounted for ($R2 = 0.14$ or 0.23 in (b) or (c), respectively).

genetic regulation involved in the development of asthma appears to be heterogeneous in relation to age at onset, as seen in other complex diseases including Alzheimer's disease (16) and breast cancer (17). Furthermore, our results represented only a small portion of the genetic contribution to total serum IgE levels, which was in accordance with the results of recent genome-wide linkage analyses of asthma or asthma-related phenotypes (2, 18) and indicated that a number of genes with minor or moderate effects were involved in the genetics of elevated total IgE levels.

We failed to find any mutation in the entire coding regions of the *FCER1B* gene in 24 patients with atopic asthma. Therefore, although −109C/T polymorphism could be in linkage disequilibrium with a causal allele that is located in a region not investigated in this study, it appears unlikely that our findings of a significant association resulted from linkage disequilibrium with coding variants including I181L/V183L and E237G. At least in the population studied here, coding variants seem to be very infrequent and seem to have a limited contribution to the development of asthma, despite the fact that another study in a Japanese population reported that 16% of asthmatic subjects studied had the coding variant of E237G (17).

We conclude that there is sufficient preliminary evidence that −109C/T polymorphism is a genetic determinant of serum total IgE levels especially in subjects with atopic asthma. Our data indicated a role for genetic heterogeneity and complex interactions between genetic and environmental components in the regulation of total serum IgE levels. Further studies will examine the functional role of the −109C/T promoter polymorphism with an emphasis on functional analysis to consider this complex gene–gene or gene–environmental interaction.

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