

FcεRIα gene (*FCER1A*) promoter polymorphisms and total serum IgE levels in Japanese atopic dermatitis patients

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Summary

Two promoter polymorphisms of the high-affinity IgE receptor α-subunit (FcεRIα) gene (*FCER1A*), –66T>C (rs2251746) and –315C>T (rs2427827), were analysed in Japanese atopic dermatitis subjects. Patients with the –315CT/TT genotype tended to have higher total serum IgE levels, while the proportion of –315CT/TT genotype or the –315T allele was significantly higher in those with highly elevated total serum IgE concentrations.

FCER1A is an important immunity-related gene that encodes the ligand-binding subunit of the high-affinity IgE receptor (FcεRI), the α-chain (FcεRIα; Zhang *et al.*, 2007). Thus, recent studies have focused not only on human FcεRIα gene variability (Shikanai *et al.*, 2002; Hasegawa *et al.*, 2003; Potaczek *et al.*, 2006, 2007, 2008; Bae *et al.*, 2007; Kanada *et al.*, 2008) but also on polymorphisms of its animal counterparts (Musilova *et al.*, 2005; Vychodilova-Krenkova *et al.*, 2005).

Two FcεRIα single-nucleotide polymorphisms, –315^aC>T (rs2427827) and –66^aT>C (rs2251746), which appear to be the only common genetic variants

localized in a region of the proximal promoter in Caucasians (Shikanai *et al.*, 2002; Potaczek *et al.*, 2006, 2008) and the only two variants present in that region in East Asians (Hasegawa *et al.*, 2003; Bae *et al.*, 2007; Kanada *et al.*, 2008; Potaczek *et al.*, 2008), have been deemed functional (Hasegawa *et al.*, 2003; Bae *et al.*, 2007; Kanada *et al.*, 2008). In brief, both mutations affect binding of the specific transcription factors to the FcεRIα gene proximal promoter, thus leading to altered transcriptional activity of the gene (Hasegawa *et al.*, 2003; Bae *et al.*, 2007; Kanada *et al.*, 2008), and thereby affecting cell surface expression of the receptor on basophils (Hasegawa *et al.*, 2003; Kanada *et al.*, 2008). The FcεRIα gene –315C>T polymorphism has been shown to be associated with total serum IgE levels in different groups of allergic subjects (Shikanai *et al.*, 2002; Potaczek *et al.*, 2006, 2007; Bae *et al.*, 2007). In addition, –66C allele-carrying status was found to be less frequent in atopic dermatitis (AD) subjects (Hasegawa *et al.*, 2003), while the –315C>T (rs2427827) polymorphism was found to be associated with aspirin-induced urticaria (Bae *et al.*, 2007). In the present study, we investigated the relationship between FcεRIα –315C>T (rs2427827) and –66T>C genetic (rs2251746) variants and total serum IgE levels in AD patients.

We recruited 129 Japanese AD patients [33.3 ± 12.2 years, mean ± SD; male:female, 85:44; total serum IgE levels, 3614.0 (9045.0) IU/mL, median (IQR); log₁₀-transformed total serum IgE levels, 3.43 ± 0.79, mean ± SD] who were diagnosed according to the Hanifin & Rajka (1980) criteria, and 105 healthy volunteers of Japanese ethnicity with no symptoms or history of any allergic disorder. The Juntendo University School of Medicine and Tokyo Medical University ethics committees approved the study. All subjects gave their written informed consent. Genomic DNA was prepared from peripheral blood leucocytes using a DNA quick kit (Dainippon Pharmaceutical, Osaka, Japan). The genotypes of the –315C>T (rs2427827) and –66T>C (rs2251746) variants were obtained by direct sequencing (Applied Biosystems,

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^a Both polymorphisms are numbered in the present study according to the FcεRIα gene (*FCER1A*) proximal promoter transcription start site; the –315C>T (rs2427827) and –66T>C (rs2251746) polymorphisms can be alternatively numbered according to the *FCER1A* translation start site as –344C>T and –95T>C, respectively.

Foster City, CA, USA) of the *FcεRIα* gene proximal promoter region encompassing both polymorphisms using the following primers: 5'-CTTAGGGGTTA-GATTTTATGTGTTTGAACCCCAA-3' (-551/-518, sense), 5'-CCATCTTCTTCATGGACTCCTGGTGCT-TAC-3' (+33/+4, antisense) and 5'-GTAGATAGTGATAGTATGTACTTTATAGG-3' (-441/-413, sequencing). Total serum IgE levels were measured in the AD patients by enzyme immunoassay (SRL, Tokyo, Japan). The genotypes of the -315C>T (rs2427827) and -66T>C (rs2251746) polymorphisms were distributed according to Hardy-Weinberg equilibrium in the AD patients ($P = 0.92$ and 0.90 respectively, chi-square test) as well as in the healthy controls ($P = 0.86$ and 0.58 respectively, chi-square test). Pairwise -315C>T/-66T>C linkage disequilibrium was analysed in the Japanese healthy volunteers using HAPVIEW_4.0 software (<http://www.broad.mit.edu/mpg/haploview/download.php>; Barrett *et al.*, 2005). D' of 1.0, r^2 of 0.02 and the presence of only three haplotypes (-315C/-66T, -315T/-66T and -315C/-66C) were observed.

The distribution of the -315C>T (rs2427827) variant was similar in the AD patients and in the healthy controls in all tested models (Table 1). However, similar to our previous observations (Hasegawa *et al.*, 2003), there were more carriers of the -66C allele among the healthy subjects than among the AD patients, although that association also did not reach statistical significance ($P = 0.10$, two-tailed Fisher exact test; Table 2). A similar trend was also observed in a genotype model ($P = 0.10$, chi-square test; Table 2).

The distribution of total serum IgE levels in the AD patients was approximated to normality by log₁₀-transformation. No significant association was observed between the -66T>C (rs2251746) polymorphism and log₁₀-transformed total serum IgE levels in the AD patients in a dominant model (TT vs TC + CC; 3.43 ± 0.80 vs 3.43 ± 0.73 , mean $\pm$ SD; $P = 0.99$, unpaired Student's *t*-test). Recessive and genotype models were not tested because of the very low frequency of -66CC homozygotes. Furthermore, the distribution of -66T>C (rs2251746) genotypes did not differ between AD patients with total serum IgE ≥ 1000 IU/mL and those with concentrations < 1000 IU/mL in genotype ($P = 0.82$, chi-square test), dominant ($P = 1.0$, two-tailed Fisher exact test), recessive ($P = 1.0$, two-tailed Fisher exact test) and allelic ($P = 1.0$, two-tailed Fisher exact test) models (data not shown). A value of 1000 IU IgE/mL can be considered the border between moderately and highly elevated total serum IgE levels in AD patients (Laske *et al.*, 2003; Akhavan & Rudikoff, 2008); therefore, this was used as the cutoff value for comparisons of the genotype/allelic distributions of the polymorphisms. However, there was a trend towards an association between the -315C>T (rs2427827) genetic variant and log₁₀-transformed total serum IgE levels in a dom-

Table 1. Analysis of the *FcεRIα* gene (*FCER1A*) -315C>T (rs2427827) polymorphism

Group of subjects studied	Genotype frequency, <i>n</i> (%)			
	Total	CC	CT	TT
AD patients	129 (100.0)	87 (67.4)	39 (30.2)	3 (2.3)
With total serum IgE levels ≥ 1000 IU/mL	94 (100.0)	58 (61.7)	33 (35.1)	3 (3.2)
With total serum IgE levels < 1000 IU/mL ^a	35 (100.0)	29 (82.9)	6 (17.1)	0 (0.0)
Healthy subjects ^b	105 (100.0)	66 (62.9)	36 (34.3)	3 (2.9)

^aCompared with atopic dermatitis (AD) patients with total serum IgE levels ≥ 1000 IU/mL: CC vs CT vs TT (genotype model), $P = 0.063$ (chi-square test); CC vs CT + TT (dominant model), $P = 0.033$ (two-tailed Fisher exact test); CC + CT vs TT (recessive model), $P = 0.56$ (two-tailed Fisher exact test); C vs T (allelic model), $P = 0.026$ (two-tailed Fisher exact test). ^bCompared with the whole ($n = 129$) group of AD patients: CC vs CT vs TT (genotype model), $P = 0.76$ (chi-square test); CC vs CT + TT (dominant model), $P = 0.49$ (two-tailed Fisher exact test); CC + CT vs TT (recessive model), $P = 1.0$ (two-tailed Fisher exact test); C vs T (allelic model), $P = 0.55$ (two-tailed Fisher exact test).

Table 2. Analysis of the *FcεRIα* gene (*FCER1A*) -66T>C (rs2251746) polymorphism

Group of subjects studied	Genotype frequency, <i>n</i> (%)			
	Total	TT	TC	CC
AD patients	129 (100.0)	114 (88.4)	14 (10.9)	1 (0.8)
Healthy subjects ^a	105 (100.0)	84 (80.0)	21 (20.0)	0 (0.0)

^aCompared with atopic dermatitis (AD) patients: TT vs TC vs CC (genotype model), $P = 0.10$ (chi-square test); TT vs TC + CC (dominant model), $P = 0.10$ (two-tailed Fisher exact test); TT + TC vs CC (recessive model), $P = 1.0$ (two-tailed Fisher exact test); T vs C (allelic model), $P = 0.17$ (two-tailed Fisher exact test).

inant model (CC vs CT + TT; 3.36 ± 0.86 vs 3.57 ± 0.61 , mean $\pm$ SD; $P = 0.12$, unpaired Student's *t*-test). A similar trend [B (SE), 0.24 (0.14); $P = 0.10$] was observed in subsequent analysis using a multiple linear regression model that included the -315C>T genotype (ordered from CC to CT + TT) as an independent variable and log-transformed total serum IgE levels as a dependent variable, and controlled for age and sex. Recessive and genotype models were not analysed because of the very low frequency of -315TT homozygotes. Moreover, the number of -315T allele carriers ($P = 0.033$, two-tailed Fisher exact test) and the frequency of the -315T allele ($P = 0.026$, two-tailed Fisher exact test) were both significantly higher in AD patients with total serum IgE levels higher or equal to 1000 IU/mL as compared with those having total serum IgE concentrations < 1000 IU/mL (Table 1). A similar trend was observed in a genotype model ($P = 0.063$, chi-square test; Table 1).

Our observation of an association between higher total serum IgE levels and the -315C>T (rs2427827)

polymorphism in AD patients concurs with previous reports (Shikanai *et al.*, 2002; Potaczek *et al.*, 2006, 2007; Bae *et al.*, 2007). Briefly, the -315CC genotype was more common among asthmatics with lower total serum IgE levels (Shikanai *et al.*, 2002), while allergic subjects with the -315TT genotype (Potaczek *et al.*, 2006, 2007) or patients suffering from aspirin-induced urticaria carrying the -315T allele (Bae *et al.*, 2007) had higher total serum IgE concentrations.

The association between the -315C>T (rs2427827) polymorphism and total serum IgE concentrations in different groups of allergic subjects, as evidenced by this report and the previous studies (Shikanai *et al.*, 2002; Potaczek *et al.*, 2006, 2007; Bae *et al.*, 2007), in combination with the functional analyses demonstrating the -315C>T (rs2427827) polymorphism to affect FcεRIα/FcεRI expression (Bae *et al.*, 2007; Kanada *et al.*, 2008) in an additive manner with the -66T>C (rs2251746) variant (Hasegawa *et al.*, 2003; Kanada *et al.*, 2008), may suggest that FcεRIα genetic polymorphisms act as important regulators of serum total IgE levels in allergic subjects.

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Conflict of interest

None declared.

References

Akhavan, A. & Rudikoff, D. (2008) Atopic dermatitis: systemic immunosuppressive therapy. *Seminars in Cutaneous Medicine and Surgery*, **27**, 151.

- Bae, J.-S., Kim, S.-H., Ye, Y.-M., Yoon, H.J., Suh, C.-H., Nahm, D.-H. *et al.* (2007) Significant association of FcεRIα promoter polymorphisms with aspirin-intolerant chronic urticaria. *Journal of Allergy and Clinical Immunology*, **119**, 449.
- Barrett, J.C., Fry, B., Maller, J. & Daly, M.J. (2005) Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*, **21**, 263.
- Hanifin, J.M. & Rajka, G. (1980) Diagnostic features of atopic eczema. *Acta Dermato-Venereologica (Stockholm)*, **92**, 44.
- Hasegawa, M., Nishiyama, C., Nishiyama, M., Akizawa, Y., Mitsuishi, K., Ito, T. *et al.* (2003) A novel -66T/C polymorphism in FcεRI α-chain promoter affecting the transcription activity: possible relationship to allergic diseases. *Journal of Immunology*, **171**, 1927.
- Kanada, S., Potaczek, D.P., Nakano, N., Maeda, K., Shimokawa, N., Niwa, Y. *et al.* (2008) Two different transcription factors discriminate the -315C>T-polymorphism of the FcεRI α-chain gene: binding of Sp1 to -315C and of a high mobility group-related molecule to -315T. *Journal of Immunology*, **180**, 8204.
- Laske, N., Bunikowski, R. & Niggemann, B. (2003) Extraordinarily high serum IgE levels and consequences for atopic phenotypes. *Annals of Allergy, Asthma and Immunology*, **91**, 202.
- Musilova, P., Kubickova, S., Vychodilova-Krenkova, L., Kralik, P., Matiasovic, J., Hubertova, D. *et al.* (2005) Cytogenetic mapping of immunity-related genes in the domestic horse. *Animal Genetics*, **36**, 507.
- Potaczek, D.P., Sanak, M., Mastalerz, L., Setkowicz, M., Kaczor, M., Nizankowska, E. *et al.* (2006) The α-chain of high-affinity receptor for IgE (FcεRIα) gene polymorphisms and serum IgE levels. *Allergy*, **61**, 1230.
- Potaczek, D.P., Sanak, M., Mastalerz, L., Milewski, M., Gawlewicz-Mroccka, A. & Szczeklik, A. (2007) Genetic polymorphisms of the novel FCER1A gene region: relation to total serum IgE levels. *Annals of Allergy, Asthma and Immunology*, **98**, 500.
- Potaczek, D.P., Sanak, M., Nishiyama, C., Okumura, K., Ogawa, H. & Szczeklik, A. (2008) FCER1A gene proximal promoter polymorphisms in Caucasians and East Asians. *International Journal of Immunogenetics*, **35**, 339.
- Shikanai, T., Silverman, E.S., Morse, B.W., Lilly, C.M., Inoue, H. & Drazen, J.M. (2002) Sequence variants in the FcεRI alpha chain gene. *Journal of Applied Physiology*, **93**, 37.
- Vychodilova-Krenkova, L., Matiasovic, J. & Horin, P. (2005) Single nucleotide polymorphisms in four functionally related immune response genes in the horse: CD14, TLR4, Cε, and Fcε R1 alpha. *International Journal of Immunogenetics*, **32**, 277.
- Zhang, M., Murphy, R.F. & Agrawal, D.K. (2007) Decoding IgE Fc receptors. *Immunologic Research*, **37**, 1.