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ORIGINAL ARTICLE

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No association between atopic asthma and a coding variant of *FcεR1β* in a Japanese population

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Abstract Susceptibility to atopic diseases is known to involve genetic factors. The Gly237 allele of a polymorphism (Glu237Gly) of the *FcεR1β* gene is reportedly associated with atopic asthma in Japanese. To confirm this association, we conducted transmission disequilibrium tests in 76 families identified through atopic asthmatics. A case-control study was also carried out in atopic asthmatic subjects and non-atopic controls. The Gly237 allele was not preferentially transmitted to atopic asthma-affected offspring. Neither the Gly237 allele nor the Gly237/Gly237 + Glu237/Gly237 genotypes were significantly more prevalent in the atopic asthmatics than in the controls. This study failed to confirm a substantial role of the Gly237Glu polymorphism of the *FcεR1β* gene in the genetic predisposition for atopic asthma in this Japanese population.

Key words Atopic asthma · IgE receptor · Polymorphism · Association · Transmission disequilibrium test

Introduction

Atopic asthma is a complex familial disease that is associated with the interaction of several genes with strong environmental factors. Atopy is characterized by exaggerated T-helper cell type 2 lymphocyte responses to common allergens with sustained, enhanced production of allergen-specific IgE. Previous studies have found linkage of atopy and bronchial hyperresponsiveness to markers on chromosome 11q13 (Sandford et al. 1993), and the beta chain of the high-affinity receptor for IgE (FcεR1β) has been identified as the most likely candidate for this linkage.

The role of the β-chain is rather obscure, although expression studies suggest that it may be involved in signal transduction and in autophosphorylation of the receptor (Paolini et al. 1991). *FcεR1β* has been shown to associate with the low-affinity IgG receptor (FcγRIII) in macrophages and mast cells (Scholl and Geha 1993). A polymorphism in exon 7 of the FcεR1β gene, which changes amino acid residue 237 from glutamic acid to glycine (Glu237Gly) in the cytoplasmic tail of the protein, has been described (Hill and Cookson 1996). The functional significance of the Glu237Gly mutation has not been elucidated. Glu237Gly is predicted to introduce a hydrophobicity change in the C-terminus of *FcεR1β* and could affect the intracellular signaling capacity of *FcεRI*, as this mutation is adjacent to the immunoreceptor tyrosine activation motif (ITAM) (Scholl and Geha 1993; Hill and Cookson 1996). Another missense variant, the Leu181 variant of the Ile181 position of *FcεR1β*, was also shown to be associated with atopy (Shirakawa et al. 1994). However, this variant was rare in many ethnic groups, including Japanese (Hizawa et al. 1995).

Hill and Cookson (1996) observed that the Gly 237 mutation was found in 5% of the Australian population and was associated with atopy and bronchial hyperresponsiveness. The relative risk of individuals with the Gly237 having asthma compared with subjects without the variant was 2.3 in the Australian population. Subsequently, the Gly237 allele was reported to be associated with atopic asthma, but not with non-atopic asthma, in a Japanese population, with an odds ratio of 3.92 (Shirakawa et al. 1996).

To confirm the role of the Gly237Glu polymorphism in the development of atopic asthma, we performed an association study, using two methods: a transmission disequilibrium test (TDT) and a case-control comparison.

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Subjects and methods

Subjects

Probands of the families studied were asthmatic children visiting the Pediatric Allergy Clinic of the University Hospital of Tsukuba. A full verbal and written explanation of the study was given to all family members interviewed, and 76 families (333 members, including 128 children with atopic asthma) gave us informed consent and participated in this study. Informed consent for subjects younger than school age was given by their parents. The mean age of the probands and their siblings was 11.2 years (range, 3–29 years); the mean age of the parents was 41.1 years (28–72 years). The families examined in this study are, in part, the same ones that participated in our previous study (Noguchi et al. 1997).

Each family member was questioned regarding allergic symptoms and underwent a physical examination by pediatricians. Asthma was diagnosed in subjects according to the criteria of the National Institutes of Health, USA, with minor modifications (National Heart Blood and Lung Insititute 1995). Patients had to show the two following characteristics: (1) two or more episodes of wheezing and shortness of breath during the past year and (2) reversibility of the wheezing and dyspnea, either spontaneously or by bronchodilator treatment. Patients treated with systemic steroids were excluded from this study. Since wheezing is often associated with viral respiratory infection in young children (Martinez et al. 1995), only subjects more than 3 years old were evaluated for the asthma phenotype. The diagnosis of asthma in this population was confirmed by physicians or pediatricians.

For the case-control study, in addition to the 76 probands of the above-mentioned families, 14 unrelated children with atopic asthma were added to the atopic asthma group. The control group consisted of unrelated subjects who lived near the University Hospital (Tsukuba controls) and in Fukuoka city (Fukuoka controls). We selected controls who met all of the following criteria.: (1) no symptoms or history of allergic diseases, (2) no detectable dust mite-specific IgE antibody, and (3) total serum IgE levels below the general population mean for their ages. This study was approved by the Committee of Ethics, the University of Tsukuba.

Total serum IgE levels and specific IgE levels to house dust mite, *Dermatophagoides farinae* (Df), were determined by the Pharmacia CAP System (Uppsala, Sweden). Atopy was defined by the presence of either or both of the following: a total serum IgE level more than one standard deviation above the geometric mean for the normal Japanese population, and/or raised specific serum IgE levels to Df (>0.35 UA/ml). Subjects whose total serum IgE exceeded 400 IU/ml were considered to have high IgE, and those having detectable Df-specific IgE (>0.35 UA/ml) were considered to be mite antibody-positive.

Molecular methods

DNA was extracted from peripheral blood leukocytes. The fragment including the Gly237Glu polymorphism was amplified by PCR, using the primer pairs 5′-CAGGTTCCAGAGGATCGT and 5′-CTTATAAATCA ATGGGAGGAAACA, which incorporate the polymorphic site into an *Xmn*1 recognition site (Shirakawa et al. 1996). The PCR conditions were described elsewhere (Shirakawa et al. 1996).

PCR fragments digested with *Xmn*1 were electrophoresed in 1.5% agarose + 3.0% NuSieve agarose gel (FMC BioProducts, Rockland, ME, USA) and visualized by ethidium bromide staining and ultraviolet transillumination. The accuracy of this genotyping method was confirmed by direct sequencing of samples from two individuals with each genotype (Gly237/Gly237,Gly237/Glu237,Glu237/Glu237).

Statistical analyses

TDT of the Gly237Glu polymorphism was performed using the SibPair program (http://www.qimr.edu.au/davidD/davidd.html). Case-control comparisons of allele and genotype numbers were carried out using the χ^2 test. This study was intended to replicate the previous findings; alpha level was set to less than 0.05 one-tailed.

Results

Table 1 shows the results of the TDT. Based on previous reports (Hill and Cookson 1996; Shirakawa et al. 1996), we assumed that the Gly237 was the atopic-asthma susceptible allele. The TDT showed that the Glu237 allele, not the Gly237, was preferentially transmitted to atopic asthmatic offspring ($P = 0.044$).

Table 2 shows the results of the case-control association study. The allele frequencies of the Gly237 were 0.11 in the atopic asthmatics, 0.10 in the controls in Tsukuba, and 0.13 in the controls in Fukuoka. Neither the Gly237 allele nor the Gly237/Gly237 + Gly237/Glu237 genotypes were significantly more prevalent in the atopic asthmatics than in the controls.

Discussion

There are considerable ethnic differences in allele frequencies of the Gly237 allele. The frequency of the Gly237 allele was reported to be 0.026 in an Australian general population sample (Hill and Cookson 1996), 0.04 in an Italian control population (Trabetti et al. 1998), and 0.20 in Black controls in South Africa (Green et al. 1998). In Japanese controls, it was previously reported to be 0.03 (Shirakawa et al. 1996). In our non-atopic control subjects in Tsukuba and Fukuoka, the frequency was 0.103 (95% confidence interval

[CI], 0.06–0.15) and 0.13 (95% CI, 0.08–0.19), respectively. Therefore, the allele frequencies were significantly different in the controls studied by Shirakawa et al. (1996) and by us ($P = 0.0005$). The difference in recruitment of the controls may have caused the difference: our controls were non-atopic children (Tsukuba) and adults (Fukuoka), and those of Shirakawa et al. were clients of a commercial-based medical examination company. Further studies are required to determine precisely the frequency of the Gly237 allele in Japanese.

We failed to replicate an association between the Gly237 allele and atopic asthma in our Japanese child subjects. The frequency of this allele in our patient and control subjects was similar to that in the atopic asthma group reported by Shirakawa et al. (1996). Shirakawa et al. (1996) reported that the Glu237/Gly237 genotype was significantly more frequent in atopic asthmatic children (20%) than in controls (6%), with an odds ratio of 3.92 (95% CI, 1.5–3.9). Although the sample size of our case-control study was slightly smaller than that of Shirakawa et al. (1996), the power to detect the association in our study was around 0.90, assuming the odds ratio to be 3.9 and the alpha level set to 0.05 one-sided. However, if the true odds ratio is as low as 1.5, the detection power would be as low as 0.36. Therefore, our case-control study only indicates that the Gly237 allele does not play a major role in the development of atopic asthma in Japanese.

In addition to a case-control comparison, we examined the association by TDT in this study. This family-based association design, in which non-transmitted alleles are treated as controls, overcomes the problems of stratification, which can lead to both false-positive and false-negative findings. Our TDT analysis indicated that the Gly237 allele was not preferentially transmitted to atopic asthmatic offspring, which is consistent with the findings of our case-control association study.

Table 1. Transmission disequilibrium test of Glu237Gly of *FcεRIβ* for atopic-asthma phenotype (all affected offpring included)

	Transmitted	Not transmitted	*P*
Gly237	20	36	0.044
Glu237	36	20	0.044

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Table 2. Association between Glu237Gly of *FcεRIβ* and atopic asthma, and specific and total IgE levels

	No of subjects	Glu237/Glu237	Glu237/Gly237	Gly237/Gly237	OR	χ^2	*P*
Controls (Fukuoka)	75	57 (0.76)	16 (0.21)	2 (0.03)			
Controls (Tsukuba)	102	81 (0.79)	21 (0.21)	0 (0.00)	1.10	0.08	0.78
Atopic asthma	90	70 (0.78)	19 (0.21)	1 (0.01)			
Very high (>1000) IgE	41	30 (0.73)	10 (0.24)	1 (0.02)	1.48	0.93	0.33
Lower (<1000) IgE	124	97 (0.78)	25 (0.20)	2 (0.02)			
Anti-Df IgE-negative	57	46 (0.81)	11 (0.19)	0 (0.00)	0.84	0.2	0.65
Anti-Df IgE-positive	108	81 (0.75)	24 (0.22)	3 (0.03)			

Numbers in parentheses are frequencies
Controls (Tsukuba); patients and their families were living in the same area. Fukuoka is 1000 km from Tsukuba
Df, *Dermatophagoides farinae*; OR, odds ratio

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