

Lack of association between atopic eczema and the genetic variants of interleukin-4 and the interleukin-4 receptor α chain gene: heterogeneity of genetic backgrounds on immunoglobulin E production in atopic eczema patients

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Summary

Background The genetic background of atopic eczema might be heterogeneous and there is a possibility that immunoglobulin (Ig)E responsiveness in patients with atopic eczema is controlled separately from the development of atopic eczema. Although both interleukin (IL)-4 and the IL-4 receptor α chain have an important role for IgE production and are therefore possible candidate genes for atopy, it has not been clarified whether these genes play any roles in atopic eczema patients who have normal IgE productivity.

Objective We aimed to assess whether the polymorphisms of the IL-4 gene and the IL-4 receptor α chain gene play any roles in atopic eczema patients, particularly in patients who have normal IgE productivity.

Method We determined the genotype with regard to polymorphisms in the genes for IL-4 and the IL-4 receptor α chain (– 589C/T of IL-4; Ile50Val, Ala375Glu and Arg551Gln of IL-4 receptor α chain) in patients with atopic eczema using the fluorogenic 5' nuclease assay.

Results IL-4 and the IL-4 receptor α chain genotypes were not significantly associated with either total patients with atopic eczema or atopic eczema patients who had normal IgE productivity. The distribution of genotypes of IL-4–589C/T differed by the serum IgE levels in patients with atopic eczema.

Conclusion These results suggest that the polymorphisms in the IL-4 gene and the IL-4 receptor α chain gene play no role in the development of atopic eczema in patients who have normal IgE productivity.

Keywords: atopic eczema, IL-4, IL-4 receptor α chain, fluorogenic 5' nuclease assay

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Introduction

Atopic eczema is a chronic inflammatory skin disease, which affects up to 30% of the Japanese population [1]. Patients with atopic eczema often have personal or family

histories of atopic respiratory disease (asthma and allergic rhinitis) [2–4], and are often characterized by heightened immunoglobulin (Ig)E responsiveness [5]. However, though patients with both atopic eczema and atopic respiratory disease usually show high IgE concentration, patients without personal and family histories of atopic respiratory disease tend to show normal IgE concentration

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[6]. Previously, we examined the genetic variants of mast cell chymase which is a serine protease secreted by mast cells, and found that there was a significant difference in the distribution of mast cell chymase genotype between patients with normal IgE concentration and patients with very high IgE concentration [7]. There was no difference in the morphological appearance of the skin lesions and the clinical course between these two groups of patients. Thus, the genetic background of atopic eczema might be heterogeneous and there is a possibility that IgE responsiveness is controlled separately from the development of atopic eczema *per se*.

Recently, a number of susceptibility genes for atopy, including the β chain of the high affinity IgE receptor (Fc ϵ RI β) and the α and the β chain of the T cell receptor, have been identified [8–12]. Marsh *et al.* have shown that chromosome 5q31 markers are linked to total serum IgE levels, and the gene encoding interleukin (IL)-4 is located in this region [13]. IL-4 plays an essential role in IgE production, and is therefore a possible candidate gene for atopy [14]. The IL-4 promoter has a common polymorphism, which is a C to T change at position –589 [15,16]. IL-4 acts through the IL-4 receptor, which consists of a 140 kDa α chain and a γ c chain, common to several cytokine receptors [14]. The IL-4 receptor α chain (IL-4R α) is also one of the candidate genes for atopy, and several polymorphisms in the IL-4R α gene have been reported to be associated with atopy [17–19]. They include single nucleotide polymorphisms (SNPs) in the coding region of IL-4R α ; Ile50Val [17], Ala375Glu [18] and Arg551Gln [19]. Recently Oiso *et al.* reported that Arg551Gln polymorphism of IL-4R α is associated with atopic eczema in the Japanese population [20]. However, the size of their samples of atopic eczema was small, and a study examining a large population is needed to confirm this association.

Both IL-4 and IL-4R α carry out important roles in IgE production [14], but it is not clear whether IL-4 and IL-4R α play any roles in atopic eczema patients who have normal IgE productivity and the development of atopic eczema *per se*. We therefore examined associations between atopic eczema and the genetic variants of IL-4 and IL-4R α to assess the possible involvement of IL-4 and IL-4R α in the development of atopic eczema, particularly in patients who have normal IgE productivity.

In this study we utilized the fluorogenic 5' nuclease (TaqMan[™]) assay, for examining the genotypes of IL-4 promoter –589C/T and three polymorphisms in IL-4R α (Ile50Val, Ala375Glu and Arg551Gln). This assay uses a probe technology that takes advantage of the 5'-3' nuclease activity of AmpliTaq Gold[™] to cause cleavage of allele-specific fluorescent-labelled probes, which are hybridized to template DNA during polymerase chain reaction (PCR)s,

and cleavage of the probes causes an increase in the fluorescence. Detecting the increase of fluorescence allows high-throughput SNP genotyping [21].

Materials and methods

Subjects

A total of 302 Japanese patients (171 males and 131 females) with atopic eczema were included in the study. They ranged in age from 16 to 60 years (mean, 24 years). The diagnosis was based on the morphological appearance of skin lesions, clinical course, and personal and family histories of atopic diseases. All patients fulfilled the criteria of Hanifin and Rajka [22]. Venous blood was taken from all subjects for DNA extraction and serum IgE measurement. One hundred and twenty-two non-atopic patients who visited Shiga University of Medical Science Hospital were selected as controls; they comprised 58 males and 64 females, ranging in age from 14 to 38 years (mean, 26 years). They had no personal and family histories of atopic eczema, asthma and rhinitis. All control subjects showed normal serum IgE concentration. All of the patients and the control subjects agreed to participate in this study and appropriate informed consent was provided. This study was approved by the Committee of Ethics, Shiga University of Medical Science.

Classification of patients with atopic eczema in IgE production

Total serum IgE was measured by enzyme immunoassay (TOSOH, Tokyo, Japan) and high IgE concentration of total serum IgE was taken to be greater than 500 IU/mL (mean plus 2SD). Atopic eczema patients who have neither personal nor family histories of atopic respiratory disease usually show serum IgE concentrations of <500 IU/mL, whereas those with personal or family histories of atopic respiratory disease often show serum IgE concentrations of >2000 IU/mL [6]. We therefore regarded total serum IgE concentrations greater than 2000 IU/mL as very high. We have previously reported that patients with atopic eczema may be classified at least two subgroups: those with atopic respiratory disease predisposition who have an enhanced IgE producing potential, and those without atopic respiratory disease predisposition who have a low or non-enhanced ability for IgE production [6,7]. Serum IgE concentrations in patients with atopic eczema are also affected by severity of eczema, and severe atopic eczema patients without personal or family histories of atopic respiratory disease sometimes show high IgE concentrations, however, they seldom show IgE concentrations over 2000 IU/mL [6,23]. We therefore classified the 302

patients with atopic eczema into three groups according to their serum IgE concentrations: (i) those with serum IgE concentrations of < 500 IU/mL (97 individuals) (ii) those with serum IgE concentrations from 500 to 2000 IU/mL (60 individuals), and (iii) those with serum IgE concentrations > 2000 IU/mL (145 individuals).

Genotype Analysis

Genomic DNA was extracted from leucocytes with an Iso Quick kit (ORCA Research, Bothell, WA, USA). For detection of the IL-4 promoter -589C/T, IL-4 α Ile50Val, Ala375Glu and Arg551Gln polymorphisms, the fluorogenic 5' nuclease (TaqManTM) assay was carried out. Amplification of genomic DNA (25 ng) was performed in 96-well optical reaction plates using a programmable thermal cycler (PTC-200; MJ Research, Inc., Watertown, MA, USA). Oligonucleotide PCR primers and double dye-labelled probes were designed using Primer ExpressTM (PE Biosystems) except IL-4R α Ile50Val. The primers for IL-4-589C/T were 5'-ACG ACC TGT CCT TCT CAA AAC ACT-3' and 5'-GCA GAA TAA CAG GCA GAC TCT CCT A-3'. The TaqMan probe for IL-4-589C was 5'-AGA ACA TTG TCC CCC AGT GCT GG-3' labelled with the fluorescent dye VIC, and for -589T 5'-AGC ACT GGG GAA CAA TGT TCT CCC-3' labelled with FAM. The primers for Ile50Val were 5'-AGG TGA CCA GCC TAA CCC AG-3' and 5'-TGA AGG AGC CCT TCC ACA G-3', and the probe for Val50 was 5'-CCC ACA CGT GTA TCC CTG AGA ACA AC-3' labelled with VIC and for Ile50 5'-AGC CCA CAC GTG TGT CCC TGA G-3' labelled with FAM [21]. Primers for Ala375Glu were 5'-TGT GCA TCG CCT GAG AGC-3' and 5'-GCA GGT CCA GGA ACA GGC T-3', and the probe for Glu375 was 5'-CCA TGC CCT CCC TTC CCT CCT G-3' labelled with VIC and for Ala375 5'-AAT GCC CGC CCT TCCCTC CT-3' labelled with FAM.

The primers for Arg551Gln were 5'-CGA AAT GTC CTC CAG CAT-3' and 5'-ACT GGC CTG GGT GCC A-3', and the probe for Gln551 was 5'-CCA CCA GTG GCT ATC AGG AGT TTG TAC A-3' labelled with VIC and for Arg551 5'-CAC CAG TGG CTA TCG GGA GTT TGT ACA T-3' labelled with FAM. PCR cocktails including both probes (200 nM each), primers (900 nM each) and 1 $\times$ TaqManTM PCR Master MixTM (PE Biosystems) were set up and were added to each well of the 96-well plate, the volume made up to 25 μ L with H₂O and the plates were sealed with optical caps. PCR amplification was carried out under the following cycling conditions: 2 min at 50°C, 10 min at 95°C, then 40 cycles of 94°C for 15 s, 62°C for 1 min. After cycling, plates were placed into the ABI PRISM 7700 Sequence Detector (PE Biosystems) and analysed for post-PCR allelic discrimination. All samples could not be utilized for each assay and samples that were not amplified by PCR were excluded.

Statistics

Contingency table analyses were performed by computerized methods (SPSS program version 9.0). Significance values were calculated by Pearson chi-square test. If the number in the column was ≤ 10 , Fisher's exact method was used.

Results

The gene frequencies of the genotypes for IL-4 and IL-4R α in this Japanese population were consistent with Hardy-Weinberg equilibrium (- 589C = 0.32, - 589T = 0.68 for IL-4, Val50 = 0.38, Ile50 = 0.62 for Ile50Val of IL-4R α , Glu375 = 0.95, Ala375 = 0.05 for Ala375Glu of IL-4R α , and Glu551 = 0.91, Arg551 = 0.09 for Arg551Gln for IL-4R α).

Table 1. Association between IL-4 - 590 genotype and atopic eczema

Subjects	Serum IgE (IU/mL)	n	IL-4 - 590				P*	
			C/C	C/T	T/T			
Controls		116	10 (9%)	55 (47%)	51 (44%)			
Atopic eczema		190	15 (8%)	92 (48%)	83 (44%)	0.822**		
	< 500	61	9 (15%)	29 (48%)	23 (37%)	0.214**		
	500-2000	38	2 (5%)	16 (42%)	20 (53%)	0.731**	0.196†	
	2000	91	4 (4%)	47 (52%)	40 (44%)	0.275**	0.037†	

* C/C vs. C/T + T/T.

** Compared to controls.

† Compared to subjects with serum IgE concentration less than 500 IU/mL.

Table 2. Association between Ile50Val of IL-4R α and atopic eczema

Subjects	Serum IgE (IU/mL)	<i>n</i>	Ile50Val			<i>P</i> *	
			Val50/Val50	Val50/Ile50	Ile50/Ile50		
Controls		119	15 (13%)	60 (50%)	44 (37%)		
Atopic eczema		231	40 (17%)	94 (41%)	97 (42%)	0.365**	
	< 500	66	12 (18%)	32 (48%)	22 (33%)	0.620**	
	500–2000	42	6 (14%)	19 (45%)	17 (41%)	0.688**	0.451†
	> 2000	123	22 (18%)	43 (35%)	58 (47%)	0.109**	0.067†

* Val50/Val50 + Val50/Ile50 vs. Ile50/Ile50.

** Compared to controls.

† Compared to subjects with serum IgE concentration less than 500 IU/mL.

The distribution of the IL-4 –589 genotype did not differ between controls and total patients with atopic eczema (Table 1). There was no significant difference in the distribution of the genotype between controls and atopic eczema patients who showed normal IgE concentrations (< 500 IU/mL). The distribution of IL-4–589 genotype differed significantly ($P = 0.037$) between patients with very high IgE concentrations (> 2000 IU/mL) and patients with normal IgE concentrations (< 500 IU/mL). There was no significant association between Ile50Val of IL-4R α and total patients with atopic eczema or atopic eczema patients who showed normal IgE concentrations (Table 2). Patients with very high IgE concentrations (> 2000 IU/mL) showed Ile50/Ile50 more frequently than patients with normal IgE concentrations (< 500 IU/mL), but the difference was not statistically significant ($P = 0.067$).

The distribution of Ala375Glu and Arg551Gln of IL-4R α did not differ between control subjects and total patients with atopic eczema or atopic eczema patients who showed normal IgE concentrations (Tables 3 and 4). There was no significant difference in the distribution of these

genotypes between patients with normal IgE concentrations (< 500 IU/mL) and patients with very high IgE concentrations (> 2000 IU/mL).

Discussion

The present study has failed to find any association of the polymorphisms in the IL-4 promoter gene and the IL-4R α gene with atopic eczema patients who have normal IgE concentrations. This result suggests that these variants of IL-4 and IL-4R α are not involved in the development of atopic eczema in patients who have normal IgE productivity.

Our result, which could find no association of the polymorphism in the IL-4 promoter gene with total patients with atopic eczema, differs from the previous reports stating that the IL-4 gene is related to the development of atopic eczema [24,25]. The true reason for this discrepancy between our results and those from the previous report is unclear. One possible explanation is the heterogeneity of genetic backgrounds in IgE productivity in atopic eczema

Table 3. Association between Ala375Glu of IL-4R α and atopic eczema

Subjects	Serum IgE (IU/mL)	<i>n</i>	Ala375Glu			<i>P</i> *	
			Glu375/Glu375	Glu375/Ala375	Ala375/Ala375		
Controls		122	112 (92%)	9 (7%)	1 (1%)		
Atopic eczema		235	221 (94%)	14 (6%)	0 (0%)	0.423**	
	< 500	75	68 (91%)	7 (9%)	0 (0%)	0.798**	
	500–2000	38	37 (97%)	1 (3%)	0 (0%)	0.462**	0.264†
	> 2000	122	116 (95%)	6 (5%)	0 (0%)	0.439**	0.248†

* Glu375/Glu375 vs. Glu375/Ala375 + Ala375/Ala375.

** Compared to controls.

† Compared to subjects with serum IgE concentration less than 500 IU/mL.

Table 4. Association between Arg551Gln of IL-4R α and atopic eczema

Subjects	Serum IgE (IU/mL)	n	Arg551Gln			P*	
			Gln551/Gln551	Gln551/Arg551	Arg551/Arg551		
Controls		122	100 (82%)	21 (17%)	1 (1%)		
Atopic eczema		277	227 (82%)	47 (17%)	3 (1%)	0.997**	
	< 500	92	74 (80%)	17 (19%)	1 (1%)	0.776**	
	500–2000	45	39 (87%)	6 (13%)	0 (0%)	0.641**	0.475†
	> 2000	140	114 (82%)	24 (17%)	2 (1%)	0.910**	0.850†

* Gln551/Gln551 vs. Gln551/Arg551 + Arg551/Arg551.

** Compared to controls.

† Compared to subjects with serum IgE concentration less than 500 IU/mL.

patients. Patients with atopic eczema and atopic respiratory disease often show high serum IgE levels [6], blood eosinophilia [26], increased deposition of eosinophil granule major basic protein in skin lesions [27], increased number of mast cells in skin lesions [28]. However, patients with atopic eczema who do not have personal and family histories of respiratory atopy [23,29] show normal IgE levels [6,30,31], normal eosinophil count [26], scant deposition of major basic protein in skin lesions [27], and a normal number of mast cells in skin lesions [28]. Our study included two groups of patients; one was the group of patients who had heightened IgE productivity, and the other was the group of patients who had normal IgE productivity. The previous studies that found the association between atopic eczema and IL-4 gene did not precisely mention IgE productivity of the patients examined.

The present study has demonstrated that patients with atopic eczema and high IgE productivity show – 589T of IL-4 promoter more frequently than patients with atopic eczema and normal IgE productivity, while there are no significant difference in the distribution of IL-4R Ile50Val genotype between patients with high IgE productivity and patients with normal IgE productivity. IL-4 acts through IL-4R and activates STAT-6 (signal transducer and activator of transcription 6), and promotes IgE production by B cells [14]. Mice deficient in IL-4, IL-4R or STAT-6 lack IgE synthesis and Th2-type reaction [14]. Thus, our result on IgE productivity and IL-4 promoter gene is consistent to the previous reports that IL-4 and the IL-4R α are key molecules in IgE production [13,15,17,24,25]. On the other hand, our results which could not show statistically significant association between IL-4R α gene and high IgE concentrations suggests that there could remain other predisposing genes to control IgE production in patients with atopic eczema. Further study is needed to clarify the dominant factor determining the potential of IgE production in patients with atopic eczema, and also the

disease specific factor in the development of atopic eczema.

On Ala375Glu and Arg551Gln in IL-4R α , the polymorphisms were rare both in control subjects and in atopic eczema patients examined, suggesting that there may exist racial difference in the distribution of these genotypes.

In conclusion, our study suggests that the polymorphisms in IL-4 and IL-4R α are not involved in the development of atopic eczema in patients with normal IgE productivity and the development of atopic eczema and IgE production may be controlled separately.

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