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ORIGINAL ARTICLE

Lack of Association between Atopic Dermatitis and –401A/G Polymorphism in the Promoter Region of the RANTES Gene in Japanese

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ABSTRACT

Background: Regulated on activation, normal T expressed and secreted (RANTES) has been known to be deeply involved in the pathophysiology of atopic dermatitis (AD). To examine whether a –401A/G polymorphism in the RANTES promoter gene is associated with the susceptibility to AD in Japanese, we determined the genotypes of the polymorphism in 145 Japanese students and 65 patients with AD.

Methods: DNA was isolated from the mononuclear cells by using SepaGene (Sanko) and analyzed by the polymerase chain reaction (PCR) technique.

Results: The frequency of –401A allele was 0.39 in the healthy subjects without AD. This was not significantly different from that in the patients with AD (0.41). We also failed to show association between the polymorphism in the RANTES promoter gene and the production of IgE.

Conclusions: These results suggest that the –401A/G polymorphism in the RANTES promoter gene does not determine the susceptibility to AD in Japanese, although larger studies could explore the exact role of the polymorphisms in AD, or Japanese might have other susceptibility genes more potent than the RANTES promoter gene.

KEY WORDS

atopic dermatitis, IgE, polymorphism, RANTES

INTRODUCTION

Chemotactic cytokines (chemokines) are small signaling proteins, which are deeply involved in the physiology and pathophysiology of acute and chronic inflammatory processes by attracting and stimulating specific subsets of leukocytes.[1] The chemokines are divided into four groups, CXC chemokine, C-C chemokine, CX3C chemokine and C chemokine by their molecular structure. Among the C-C chemokines, regulated on activation, normal T expressed and secreted (RANTES) has been considered to play a crucial role in allergic diseases because of its potent activity for attracting eosinophils and memory T cells. In fact, an enhanced production of RANTES was demonstrated in many of the allergic diseases suggesting the involvement of the chemokine in the pathogenesis of these diseases.[2-5] Recently, a single-nucleotide mutation in the RANTES promoter gene (–401A/G, refSNP ID: rs2107538) has been reported to affect the transcriptional-rate of the gene by constructing an additional binding site of GATA transcription factor, and this gain-of-mutation function has been shown to be associated with atopic dermatitis (AD) in German children. In the human mast cell line HMC-1 and the T cell line the –401A allele was shown to have higher promoter activity of the RANTES gene compared with the more frequent allele –401G, suggesting that overproduction of RANTES underlies the development of AD. However, great differences in the frequency of –401A allele were also reported among races.[6]

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Table 1 Distribution of RANTES promotor genotypes (− 401A/G) and total serum IgE*

Subjects	Number	Genotype AA		AG		GG		Frequency of A
		Number	IgE (mean ± SD)	Number	IgE (mean ± SD)	Number	IgE (mean ± SD)	
AD	65	10	4.08 ± 0.56	33	3.66 ± 0.64	22	3.80 ± 0.58	0.41
Students (with AD)	28	1	1.146	9	2.60 ± 0.72	18	2.33 ± 0.57	0.20
Students (without AD)	117	16	2.13 ± 0.61	59	2.07 ± 0.64	42	2.00 ± 0.66	0.39

*data was expressed as log (IgE)

In order to ascertain the frequency of −401A allele in the RANTES promoter gene and to investigate whether the polymorphism determines susceptibility to AD in Japanese, the genotypes of the RANTES −401A/G polymorphism in 65 patients with AD and 145 students who had been randomly selected, were determined and the relation between AD and genotype was analyzed.

METHODS

SUBJECTS

Sixty-five patients with AD who were being treated at the Hiroshima University Hospital Department of Dermatology and 145 students of the Hiroshima University Faculty of Medicine were enrolled in the study. The patients with AD were aged 18 to 50 years, and the students were aged 22 to 30 years. The diagnosis of AD was made using the criteria of the Japanese Dermatological Association.[7] Informed consent was obtained from all subjects according to the criteria established by the ethics committee of Hiroshima University. Total serum IgE and specific IgE antibodies for cedar pollen, candida, pityrosporium, dermatophagoides farinae, wheat and rice in serum were determined by a fluoroenzyme immunoassay (CAP system, Pharmacia Upjohn, Uppsala, Sweden).[8]

GENOTYPING OF RANTES GENE

Mononuclear cells were separated from 2 ml of anticoagulated peripheral blood. DNA was isolated from the mononuclear cells by using SepaGene (Sanko) and analyzed by the polymerase chain reaction (PCR) technique. The PCR mixture (in a volume of 50 µl) contained 160 µM of dNTPs, 1 µM of specific primer (see below), 1 unit of Taq DNA polymerase and 1 µl of DNA sample. Primers were as follows: the sense primers for RANTES were −401A 5'-CATGGATGAGGGAAAGGAGA-3' and −401G 5'-CATGGATGAGGGAAAGGAGG-3', and the antisense primer was 5'-ATCCTCTGCAGGAATCCTCTG-3'. The reaction conditions were: denaturation, 94℃, 1 minute; annealing, 62℃, 2 minutes; extension, 72℃, 3 minutes for 35 cycles in a DNA thermal cycle (MIR-D40, Sanyo).

The PCR product (5 µl) was mixed with 1 µl dye solution (1% SDS, 50% glycerol and 0.05% bromophenol blue) and electrophoresed in 2.5% agar gels in 1 × TAE buffer (40 mM Tris, 8 mM acetate, 0.4 mM EDTA) at a constant 100 V for 1 hour. Products were visualized by ethidium bromide over a UV transilluminator and then photographed.

STATISTICAL ANALYSIS

Wilcoxon nonparametric tests were performed to assess differences in the total serum IgE levels of the subject groups. The χ^2 test was used to determine the significance in the frequency of −401A allele of the RANTES promoter gene among the subject groups.

RESULTS

In our study, 28 subjects of 145 students were diagnosed as having or having had AD, and 117 subjects were found to have neither symptoms nor a history of AD. The distribution of RANTES promoter genotypes (−401A/G) is shown in Table 1. Frequency of −401A allele of the RANTES promoter gene in the subjects without AD ($n = 117$) was 0.39, and that in the patients with AD was 0.41. There was no significant difference in the allele frequency between the subjects without AD and the AD patients ($p = 0.93$).

To investigate the influence on serum IgE levels of the genotype, total serum IgE levels were compared among the genotypes (Table 1). There was no significant difference among the genotypes in the patients with AD or in the students without AD ($p = 0.99$), although the levels of total serum IgE in the patients with AD were significantly higher than those found in the students. The influence of the genotypes on antigen-specific IgE production was also examined in the students ($n = 145$). Table 2 shows the distribution of RANTES promoter-genotypes in antigen-specific IgE-positive and negative groups. There was no significant difference in frequency of the polymorphisms of the RANTES promoter gene in any group.

DISCUSSION

In the present study, we found that the frequency of the mutated gene was 0.35 in Japanese students. This was higher than that in Caucasians (0.14) and Colombians (0.30), but lower than that in African Americans

Table 2 Distribution of RANTES promotor genotypes (− 401A/G) in antigen-specific IgE-positive and negative groups

antigen	specific IgE	number	Genotype			Frequency of A
			AA	AG	GG	
cedar	(+)	83	9	37	37	0.33
	(−)	62	8	31	23	0.38
candida	(+)	17	1	7	9	0.26
	(−)	128	16	61	51	0.36
pityrosporium	(+)	10	1	4	5	0.30
	(−)	135	16	64	55	0.36
dermatophagoides farinae	(+)	81	11	40	30	0.38
	(−)	64	6	28	30	0.31
wheat	(+)	12	1	3	8	0.21
	(−)	133	16	65	52	0.36
rice	(+)	10	1	2	7	0.20
	(−)	135	16	66	53	0.36

(0.43) and Afro-Caribbeans (0.44).[6] Because the students were randomly selected and contained subjects with AD and a history of AD, the frequency of the mutated gene was then recalculated in the subjects without AD and a history of AD. The frequency in the healthy subjects was 0.39, and this was not significantly different from that in the patients with AD (Table 1). This supports the data obtained by Kozma *et al.* who calculated the frequency of −401A allele for 0.18 in Hungarian AD patients, and this was not significantly different from that in Hungarian children without allergic disorders.[9] Our results also showed that −401A/G polymorphism of the RANTES promoter gene is not associated with total serum IgE levels and production of antigen-specific IgE in Japanese subjects.

Recently, a promoter gene polymorphism of another C-C chemokine, thymus and activation-regulated chemokine (TARC), was shown to be a gain-of-function mutation.[10] In fact, a single nucleotide polymorphism in the 5'-flanking region of the TARC gene (−431C/T) was reported to be associated with serum levels of TARC in patients with AD.[10] However, in Japanese, no significant difference was found in the allele frequencies between AD patients and controls. These results suggest that the -431C/T polymorphism in the TARC gene is not associated with the susceptibility to AD in Japanese population, either.[11]

The discrepancy in the data among these counties is unclear at the moment. Since AD develops as a cumulative effect of genetic factors and environmental factors, we might consider the difference in environmental factors between Europe and Asia: a possibility that there are not enough environmental factors to trigger the production of RANTES in Japan. This is unlikely because we have almost the same frequency of AD patients in Japan as in European countries.[12,13] The results obtained in this study suggest that the −401A/G polymorphism in the RANTES gene does not determine the susceptibility to AD in Japanese, although larger studies could explore the exact role of the polymorphism on the development of AD in Japanese. Japanese might have other genes that predominantly influence the susceptibility to AD to a greater extent than the RANTES promoter gene.

AD develops mostly in families with atopic diathesis together with allergic rhinitis and allergic bronchial asthma. Atopic diathesis is a complex genetic background characterized by the excessive production of total and allergen-specific IgE. Involvement of genetic factors in the development of AD was clearly determined by a twin-study performed by Larsen *et al.*[14] Monozygotic twins run a risk of 0.86 of having AD if the twin partner has the disease, whereas the disease risk of 0.21 by dizygotic partners does not differ from the frequency seen in ordinary brothers and sisters.[14] A great deal of investigation has focused on polymorphisms in the genome to clarify the genetic background susceptibility to AD including genes for FcεRI-β,[15] mast cell chymase,[16] interleukin-4 (IL-4)[17] and IL-4 receptor.[18] However, the associations of these genes with AD still remain controversial. Further studies will be necessary to clarify the genetic background in AD.

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