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INVESTIGATIVE REPORT

Lack of Association of *CCR4* Single Nucleotide Polymorphism with Atopic Dermatitis in Japanese Patients

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CCR4, a member of the CC chemokine receptor family, is believed to play an important role in the pathogenesis of atopic dermatitis. To examine whether *CCR4* single nucleotide polymorphism (SNP) is associated with susceptibility to atopic dermatitis, we investigated the allele and genotype frequencies of C1014T SNP of *CCR4* in 198 Japanese patients with atopic dermatitis and controls by a PCR-restriction fragment length polymorphism method. There was no significant difference in allele or genotype frequencies between patients with atopic dermatitis and controls. Serum IgE levels and peripheral blood eosinophil counts were not significantly different among genotypes. There was also no significant difference in allele or genotype frequencies between the patient subgroup with and without asthma, with mild or moderate disease, with and without family history of atopic dermatitis, or with and without family history of atopic disorders. C1014T SNP of *CCR4* does not appear to be associated with susceptibility to atopic dermatitis in Japanese patients. *Key words: atopic dermatitis; CCR4; single nucleotide polymorphism.*

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Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disease associated with elevated serum IgE levels and tissue and peripheral blood eosinophilia (1), and is characterized by expansion of Th2 cells and a decrease of Th1 cells, at least in the initial stages (1, 2). The pathogenesis of AD involves various cytokines, chemokines and their receptors (1).

CCR4 is a member of the CC chemokine receptor family (3–5). It was shown to be predominantly expressed on Th2 lymphocytes, eosinophils and basophils (3–9), and is a receptor for two chemokines, thymus and activation-regulated chemokine (TARC) (10) and macrophage-derived chemokine (MDC) (11), which act specifically on activated Th2 lymphocytes (4, 5, 7–9).

Recent studies indicated that TARC, MDC and CCR4 play a vital role in the pathogenesis of AD, inducing Th2 response by attracting CCR4+ Th2 cells to the lesional skin (12–20).

The biological activities of CCR4 could indicate that polymorphism of *CCR4* is a candidate as one of the genetic factors in AD. Variations in *CCR4* have been reported (21), including silent mutations: G498C (V166V), C1014T (Y338Y) and missense mutations: C388G (L130V), G533C (C178S) (counting from the ATG start codon). While variations other than C1014T are rare in the Japanese population, the frequency of 1014T allele was reported to be 3.6% (21), and this variation was considered a single nucleotide polymorphism (SNP).

In this study, we examined whether this SNP in *CCR4* is associated with susceptibility to AD in Japanese patients using case-control analysis. We also compared the allele and genotype frequencies between patient subgroups with and without asthma, with mild or with moderate disease and with and without a family history of AD or any type of atopic disorders.

MATERIALS AND METHODS

We evaluated 198 unrelated Japanese patients with AD who were diagnosed according to the generally accepted criteria of Hanifin and Rajka (22). The patient group consisted of 139 male and 59 female subjects, aged 11–61 years (mean ± SD, 27.4 ± 7.7) with serum IgE levels in the range of 5–84 000 U/ml (median [interquartile range]: 7000 [1600–15 000]) and peripheral blood eosinophil counts in the range of 0–2246/μl (421 [271–649]). IgE levels and peripheral blood eosinophil counts were examined prior to therapies. One hundred and eighty-three Japanese individuals served as control subjects: 104 male and 79 female subjects, aged 18–82 years (29.2 ± 13.4).

Genomic DNA was extracted from peripheral blood leukocytes using a QIAamp blood kit (QIAGEN, Hilden, Germany). Genotyping was carried out by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. We amplified the region of *CCR4*, which includes a SNP site C1014T, by PCR using a set of specific primers: 5′-TGTGGGCTCCTCCAAATGTA-3′ (sense: 1011T>G, mismatch primer) and 5′-TGTAAGCCTTCCTC CTGACA-3′ (antisense). A mismatch primer was used to

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create the *Rsa*I restriction endonuclease polymorphism site. The SNP site was detected by digestion with *Rsa*I. The PCR product was 206 bp. The product from 1014C allele was cut into 187- and 19-bp fragments while that of 1014T allele was uncut and remained 206 bp.

We compared the allele and genotype frequencies between patients with AD and controls, and compared serum IgE levels and peripheral blood eosinophil counts among genotypes in the patients with AD. We also compared the allele and genotype frequencies between patients with AD with asthma and those without asthma (information about whether asthma coexisted or not was available in 134 patients with AD). Asthma was diagnosed according to accepted criteria (23). Comparison was also made of the allele and genotype frequencies between patients with AD with mild disease (SCORAD <15) (24, 25) and those with moderate disease ($15<$SCORAD<40), between those with a family history of AD and those without it, and between those with a family history of atopic disorders (AD, asthma, allergic rhinitis, allergic conjunctivitis or pollinosis) and those without it (information about disease severity, family history of AD and that of atopic disorders was available in 71, 114 and 81 patients with AD, respectively). There was no patient with SCORAD >40, which is severe AD.

Statistical significance was determined by χ^2 test (with Yates' correction if necessary) for differences of allele and genotype frequencies, and by Mann–Whitney's U test for differences of serum IgE levels and peripheral blood eosinophil counts among genotypes.

All studies were approved by the ethics committee for genome research of the Faculty of Medicine, University of Tokyo. All patients and controls involved gave written informed consent for the genetic studies.

RESULTS

The frequencies of allele and genotype at C1014T SNP are listed in Tables I and II. There was no significant difference between patients with AD and controls. There was also no significant difference in serum IgE levels or peripheral blood eosinophil counts among genotypes (Table I). We then compared the allele and genotype frequencies between patients with AD with asthma and those without asthma, and found no difference between the two subgroups (Table II). Nor was any significant difference observed in allele or genotype frequencies between patients with AD with mild disease and those with moderate disease, between those with a family history of AD and those without it, or between those with a family history of atopic disorders and those without such history (Table II).

DISCUSSION

C1014T SNP is located at the C-terminal tail of the CCR4. The functional effect of the SNP has not been studied. The function of the CCR4 will not be affected by this SNP because this is a synonymous substitution.

Table I. *Genotype frequencies of C1014T SNP of CCR4 in patients with atopic dermatitis (AD) and control subjects, and serum IgE levels and peripheral blood eosinophil counts in each genotype in patients with AD*

Genotype	AD ($n=198$)	Control ($n=183$)	*P* value	Serum IgE* (IU/ml)	*P* value	Eosinophil counts* (/μl)	*P* value
C/C	188 (94.9)	173 (94.5)	0.58	7700 [1700–15700]	0.18	421 [270–650]	0.84
C/T	9 (4.5)	10 (5.5)		3100 [1400–5200]		420 [299–653]	
T/T	1 (0.5)	0 (0.0)		35		493	

No significant differences were found. Numbers in parentheses are percentages. C/C, C/T and T/T are the subjects who have C/C, C/T or T/T genotype at the position of 1014 in *CCR4*, respectively.
*Median [interquartile range].

Table II. *Analysis of association of allele and genotype frequencies with asthma, disease severity, or family history of atopic dermatitis (AD) or atopic disorders in C1014T SNP in CCR4*

		Asthma *n* (%)		Severity *n* (%)		Family history of AD *n* (%)		Family history of atopic disorders, *n* (%)		
	AD (total) *n* (%)	Yes	No	Mild	Moderate	Yes	No	Yes	No	Control *n* (%)
C/C	188 (94.9)[b]	71 (100.0)	56 (88.9)	33 (91.7)	33 (94.3)	59 (95.2)	50 (96.2)	41 (93.2)	35 (94.6)	173 (94.5)
C/T	9 (4.5)	0 (0.0)	7 (11.1)	3 (8.3)	2 (5.7)	3 (4.8)	2 (3.8)	3 (6.8)	2 (5.4)	10 (5.5)
T/T	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
P value		0.099	0.22	0.78	0.73	0.89	0.91	0.98	0.70	
C	385 (97.2)	142 (100.0)	119 (94.4)	69 (95.8)	68 (97.1)	121 (97.6)	102 (98.1)	85 (96.6)	72 (97.3)	356 (97.3)
T	11 (2.8)	0 (0.0)	7 (5.6)	3 (4.2)	2 (2.9)	3 (2.4)	2 (1.9)	3 (3.4)	2 (2.7)	10 (2.7)
P value		0.10	0.22	0.78	0.73	0.89	0.91	0.98	0.70	

C and T mean the subjects who have C or T allele at the position of 1014 in *CCR4*, respectively. C/C, C/T and T/T mean the subjects who have C/C, C/T or T/T genotype at the position of 1014 in *CCR4*, respectively. *P* values were calculated by χ^2 test with Yates' correction in 2×2 contingency table (vs control). No significant difference was found.

There is, however, a possibility that the substitution of the nucleotide in mRNA might lead to a change of mRNA stability. This SNP might, therefore, be a susceptibility gene for AD.

The results of this study show that C1014T SNP of *CCR4* is not associated with susceptibility to AD, at least in Japanese patients. We also examined the association of this SNP with AD subgroups with and without asthma, with mild diseases and moderate disease, with and without a family history of AD, and with and without a family history of atopic disorders, but observed no association there either. Indeed, there is a possibility that the association could not be detected due to the lower power of this study because only 198 patients with AD and 183 controls were included, and the frequency of the 1014T allele was low in these subjects (2.7%). However, when we estimated the statistical significance for a larger sample size (power calculation), *P* value would reach 0.05 if the sample size for both the patients and healthy individuals were increased 2609-fold. Thus there is very little possibility that the lower power of this study was the reason we were unable to detect the association. We also found that C1014T SNP demonstrated no significant association with asthma in the Japanese population (manuscript in preparation). *CCR4* SNP seems not to play a role in the pathogenesis of allergic diseases in the Japanese population. It would be of interest if the same study could be performed in patients with AD in other populations.

Kato et al. reported that the frequency of 1014T allele was 3.6% (22/608) in healthy individuals (21). In this study, the frequency was 2.7% (10/366). There is no significant difference between these two frequencies ($P = 0.45$). Putting the results together, the frequency of 1014T allele in Japanese population would be 3.3% (32/974).

REFERENCES

1. Leung DY. Atopic dermatitis: new insights and opportunities for therapeutic intervention. J Allergy Clin Immunol 2000; 105: 860–876.
2. Grewe M, Bruijnzeel-Koomen CA, Schopf E, Thepen T, Langeveld-Wildschut AG, Ruzicka T, et al. A role for Th1 and Th2 cells in the immunopathogenesis of atopic dermatitis. Immunol Today 1998; 19: 359–361.
3. Zlotnik A, Yoshie O. Chemokines: a new classification system and their role in immunity. Immunity 2000; 12: 121–127.
4. Nickel R, Beck LA, Stellato C, Schleimer RP. Chemokines and allergic disease. J Allergy Clin Immunol 1999; 104: 723–742.
5. Sallusto F, Lanzavecchia A, Mackay CR. Chemokines and chemokine receptors in T-cell priming and Th1/Th2-mediated responses. Immunol Today 1998; 19: 568–574.
6. Bonecchi R, Bianchi G, Bordignon PP, D'Ambrosio D, Lang R, Borsatti A, et al. Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. J Exp Med 1998; 187: 129–134.
7. Sallusto F, Lenig D, Mackay CR, Lanzavecchia A. Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. J Exp Med 1998; 187: 875–883.
8. Imai T, Nagira M, Takagi S, Kakizaki M, Nishimura M, Wang J, et al. Selective recruitment of CCR4-bearing Th2 cells toward antigen-presenting cells by the CC chemokines thymus and activation-regulated chemokine and macrophage-derived chemokine. Int Immunol 1999; 11: 81–88.
9. D'Ambrosio D, Iellem A, Bonecchi R, Mazzeo D, Sozzani S, Mantovani A, et al. Selective up-regulation of chemokine receptors CCR4 and CCR8 upon activation of polarized human type 2 Th cells. J Immunol 1998; 161: 5111–5115.
10. Imai T, Baba M, Nishimura M, Kakizaki M, Takagi S, Yoshie O. The T cell-directed CC chemokine TARC is a highly specific biological ligand for CC chemokine receptor 4. J Biol Chem 1997; 272: 15036–15042.
11. Imai T, Chantry D, Raport CJ, Wood CL, Nishimura M, Godiska R, et al. Macrophage-derived chemokine is a functional ligand for the CC chemokine receptor 4. J Biol Chem 1998; 273: 1764–1768.
12. Vestergaard C, Yoneyama H, Murai M, Nakamura K, Tamaki K, Terashima Y, et al. Overproduction of Th2-specific chemokines in NC/Nga mice exhibiting atopic dermatitis-like lesions. J Clin Invest 1999; 104: 1097–1105.
13. Kakinuma T, Nakamura K, Wakugawa M, Mitsui H, Tada Y, Saeki H, et al. Thymus and activation-regulated chemokine in atopic dermatitis: serum thymus and activation-regulated chemokine level is closely related with disease activity. J Allergy Clin Immunol 2001; 107: 535–541.
14. Kakinuma T, Nakamura K, Wakugawa M, Mitsui H, Tada Y, Saeki H, et al. Serum macrophage-derived chemokine (MDC) levels are closely related with the disease activity of atopic dermatitis. Clin Exp Immunol 2002; 127: 270–273.
15. Galli G, Chantry D, Annunziato F, Romagnani P, Cosmi L, Lazzeri E, et al. Macrophage-derived chemokine production by activated human T cells in vitro and in vivo: preferential association with the production of type 2 cytokines. Eur J Immunol 2000; 30: 204–210.
16. Zheng X, Nakamura K, Furukawa H, Nishibu A, Takahashi M, Tojo M, et al. Demonstration of TARC and CCR4 mRNA expression and distribution using in situ RT-PCR in the lesional skin of atopic dermatitis. J Dermatol 2003; 30: 26–32.
17. Vestergaard C, Bang K, Gesser B, Yoneyama H, Matsushima K, Larsen CG. A Th2 chemokine, TARC, produced by keratinocytes may recruit CLA+CCR4+ lymphocytes into lesional atopic dermatitis skin. J Invest Dermatol 2000; 115: 640–646.
18. Wakugawa M, Nakamura K, Kakinuma T, Onai N, Matsushima K, Tamaki K. CC chemokine receptor 4 expression on peripheral blood CD4+ T cells reflects disease activity of atopic dermatitis. J Invest Dermatol 2001; 117: 188–196.
19. Yamamoto J, Adachi Y, Onoue Y, Adachi YS, Okabe Y, Itazawa T, et al. Differential expression of the chemokine receptors by the Th1- and Th2-type effector populations within circulating CD4+ T cells. J Leukoc Biol 2000; 68: 568–574.
20. Campbell JJ, Haraldsen G, Pan J, Rottman J, Qin S, Ponath P, et al. The chemokine receptor CCR4 in

vascular recognition by cutaneous but not intestinal memory T cells. Nature 1999; 400: 776–780.
21. Kato H, Tsuchiya N, Izumi S, Miyamasu M, Nakajima T, Kawasaki H, et al. New variations of human CC-chemokine receptors CCR3 and CCR4. Genes Immun 1999; 1: 97–104.
22. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. Acta Derm Venereol 1980; Suppl. 92: 44–47.
23. National Asthma Education and Prevention Program. Expert panel report 2: guidelines for the diagnosis and management of asthma. Publication No. 97–4051. Bethesda, MD: National Institutes of Health, 1997.
24. European Task Force on Atopic Dermatitis. Severity scoring of atopic dermatitis: the SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. Dermatology 1993; 186: 23–31.
25. Kunz B, Oranje AP, Labreze L, Stalder JF, Ring J, Taieb A. Clinical validation and guidelines for the SCORAD index: consensus report of the European Task Force on Atopic Dermatitis. Dermatology 1997; 195: 10–19.