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Analysis of the polymorphisms in eotaxin gene family and their association with asthma, IgE, and eosinophil

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Abstract

The eotaxin gene family (*eotaxin*, *eotaxin-2*, and *eotaxin-3*) has been implicated in the recruitment of eosinophils, basophiles, and Th2 lymphocytes that is a central aspect of allergic diseases such as asthma. To determine whether the single nucleotide polymorphisms (SNPs) of eotaxin gene family are associated with susceptibility to asthma, we scanned 225 asthma patients and 294 non-asthmatic controls using the direct sequencing method. We further investigated the relationships among each SNP, eosinophils, and serum total IgE levels in asthma patients. Eleven SNPs were identified in the eotaxin gene family. We found that $EoB179T > C$ ($P = 0.0001$), $EoB275C > T$ ($P = 0.018$) of the *eotaxin-2* and $EoA2497T > G$ ($P = 0.003$) of the *eotaxin-3* were significantly associated with the susceptibility of asthma. Furthermore, our data demonstrated for the first time that $EoA2497T > G$ ($P = 0.005$) is related to serum total IgE level while $EoA77C > T$ ($P = 0.035$) and $EoA2497T > G$ ($P = 0.033$) are related to the peripheral blood eosinophil counts in asthma. Our results suggest that the polymorphisms of the eotaxin gene family are associated with the susceptibility of asthma and Eotaxin-3 might play the critical role for the recruitment of eosinophils and the maintenance of IgE levels.

Keywords: Eotaxin; Eotaxin-2; Eotaxin-3; Single nucleotide polymorphism; IgE; Eosinophil; Asthma

Asthma is a multi-complex inflammatory disorder of the airways, characterized by reversible airflow obstruction and airway inflammation, persistent airway hyper-reactivity (AHR), and airway remodeling [1]. Eosinophils accumulate in high numbers in the lungs of asthmatic patient, and are believed to be important in the pathogenesis of asthma. The inflammation accompanied by the accumulation of eosinophils within the bronchial wall is a characteristic feature of asthma [2]. Etiology of asthma involves the interaction between genetic factors and environmental stimuli. Asthmatic persons were reported to have a high concentration of *eotaxin* in the bronchoalveolar lavage (BAL) fluid as well as increased level of *eotaxin* mRNA and protein in the epithelium and submucosa of their airways compared with healthy controls. In the BAL cells, the *eotaxin* immunoreactivity co-localizes mainly to macrophages, with a lesser contribution of T cells and eosinophils [3]. The number of cells that expressed *eotaxin* mRNA in the bronchial mucosa of asthmatic patients is significantly correlated with both airway eosinophilia and bronchial hyper-reactivity [4]. The levels of *eotaxin* are increased in the sputum of atopic and non-atopic asthmatic persons [5]. Increased *eotaxin*

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mRNA and protein levels are also observed in chronic sinusitis and allergic rhinitis [6]. It is estimated that at least a dozen polymorphic genes regulate asthma, controlling the inflammatory response, immunoglobulin E (IgE), cytokine, and chemokine productions [7].

Eotaxin (CCL11) is a CC chemokine that stimulates the migration of eosinophils from the small blood vessels in the lungs by acting on the CC chemokine receptor CCR3. The human eotaxin has been identified to be located on chromosome 17 at q21 and contain three exons [8]. Tumor necrosis factor-α (TNF-α) and interleukin-4 (IL-4) are known to stimulate transcription of this gene in lung fibroblasts [9] and human airway epithelial cells [10]. The promoter region of *eotaxin* gene contains consensus binding sites for the transcription factors nuclear factor (NF)-κB and signal transducer and activator of transcription (STAT-6), which appear to mediate the responses to TNF-α and IL-4 [11]. The homologues to *eotaxin* gene, *eotaxin-2* (CCL24), and *eotaxin-3* (CCL26), have recently been identified [12,13] and have similar eosinophil-selective properties. In the assays of eosinophil chemotaxis, eotaxin and Eotaxin-2 exhibit similar properties while Eotaxin-3 showed much less potency [14]. Ying et al. [15] reported that *eotaxin-2* mRNA levels increased in bronchial biopsies taken from atopic and non-atopic asthmatics. The *eotaxin-3* mRNA is upregulated by the cytokines IL-13 and IL-4 in human umbilical vein endothelial cell culture [13].

To determine whether the single nucleotide polymorphisms (SNPs) of eotaxin gene family are associated with susceptibility to asthma, we scanned the chromosomes of 225 asthma patients and 294 non-asthmatic controls for the identification of SNPs in the eotaxin gene family (*eotaxin*, *eotaxin-2*, and *eotaxin-3*) and investigated their relationships among each SNP, peripheral blood eosinophil count, and serum total IgE levels in asthma patients.

Materials and methods

Subjects and DNA samples. Blood samples were obtained from 225 unrelated asthma patients and 294 unrelated non-asthmatic controls. Genomic DNA was extracted from leukocytes in peripheral blood by a standard phenol–chloroform method or by Invisorb spin blood Maxi kit, according to the manufacturer's directions. The asthma patients were recruited from our outpatient clinic at Chonbuk National University Hospital. Asthma was diagnosed according to the criteria of the American Thoracic Society [16]. Of the 225 patients, 97 were non-atopic, as determined by their clinical history and a skin prick test that was performed with common aeroallergens. We excluded patients who had a history of other atopy-related diseases, such as allergic rhinitis or atopy dermatitis. The non-asthmatic control subjects were recruited from the general population who took a comprehensive medical testing. All subjects in this study were Koreans who were living in the same area. Blood eosinophil counts and serum total IgE levels were measured within the Department of Hematology at Chonbuk National University Hospital using a Coulter GenSTM Hematology Analyzer (Florida, USA) and Roche COBAS-CORE II (Roche Diagnostics, Basal, Switzerland), respectively. The clinical profiles of asthma patients and non-asthmatic controls are shown in Table 1.

Table 1
Clinical profile of the subjects used in this study

	Non-asthmatic controls	Asthma patients
Number of subjects	294	225
Age (mean)	43.7	39.8
FVC1 (% predicted)	81.9 ± 1.9	81.3 ± 1.1
FEV1 (% predicted)	86.1 ± 1.3	78.2 ± 0.9
Eosinophile (% predicted)	4.72 ± 0.1	5.08 ± 0.1
Total IgE (IU/ml)	98.0 ± 23.1[a]	312.0 ± 42.5

[a] The value was determined by the limited numbers ($n = 108$) of subject.

Primers and polymerase chain reaction. The entire coding regions and the part of the intron regions of *eotaxin*, *eotaxin-2*, and *eotaxin-3* genes were amplified by polymerase chain reaction (PCR). Primer sequences used in this study for amplifying and sequencing of the *eotaxin*, *eotaxin-2*, and *eotaxin-3* genes are shown in Table 2. PCRs were performed using LA *Taq* polymerase (TaKaRa) and using 50 ng total genomic DNA per reaction. Amplification was carried out in a GeneAmp PCR system 9700 thermocycler (Applied Biosystem) for 94 °C at 2 min, followed by 10 cycles at 92 °C for 10 s, 68 °C for 45 s, and 68 °C for 10 min. Then 20 cycles at 92 °C for 10 s, 68 °C for 45 s, and 68 °C for 10 min (with 10 s incremental increases per cycle) were followed by a final extension at 68 °C for 7 min.

Genotyping. The genotypes of each SNP in eotaxin gene family were determined by direct sequencing. PCR products purified by PCR purification kit (Millipore, USA) were used as the template DNA for cycle sequencing. Purified PCR products were sequenced using the ABI Prism BigDye Terminator cycle sequencing system (PE Applied Biosystems, Foster City, CA, USA) on the ABI 3100 automatic sequencer (PE Applied Biosystem). Both sense and antisense strands of PCR products were directly sequenced. The reference sequence was based on the sequence of *Homo sapiens* BAC clone CTA-356E1 for *eotaxin-2* and *eotaxin-3* and *H. sapiens* chromosome 17 clone hRPK.215_E_13 for *eotaxin*.

Statistical analysis. χ^2 tests were used to estimate the Hardy–Weinberg equilibrium (HWE). Logistic regression analyses were used for calculating odds ratio (95% confidence interval) for SNP sites. Linkage disequilibrium (LD) analyses by pair-wise comparison of bi-allelic loci and haplotype and their frequencies were constructed by EM algorithm with genotyped SNPs. The asthma patients and controls were compared using case–control association analysis. Allele carrier frequency was defined as the percentage of the individuals carrying the allele among the total number of individuals. χ^2 test from contingency table was applied to analyze the comparison of the frequency of discrete variables between unrelated asthma patients and unrelated healthy controls. ANOVA using Bonferroni multiple comparison procedure or t test was applied to analyze the difference between the combined genotype and the serum total IgE and the peripheral blood eosinophil counts in asthma patients. A P value less than 0.05 was considered to indicate statistical significance.

Results

We scanned 225 asthma patients and 294 non-asthmatic controls using direct sequencing for polymorphism analysis in eotaxin gene family. The entire coding region and the intron/exon boundaries of eotaxin gene family were amplified by PCR and sequenced, respectively. Eleven single nucleotide polymorphisms (SNPs) were

Table 2
Primer sequences for amplifying and sequencing of the eotaxin gene family

Amplified region	Primer name	Primer sequence (5′ → 3′)	Product size (bp)
PCR			
Eotaxin (EoG)	EoGLF	AGGCTTCCCTGGAATCTCCCACACTGTCTG	2112
	EoGLR	AACCCATGCCCTTTGGACTGATAATGAGGT	
Eotaxin-2 (EoB)	EoBLF	GTTGCCCCATAAAGGGCAGAGGGAGGGAGT	2187
	EoBLR	AGAAGAGACACATCCCTGGAGAGTGTTGCT	
Eotaxin-3 (EoA)	EoALF	GGGTCAAAAGTGCTGCTTCTGTTCCCAACC	2750
	EoALR	TAACTCTGGGAGGAAACACCCTCTCCTCCC	
Sequencing			
EoG			
Exon 1	EoGSF1	CACCACCTCTCACGCCAAAG	159
	EoGSR1	GGTGGTTACCTTACCTTTCC	
Exon 2	EoGSF2	GTGGCTCATTTTTTTCTCTG	190
	EoGSR2	TTGTCTAGGGGAGGGTGAAC	
Exon 3	EoGSF3	CCTTCCAATTGCATCCTGTA	172
	EoGSR3	TCAGGCTCTGGTTTGGTTTC	
EoB			
Exon 1	EoBSF1	AGCCCCGAGCTGGTGCTTCT	138
	EoBSR1	GCCCTTGGAACTGCATCCTG	
Exon 2	EoBSF2	AGACGTGGCTCTTCTCTCTC	179
	EoBSR2	TCCTGCCACGTGCCCATGAA	
Exon 3	EoBSF3	CAGCTGATCCCCTGTCCCTT	241
	EoBSR3	GCAGCTCAGGCCCAAACTCA	
EoA			
Exon 1	EoASF1	AAACCTGAGAAGGGCCTGAT	152
	EoASR1	CAGGCGGTCCGGGAAGGAAC	
Exon 2	EoASF2	ATCTCCAAACCCTTCCTCTC	202
	EoASR2	CCATCCTGTTGCATGACTGA	
Exon 3	EoASF3	CCAGGAGGCAAAGAGTCTCA	181
	EoASR3	GTCCAAGCGTCCTCGGATGA	

identified in this study; three SNPs (67G > A, 1820A > C, and 1834T > G) in *eotaxin* gene, six SNPs (96G > A, 179T > C, 275C > T, 304A > C, 1923C > A, and 1926G > A) in *eotaxin-2* gene, and two SNPs in *eotaxin-3* gene (Table 3). The amino acids of *EoG67G > A*, *EoG1820A > C*, *EoG1834T > G*, and *EoB304A > C* located in the coding region were substituted to Ala23Thr, Gln82Pro, Tyr87Asp, and Ile29Leu, respectively. Generally, the amino acid substitutions in chemokine can result in loss or change of function in the expressed protein as a result of structural change. But the genotypes and allele frequencies at these SNPs were not significantly different between asthma patients and non-asthmatic controls (Table 4). Among the SNPs identified in this study, eight SNPs, one (67G > A) from *eotaxin*, five (179T > C, 275C > T, 304A > C, 1923C > A, and 1926G > A) from *eotaxin-2*, and two (77C > T and 2497T > G) from *eotaxin-3*, were selected for genotype, haplotype, and linkage disequilibrium analysis in the asthma patients and non-asthmatic controls. All geno-

Table 3
The variation sites of eotaxin gene family identified in this study

Gene	Position[a]	Amino acid	Region	Frequency[b]
Eotaxin (*EoG*)	67G > A (rs3744508)	Ala–Thr	Exon 1	0.18
	1820A > C	Gln–Pro	Exon 3	0.01
	1834T > G	Tyr–Asp	Exon 3	0.01
Eotaxin-2 (*EoB*)	96G > A		Intron 1	0.09
	179T > C (rs2302004)		Intron 1	0.30
	275C > T (rs2302005)		Intron 1	0.33
	304A > C (rs2302006)	Ile–Leu	Exon 2	0.46
	1923C > A		3′UTR	0.16
	1926G > A		3′UTR	0.16
Eotaxin-3 (*EoA*)	77C > T(rs2240478)		Intron 1	0.37
	2497T > G (rs23020009)		3′UTR	0.07

[a] Calculated from the translational start site.
[b] Frequencies of rare alleles.

Table 4
Genotype and allele frequencies of the SNPs of the eotaxin gene family in asthma patients and non-asthmatic controls

Gene	Position	Genotype	Control *n* (%)	Asthma *n* (%)	Odds ratio[a] (95% CI)	P[b]
EoG	67G > A	GG	183 (71.8)	129 (66.8)	1.00	0.604
		GA	62 (24.3)	51 (26.5)	0.86 (0.56–1.32)	
		AA	10 (3.9)	13 (6.7)	0.54 (0.23–1.27)	
EoB	179T > C	TT	37 (15.7)	19 (8.8)	1.00	0.0008
		TC	94 (39.8)	64 (29.5)	0.75 (0.40–1.43)	
		CC	105 (44.5)	134 (61.7)	0.40 (0.22–0.74)	
		T	168 (35.6)	102 (23.5)	1.00	0.0001
		C	304 (64.4)	332 (76.5)	1.80 (1.34–2.41)	
	275C > T	CC	39 (16.8)	21 (9.8)	1.00	0.058
		CT	90 (38.8)	81 (37.7)	0.60 (0.33–1.10)	
		TT	103 (44.4)	113 (52.5)	0.49 (0.27–0.89)	
		C	168 (36.2)	123 (28.6)	1.00	0.018
		T	296 (63.8)	307 (71.4)	1.42 (1.07–1.88)	
	304A > C	AA	44 (18.8)	44 (20.5)	1.00	0.340
		AC	129 (55.1)	104 (48.4)	1.24 (0.76–2.03)	
		CC	61 (26.1)	67 (31.1)	0.91 (0.53–1.57)	
	1923C > A	CC	5 (2.2)	2 (1.1)	1.00	0.616
		CA	62 (27.0)	54 (29.5)	0.46 (0.09–2.47)	
		AA	163 (70.8)	127 (69.4)	0.51 (0.10–2.69)	
	1926G > A	GG	5 (2.2)	3 (1.6)	1.00	0.799
		GA	62 (27.0)	54 (29.5)	0.69 (0.16–3.02)	
		AA	163 (70.8)	126 (68.9)	0.78 (0.18–3.31)	
EoA	77C > T	CC	111 (37.8)	86 (39.1)	1.00	0.819
		CT	148 (50.3)	105 (47.7)	1.09 (0.75–1.59)	
		TT	35 (11.9)	29 (13.2)	0.94 (0.53–1.65)	
	2497T > G	TT	254 (90.4)	171 (80.7)	1.00	0.0001
		TG	27 (9.6)	41 (19.3)	0.44 (0.26–0.75)	
		GG	0 (0.0)	0 (0.0)	–	
		T	535 (95.2)	383 (90.3)	1.00	0.003
		G	27 (4.8)	41 (9.7)	2.12 (1.28–3.51)	

[a] Logistic regression analyses were used for calculating odds ratio (95% CI; confidence interval).
[b] Values were analyzed by χ^2 test.

type frequencies were in HWE, except 179T > C in asthma patients, which had a borderline P value of 0.03 for deviation from HWE (data not shown). Although an absolute LD ($|D'| = 1$ and $d^2 = 1$) was observed between 1923C > A and 1926G > A, significant breakdown of LDs was apparent in eotaxin-2 as shown by Shin et al. [17]. The strong LDs between 77C > T and 2497T > G of eotaxin-3 were also observed (data not shown). While 16 haplotypes were identified with three major haplotypes, explaining more than 72% of distribution in *eotaxin-2* of non-asthmatic controls, 25 haplotypes in *eotaxin-2* of asthma patients were identified out of 32 possible haplotypes (data not shown). Three haplotypes were identified with two major haplotype, explaining more than 96% and 91% of distribution in non-asthmatic controls and asthma patients, respectively (data not shown).

The allele frequencies of *EoB179T > C*, *EoB275C > T*, and *EoA2497T > G* were significantly different between the patients with asthma and non-asthmatic controls ($P = 0.0001$, 0.018, and 0.003, respectively) and the genotype frequencies of these SNPs were also significantly different (Table 4), suggesting that the *EoB179T > C*, *EoB275C > T*, and *EoA2497T > G* polymorphisms were associated with the susceptibility of asthma. The serum total IgE levels and peripheral blood eosinophil counts among the genotypes of SNPs in eotaxin gene family were analyzed in asthma patients (Table 5 and Fig. 1). *EoA2497T > G* showed significant association with serum total IgE level in asthma patients ($P = 0.005$, Table 5). The serum total IgE level of TG genotype (mean 457.6 IU/ml) on *EoA2497T > G* was higher than that of TT genotype (mean 284.1 IU/ml). The distribution range of serum total IgE in the TG genotype of *EoA2497T > G* (SD 465.0; 95% CI 346.1–569.0) was significantly higher than that in the TT genotype (SD 192.0; 95% CI 236.6–331.6, Fig. 1A). Significant difference was observed in the peripheral blood eosinophil counts among the genotypes of *EoA77C > T* and *EoA2497T > G* in asthma patients (Table 5 and Fig. 1B). In *EoA77C > T*, the eosinophil count was lower in TT genotype (mean 3.3%) than in CC and CT genotype (mean 5.2% and

Table 5
Analysis of serum total IgE and peripheral blood eosinophil counts among the genotype of each SNP of eotaxin gene family in asthma patients

Position	Genotype	IgE (IU/ml)			P^a	Eosinophil (%)[b]			P^a
		n	Mean	SD		*n*	Mean	SD	
EoG67G > A	GG	75	276.1	194.0	0.083	104	5.3	4.9	0.611
	GA	34	361.7	341.0		50	4.6	4.1	
	AA	8	456.5	404.6		13	5.4	3.9	
EoB179T > C	TT	12	230.5	181.1	0.461	14	6.1	4.6	0.677
	TC	43	297.6	203.9		55	4.9	3.5	
	CC	78	327.0	293.8		114	5.1	4.9	
EoB275C > T	CC	15	279.9	190.9	0.880	17	6.2	4.5	0.573
	CT	52	318.5	247.8		69	4.7	3.8	
	TT	69	310.3	281.5		100	6.6	15.4	
EoB304A > C	AA	29	300.9	191.6	0.830	34	6.2	4.5	0.232
	AC	63	303.8	291.0		89	4.7	4.2	
	CC	38	334.2	260.8		59	5.5	4.8	
EoA77C > T	$CC^{(p)}$	56	337.2	296.6	0.778	77	5.2	4.7^r	0.035
	$CT^{(q)}$	62	304.0	239.5		90	5.5	4.6^r	
	$TT^{(r)}$	31	339.9	362.9		38	3.3	$3.1^{p,q}$	
EoA2497T > G	TT	110	284.1	192.0	0.005	148	4.7	4.0	0.033
	TG	20	457.6	464.7		35	6.5	5.9	
	GG	0	0.0	0.0		0	0.0	0.0	

[a] ANOVA or *t* test (for *EoA2497T > G*) was applied. Significant differences ($p < 0.05$) between two groups found by *t* test or Bonferroni multiple comparisons are indicated by values which have the same letter (p–r).
[b] Values were determined by the eosinophil numbers per total cell numbers on mm^3.

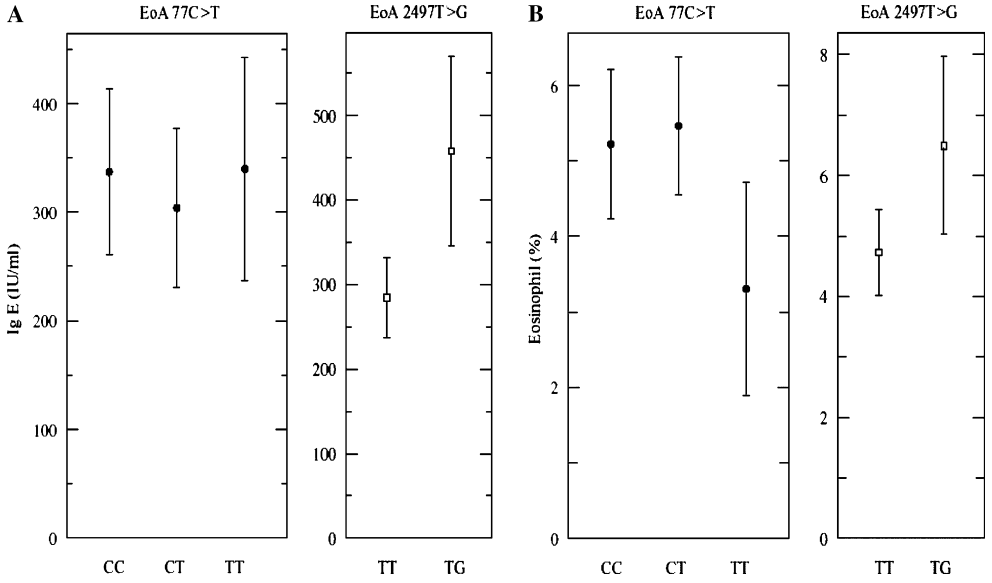

Fig. 1. The distribution range of serum total IgE levels (A) and peripheral blood eosinophil counts (B) among the genotypes of *EoA77C > T* and *EoA2497T > G* in asthma patients. The results were analyzed at http://www.physics.csbsju.edu/stats/anova.html. Bars indicate 95% confidence intervals (CIs).

5.5%, respectively) with significant frequency ($P = 0.035$, Table 5). The distribution range of their eosinophil counts was also lower in TT genotype (SD 3.1; 95% CI 1.89–4.71) than that in CC genotype (SD 4.7; 95% CI 4.23–6.21) and CT genotype (SD 4.6; 95% CI 4.23–6.21) with significant frequency (Fig. 1B). As in the analysis of the serum total IgE levels, the peripheral blood eosinophil counts in *EoA2497T > G* were also higher in TG genotype (mean 6.5%) than in TT genotype (mean 4.7%) with significant frequency ($P = 0.033$, Table 5) and the distribution range of their eosinophil counts was also higher in TG genotype (SD 5.9; 95% CI 5.03–7.96) than in CC genotype (SD 4.0; 95% CI 4.02–5.44, Fig. 1B).

Discussion

The SNPs of many cytokines and their receptor genes, such as IL-10, IL-12, IFNγ, RANTES, CCR2, and CCR5, were identified and indicated that these polymorphisms were associated with immune disorders [18,19]. Contrary to these cytokines, only a few studies about the SNPs of eotaxin gene family were reported [20–22]. *Eotaxin-2* and *eotaxin-3* genes are located on chromosome 7q11.2, distinct from the *eotaxin* gene on chromosome 17q21.1–q21.2 although they possess similar eosinophil-selective properties and signal exclusively via CCR3. We identified a total of eleven SNPs in eotaxin gene family; three SNPs in *eotaxin*, six SNPs in *eotaxin-2*, and two SNPs in *eotaxin-3* (Table 3). We think that $EoG1820A > C$ and $EoG1834T > G$ among the SNPs identified in this study exist in Korean population with very low frequency. Our results show the significant breakdowns of LDs in eotaxin-2, consistent with other study [17]. However, our results suggest that an absolute LD ($|D'| = 1$ and $d^2 = 1$) between 1923C > A and 1926 G > A of Eotaxin-2 and a strong LDs between 77C > T and 2497T > G of Eotaxin-3 might be in the same block (data not shown). It is very likely, considering that these genes are located on the same chromosome at a distance of about 40 kb.

Our results suggest that the SNPs of the *eotaxin-2* gene, $EoB179T > C$ and $EoB275C > T$, and a polymorphism of the *eotaxin-3* gene, $EoA2497T > G$, might be associated with asthma susceptibility (Table 4). It is a novel finding revealing the association between these SNPs of eotaxin gene family and asthma susceptibility for the first time. It would also be interesting to analyze the allele frequency in intron or 3′-UTR where these SNPs are located since it is shown that some of the asthma-associated SNPs were known to be located in introns and 3′-UTR of *Adam33* gene [23]. Actually, 3′-UTR sequence was reported to control mRNA stability and mRNA translation efficiency [24,25].

In addition, our finding showed for the first time that $EoA2497T > G$ might be related to serum total IgE and $EoA77C > T$ and $EoA2497T > G$ might be related to the peripheral blood eosinophil counts in asthma (Table 5). This study also demonstrates the relationship between *eotaxin-3* and asthma for the first time. IgE is thought to play a direct role in mediating not only the early asthmatic response but also the late asthmatic response due to the central role of IgE in the induction of lung eosinophil infiltration and Th2 cell cytokine production [26,27]. The *eotaxin* deficient mice showed impairment in the recruitment of eosinophils during the early part of the late phase response in the lung [28]. The *eotaxin* and *eotaxin-2* mRNA were increased in patients with asthma in comparison with normal controls, although there was no further increase after allergen challenge. In contrast, *eotaxin-3* mRNA was dramatically increased 24 h after allergen challenge [29]. This result demonstrates that Eotaxin-3 may mainly act for the eosinophil recruitment in the later stage of asthmatic response. In fact, our results indicated that Eotaxin-3, rather than Eotaxin or Eotaxin-2, may account for the eosinophil recruitment to asthmatic response (Table 5). These results also suggest that Eotaxin-3 might be associated with asthma development and susceptibility. It will be important in future studies to determine whether these newly described polymorphisms in *eotaxin-3* gene are associated with some functions and expression of Eotaxin-3.

Polymorphisms in *eotaxin* gene are likely to influence the course of asthma. One SNP, $67G > A$ of *eotaxin* gene, has been shown to be associated with decreased level of eosinophil and higher level of lung function in subjects with asthma [17]. Recently, Tsunemi et al. [22] reported that $EoG\text{-}426C > T$ and $EoG\text{-}384A > G$ were associated with serum total IgE levels but not to $EoG67G > A$ in atopic dermatitis. More recently, a research group examined the association of eotaxin gene family with asthma and serum total IgE levels [17]. They demonstrated that one of the SNPs of the *eotaxin-2* gene, $EOT2 + 1265A > G$, was associated with asthma development, and that $eotaxin\text{-}1 + 123G > A$ was related to serum total IgE levels in asthmatics. They did not examine the relationship between the eosinophil counts and SNPs in asthma. They also failed to demonstrate the association in $EoB179T > C$, $EoB275C > T$, and $EoA2497T > G$ and the relation with serum total IgE levels in $EoA2497T > G$ that revealed high association and relation in our study. They did not analyze the association in $EoB179T > C$, $EoB275C > T$, and $EoA2497T > G$.

The data from asthma patients with T/G genotype at $EoA2497T > G$ revealed that the serum total IgE levels and the peripheral blood eosinophil counts were significantly higher than those in the asthma patients with T/T genotype (Table 5 and Fig 1). Interestingly, the G/G genotype in $EoA2497T > G$ was not detected in this study ($n = 493$, Table 4) and another atopic such as allergic rhinitis and inflammatory bowel diseases ($n = 421$) as well as this polymorphism is associated with the susceptibility of allergic rhinitis [unpublished our data]. In summary, our results suggest that the polymorphisms of the eotaxin gene family are associated with the susceptibility of asthma and Eotaxin-3 might play the critical role for the recruitment of eosinophils and the maintenance of IgE levels.

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