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Association of *Eotaxin* gene family with asthma and serum total IgE

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The *Eotaxin* gene family (*Eotaxin1*, *Eotaxin2* and *Eotaxin3*) recruits and activates CCR3-bearing cells such as eosinophils, mast cells and Th2 lymphocytes that play a major role in allergic disorders. To date, the effect of polymorphisms of *Eotaxin* genes on asthma phenotypes has not been thoroughly examined. In our research, we sequenced whole regions of the *Eotaxin* gene family to identify polymorphisms, which may be involved in the development of asthma and total serum IgE. We have identified 37 SNPs in the *Exotaxin* gene family (*Exotaxin1*, *2* and *3*), and 17 common polymorphic sites were selected for genotyping in our asthma cohort (*n* = 721). Statistical analysis revealed that the *EOT2+1265A>G G allele showed significantly lower frequency in asthmatics than in normal healthy controls (0.14 versus 0.23, *P* = 0.002), and that distribution of the *EOT2+1265A>G G** allele-containing genotypes was also much lower in asthmatics (26.3 versus 40.8%, *P* = 0.003). In addition, a non-synonymous SNP in *Eotaxin*1, *EOT1+123Ala>Thr* showed significant association with total serum IgE levels (*P* = 0.002–0.02). The effect of *EOT1+123Ala>Thr* on total serum IgE appeared in a gene-dose-dependent manner. Our findings suggest that the development of asthma may be associated with *EOT2+1265A>G* polymorphisms, and the susceptibility to high IgE production may be attributed to the *EOT1+123Ala>Thr* polymorphism. *Eotaxin* variation/haplotype information identified in this study might provide valuable insights into strategies for the control of asthma.**

INTRODUCTION

Asthma is a common and heterogeneous respiratory disease characterized by reversible airway obstruction that is caused by chronic allergic inflammation of the airways. Bronchial hyperresponsiveness is a characteristic feature of asthma, and serum IgE levels are closely associated with asthma development. The development of asthma is determined by the interaction between host susceptibility (genetics) and a variety of environmental exposures (1–4).

The human *Eotaxin* gene family is located on chromosomes 17 and 7: *Eotaxin1* (MIM no. 601156) on 17q21.1–q21 with three exons (~8 kb) (5,6), *Eotaxin2* (MIM no. 602495) on 7q11.23 with three exons (~6.5 kb) (7), and *Eotaxin3* (MIM no. 604697) on 7q11.23 with three exons (~6 kb) (8), respectively (Fig. 1). The identification of three Eotaxins, each acting via CCR3 receptors, raises the issue of the differential roles of these functionally analogous CC chemokines. The expression of Eotaxin1 mRNA and protein was found to be increased in the bronchial epithelium and submucosal layer of the airways of chronic asthmatics. Furthermore, the elevation of Eotaxin1 levels was proportional to eosinophil infiltration and bronchial hyperreactivity (9,10). The relevance of Eotaxin1 to asthma was evidenced by an association of increased plasma Eotaxin1 levels with impaired lung function of the asthmatics in a large population study (11). In addition, subjects with acutely

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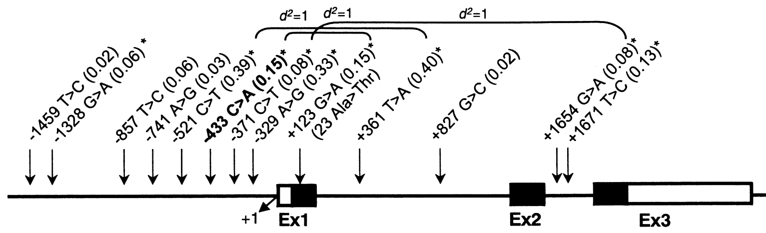
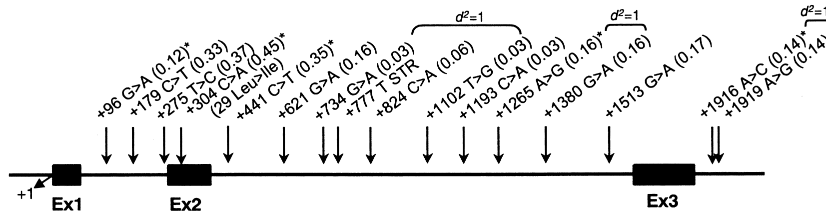
A1 ***Eotaxin1* on chr. 17q21.1-q21.2**



A2 ***Eotaxin1* Haplotypes**

Haplotype	-1328 G>A	-521 C>T	-433 C>A	-371 C>T	-329 A>G	+123 G>A	+361 T>A	+1654 G>A	+1671 T>C	Freq.
EOT1-ht1 (GCCCAGTGT)	G	C	C	C	A	G	T	G	T	0.47
EOT1-ht2 (GTACGAAGT)	.	T	A	.	G	A	A	.	.	0.16
EOT1-ht3 (GCCCAGTGC)	.	.	.	.	.	.	.	.	C	0.14
EOT1-ht4 (GTCCGGAGT)	.	T	.	.	G	.	A	.	.	0.09
EOT1-ht5 (GTCTGGAAT)	.	T	.	T	G	.	A	A	.	0.08
EOT1-ht6 (ATCCAGAGT)	A	T	.	.	.	.	A	.	.	0.06

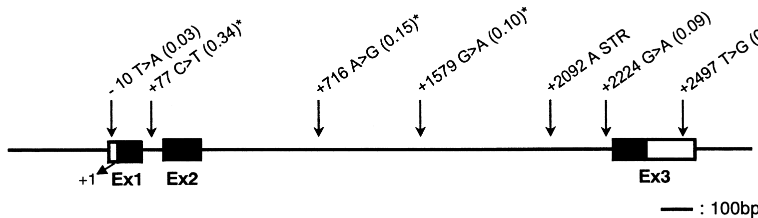
B1 ***Eotaxin2* on chr. 7q11.23**



B2 ***Eotaxin2* Haplotypes**

Haplotype	+96 G>A	+304 C>A	+441 C>T	+1265 A>G	+1916 A>C	Freq.
EOT2-ht1 (GCCAA)	G	C	C	A	A	0.31
EOT2-ht2 (GATAA)	.	A	T	.	.	0.21
EOT2-ht3 (AACAA)	A	A	.	.	.	0.12
EOT2-ht4 (GACAA)	.	A	.	.	.	0.06
EOT2-ht5 (GCCGC)	.	.	.	G	C	0.06
EOT2-ht6 (GCTGA)	.	.	T	G	.	0.06
EOT2-ht7 (GCTAA)	.	.	T	.	.	0.05
EOT2-ht8 (GCCAC)	.	.	.	.	C	0.04
EOT2-ht9 (GCCGA)	.	.	.	G	.	0.03
EOT2-ht10 (GACAC)	.	A	.	.	C	0.02
Others(1)	~	~	~	~	~	0.05

C1 ***Eotaxin3* on chr. 7q11.2**



C2 ***Eotaxin3* Haplotypes**

Haplotype	+77 C>T	+716 A>G	+1579 G>A	Freq.
EOT3-ht1 (CAG)	C	A	G	0.50
EOT3-ht2 (TAG)	T	.	.	0.31
EOT3-ht3 (CGA)	.	G	A	0.07
EOT3-ht4 (CGG)	.	G	.	0.07
EOT3-ht5 (CAA)	.	.	A	0.03
EOT3-ht6 (TGG)	T	G	.	0.02

Figure 1. Gene maps and haplotypes of the *Eotaxin* gene family (*Eotaxin1*, *2* and *3*). Coding exons are marked by shaded blocks and 5′- and 3′-UTR by white blocks. Asterisks indicate SNPs which were genotyped in larger population. The frequencies of SNPs without large-scale genotyping were based on sequencing data ($n = 24$). First base of the transcription start site (*Eotaxin1*) or first base of the translation start site (*Eotaxin2* and *Eotaxin3*) was denoted as nucleotide +1. Dots in A2, B2 and C2 represent the alleles that are found on the most common haplotype. (**A1**) Polymorphisms identified in *Eotaxin1* on 7q21.1–q21.2 (reference sequence Z92709). (**A2**) Haplotypes of *Eotaxin1*. (**B1**) Polymorphisms identified in *Eotaxin2* on 7q11.23 (reference sequence of *Eotaxin2* mRNA: NM_002991). (**B2**) Haplotypes of *Eotaxin2*. Haplotypes with frequency >0.02 are presented. Others (1) contain rare haplotypes: GATAC, GATGA, GACGC, AACGC, GCTAC, GACGA, GATGC and ACTAA. (**C1**) Polymorphisms identified in *Eotaxin3* on 7q11.2 (reference sequence of *Eotaxin3* mRNA: AB010447). (**C2**) Haplotypes of *Eotaxin3*.

exacerbated asthma symptoms and airflow obstruction had higher plasma and sputum Eotaxin1 levels than subjects with stable asthma, and higher levels were associated with less airflow reversibility after treatment (12–14), which suggested that Eotaxin1 was induced locally in a certain state of asthma. In addition, Eotaxin1 is important for eosinophilic inflammation in the early phase of the asthmatic response, while Eotaxin3 may account for the eosinophil recruitment to the asthmatic airway in the later stage of asthmatic response (15). Based on the biological properties involved in allergic inflammatory reactions, it is hypothesized that the Eotaxins play an important role in coordinating the recruitment of inflammatory cells to the sites of allergic inflammation that leads to the development of allergic diseases such as asthma.

The known biological effects of Eotaxins in allergic inflammation and the positive signals from genome-wide studies for atopy and/or total IgE on the region of chromosome 17q (16,17), on which Eotaxin genes are located, facilitated systemic study of this gene family. Here we describe genetic polymorphisms in the *Eotaxin* gene family (*Eotaxin1*, *2* and *3*) and their relationship with the development of asthma and serum total IgE.

RESULTS

By direct DNA sequencing, 37 single-nucleotide polymorphisms (SNPs) in the *Eotaxin* gene family (*Exotaxin1*, *2* and *3*) were identified: 14 in *Eotaxin1* [eight in the 5′ flanking region, one in the coding region (exon1; Ala>Thr) and five in the intron region, including three known polymorphisms], 16 in *Eotaxin2* [one in the coding region (exon1; Leu>Ile), 13 in the intron region and two in 3′ end], and seven in *Eotaxin3* (one in 5′-UTR, five in the intron region and one in 3′-UTR), respectively. Among the SNPs identified, 17 common

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Table 1. Rare allele frequencies of SNPs in the *Eotaxin* gene family (*Eotaxin1*, *Eotaxin2* and *Eotaxin3*), and distributions in asthmatics ($n = 550$) and normal subjects ($n = 171$) in the Korean population

Gene	Locus	Location	Bronchial asthma	Normal controls	P^a
Eotaxin1	*−1328G>A*	5′ flanking	0.06	0.07	0.16
	−329A>G	5′ flanking	0.32	0.35	1.00
	+123G>A (*Ala>Thr*)	Exon1	0.15	0.16	0.99
	+361T>A	Intron1	0.40	0.43	0.72
	+1654G>A	Intron2	0.07	0.08	0.86
	+1671T>C	Intron2	0.14	0.14	0.81
	ht1	.	0.45	0.41	0.33
	ht4	.	0.06	0.07	0.40
Eotaxin2	*+96G>A*	Intron1	0.11	0.11	0.88
	+304C>A (Leu>Ile)	Exon1	0.45	0.43	0.48
	+441C>T	Intron1	0.34	0.37	0.54
	+1265A>G	Intron1	**0.14**	**0.23**	**0.002**
	+1916A>C	Intron2	0.13	0.19	0.05
	ht1	.	0.33	0.32	0.81
	ht2	.	0.06	0.05	0.99
	ht3	.	0.52	0.52	0.15
Eotaxin3	*+77C>T*	Intron1	0.34	0.29	0.19
	+716A>G	Intron2	0.16	0.15	0.25
	+1579G>A	Intron2	0.11	0.14	0.48
	ht1	.	0.47	0.51	0.27
	ht2	.	0.33	0.30	0.18

[a]*P*-values for logistic analyses of co-dominant models controlling age and sex as co-variables. Haplotypes and their frequencies were inferred using the algorithm developed by Stephens *et al.* (41). Missing genotype data were omitted for exact haplotyping. No statistical analyses were performed with *EOT1−521C>T*, *EOT1−433C>A* and *EOT1−371C>T* because of absolute LD ($|D'| = 1$ and $d^2 = 1$) with *EOT1+361T>A*, *EOT1+123G>A* and *EOT1+1654G>A*, respectively. *EOT1-ht2*, *ht3*, *ht5* and *ht6* were not analyzed because they were equivalent models with *EOT1+123G>A* (*= −433C>A*), *EOT1+1671T>C*, *EOT1+1654G>A* (*= −371C>T*) and *EOT1+1328G>A*, respectively (see Fig. 1, A2). Additional information about primers, LD coefficients between SNPs, genotype distributions, Hardy–Weinberg equilibrium and heterozygosity of each SNP are available at www.snp-genetics.com/user/news_list.asp.

Table 2. Logistic analysis of *EOT2+1265A>G* in asthmatic and normal subjects

Subgroups	Genotype			*P*-value		
	A/A	AG	G/G	Co-dominant	Dominant	Recessive
BA	336 (73.7%)	108 (23.7%)	12 (2.6%)	0.002	0.003	0.08
NC	96 (59.3%)	56 (34.6%)	10 (6.2%)			
Atopic BA	230 (74.0%)	74 (23.8%)	7 (2.3%)	0.0007	0.002	0.04
Atopic NC	39 (50.0%)	32 (41.0%)	7 (9.0%)			
Non-atopic BA	104 (72.3%)	34 (23.8%)	5 (3.5%)	0.71	0.72	0.82
Non-atopic NC	57 (67.9%)	24 (28.6%)	3 (3.6%)			

Genotype distributions and *P*-values for logistic analyses of three alternative models (co-dominant, dominant and recessive models) are shown.

polymorphic sites were selected for genotyping in our asthma cohort, in consideration of the location, linkage disequilibrium (LD) with other sites and frequency: nine from *Eotaxin1*, five from *Eotaxin2* and three from *Eotaxin3*, respectively. The frequencies of SNPs are shown in Table 1. The distributions of all 17 SNPs were in Hardy–Weinberg equilibrium (HWE: $P > 0.05$, data not shown). Several absolute LDs ($|D'| = 1$ and $d^2 = 1$) and complete LDs ($|D'| = 1$ and $d^2 \neq 1$) were observed in each gene. Haplotypes of each gene were constructed by EM algorism with genotyped SNPs (Fig. 1). Although complete and/or absolute LDs were observed between all SNPs in *Eotaxin1* (chr. 17q21.1–21.2), significant breakdowns of LDs were apparent in *Eotaxin2* (chr. 7q11.23) and *Eotaxin3* (chr. 7q11.2 Fig. 1).

Allele frequencies of each SNP and common haplotypes (frequency > 0.1) were compared between the patients and the normal controls using logistic regression models (Table 1). The *EOT2+1265A>G G** allele showed a significantly lower frequency in the asthma patients than in the normal controls (0.14 versus 0.23, $P = 0.002$), and the distribution of *EOT2+1265A>G G** allele-containing genotypes was also much lower in asthma patients than in the normal controls (26.3 versus 40.8%, $P = 0.002$; Table 2). The protective effect of the *EOT2+1265A>G G** allele was clearer in the atopic population (0.0007–0.04, Table 2). The *P*-values of this *EOT2+1265A>G* ($P = 0.002$ by co-dominant model; see Table 1) retained significance after the strict correction of multiple comparisons. A significant difference in allele

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Table 3. Regression analyses for age, sex, smoking and atopy adjusted log(total IgE) with 17 SNPs and haplotypes among bronchial asthma patients

Gene	Locus	*n* (mean ± SD)			*P*
		C/C[a]	C/R[a]	R/R[a]	
Eotaxin1	*−1328G>A*	450 (2.24 ± 0.62)	55 (2.31 ± 0.63)	1 (2.31 ± .)	0.87
	−329A>G	238 (2.21 ± 0.63)	212 (2.3 ± 0.56)	60 (2.3 ± 0.71)	0.08
	+123G>A (Ala>Thr)	**356 (2.19 ± 0.62)**	**128 (2.35 ± 0.6)**	**12 (2.63 ± 0.49)**	**0.002**
	+361T>A	165 (2.15 ± 0.64)	226 (2.3 ± 0.6)	72 (2.28 ± 0.66)	0.09
	+1654G>A	410 (2.27 ± 0.61)	72 (2.17 ± 0.68)	1 (2.29 ± .)	0.70
	+1671T>C	375 (2.25 ± 0.61)	113 (2.28 ± 0.63)	12 (2.1 ± 0.83)	0.75
	ht1	115 (2.28 ± 0.68)	224 (2.27 ± 0.6)	91 (2.11 ± 0.61)	0.40
	ht4	382 (2.24 ± 0.63)	47 (2.26 ± 0.64)	1 (2.31 ± .)	0.70
Eotaxin2	*+96G>A*	335 (2.25 ± 0.63)	85 (2.22 ± 0.66)	4 (2.32 ± 0.43)	0.70
	+304C>A (Leu>Ile)	127 (2.27 ± 0.65)	207 (2.2 ± 0.63)	84 (2.27 ± 0.63)	0.67
	+441C>T	228 (2.28 ± 0.62)	212 (2.27 ± 0.62)	65 (2.08 ± 0.65)	0.18
	+1265A>G	326 (2.24 ± 0.62)	107 (2.27 ± 0.63)	12 (2.24 ± 0.68)	0.55
	+1916A>C	324 (2.22 ± 0.63)	98 (2.31 ± 0.63)	9 (2.31 ± 0.34)	0.18
	ht1	159 (2.21 ± 0.65)	166 (2.25 ± 0.66)	35 (2.22 ± 0.52)	0.57
	ht2	323 (2.25 ± 0.64)	32 (2.07 ± 0.68)	5 (1.98 ± 0.67)	0.47
	ht3	338 (2.22 ± 0.64)	22 (2.34 ± 0.65)	0	0.63
Eotaxin3	*+77C>T*	197 (2.25 ± 0.62)	217 (2.26 ± 0.6)	49 (2.22 ± 0.76)	0.80
	+716A>G	310 (2.21 ± 0.65)	108 (2.3 ± 0.54)	16 (2.37 ± 0.83)	0.14
	+1579G>A	319 (2.22 ± 0.64)	73 (2.36 ± 0.52)	6 (2.57 ± 0.68)	0.09
	ht1	101 (2.28 ± 0.69)	183 (2.3 ± 0.61)	89 (2.17 ± 0.59)	0.63
	ht2	174 (2.28 ± 0.61)	166 (2.26 ± 0.61)	33 (2.17 ± 0.8)	0.35

[a]C/C, C/R and R/R represent homozygotes for common allele (C/C), heterozygotes (C/R) and homozygotes for rare allele (R/R). Genotype and haplotype distributions, means, and standard deviations (SD) of log(total IgE), and *P*-values of co-dominant models for regression analyses are shown. Log(total IgE) was highly associated with age, sex and atopy among bronchial asthma patients ($P < 0.0001$). Male, atopic and younger patients showed higher levels of log(total IgE). Smoking (categorized as non-smoker, ex-smoker and smoker) was not added into the analysis because it had no significant association with log(total IgE) ($P = 0.07$).

Table 4. Regression analyses of log(total IgE) as functions of *EOT1+123G>A* (*Ala>Thr*) among bronchial asthma patients

Subgroups	Genotype	*n*	Mean	SD	*P*-value		
					Co-dominant	Dominant	Recessive
BA	Ala/Ala	356	2.19	0.62			
	Ala/Thr	128	2.35	0.60	0.002	0.006	0.02
	Thr/Thr	12	2.63	0.49			
Atopic BA	Ala/Ala	241	2.45	0.51			
	Ala/Thr	95	2.54	0.51	0.02	0.05	0.05
	Thr/Thr	9	2.83	0.39			
Non-atopic BA	Ala/Ala	115	1.66	0.47			
	Ala/Thr	33	1.79	0.50	0.10	0.17	0.16
	Thr/Thr	3	2.04	0.11			

Genotype distributions, means and standard deviations (SD) of log(total IgE), and *P*-values for regression analyses of three alternative models (co-dominant, dominant and recessive models) are shown.

frequency of neighboring *EOT2+1916A>C* ($P = 0.05$) could be the result of linkage with *EOT2+1265A>G*.

All single SNPs and common haplotypes (frequency >0.1) were also analyzed for their association with serum total IgE among asthma patients, using multiple regression models. The serum levels of total IgE were highly associated with age, sex and atopy among asthma patients ($P < 0.0001$). Male, atopic and younger patients showed higher levels of total IgE, as expected (data not shown). Among the 21 loci analyzed, *EOT1+123Ala>Thr* showed significant association with log-transformed total IgE level in the asthma patients ($P = 0.002$, Table 3). The effect of *EOT1+123Ala>Thr* on total serum IgE was apparent in a gene-dose-dependent manner, i.e. highest in homozygotes (Thr–Thr 2.63), intermediate in heterozygotes (Ala–Thr 2.35), and lowest in wild-type homozygotes (Ala–Ala 2.19; Tables 3 and 4). In further analyses with subgroups of patients, *EOT1+123Ala>Thr* revealed a similar association with total serum IgE in atopic asthmatic patients ($P = 0.02$–0.05, Table 4). Although it was not statistically significant in non-atopic patients, the levels of total serum IgE showed the same trend of increasing in proportion to the number of Thr allele of *EOT1+123Ala>Thr* (Table 4).

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DISCUSSION

Eotaxins are a family of CC chemokines that coordinate the recruitment of inflammatory cells bearing the CCR3 receptor to the sites of allergic inflammation (18). During the last decade there has been intensive investigation into the biological effects of Eotaxins. To date, three members of this family: Eotaxin1 [CC chemokine ligand (CCL)[3] 11], Eotaxin2 (CCL24) and Eotaxin3 (CCL26) have been identified. Although there is low sequence homology between the Eotaxins, all the Eotaxins have been shown to signal via the chemokine receptor, CCR3 (6,19–23). CCR3 is highly expressed on eosinophils (6,24,25), and all three Eotaxins have been known to activate eosinophils with the same potentials (6,19,21,22,26,27). CCR3 has also been detected on other cell types, including basophils, mast cells and a subpopulation of Th2 cells (27–29).

In this study, we demonstrated that one of the SNPs of the *Eotaxin2* gene, *EOT2+1265A>G*, was associated with asthma development, and that *Eotaxin1+123G>A* (*Ala>Thr*) was related to total serum IgE in asthmatics. To our knowledge, an association study has not been performed to examine the relationship between Eotaxin2 and Eotaxin3 and asthma development. Our result concerning the association of the SNP of the Eotaxin2 gene (*EOT2+1265A>G*) with asthma development is a novel finding. A non-synonymous SNP of *Eotaxin1* [*+123G>A* (*Ala>Thr*)] has been reported to reduce the secretion of Eotaxin protein (30). This non-synonymous SNP alters the predicted Eotaxin1 amino acid sequence by substituting a polar threonine residue for the hydrophobic alanine residue. As a secreted protein, Eotaxin1 contains an amino-terminal sequence of hydrophobic amino acids that are a substrate for signal peptidases, which process proteins for transport to the cell surface and secretion (31,32). The substitution of threonine for alanine substantially reduced the predicted signal peptidase activity for the threonine variant of *EOT+123Ala>Thr*, and subsequently secreted significantly less than did the alanine form. This was confirmed in an *in vivo* study. Asthmatic subjects who were homozygous for the threonine form of Eotaxin1 had significantly lower plasma Eotaxin1 levels than asthmatic individuals with the alanine form (30). Although biological action of Eotaxin1 in IgE production has not been well studied, we found the expression of Eotaxin1 to be decreased markedly in peripheral blood mononuclear cells of the patients with hyper-IgE syndrome (33). Two other single nucleotide substitutions of *Eotaxin1*, i.e. C to T at position −426 (−426C>T, −371C>T in this study calculated from the transcriptional start site) and A to G at position −384 (−329 A>G in this study) from the translation start site, were associated with serum IgE levels in patients with atopic dermatitis (34), but not with susceptibility to asthma (35). Our study showed a positive association of *Eotaxin1* (*+123G>A*) SNP with serum IgE levels in asthma patients, and a result of no association with asthma development by the *Eotaxin1 + 123G>A* (*Ala>Thr*), which is concordant with that shown in the previous study (31).

The presence of an association between asthma development and the *Eotaxin2* polymorphism, and between IgE production and the *Eotaxin1* polymorphism, could raise questions regarding the differential roles of Eotaxin1 and Eotaxin2, which are functionally analogous chemokines, by acting via CCR3 receptors. The plausible explanations for this are the differential expression of Eotaxins and the differential modulation of them. Eotaxin1 is induced mainly by inflammatory cytokines, while Eotaxin2 and Eotaxin3 are up-regulated by Th2 cytokine such as IL4 and IL13 (23,36). Although Eotaxin1 is important for eosinophilic inflammation in the early phase of the asthmatic response, it does not account for ongoing late asthmatic response (37). A comparative study regarding the different roles among Eotaxins showed that Eotaxin3 rather than Eotaxin1 or Eotaxin2 may account for the eosinophil recruitment to the asthmatic airway in the later stage of asthmatic response (15). However, the specific relation of Eotaxin2 and Eotaxin3 with a Th2 environment remains unproven. The role of Eotaxins in IgE production still lacks supporting biological data. However, the expression of the CCR3 receptor on human dendritic cells (38) and Th2 cells (17) raises a possibility that Eotaxins may promote or down-regulate IgE production by acting on these two main cells in the immune response of allergic inflammation.

In summary, we have identified 37 SNPs in the *Exotaxin* gene family (*Exotaxin1*, *2* and *3*), and 17 common polymorphic sites were selected for genotyping in our asthma cohort. The *EOT2+1265A>G G** allele showed decreased risk of asthma. In addition, *EOT1+123Ala>Thr* showed significant association with total IgE level in a gene-dose dependent manner. Further studies would be needed to elucidate the functions of the variants, which showed significant association with asthmatic phenotypes.

MATERIAL AND METHODS

Subjects

Subjects were recruited from the Asthma Genome Research Center that consists of four tertiary hospitals located around Seoul, Korea (Soonchunhyang University Hospital, Ajuo University Hospital, Choong-Ang University Hospital and Ulsan University Hospital). Ethical approvals were obtained from the institutional review board of each hospital. All subjects in this study were ethnically Korean. All patients with asthma had currently the one or more asthma symptoms and the physical examination compatible with asthma definition by the American Thoracic Society (39). Normal subjects were recruited from spouses of the patients and the general population who answered negatively to a screening questionnaire for respiratory symptoms and had FEV1 greater than 75% predicted, PC20 methacholine greater than 10 mg/ml, and normal findings on a simple chest radiogram. Total IgE and specific IgE to *Dermatophagoides farinae* (Df) and *D. pteronyssinus* (Dp) were measured using CAP system (Pharmacia Diagnostics, Sweden). Twenty-four common inhalant allergens were used for the skin prick test. Atopy was defined as having wheal reaction equal to or greater than a histamine of 3 mm in diameter and/or positive response of specific IgE to Dp and Df. The clinical parameters are summarized in Table 5.

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Table 5. Clinical profile of the study subjects

	Normal controls	Asthmatics
Number of subjects	171	550
Age [mean (range)]	28.7 (7–75)	35.2 (7–80)
Sex (male/female)	85/86	248/302
Current smoker	30.8%	19.6%[a]
FVC1%, predicted	89.0 ± 1.7	87.9 ± 0.7
FEV1%, predicted	93.7 ± 1.2	83.0 ± 0.9[a]
PC_{20}, methacholine (mg/ml)	24.0 ± 1.4	2.8 ± 0.7[a]
Total IgE (IU/ml)	212.0 ± 41.7	537.0 ± 52.4[a]
Positive rate of specific IgE (Df)	32.2%	45.5%[a]
Positive rate of specific IgE (Dp)	36.3%	49.8%[a]
Positive rate of atopy	46.7%	65.1%[a]

[a]*P*-value < 0.001; asthmatics versus normal controls.

Sequencing analysis of the human *Eotaxin* gene family

Genomic DNAs for sequencing were isolated from 24 healthy volunteers. We sequenced the whole gene, including the 5′ flanking region (~1.5 kb), to discover single nucleotide polymorphisms (SNPs) using the ABI PRISM 3700 DNA analyzer (Applied Biosystems, Foster City, CA).

Genotyping by single-base extension (SBE) and electrophoresis

Primer extension reactions were performed with the SNaPshot ddNTP Primer Extension Kit (Applied Biosystems, Foster City, CA, USA). To clean up the primer extension reaction, one unit of SAP was added to the reaction mixture, and the mixture was incubated at 37°C for 1 h, followed by 15 min at 72°C for enzyme inactivation. The DNA samples, containing extension products, and Genescan 120 Liz size standard solution were added to Hi-Di formamide (Applied Biosystems, Foster City, CA, USA) at the recommendation of the manufacturer. The mixture was incubated at 95°C for 5 min, followed by 5 min on ice, and then electrophoresis was performed by using the ABI Prism 3100 Genetic Analyzer. The results were analyzed using the program ABI Prism GeneScan and Genotyper (Applied Biosystems, Foster City, CA, USA).

Statistics

We examined widely used measures of linkage disequilibrium between all pairs of biallelic loci, Lewontin's D' ($|D'|$) (40). Haplotypes and their frequencies were inferred using the algorithm developed by Stephens *et al.* (41). Logistic regression models were used for calculating odds ratios (95% confidential interval) and corresponding *P*-values for SNP sites and haplotypes controlling age and sex as co-variables (Table 1). Means and standard deviations (SD) of log(total IgE) and *P*-values for regression analyses of three alternative models (co-dominant, dominant and recessive models) were calculated using multiple regression analyses controlling age, sex and atopy as co-variables. The values of total serum IgE were log transformed.

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REFERENCES

1. Ahmadi, K.R. and Goldstein, D.B. (2002) Multifactorial diseases: asthma genetics point the way. *Curr. Biol.*, **12**, R702–704.
2. Burrows, B., Martinez, F.D., Halonen, M., Barbee, R.A. and Cline, M.G. (1989) Association of asthma with serum IgE levels and skin-test reactivity to allergens. *New Engl. J. Med.*, **320**, 271–277.
3. Kim, Y.K., Cho, S.H., Koh, Y.Y., Son, J.W., Jee, Y.K., Lee, M.H., Min, K.U. and Kim, Y.Y. (1999) Skin reactivity to inhalant allergens, total serum IgE levels, and bronchial responsiveness to methacholine are increased in parents of nonatopic asthmatic children. *J. Allergy Clin. Immunol.*, **104**, 311–316.
4. Koh, Y.Y., Jeong, J.H., Kim, C.K., Kim, Y.K., Jee, Y.K., Cho, S.H., Min, K.U. and Kim, Y.Y. (2000) Atopic status and level of bronchial responsiveness in parents of children with acute bronchiolitis. *J. Asthma*, **37**, 709–717.
5. Garcia-Zepeda, E.A., Rothenberg, M.E., Weremowicz, S., Sarafi, M.N., Morton, C.C. and Luster, A.D. (1997) Genomic organization, complete sequence, and chromosomal location of the gene for human eotaxin (SCYA11), an eosinophil-specific CC chemokine. *Genomics*, **41**, 471–476.
6. Kitaura, M., Nakajima, T., Imai, T., Harada, S., Combadiere, C., Tiffany, H.L., Murphy, P.M. and Yoshie, O. (1996) Molecular cloning of human eotaxin, an eosinophil-selective CC chemokine, and identification of a specific eosinophil eotaxin receptor, CC chemokine receptor 3. *J. Biol. Chem.*, **271**, 7725–7730.
7. Nomiyama, H., Osborne, L.R., Imai, T., Kusuda, J., Miura, R., Tsui, L.C. and Yoshie, O. (1998) Assignment of the human CC chemokine MPIF-2/ eotaxin-2 (SCYA24) to chromosome 7q11.23. *Genomics*, **49**, 339–340.
8. Guo, R.F., Ward, P.A., Hu, S.M., McDuffie, J.E., Huber-Lang, M. and Shi, M.M. (1999) Molecular cloning and characterization of a novel human CC chemokine, SCYA26. *Genomics*, **58**, 313–317.
9. Ying, S., Robinson, D.S., Meng, Q., Rottman, J., Kennedy, R., Ringler, D.J., Mackay, C.R., Daugherty, B.L., Springer, M.S., Durham, S.R. *et al.* (1997) Enhanced expression of eotaxin and CCR3 mRNA and protein in atopic asthma. Association with airway hyperresponsiveness and predominant co-localization of eotaxin mRNA to bronchial epithelial and endothelial cells. *Eur. J. Immunol.*, **27**, 3507–3516.
10. Lamkhioued, B., Renzi, P.M., Abi-Younes, S., Garcia-Zepada, E.A., Allakhverdi, Z., Ghaffar, O., Rothenberg, M.D., Luster, A.D. and Hamid, Q. (1997) Increased expression of eotaxin in bronchoalveolar lavage and airways of asthmatics contributes to the chemotaxis of eosinophils to the site of inflammation. *J. Immunol.*, **159**, 4593–4601.
11. Nakamura, H., Weiss, S.T., Israel, E., Luster, A.D., Drazen, J.M. and Lilly, C.M. (1999) Eotaxin and impaired lung function in asthma. *Am. J. Respir. Crit. Care Med.*, **160**, 1952–1956.
12. Taha, R.A., Laberge, S., Hamid, Q. and Olivenstein, R. (2001) Increased expression of the chemoattractant cytokines eotaxin, monocyte chemotactic protein-4, and interleukin-16 in induced sputum in asthmatic patients. *Chest*, **120**, 595–601.
13. Yamada, H., Yamaguchi, M., Yamamoto, K., Nakajima, T., Hirai, K., Morita, Y. and Sano, Y. (2000) Eotaxin in induced sputum of asthmatics: relationship with eosinophils and eosinophil cationic protein in sputum. *Allergy*, **55**, 392–397.
14. Lilly, C.M., Woodruff, P.G., Camargo, C.A., Jr., Nakamura, H., Drazen, J.M., Nadel, E.S. and Hanrahan, J.P. (1999) Elevated plasma eotaxin levels in patients with acute asthma. *J. Allergy Clin. Immunol.*, **104**, 786–790.
15. Berkman, N., Ohnona, S., Chung, F.K. and Breuer, R. (2001) Eotaxin-3 but not eotaxin gene expression is upregulated in asthmatics 24 hours after allergen challenge. *Am. J. Respir. Cell. Mol. Biol.*, **24**, 682–687.
16. Koppelman, G.H., Stine, O.C., Xu, J., Howard, T.D., Zheng, S.L., Kauffman, H.F., Bleecker, E.R., Meyers, D.A. and Postma, D.S. (2002) Genome-wide search for atopy susceptibility genes in Dutch families with asthma. *J. Allergy Clin. Immunol.*, **109**, 498–506.

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17. Barnes, K.C., Mathias, R.A., Nickel, R., Freidhoff, L.R., Stockton, M.L., Xue, X., Naidu, R.P., Levett, P.N., Casolaro, V. and Beaty, T.H. (2001) Testing for gene-gene interaction controlling total IgE in families from Barbados: evidence of sensitivity regarding linkage heterogeneity among families. *Genomics*, **71**, 246–251.
18. Rankin, S.M., Conroy, D.M. and Williams, T.J. (2000) Eotaxin and eosinophil recruitment: implications for human disease. *Mol. Med. Today*, **6**, 20–27.
19. Ponath, P.D., Qin, S., Ringler, D.J., Clark-Lewis, I., Wang, J., Kassam, N., Smith, H., Shi, X., Gonzalo, J.A., Newman, W. *et al.* (1996) Cloning of the human eosinophil chemoattractant, eotaxin. Expression, receptor binding, and functional properties suggest a mechanism for the selective recruitment of eosinophils. *J. Clin. Invest.*, **97**, 604–612.
20. Patel, V.P., Kreider, B.L., Li, Y., Li, H., Leung, K., Salcedo, T., Nardelli, B., Pippalla, V., Gentz, S., Thotakura, R. *et al.* (1997) Molecular and functional characterization of two novel human C-C chemokines as inhibitors of two distinct classes of myeloid progenitors. *J. Exp. Med.*, **185**, 1163–1172.
21. Forssmann, U., Uguccioni, M., Loetscher, P., Dahinden, C.A., Langen, H., Thelen, M. and Baggiolini, M. (1997) Eotaxin-2, a novel CC chemokine that is selective for the chemokine receptor CCR3, and acts like eotaxin on human eosinophil and basophil leukocytes. *J. Exp. Med.*, **185**, 2171–2176.
22. White, J.R., Imburgia, C., Dul, E., Appelbaum, E., O'Donnell, K., O'Shannessy, D.J., Brawner, M., Fornwald, J., Adamou, J., Elshourbagy, N.A. *et al.* (1997) Cloning and functional characterization of a novel human CC chemokine that binds to the CCR3 receptor and activates human eosinophils. *J. Leukoc. Biol.*, **62**, 667–675.
23. Kitaura, M., Suzuki, N., Imai, T., Takagi, S., Suzuki, R., Nakajima, T., Hirai, K., Nomiyama, H. and Yoshie, O. (1999) Molecular cloning of a novel human CC chemokine (Eotaxin-3) that is a functional ligand of CC chemokine receptor 3. *J. Biol. Chem.*, **274**, 27975–27980.
24. Daugherty, B.L., Siciliano, S.J., DeMartino, J.A., Malkowitz, L., Sirotina, A. and Springer, M.S. (1996) Cloning, expression, and characterization of the human eosinophil eotaxin receptor. *J. Exp. Med.*, **183**, 2349–2354.
25. Ponath, P.D., Qin, S., Post, T.W., Wang, J., Wu, L., Gerard, N.P., Newman, W., Gerard, C. and Mackay, C.R. (1996) Molecular cloning and characterization of a human eotaxin receptor expressed selectively on eosinophils. *J. Exp. Med.*, **183**, 2437–2448.
26. Jose, P.J., Griffiths-Johnson, D.A., Collins, P.D., Walsh, D.T., Moqbel, R., Totty, N.F., Truong, O., Hsuan, J.J. and Williams, T.J. (1994) Eotaxin: a potent eosinophil chemoattractant cytokine detected in a guinea pig model of allergic airways inflammation. *J. Exp. Med.*, **179**, 881–887.
27. Garcia-Zepeda, E.A., Rothenberg, M.E., Ownbey, R.T., Celestin, J., Leder, P. and Luster, A.D. (1996) Human eotaxin is a specific chemoattractant for eosinophil cells and provides a new mechanism to explain tissue eosinophilia. *Nat. Med.*, **2**, 449–456.
28. de Paulis, A., Annunziato, F., Di Gioia, L., Romagnani, S., Carfora, M., Beltrame, C., Marone, G. and Romagnani, P. (2001) Expression of the chemokine receptor CCR3 on human mast cells. *Int. Arch. Allergy Immunol.*, **124**, 146–150.
29. Sallusto, F., Mackay, C.R. and Lanzavecchia, A. (1997) Selective expression of the eotaxin receptor CCR3 by human T helper 2 cells. *Science*, **277**, 2005–2007.
30. Nakamura, H., Luster, A.D., Nakamura, T., In, K.H., Sonna, L.A., Deykin, A., Israel, E., Drazen, J.M. and Lilly, C.M. (2001) Variant eotaxin: its effects on the asthma phenotype. *J. Allergy Clin. Immunol.*, **108**, 946–953.
31. Nielsen, H., Engelbrecht, J., Brunak, S. and von Heijne, G. (1997) A neural network method for identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. *Int. J. Neural. Syst.*, **8**, 581–599.
32. Nielsen, H., Engelbrecht, J., Brunak, S. and von Heijne, G. (1997) Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. *Protein Eng.*, **10**, 1–6.
33. Ganzalo, J.A., Jia, G.Q., Aguirre, V., Friend, D., Coyle, A.J., Jenkins, N.A., Lin, G.S., Katz, H., Lichtman, A., Copeland, N. *et al.* (1996) Mouse Eotaxin expression parallels eosinophil accumulation during lung allergic inflammation but it is not restricted to a Th2-type response. *Immunity*, **4**, 1–14.
34. Tsunemi, Y., Saeki, H., Nakamura, K., Sekiya, T., Hirai, K., Fujita, H., Asano, N., Tanida, Y., Kakinuma, T., Wakugawa, M. *et al.* (2002) Eotaxin gene single nucleotide polymorphisms in the promoter and exon regions are not associated with susceptibility to atopic dermatitis, but two of them in the promoter region are associated with serum IgE levels in patients with atopic dermatitis. *J. Dermatol. Sci.*, **29**, 222.
35. Miyamasu, M., Sekiya, T., Ohta, K., Ra, C., Yoshie, O., Yamamoto, K., Tsuchiya, N., Tokunaga, K. and Hirai, K. (2001) Variations in the human CC chemokine eotaxin gene. *Genes Immun.*, **2**, 461–463.
36. Hoeck, J. and Woisetschlager, M. (2001) Activation of eotaxin-3/CCLl26 gene expression in human dermal fibroblasts is mediated by STAT6. *J. Immunol.*, **167**, 3216–3222.
37. Brown, J.R., Kleimberg, J., Marini, M., Sun, G., Bellini, A. and Mattoli, S. (1998) Kinetics of eotaxin expression and its relationship to eosinophil accumulation and activation in bronchial biopsies and bronchoalveolar lavage (BAL) of asthmatic patients after allergen inhalation. *Clin. Exp. Immunol.*, **114**, 137–146.
38. Beaulieu, S., Robbiani, D.F., Du, X., Rodrigues, E., Ignatius, R., Wei, Y., Ponath, P., Young, J.W., Pope, M., Steinman, R.M. *et al.* (2002) Expression of a functional eotaxin (CC chemokine ligand 11) receptor CCR3 by human dendritic cells. *J. Immunol.*, **169**, 2925–2936.
39. (1987) Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, November 1986. *Am. Rev. Respir. Dis.*, **136**, 225–244.
40. Hedrick, P.W. (1987) Gametic disequilibrium measures: proceed with caution. *Genetics*, **117**, 331–341.
41. Stephens, M., Smith, N.J. and Donnelly, P. (2001) A new statistical method for haplotype reconstruction from population data. *Am. J. Hum. Genet.*, **68**, 978–989.

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