Ji-Won Min · June-Hyuk Lee · Choon-Sik Park
Hun Soo Chang · Tai Youn Rhim · Sung-Woo Park
An-Soo Jang · Hyoung-Doo Shin

Association of eotaxin-2 gene polymorphisms with plasma eotaxin-2 concentration

Received: 11 November 2004 / Accepted: 22 December 2004 / Published online: 3 March 2005
© The Japan Society of Human Genetics and Springer-Verlag 2005

Abstract Chemokine (C–C motif) ligand 24 (CCL24, eotaxin-2) is a CC chemokine that recruits and activates cells bearing the CC chemokine receptor 3, which play a major role in asthma. Previously, we observed a significant association between a single nucleotide polymorphism (SNP) in eotaxin-2 (*CCL24+1272A* → *G*) and a lower risk of asthma. Consequently, this study has followed up on those genetic effects by evaluating the association between the SNP and plasma eotaxin-2 concentration in 172 asthmatics and 135 normal controls. Asthmatics had significantly higher plasma eotaxin-2 levels than did normal controls ($P=0.02$). The SNP (*CCL24+1272A* → *G*) and two haplotypes (*ht2* and *ht6*) were strongly associated with plasma eotaxin-2 levels in asthmatics (*CCL24+1272A* → *G*: $P=0.006$, *ht2*: $P=0.006$, and *ht6*: $P=0.002$). The *CCL24+1272A* → *G* allele and the *ht2* and *ht6* haplotypes showed a gene-dose effect on the plasma eotaxin-2 levels in asthmatics ($P=0.005–0.032$). In conclusion, the susceptibility of patients with asthma to high eotaxin-2 production may be due to genetic effects of the *CCL24+1272A* → *G* polymorphism, *ht2* and *ht6* haplotypes.

Keywords Asthma · Eotaxin-2 · Haplotype · Polymorphisms · SNP

Introduction

Eotaxins belong to the family of CC chemokines that coordinates the recruitment of inflammatory cells bearing chemokine (C–C motif) receptor 3 (CCR3) to sites of allergic inflammation (Rankin et al. 2000). CCR3 is expressed on eosinophils, Th2 lymphocytes, basophils, and mast cells (Daugherty et al. 1996; Ponath et al. 1996a, 1996b), which are key cells in the development of asthma. To date, three members of this family have been identified: eotaxin-1 [CC chemokine ligand (CCL) 11] (Kitaura M et al. 1996; Garcia-Zepeda et al. 1997), eotaxin-2 (CCL24) (Nomiyama et al. 1998), and eotaxin-3 (CCL26) (Guo et al. 1999). Although there is low sequence homology between the eotaxins, all the eotaxins signal via the CCR3 (Ponath et al. 1996a, 1996b; Forssmann et al. 1997; Patel et al. 1997; White et al. 1997; Kitaura et al. 1999). Many human studies have examined the biological activity and clinical relevance of eotaxin-1 in asthma and airway eosinophilia (Lamkhieoued et al. 1997; Mould et al. 1997; White et al. 1997). Since the three eotaxins share the same CCR3 and have the potential to activate eosinophils in vitro (Ponath et al. 1996a, 1996b; Patel et al. 1997; White et al. 1997), eotaxin-2 or eotaxin-3 may play important roles in the development of asthma and intermediate phenotypes, as does eotaxin-1. The protein and mRNA of eotaxin-2 increase in the airway of asthmatics (Ying et al. 1999; Rojas-Ramos et al. 2003). Local eotaxin-2 promotes eosinophil accumulation into the airway, IL-13 production, and airway hyperreactivity in cooperation with IL-5 (Yang et al. 2003) and recruits newly produced and CD34⁺ eosinophils after allergen exposure in animal study (Radinger et al. 2004). We previously identified 16 novel single nucleotide polymorphisms (SNPs) of the eotaxin-2 gene and demonstrated a significant association between one SNP in eotaxin-2 (*CCL24+1272A* → *G* G^* allele) and a lower risk of asthma (Shin et al. 2003). The known biological effects of eotaxin-2

J.-W. Min · J.-H. Lee · C.-S. Park (✉) · H. S. Chang
T. Y. Rhim · S.-W. Park · A.-S. Jang
Division of Allergy and Respiratory Medicine,
Department of Internal Medicine,
Soonchunhyang University Bucheon Hospital,
1174, Jung Dong, Wonmi Ku, Bucheon,
Gyeonggi Do, 420-021, Korea
E-mail: mdcsipark@unitel.co.kr
Tel.: +82-32-621-5105
Fax: +82-32-621-5023

H.-D. Shin
Department of Genetic Epidemiology, SNP Genetics, Inc.,
11th Floor, MaeHun B/D, 13 Chongro 4 Ga,
Chongro-Gu, Seoul, Korea, 110-834

(Ying et al. 1999; Rojas-Ramos et al. 2003; Yang et al. 2003; Radinger et al. 2004) and the protective effect of the *CCL24+1272 A → G G** allele (Shin et al. 2003) prompted us to compare the plasma eotaxin-2 levels in asthmatics and normal controls to validate functional evidence for the association between the genetic polymorphism of the eotaxin-2 gene and asthma. Here, we evaluated whether the plasma eotaxin-2 level is dependent on the polymorphism of the eotaxin-2 gene.

Materials and methods

Subjects

The study examined 135 normal healthy subjects and 172 asthmatics recruited from the Genome Research Center for Allergy and Respiratory Diseases of Soonchunhyang University Hospitals. The study was approved by the institutional review board of the hospital. All subjects were Korean. They were selected from patients who had not received steroid therapy and had not experienced an acute exacerbation within 6 weeks before the study and who experienced mild or intermittent-to-moderate persistent asthma based on their symptom severity and initial forced expiratory volume in 1 s (FEV1) (National Institutes of Health, 2002). All patients had clinical symptoms of asthma and a positive bronchodilator test ($> 15\%$ increase of FEV1) and/or airway hyperreactivity (< 10 mg/ml of methacholine) (American Thoracic Society, 1987) measured using Chai's method (Chai et al. 1975). Normal subjects were recruited from the spouses of patients and the general population who gave negative answers to a screening questionnaire for respiratory symptoms (Ferris 1978). They had no asthmatics as first-degree relatives, FEV1 $> 75\%$ of the predicted value, PC20 methacholine > 10 mg/ml, and normal findings on a simple chest radiogram. CBC and differential count were done automatically using a Coulter counter. Total IgE was measured using the CAP system (Pharmacia Diagnostics, Sweden). Atopy was defined as the presence of an immediate skin reaction > 3 mm in diameter to one or more of 24 common aeroallergens (Park et al. 1984).

Measurement of eotaxin-2 by ELISA

Plasma eotaxin-2 was measured using a quantitative sandwich enzyme immunoassay kit (R&D, San Diego, CA, USA). The lower limit of detection for eotaxin-2 was 15.6 pg/ml. Values below this limit were assumed to be 15.6 pg/ml for the statistical analysis. The inter- and intra-assay coefficients of variance were below 10%.

Genotyping by single-base extension (SBE) and electrophoresis

Primer extension reactions were performed with a SNaPshot ddNTP Primer Extension Kit (Applied

Biosystems, Foster City, CA, USA), as described (Shin et al. 2003). The DNA samples containing extension products and GeneScan 120-Liz-size standard solutions were added to Hi-Di formamide (Applied Biosystems). The mixture was incubated at 95°C for 5 min and then on ice for 5 min; following this, electrophoresis was performed using an ABI Prism 3100 Genetic Analyzer. The results were analyzed using the programs ABI Prism GeneScan and Genotyper (Applied Biosystems).

Statistics

The eotaxin-2 levels were log transformed to normalize their left-skewed distributions. Differences of clinical parameters between asthma and control groups were compared using Student's *t* test or χ^2 test. Simple linear regression analysis was used to assess correlations between the parameters before multiple regression test for searching potential confounding factors. The χ^2 test was used to compare the observed numbers of each genotype with those expected for a population in Hardy-Weinberg equilibrium. Haplotypes were inferred using the algorithm (Excoffier and Slatkin 1995). Missing genotype data were omitted for exact haplotyping. A rare allele was coded as a dominant in a dominant model and a recessive in a recessive model. A haplotype was also coded in same manner. Odds ratio and 95% confidence intervals were calculated for each genotype and haplotype using logistic regression models. The multiple regression models for eotaxin-2 levels were adjusted for age. We did not perform hierarchical regression analyses because we considered the hypothesis that different polymorphisms existed in a same gene could be influenced by same factors. If some effect selection method was carried out, better fitting models could be taken, which had different covariants between each other in spite of existing in a same gene. Differences were considered significant when $P < 0.05$. These statistical analyses were performed with SAS (version 8.01, SAS institute, Cary, NC, USA).

Results

Clinical characteristics of the study subjects and correlation of plasma eotaxin-2 level with peripheral blood eosinophil count and total IgE

The clinical parameters of the study subjects are summarized in Table 1. The levels of total IgE, positive rate of skin test, and blood eosinophil number were significantly higher in asthmatics than in normal controls (Table 1). Plasma eotaxin-2 levels were also significantly higher in asthmatics than in normal controls ($P=0.02$). The log(plasma eotaxin-2 level) was analyzed with possible confounding factors using regression analysis. Age was related to the log (plasma eotaxin-2 level)

Table 1 Clinical characteristics of study subjects. Values are mean $\pm$ SE. *FVC1* forced vital capacity in 1 s, *FEV1* forced expiratory volume in 1 s

	Asthma	Control	<i>P</i> value
No. of subjects	172	135	
Age: median (range)	48 (20–81)	37 (20–81)	0.07
Gender (male/female)	68/104	73/62	0.01
Smoker %	33.1	37.2	0.4
FVC %, predicted	85.6 $\pm$ 1.0	96.4 $\pm$ 1.2	<0.0001
FEV1 %, predicted	76.8 $\pm$ 1.2	96.1 $\pm$ 1.2	<0.0001
% changes of FEV1 by bronchodilator	10.3 $\pm$ 0.6	3.2 $\pm$ 0.5	<0.0001
PC ₂₀ , methacholine (mg/ml)	3.3 $\pm$ 0.3	24.1 $\pm$ 0.4	<0.0001
Total IgE (IU/ml)	349 $\pm$ 34.0	188 $\pm$ 26.3	<0.0001
Positive skin test %	33.4	22.1	0.01
Eotaxin-2 (pg/ml) ^a	248.2 $\pm$ 16.2	196.2 $\pm$ 16.9	0.02
Log (eotaxin-2) (pg/mL) ^a	2.26 $\pm$ 0.02	2.16 $\pm$ 0.03	0.03
Blood eosinophil (/ μ l)	435 $\pm$ 27.5	158 $\pm$ 14.9	0.003

^a Plasma eotaxin-2 was measured in 172 asthmatics and 135 normal controls

($R^2=0.0768$, $P=0.0002$), but not to gender or status of atopy in the asthmatics (Table 2).

Association of SNPs and haplotypes of eotaxin-2 gene with the plasma eotaxin-2 level

Of the 16 SNPs identified in our previous study (Shin et al. 2003), five SNPs were selected for larger-scale genotyping based on linkage disequilibriums (LDs) among SNPs and frequencies. Four polymorphisms (+734G $\rightarrow$ A, +824C $\rightarrow$ A, +1102T $\rightarrow$ G, and +1193 $\rightarrow$ A) showing low frequencies (<0.10) were excluded for further analysis. In addition, SNPs showing high LD values ($|D'| \approx 1$ and $d^2 \approx 1$) were also excluded for genotyping; +179C $\rightarrow$ T (LD with +441C $\rightarrow$ T), +275T $\rightarrow$ C (with +304C $\rightarrow$ A), +621G $\rightarrow$ A and +1380G $\rightarrow$ A (with +1265A $\rightarrow$ G), and +1513G $\rightarrow$ A $\rightarrow$ G and +1919A $\rightarrow$ C (with +1916A $\rightarrow$ C). The original nomenclature of SNPs of eotaxin-2 was corrected as follows: *CCL24*+441 C $\rightarrow$ T to *CCL24*+447 C $\rightarrow$ T, *CCL24*+1265 A $\rightarrow$ G to *CCL24*+1272 A $\rightarrow$ G, and *CCL24*+1916 A $\rightarrow$ C to *CCL24*+1923 A $\rightarrow$ C (Shin et al. 2003). The distributions of all five SNPs were in Hardy–Weinberg equilibrium (HWE; $P > 0.05$). All single SNPs and the seven haplotypes were analyzed for associations with the log (plasma eotaxin-2 level) using multiple regression models in asthmatics and normal

controls (Table 3). The association analysis revealed that one (*CCL24*+1272 A $\rightarrow$ G G* allele) of the five SNPs ($P=0.006$) and two (*ht2* and *ht6*) of the seven haplotypes were strongly associated with plasma eotaxin-2 level in asthmatics ($P=0.006$ and 0.002, respectively). The *CCL24*+1272 A $\rightarrow$ G allele polymorphism had the gene–dose effect on the plasma eotaxin-2 level in asthmatics. Asthmatics carrying the *CCL24*+1272 A $\rightarrow$ G G* allele had higher eotaxin-2 levels than those having *CCL24*+1272 A $\rightarrow$ G A* homozygote [log (plasma eotaxin-2)=2.36 $\pm$ 0.03 versus 2.21 $\pm$ 0.02, $P=0.005$]. The *ht2* and *ht6* haplotypes also showed the gene–dose effect on plasma eotaxin-2 levels in asthmatics. Asthmatics carrying the *ht2* haplotype (GATAA) homozygote (*ht2*+ / +) had the lowest eotaxin-2 level, and those carrying none of the *ht2* haplotype (*ht2*- / -) had the highest level. Asthmatics having one of the *ht2* haplotypes showed the intermediate level. There were significant differences between the three groups ($P=0.006$ –0.026) (Table 3). In contrast, asthmatics carrying the *ht6* haplotype (GCTGA) homozygote (*ht6*+ / +) had the highest eotaxin-2 level, and those carrying none of the *ht6* haplotypes (*ht6*- / -) had the lowest level. Asthmatics having one of the *ht6* haplotypes showed the intermediate level. There were significant differences between the three groups ($P=0.002$ –0.032) (Table 3). However, *ht5* (GCCGA) having the same + 1272A $\rightarrow$ G polymorphism was not associated

Table 2 Regression analyses between log (plasma eotaxin-2 level) and possible confounding factors

Subject	Variables	Log (plasma eotaxin-2 level)			
		<i>P</i> value	R^2	<i>B</i>	S.E
Asthma	Age	0.0002	0.0768	0.00567	0.00151
	Gender	0.5878	0.0017	0.02757	0.05077
	Atopy	0.7050	0.0009	-0.01909	0.05034
Normal control	Age	0.0673	0.0249	-0.00392	0.00212
	Gender	0.2466	0.0101	-0.05311	0.04564
	Atopy	0.7906	0.0006	0.01232	0.04632

Table 3 Regression analyses for age-adjusted log (plasma eotaxin-2) with five single nucleotide polymorphisms (SNPs) and three haplotypes in bronchial asthma ($n = 172$) and normal controls ($n = 135$). Genotypic and haplotypic distributions, mean, and standard error (SE) of log (plasma eotaxin-2) are shown. Rare allele (or haplotype) is dominant and recessive in dominant and recessive models, respectively. BA bronchial asthma, NC normal control, C/C homozygotes for common allele, C/R heterozygote, R/R homozygote for rare allele, ht+ bearing of haplotype

Subject	Polymorphism	n (mean $\pm$ SE)			P value of alternative models*		
					Codominant	Dominant	Recessive
BA		C/C	C/R	R/R			
	<i>CCL24</i> +96 <i>G</i> $\rightarrow$ <i>A</i>	117 (2.24 $\pm$ 0.03)	31 (2.32 $\pm$ 0.02)	2 (2.47 $\pm$ 0.04)	0.23	0.22	0.72
	<i>CCL24</i> +304 <i>C</i> $\rightarrow$ <i>A</i>	40 (2.25 $\pm$ 0.03)	78 (2.26 $\pm$ 0.03)	29 (2.29 $\pm$ 0.02)	0.81	0.17	0.89
	<i>CCL24</i> + 447 <i>C</i> $\rightarrow$ <i>T</i>	78 (2.29 $\pm$ 0.03)	64 (2.23 $\pm$ 0.03)	22 (2.27 $\pm$ 0.03)	0.38	0.79	0.96
	<i>CCL24</i> + 1272 <i>A</i> $\rightarrow$ <i>G</i>	105 (2.21 $\pm$ 0.02)	40 (2.34 $\pm$ 0.03)	7 (2.36 $\pm$ 0.03)	0.006	0.005	0.23
	<i>CCL24</i> + 1923 <i>A</i> $\rightarrow$ <i>C</i>	110 (2.28 $\pm$ 0.03)	36 (2.27 $\pm$ 0.03)	5 (2.09 $\pm$ 0.01)	0.62	0.85	0.32
		ht-/-	ht+/-	ht+/+			
	<i>ht1</i> (G-C-C-A-A)	75 (2.29 $\pm$ 0.04)	77 (2.25 $\pm$ 0.04)	20 (2.27 $\pm$ 0.08)	0.65	0.49	0.91
	<i>ht2</i> (G-A-T-A-A)	98 (2.31 $\pm$ 0.03)	67 (2.24 $\pm$ 0.04)	7 (2.00 $\pm$ 0.09)	0.006	0.026	0.017
	<i>ht3</i> (A-A-C-A-A)	139 (2.26 $\pm$ 0.03)	31 (2.31 $\pm$ 0.05)	2 (2.47 $\pm$ 0.28)	0.34	0.33	0.79
	<i>ht4</i> (G-A-C-A-A)	152 (2.25 $\pm$ 0.03)	18 (2.42 $\pm$ 0.07)	2 (2.35 $\pm$ 0.08)	0.06	0.039	0.71
	<i>ht5</i> (G-C-C-G-C)	152 (2.27 $\pm$ 0.03)	18 (2.22 $\pm$ 0.09)	2 (2.15 $\pm$ 0.05)	0.90	0.91	0.92
	<i>ht6</i> (G-C-T-G-A)	155 (2.24 $\pm$ 0.03)	14 (2.46 $\pm$ 0.07)	3 (2.60 $\pm$ 0.26)	0.002	0.004	0.032
	<i>ht7</i> (G-C-T-A-A)	164 (2.27 $\pm$ 0.03)	7 (2.09 $\pm$ 0.09)	1 (2.55 $\pm$ 0)	0.74	0.37	0.16
		C/C	C/R	R/R			
NC	<i>CCL24</i> +96 <i>G</i> $\rightarrow$ <i>A</i>	99 (2.17 $\pm$ 0.02)	29 (2.29 $\pm$ 0.02)	0 (. $\pm$.)	0.02	0.02	–
	<i>CCL24</i> +304 <i>C</i> $\rightarrow$ <i>A</i>	38 (2.19 $\pm$ 0.03)	65 (2.19 $\pm$ 0.02)	23 (2.22 $\pm$ 0.01)	0.72	0.89	0.64
	<i>CCL24</i> +447 <i>C</i> $\rightarrow$ <i>T</i>	51 (2.23 $\pm$ 0.02)	67 (2.18 $\pm$ 0.02)	17 (2.15 $\pm$ 0.03)	0.13	0.13	0.42
	<i>CCL24</i> + 1272 <i>A</i> $\rightarrow$ <i>G</i>	74 (2.18 $\pm$ 0.02)	45 (2.21 $\pm$ 0.03)	8 (2.34 $\pm$ 0.01)	0.23	0.49	0.1
	<i>CCL24</i> + 1923 <i>A</i> $\rightarrow$ <i>C</i>	84 (2.17 $\pm$ 0.02)	43 (2.27 $\pm$ 0.02)	3 (2.1 $\pm$ 0.01)	0.11	0.05	0.56
		ht-/-	ht+/-	ht+/+			
	<i>ht1</i> (G-C-C-A-A)	73 (2.20 $\pm$ 0.03)	48 (2.22 $\pm$ 0.04)	14 (2.09 $\pm$ 0.08)	0.34	0.76	0.11
	<i>ht2</i> (G-A-T-A-A)	77 (2.24 $\pm$ 0.03)	55 (2.15 $\pm$ 0.03)	3 (2.08 $\pm$ 0.10)	0.047	0.049	0.51
	<i>ht3</i> (A-A-C-A-A)	106 (2.17 $\pm$ 0.03)	29 (2.29 $\pm$ 0.04)	0 (. $\pm$.)	0.039	0.039	–
	<i>ht4</i> (G-A-C-A-A)	124 (2.19 $\pm$ 0.02)	11 (2.27 $\pm$ 0.06)	0 (. $\pm$.)0	0.350	0.35	–
	<i>ht5</i> (G-C-C-G-C)	107 (2.17 $\pm$ 0.03)	28 (2.28 $\pm$ 0.05)	0 (. $\pm$.)	0.051	0.51	–
	<i>ht6</i> (G-C-T-G-A)	106 (2.20 $\pm$ 0.02)	27 (2.19 $\pm$ 0.07)	2 (2.35 $\pm$ 0.26)	0.79	0.98	0.36
	<i>ht7</i> (G-C-T-A-A)	132 (2.20 $\pm$ 0.02)	3 (2.13 $\pm$ 0.04)	0 (. $\pm$.)	0.22	0.22	–

*. P values of three alternative models (codominant, dominant, and recessive) are demonstrated

with eotaxin-2 levels. These data indicate that the gene-dose effect of + 1272A $\rightarrow$ G polymorphism and *ht6* may be partially dependent. P values were corrected for multiple comparisons by using the Bonferroni method (Altman 1991). When multiple comparisons were performed by multiplying by 5 for SNPs and by 7 for haplotypes, the P values remained significant (*CCL24* + 1272A $\rightarrow$ G: $P = 0.03$, *ht2*: $P = 0.042$, and *ht6*: $P = 0.014$ in the codominant model). This gene-dose effect was also observed in the *CCL24* + 1272A $\rightarrow$ G G^* allele and the *ht2* haplotype of normal controls although statistical significance after Bonferroni adjustment was not observed in normal controls due to small sample size (Table 3).

Discussion

Eotaxins are a family of CC chemokines that coordinate the recruitment of inflammatory cells bearing the CCR3 to sites of allergic inflammation (Rankin et al. 2000). Among the SNPs and haplotypes for the three genes of this family, we previously found a significant association between one polymorphic allele of the eotaxin-2 gene

(*CCL24* + 1272 A $\rightarrow$ G G^* allele) and a lower risk of asthma (Shin et al. 2003). Nevertheless, the association between the SNP of eotaxin-2 and asthma development needs confirmation with a functional analysis of the SNP. In this study, we demonstrated that plasma eotaxin-2 levels are significantly higher in asthmatics than in normal controls (Table 1) and are proportional to the number of *CCL24* + 1272 A $\rightarrow$ G G^* alleles and the *ht6* haplotype inversely to that of the *ht2* haplotype in asthmatics (Table 3). This functional evidence of eotaxin-2 gene polymorphism is a novel finding to our knowledge. The polymorphic locus *CCL24* + 1272 A $\rightarrow$ G falls within an intron (Shin et al. 2003). The functional consequences of intronic variation are unclear. Theoretically, mechanisms by which the polymorphism within intron could affect eotaxin-2 production include enhanced mutability due to altered DNA sequence context, increased cryptic splicing events, and altered transcript stability or tissue-specific expression (Lozano and Levine 1991; Matrick 1994). However, the supporting experimental data for such mechanisms are lacking at present.

Since the plasma eotaxin-2 level and peripheral blood eosinophil count may depend on the severity and acute

exacerbation of asthma or on steroid treatment, as with eotaxin-1 (Lampinen et al. 2004), we measured eotaxin-2 concentration in plasma from the selected patients with intermittent and mild-to-moderate asthma who had not received steroid therapy and had not experienced an acute exacerbation in the 6 weeks before the study. These selection criteria excluded a possible sampling bias of the study population due to asthma severity such as acute exacerbation and steroid treatment.

Clinical relevance of eotaxin-1 has been revealed in asthma and airway eosinophilia (Lamkhioed et al. 1997; Mould et al. 1997; White et al. 1997). Although the protein and mRNA of eotaxin-2 increase in the airway of asthmatics (Ying et al. 1999; Rojas-Ramos et al. 2003), the clinical relevance of eotaxin-2 and -3 are still lacking. In recruitment of eosinophils into the airways, several chemokines, such as RANTES, eotaxin-1, -2, -3, MCP-3, and M-4, attract the cells to the inflammatory site in the tissue (Lampinen et al. 2004). Like eotaxin-1 (Leckie et al. 2000; van Rensen et al. 2001), local eotaxin-2 promotes eosinophil accumulation into the airway in cooperation with IL-5 (Yang et al. 2003). In the present study, asthmatics with the rare allele ($CCL24 + 1272 A \rightarrow G G^*$) have higher plasma eotaxin-2 levels than do those with the homozygote of wild allele ($CCL24 + 1272 A > G A^*$). This suggests that plasma eotaxin-2 may have a protective effect on asthma development. Thus, how does the eotaxin-2 in plasma exert the protective effect? One possible explanation would be desensitization via CCR3 between eotaxin families. Eotaxin-2 in peripheral blood may desensitize circulating CCR3-bearing cells, which in turn become less susceptible to chemotactic signals of CC chemokines, such as eotaxin-1, in the airways. This desensitization has been well demonstrated in in vivo and in vitro studies. In IL-5 transgenic mice, systemic eotaxin-1 pretreatment significantly inhibited the recruitment of eosinophils to the lung by local eotaxin-2 treatment (Yang et al. 2003). This data indicate that prior exposure of eosinophils to eotaxin-1 induces internalization of CCR3 (Zimmermann et al. 1999). This process occurs in the circulation when eosinophils are exposed to intravenous eotaxin-1, thus inhibiting the ability of eotaxin-2 to signal and recruit CCR3-bearing cells from the blood to the lung (Yang et al. 2003). Such a complete cross-desensitization has been clearly demonstrated in vitro between eotaxin-2 and -1 (Forssmann et al. 1997). The profiles of the in vitro protein production of eotaxin subfamily members clearly vary among the individual cell types and sites. Eotaxin-1 is mainly produced by epithelial and mesenchymal cells, including fibroblast and smooth muscle in the airway (Ying et al. 1999; Miyamasu et al. 2000; Moore et al. 2002). However, a large amount of eotaxin-2 is produced by peripheral blood monocytes while eotaxin-1 is not detected in the supernatants of monocytes (Komiya et al. 2003). The plasma level of eotaxin-2 (248.2 ± 16.2 pg/ml) is 10 times higher than that of eotaxin-1 (26.2 ± 1.1 pg/ml) in asthmatics of our study (data not shown). Thus, in peripheral blood, eotaxin-2 may exert the biologic activity on CCR3-bearing cells more than does eotaxin-1.

In summary, we validate the association study of the $CCL24 + 1272 A \rightarrow G$ allele polymorphism and the *ht2* and *ht6* haplotypes with asthma development by demonstrating that the plasma eotaxin-2 level appears to be gene-dose dependent on the effect of $CCL24 + 1272 A \rightarrow GG^*$ and *ht2* and *ht6* haplotypes. Our findings suggest that the susceptibility of patients with asthma to high eotaxin-2 production may be due to genetic effects of the $CCL24 + 1272 A \rightarrow G$ polymorphism and *ht2* and *ht6* haplotypes.

Acknowledgements This work was supported by a grant from the Korea Health 21 R&D Project, Ministry of Health and Welfare, Republic of Korea (01-PJ3-PG6-01GN04-003).

References

- Altman DG (1991) Practical statistics for medical research. Chapman and Hall, London
- American Thoracic Society (1987) Standards for the diagnosis and treatment of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 136:255–244
- Chai H, Farr RS, Froehlich LA, Mathison DA, McLean JA, Rosenthal RR, Sheffer AL, Spector SL, Townley RG (1975) Standardization of bronchial inhalation challenge procedures. *J Allergy Clin Immunol* 56:323–327
- Daugherty BL, Siciliano SJ, DeMartino JA, Malkowitz L, Sirotna A, Springer MS (1996) Cloning, expression, and characterization of the human eosinophil eotaxin receptor. *J Exp Med* 183:2349–2354
- Excoffier L, Slatkin M (1995) Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. *Mol Biol Evol* 12:21–27
- Ferris BG (1978) Epidemiology standardization project II. Recommended respiratory disease questionnaires for use with adults and children in epidemiological research. *Am Rev Respir Dis* 118:10–35
- Forssmann U, Uguccioni M, Loetscher P, Dahinden CA, Langen H, Thelen M, 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
- Garcia-Zepeda EA, Rothenberg ME, Weremowicz S, Saraf MN, Morton CC, Luster AD (1997) Genomic organization, complete sequence, and chromosomal location of the gene for human eotaxin (SCYA11), an eosinophil-specific CC chemokine. *Genomics* 41:471–476
- Guo RF, Ward PA, Hu SM, McDuffie JE, Huber-Lang M, Shi MM (1999) Molecular cloning and characterization of a novel human CC chemokine, SCYA26. *Genomics* 58:313–317
- Kitaura M, Nakajima T, Imai T, Harada S, Combadiere C, Tiffany HL, Murphy PM, 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
- Kitaura M, Suzuki N, Imai T, Takagi S, Suzuki R, Nakajima T, Hirai K, Nomiyama H, 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
- Komiya A, Nagase H, Yamada H, Sekiya T, Yamaguchi M, Sano Y, Hanai N, Furuya A, Ohta K, Matsushima K, Yoshie O, Yamamoto K, Hirai K (2003) Concerted expression of eotaxin-1, eotaxin-2, and eotaxin-3 in human bronchial epithelial cells. *Cell Immunol* 225:91–100
- Lamkhioed B, Renzi PM, Abi-Younes S, Garcia-Zepeda EA, Allakhverdi Z, Ghaffar O, Rothenberg MD, Luster AD, 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
- Lampinen M, Carlson M, Hakansson LD, Venge P (2004) Cytokine-regulated accumulation of eosinophils in inflammatory disease. *Allergy* 59:793–805
- Leckie MJ, ten Brinke A, Khan J, Diamant Z, O'Connor BJ, Walls CM, Mathur AK, Cowley HC, Chung KF, Djukanovic R, Hansel TT, Holgate ST, Sterk PJ, Barnes PJ (2000) Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 356:2144–2148
- Lozano G, Levine AJ (1991) Tissue-specific expression of p53 in transgenic mice is regulated by intron sequences. *Mol Carcinog* 4:3–9
- Matrick JS (1994) Introns: evolution and Function. *Curr Opin Genet Dev* 4:823–831
- Miyamasu M, Misaki Y, Yamaguchi M, Yamamoto K, Morita Y, Matsushima K, Nakajima T, Hirai K (2000) Regulation of human eotaxin generation by Th1-/Th2-derived cytokines. *Int Arch Allergy Immunol* 122:54–58
- Moore PE, Church TL, Chism DD, Panettieri RA Jr, Shore SA (2002) IL-13 and IL-4 cause eotaxin release in human airway smooth muscle cells: a role for ERK. *Am J Physiol Lung Cell Mol Physiol* 282:L847L–853
- Mould AW, Matthaei KI, Young LG, Foster PS (1997) Relationship between interleukin-5 and eotaxin in regulating blood and tissue eosinophilia in mice. *J Clin Invest* 99:1064–071
- National Institutes of Health (2002) Global strategy for asthma management and prevention, NHLBI/WHO workshop report, National Heart, Lung, and Blood Institute Publication No. 02–3659
- Nomiyama H, Osborne LR, Imai T, Kusuda J, Miura R, Tsui LC, Yoshie O (1998) Assignment of the human CC chemokine MPIF-2/eotaxin-2 (SCYA24) to chromosome 7q11.23. *Genomics* 49:339–340
- Park CS, Kim YY, Kang SY (1984) Collection between RAST and skin test for inhalant offending allergens. *J Kor Soc Allergol* 983(3):1–9
- Patel VP, Kreider BL, Li Y, Li H, Leung K, Salcedo T, Nardelli B, Pippalla V, Gentz S, Thotakura R, Parmelee D, Gentz R, Garotta G (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
- Ponath PD, Qin S, Post TW, Wang J, Wu L, Gerard NP, Newman W, Gerard C, Mackay CR (1996a) Molecular cloning and characterization of a human eotaxin receptor expressed selectively on eosinophils. *J Exp Med* 183:2437–2448
- Ponath PD, Qin S, Ringler DJ, Clark-Lewis I, Wang J, Kassam N, Smith H, Shi X, Gonzalo JA, Newman W, Gutierrez-Ramos JC, Mackay CR (1996b) 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
- Radinger M, Johansson AK, Sitkauskiene B, Sjostrand M, Lotvall J (2004) Eotaxin-2 regulates newly produced and CD34 airway eosinophils after allergen exposure. *J Allergy Clin Immunol* 113:1109–1116
- Rankin SM, Conroy DM, Williams TJ (2000) Eotaxin and eosinophil recruitment: implications for human disease. *Mol Med Today* 6:20–27
- van Rensen EL, Stirling RG, Scheerens J, Staples K, Sterk PJ, Barnes PJ, Chung KF (2001) Evidence for systemic rather than pulmonary effects of interleukin-5 administration in asthma. *Thorax* 56:935–940
- Rojas-Ramos E, Avalos AF, Perez-Fernandez L, Cuevas-Schacht F, Valencia-Maqueda E, Teran LM (2003) Role of the chemokines RANTES, monocyte chemoattractant proteins-3 and -4, and eotaxins-1 and -2 in childhood asthma. *Eur Respir J* 22:310–316
- Shin HD, Kim LH, Park BL, Jung JH, Kim JY, Chung IY, Kim JS, Lee JH, Chung SH, Kim YH, Park HS, Choi JH, Lee YM, Park SW, Choi BW, Hong SJ, Park CS (2003) Association of Eotaxin gene family with asthma and serum total IgE. *Hum Mol Genet* 12:1279–1285 and 2083(Corrigendum)
- White JR, Imburgia C, Dul E, Appelbaum E, O'Donnell K, O'Shannessy DJ, Brawner M, Fornwald J, Adamou J, Elshourbagy NA, Kaiser K, Foley JJ, Schmidt DB, Johanson K, Macphee C, Moores K, McNulty D, Scott GF, Schleimer RP, Sarau HM (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
- Yang M, Hogan SP, Mahalingam S, Pope SM, Zimmermann N, Fulkerson P, Dent LA, Young IG, Matthaei KI, Rothenberg ME, Foster PS (2003) Eotaxin-2 and IL-5 cooperate in the lung to regulate IL-13 production and airway eosinophilia and hyperreactivity. *J Allergy Clin Immunol* 112:935–943
- Ying S, Meng Q, Zeibecoglou K, Robinson DS, Macfarlane A, Humbert M, Kay AB (1999) Eosinophil chemotactic chemokines (eotaxin, eotaxin-2, RANTES, monocyte chemoattractant protein-3 (MCP-3), and MCP-4), and C–C chemokine receptor 3 expression in bronchial biopsies from atopic and nonatopic (intrinsic) asthmatics. *J Immunol* 163:6321–6329
- Zimmermann N, Conkright JJ, Rothenberg ME (1999) CC chemokine receptor-3 undergoes prolonged ligand-induced internalization. *J Biol Chem* 274:12611–12618