Eotaxin polymorphisms and serum total IgE levels in children with asthma

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Background: Eotaxin (chemokine, CC motif, ligand; CCL11) is a potent eosinophil chemoattractant strongly implicated in the pathobiology of asthma. Genetic variation at the CCL11 locus has been correlated with serum total IgE, blood eosinophil counts, and circulating eotaxin protein levels in several case-control asthma studies. Family-based association studies of CCL11 genetic variants have not been reported to date.
Objective: To evaluate 9 common CCL11 single nucleotide polymorphisms (SNPs) in nuclear families ascertained through patients with asthma participating in the Childhood Asthma Management Program study.
Methods: Single nucleotide polymorphism genotyping was performed by using minisequencing and probe hybridization platforms. Family-based association analysis for asthma and 4 asthma-related intermediate quantitative phenotypes was performed by using FBAT.
Results: One SNP, −384A>G, was associated with asthma among African American families ($P = .01$). CCL11 SNPs and haplotypes were not associated with asthma among white or Hispanic families. Two low-frequency alleles in strong pairwise linkage disequilibrium, −426C and IVS2+199A, were associated with lower serum total IgE levels ($P = .0006$ and $P = .009$, respectively) in white families, whereas 2 more common variants, −576C and g.4438C, were associated with higher IgE levels in African American families ($P = .01$-$.04$). Haplotype analysis in the white cohort provided additional evidence of association with serum total IgE, implicating 2 haplotypes. No single SNP or haplotype associations were observed with blood eosinophil levels, FEV_1, or airway responsiveness.
Conclusion: These findings provide further evidence that genetic variation at the CCL11 locus is an important determinant of serum total IgE levels among patients with asthma. (J Allergy Clin Immunol 2006;117:298-305.)

Key words: *Eotaxin, CCL11, single nucleotide polymorphism, IgE, genetics, asthma, association*

Eotaxin (chemokine, CC motif, ligand; CCL11), the most selective chemoattractant for eosinophils, contributes to the recruitment of peripheral blood eosinophils into the lung during acute allergic inflammation.[1,2] Initially isolated from guinea pig bronchoalveolar lavage fluid (BAL) after allergen challenge,[3] eotaxin has been found at high levels in clinical samples from patients with asthma.[4,5] Plasma eotaxin levels during asthma exacerbations are elevated compared with levels in patients with stable asthma[6] and have been associated with various measures of asthma severity.[6-8] Eotaxin mRNA and protein levels are increased in the bronchial epithelium and airway wall in patients with chronic asthma and in BAL samples after inhalational allergen challenge.[9-11] BAL eotaxin levels also correlate with both airway eosinophilia and airway hyperresponsiveness.[9-11] Eotaxin binds to its primary ligand, the chemokine receptor CCR3, located on the eosinophil cell surface.[12] CCR3 expression has also been detected on the surface of basophils,[13,14] mast cells,[15] dendritic cells,[16] and T_H2 $CD4^+$ lymphocytes.[17] Subpopulations of these cell types bearing CCR3 receptors are activated by eotaxin, suggesting that eotaxin's role in asthma is not limited to eosinophil recruitment but also extends to other aspects of the allergic immune response.[18,19]

CCL11 maps to chromosome 17q21.1, a region occasionally linked to asthma in genome-wide linkage studies.[20-22] Several groups have examined the effect of genetic variation at the CCL11 locus on asthma and related phenotypes. Five case-control studies found no association between CCL11 variants and asthma.[23-27] However, significant associations with several asthma-related intermediate phenotypes have been described, including plasma eotaxin levels, circulating eosinophil counts, lung function, and serum total IgE levels.[24,25,27,28] Of these, the most frequently observed are with serum

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Disclosure of potential conflict of interest: E. Silverman has received grants from GlaxoSmithKline. S. Weiss has consultant arrangements with Roche Pharmaceuticals, Millennium Pharmaceuticals, Genentech, Schering-Plough, Variagenics, Genome Therapeutics, and Merck Frost, and has received grants from Glaxo Wellcome, Astra Zeneca, and Pfizer. The rest of the authors have no conflict of interest to disclose.

The CAMP Genetics Ancillary Study was supported by the National Heart, Lung, and Blood Institute, NO1-HR-16049. Additional support for this research came from grants P50 HL67664 and T32 HL07427 from the National Institutes of Health and the National Heart, Lung and Blood Institute. B. Raby is a recipient of a Clinician Scientist Award from the Canadian Institutes of Health Research (MC1-40745) and a Mentored Clinical Scientist Development Award from the National Institutes of Health/National Heart, Lung, and Blood Institute (K08 HL074193).

Received for publication July 1, 2005; revised October 14, 2005; accepted for publication October 19, 2005.

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0091-6749/$32.00

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doi:10.1016/j.jaci.2005.10.041

Abbreviations used
BAL: Bronchoalveolar lavage
CAMP: Childhood Asthma Management Program
CCL: Chemokine, CC motif, ligand
IVS: Intervening sequence/intron
LD: Linkage disequilibrium
SNP: Single nucleotide polymorphism

total IgE, although the associated single nucleotide polymorphisms (SNPs) and the direction of association differ across cohorts.[24,25,28] Although these studies suggest that genetic variation at CCL11 may affect intermediate allergic phenotypes, the inconsistencies across studies raise doubts regarding their biological significance. Among various possible explanations for such inconsistencies in case-control studies (not addressed in any of the aforementioned eotaxin studies) is population substructure, when differences in allele or haplotype frequencies between cases and controls result from the populations' ethnic composition, rather than reflecting true relationships between genetic variation and disease pathogenesis.[29] This form of bias can be addressed in several ways, including careful matching of cases and controls by genetic ancestry, adjusting association tests for detected population substructure using a set of randomly selected polymorphic markers,[30] or using a family-based study design.[31] Family-based genetic association studies control for potential substructure by testing for allelic association among affected offspring while adjusting for parental genotype. We performed a family-based analysis of association between 9 CCL11 variants and asthma-related phenotypes in nuclear families ascertained through asthmatic probands participating in the Childhood Asthma Management Program (CAMP).

METHODS

Study population

The CAMP is a multicentered North American clinical trial designed to investigate the long-term effects of inhaled anti-inflammatory medications in children with mild to moderate asthma.[32,33] Of the 1041 children originally enrolled, 968 children and 1518 parents contributed DNA samples. A total of 582 complete nuclear families (including 55 families with additional affected offspring) are included in the analysis presented here: 471 of non-Hispanic white descent, 64 of African American descent, and 47 of Hispanic descent. A diagnosis of asthma was based on methacholine hyperreactivity ($PC_{20} \leq$ 12.5 mg/mL) and 1 or more of the following for at least 6 months in the year before recruitment: (1) asthma symptoms at least 2 times per week, (2) at least 2 usages per week of an inhaled bronchodilator, and (3) use of daily asthma medication. Phenotypic data were collected at baseline and during the course of the clinical trial as previously described.[32,33] Spirometry was performed according to American Thoracic Society recommendations with a volume-displacement spirometer, and airway responsiveness was assessed by methacholine challenge with the Wright nebulizer tidal breathing technique.[32] Total blood eosinophils were counted by center-specific methods. Serum total IgE was measured by radioimmunosorbent assays from blood samples collected during the CAMP screening sessions.

Human subjects

The Institutional Review Board of the Brigham and Women's Hospital, as well as those of the other CAMP study centers, approved this study. Informed assent and consent were obtained from the study participants and their parents to collect DNA for genetic studies.

Polymorphism genotyping

SNPs were genotyped by using unlabeled minisequencing reactions and mass spectrometry analysis as implemented in the SEQUENOM platform (Sequenom, San Diego, Calif), and by the 5′ → 3′ exonuclease assay as implemented in the TaqMAN assay (PE Biosystems, Foster City, Calif).[34] Protocol details, SNP flanking sequence, and primer data are available at http://wchanning.bwh.harvard.edu/epigenetics/Projects. We developed reliable genotype assays for 9 CCL11 variants. Genotype data quality control was assessed by several methods. Duplicate genotyping was performed on approximately 10% of the sample to assess genotype reproducibility, with >> 1% discordance between runs for all SNPs. Genotype completion rates were at least 95% for all loci (range, 95.0% to 99.4%), and genotype patterns not compatible with mendelian transmission were observed only 7 times (~0.1% of all marker genotypes).

Statistical analysis

Parental genotypic data were assessed for Hardy-Weinberg equilibrium by an exact method.[35] Linkage disequilibrium (LD) was evaluated by a maximum likelihood method to infer phase for dual heterozygotes and was expressed as D′.[36,37] Evidence of association with asthma and quantitative trait analysis, stratified by ethnic group, was performed with FBAT v1.5.3.[38,39] Four asthma-related intermediate phenotypes were evaluated: postbronchodilator FEV_1 (% predicted), airway responsiveness to methacholine (log-transformed PC_{20}), log-transformed serum total IgE levels, and log-transformed blood eosinophil counts. Analysis was restricted to postbronchodilator (versus prebronchodilator) FEV_1 because of previous observations suggesting greater genetic influences on postbronchodilator measurements of lung function.[40] Haplotype inference and association testing were performed by using the haplotype analysis feature in FBAT.[41] A generalized linear model was implemented by using PROC GLM in SAS (SAS Institute, Cary, NC) to obtain age-adjusted and sex-adjusted genotype-specific IgE residuals.

RESULTS

We genotyped 9 polymorphic variants at the CCL11 locus in 582 nuclear families. Genotyped markers were selected from among those previously reported in populations with asthma and those validated in dbSNP (www.ncbi.nlm.nih.gov/projects/SNP) to include variants spanning the CCL11 locus. The markers included 3 upstream variants (positions −576, −426, and −384 relative to the ATG initiation codon), 2 variants localizing to the CCL11 transcript (the nonsynonymous c.67 variant and c.705, localizing to the 3′ untranslated region), 3 intronic variants (IVS1+229, IVS2+199, and IVS2+216), and 1 downstream genomic variant (g.4438). Parental genotype data were in Hardy-Weinberg equilibrium at all loci

TABLE I. Eotaxin polymorphism allele frequencies and family-based tests of association with asthma in CAMP

Transcript position	rs number	Chr.17 position*	Alleles	Minor allele frequency†			White families		African Americans families		Hispanic families	
				White (n = 923)	African American (n = 127)	Hispanic (n = 92)	T:U ratio	FBAT *P* value	T:U ratio	FBAT *P* value	T:U ratio	FBAT *P* value
c.−576	rs4795896	29636365	T>C	0.134	0.068	0.261	110:119	.55	6:9	.44	17:13	.47
c.−426	rs16969415	29636515	C>T	0.047	0.012	0.060	33:45	.22	2:1	.56	7:5	.56
c.−384	rs17809012	29636557	A>G	0.424	0.350	0.330	228:224	.85	14:31	.01	21:19	.75
c.67‡	rs3744508	29637007	G>A	0.168	0.092	0.121	131:128	.85	8:12	.37	10:10	1
IVS1+229	rs1860184	29637245	A>T	0.308	0.303	0.385	199:203	.92	27:17	.13	18:20	.75
IVS2+199	rs3815341	29638538	G>A	0.045	0.012	0.060	32:46	.14	1:1	1	7:5	.56
IVS2+216	rs4795898	29638555	T>C	0.085	0.040	0.198	76:69	.56	3:5	.48	15:11	.43
c.705	rs1019109	29639233	A>G	0.212	0.037	0.139	161:157	.82	1:5	.10	13:12	.84
g.4438	rs714910	29641378	A>C	0.308	0.292	0.390	186:188	.96	21:13	.17	20:19	.87

T:U, Ratio of transmitted to untransmitted minor alleles.
*Chromosome position in bp from pter, based on Human Genome Browser, May 2004 release (http://genome.ucsc.edu).
†Minor allele frequencies based on all available parental chromosomes; *n,* number of parents.
‡c.67 G>A results in nonsynonymous amino acid substitution of alanine to threonine at codon 23 (Ala23Thr).

in each ethnic group. On the basis of the observed mating patterns, 544 families were informative for family-based association tests with at least 1 CCL11 marker: 445 non-Hispanic white families (including 31 families with 2 offspring with asthma and 2 families with 3 affected offspring), 55 African American families (including 1 with 2 affected offspring), and 44 Hispanic families (including 6 with 2 affected offspring). Phenotypic characteristics of 586 affected offspring from these 544 informative families are presented as Table E1 in the Online Repository at www.jacionline.org.

Ethnic-specific allele frequencies and family-based tests of association with asthma for the 9 genotyped CCL11 variants are presented in Table I. All 9 variants were polymorphic in the three ethnic groups, although differences in allele frequencies were detected. However, LD was very strong across the CCL11 locus in all 3 ethnic groups, with more than 95% of all SNP-pair comparisons having D′ values greater than 0.90 (see this article's Fig E1, *A-C,* in the Online Repository at www.jacionline.org). Family-based tests of association with asthma, stratified by ethnicity, were performed by using FBAT.[38,39] No evidence of association with asthma was observed for the 9 tested polymorphisms among white subjects. Among the much smaller African-American sample, SNP −384A>G demonstrated association with asthma: the −384 G allele was undertransmitted to affected offspring at a ratio of ~1:2 ($P = .01$). This association was not significant when adjusted for multiple comparisons. No variants were associated with asthma in the Hispanic cohort.

Family-based tests of association were performed with 4 quantitative asthma-related intermediate phenotypes: postbronchodilator FEV_1 (percent predicted), airways responsiveness (as measured by methacholine PC_{20}), serum total IgE levels, and blood eosinophil counts. Among white families, 2 variants in complete LD (both D′ and $r^2 = 1.00$), c.−426 and IVS2+199, demonstrated strong associations with serum total IgE levels (Table II). The respective minor alleles at these loci (T and A) were associated with significantly lower serum total IgE levels ($P = .0006$ and .009, respectively). For c.−426, this relationship remained statistically significant ($P < .03$) after an overly conservative Bonferroni correction, assuming complete independence of all markers and phenotypes (ie, 9 independent markers × 5 independent phenotypes). The distribution of residual values of serum total IgE levels among informative white probands adjusted for age and sex, according to c.−426 genotype, is presented in Fig 1. Among African Americans, variants c.−426T and IVS2+199A were rare (minor allele frequency ~1%), precluding analysis for quantitative traits. However, two other common variants (c.−576T>C and g.4438A>C) demonstrated significant associations with serum total IgE levels among African Americans ($P = .01$ and .04, respectively; Table III). In both instances, the minor alleles were associated with higher IgE levels. No association with serum total IgE levels was observed with c.−384 (the SNP associated with asthma in the African American families). No variants were associated with serum total IgE in the Hispanic trios, although the sample size was small (see this article's Table E2 in the Online Repository at www.jacionline.org). Aside from a marginal association between IVS2+216T>C and airways responsiveness ($P = .05$) in white subjects, no associations were seen with blood eosinophils, airways responsiveness, or FEV_1 in any ethnic group.

As stated, LD across the CCL11 locus is very strong. Among white subjects, all SNP-pairs exhibited D′ values greater than 0.90, suggesting limited haplotype diversity. Haplotype imputation in FBAT using the family data at all 9 CCL11 loci confirmed this: among white subjects, 7 haplotypes ranging in frequency from 4.4% to 26.8% accounted for ~99% of chromosomes (Table IV). Limited haplotype diversity was also detected among the African American and Hispanic chromosomes, although important

TABLE II. Family-based association tests of eotaxin polymorphisms with asthma-related quantitative traits in CAMP white families

			Serum total IgE			Blood eosinophils			Methacholine PC_{20}			Postbronchodilator FEV_1, % predicted		
SNP	Allele	Allele frequency	n	*z* Score	FBAT *P* value	n	*z* Score	FBAT *P* value	n	*z* Score	FBAT *P* value	n	*z* Score	FBAT *P* value
c.−576	C	0.132	180	0.71	.48	172	1.16	.24	185	−1.54	.12	185	−1.02	.31
c.−426	T	0.051	73	−3.40	.0006*	72	−1.31	.19	72	1.70	.09	73	−1.76	.08
c.−384	G	0.398	320	−1.64	.10	310	−1.04	.30	325	1.63	.10	324	−0.22	.83
c.67	A	0.148	197	1.12	.26	190	−0.02	.98	199	0.69	.49	200	0.65	.51
IVS1+229	T	0.301	291	0.14	.89	282	0.53	.60	296	−1.25	.21	296	−0.19	.85
IVS2+199	A	0.054	72	−2.54	.009**	72	−0.83	.41	71	1.05	.29	72	−1.68	.09
IVS2+216	C	0.088	118	0.55	.58	115	0.71	.48	121	−2.00	.05	121	0.33	.74
c.705	G	0.198	240	−1.06	.29	230	−0.47	.64	242	1.02	.31	242	0.03	.98
g.4438	C	0.300	268	0.29	.77	256	0.63	.53	271	−1.25	.21	271	−0.29	.77

n, Number of informative families.
*Bonferroni *P* value .027 (correction for 9 markers × 5 traits).
**Bonferroni *P* value .40 (correction for 9 markers × 5 traits).

differences in haplotype frequencies were observed across ethnic groups. For example, H8 is the third most common haplotype among African American subjects (frequency 0.193) but was rarely found elsewhere. Comparisons of the observed haplotype patterns in the CAMP families with Korean haplotypes reported by Shin et al[25] show similar differences (Table IV).

Family-based haplotype association analysis in the white families was consistent with the single SNP analysis, and provided further support for the association between CCL11 variants and serum total IgE levels (Table IV). An omnibus multiallelic test of haplotype association demonstrated a significant association between CCL11 haplotypes and IgE levels (z score = 17.3; 7 degrees of freedom; $P = .02$). SNPs c.−426 and IVS2+199, associated with serum total IgE in the white cohort, are uniquely represented on 1 haplotype (H7), which demonstrated strong associations with lower serum total IgE in a biallelic haplotype test ($P = .0005$). In addition, a second haplotype composed of the common allele at each locus (H1) demonstrated significant association with higher serum total IgE (biallelic $P = .03$), suggesting that the effect on the IgE phenotype may not be attributable to the effects of SNPs c.−426 and IVS2+199 alone. Like the single SNP analysis, CCL11 haplotypes were not associated with asthma, blood eosinophils, airways responsiveness, or FEV_1 (data not shown). Because of the very limited sizes of the African American and Hispanic cohorts, haplotype association analysis could not be reliably performed.

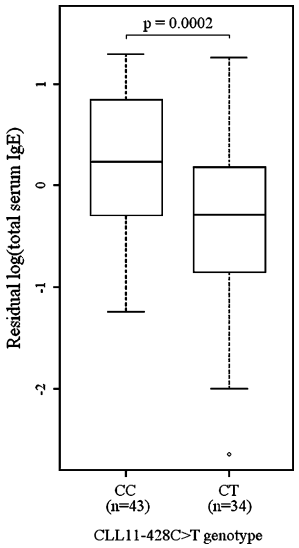

FIG 1. Relationship of CCL11 −426C>T with serum total IgE levels in white informative probands. Residual values for log10 serum total IgE levels adjusted for age and sex. *CC* and *CT* refer to individuals with CC homozygous and CT heterozygous genotypes, respectively. TT homozygous probands were not detected in this cohort.

DISCUSSION

The first genetic association study of eotaxin polymorphism and asthma was reported in a US population of mixed ethnicity.[27] The c.67A allele (coding for a nonsynonymous substitution of alanine by threonine at amino acid 23—Ala23Thr) was associated with significantly lower plasma eotaxin levels, lower circulating eosinophil levels, and higher lung function. Importantly, no relationship with serum IgE levels was detected. Subsequently, associations between eotaxin polymorphisms and serum total IgE have been reported in 3 other populations (Table V). In a Korean study of asthma, Shin et al[25] demonstrated a significant relationship between the c.67A allele and higher IgE levels, with trends of association noted with SNPs c.−384 and IVS1+229. A second Korean asthma cohort that evaluated the c.67 SNP detected a nonsignificant trend for association with IgE.[24] c.67 Was not associated with IgE levels in a Japanese study of atopic dermatitis, although the minor alleles at 2 other loci, −428 and −384, were associated

TABLE III. Family-based association tests of eotaxin polymorphisms with asthma-related quantitative traits in CAMP African American families*

			Total serum IgE levels			Blood eosinophils			Methacholine PC_{20}			Postbronchodilator FEV_1, % predicted		
SNP	Allele	Allele frequency	n	z Score	FBAT P value	n	z Score	FBAT P value	n	z Score	FBAT P value	n	z Score	FBAT P value
c.−576	C	0.047	13	2.46	.01	12	1.20	.23	13	−1.17	.24	13	0.23	.82
c.−426	T	0.008	3	—	—	3	—	—	3	—	—	3	—	—
c.−384	G	0.409	38	−0.79	.43	38	−1.20	.23	38	0.29	.77	38	0.05	.96
c.67	A	0.132	18	−0.04	.97	19	1.45	.15	19	−0.35	.72	19	−0.83	.40
IVS1+229	T	0.296	35	1.91	.06	34	1.89	.06	35	−1.03	.30	35	0.61	.54
IVS2+199	A	0.019	2	—	—	2	—	—	2	—	—	2	—	—
IVS2+216	C	0.056	7	—	—	6	—	—	7	—	—	7	—	—
c.705	G	0.039	6	—	—	6	—	—	6	—	—	6	—	—
g.4438	C	0.284	29	2.06	.04	28	1.66	.10	29	−1.15	.25	29	0.82	.41

n, Number of informative families.
*SNPs c.−426, IVS2+199, IVS2+216, and c.705 were not evaluated because of insufficient numbers of informative families (ie, <10).

TABLE IV. Eotaxin ethnic-specific haplotype frequencies and family-based association analysis serum of total IgE levels in CAMP white subjects§

	SNPs									Haplotype frequency*				Family-based HBAT analysis serum total IgE in CAMP white trios‡		
	c.−576	c.−426	c.−384	c.67	IVS1 +229	IVS2 +199	IVS2 +216	c.705	g.4438	White	African American	Hispanic	Korean† (n = 24 subjects)	Families, n	z Score	HBAT P value
H1	T	C	A	G	A	G	T	A	A	0.268	0.356	0.307	0.06 (Ht6)	190	2.16	.03
H2	T	C	G	G	A	G	T	G	A	0.206	0.041	0.146	0.09 (Ht4)	164	−0.48	.63
H3	T	C	A	G	T	G	T	A	C	0.172	0.238	0.110	0.47 (Ht1)	145	−0.56	.57
H4	T	C	G	A	A	G	T	A	A	0.167	0.094	0.099	0.16 (Ht2)	132	0.34	.73
H5	C	C	A	G	T	G	C	A	C	0.080	0.038	0.192	0.14 (Ht3)	81	−0.45	.65
H6	C	C	A	G	T	G	T	A	C	0.051	0.025	0.070	0.47 (Ht1)	61	0.42	.67
H7	T	T	G	G	A	A	T	A	A	0.044	0.008	0.064	0.08 (Ht5)	52	−3.46	.0005
H8	T	C	G	G	A	G	T	A	A	0.002	0.193	0.006	Not reported	—	—	—

*For white subjects, 27 total haplotypes were inferred from the family data. Twenty (representing only 1.3% of all chromosomes) were inferred at a frequency of less than 1%. For African American subjects, 12 total haplotypes were inferred from the family data. Five (representing only 1.5% of all chromosomes) were inferred at a frequency of less than 1%. For Hispanic subjects, 10 total haplotypes were inferred from the family data. Three (representing only 1.2% of all chromosomes) were inferred at a frequency of less than 1%.
†Korean haplotypes based on resequencing of 24 subjects abstracted from Shin et al[25] for comparison. Korean haplotype Ht1 at 0.47 frequency is subdivided in 2 by SNP rs4795896 into haplotypes H3 and H6 in CAMP.
‡Twenty inferred white haplotypes at frequency of less than 0.5% were not tested for association.
§Multiallelic omnibus test of haplotype association was significant (z score = 17.3; 7 degrees of freedom; $P = .02$).

with lower serum total IgE levels.[28] The results of this last study resemble our data on white families, in which carriers of the −428 T allele had significantly lower total IgE levels than noncarriers ($P = .0006$), and a trend toward lower total IgE was also found with −384 ($P = .10$). Like the Japanese atopic dermatitis study, we found no association with the coding SNP at position c.67. We did find significant association of IgE levels with IVS2+199, but not with IVS1+229, in contrast with Shin et al.[25] We also found evidence of association with IgE levels in African American families, suggesting that eotaxin variants affect IgE levels in this ethnic group as well, although with different related variants than in the white subjects. Therefore, although replication across these studies is imperfect, the recurrent association with serum total IgE at the CCL11 locus suggests that this locus affects the IgE phenotype among atopic (asthmatic or atopic dermatitis) subjects. It is notable that the CCL11 locus is flanked by 5 other CC chemokines (CCL1, CCL2, CCL7, CCL8, and CCL13). It is possible that the associations with IgE observed with SNPs at the CCL11 locus may be a result of LD with functional variants in 1 of these adjacent genes. None of the genetic studies published to date evaluated this possibility.

Inconsistent findings in genetic studies are common. There are many possible explanations for such discrepancies, including differences with regard to sample size, phenotype definition, cohort demographics, environmental exposures, underlying genetic architecture, and population substructure. Our cohort is composed of children

TABLE V. Cross-study comparison of CCL11 SNP associations with serum total IgE levels*

		Case-control studies				Family-based study (current study)	
SNP	Alleles	US asthma[27] (n = 806/505)	Korean asthma[25] (n = 550/171)	Korean asthma[24] (n = 225/294)	Japanese atopic dermatitis[28] (n = 140/140)	White families (n = 445)	African American families (n = 55)
c.−576	T>C	—	—	—	—	NS	Higher (P = .01)
c.−426	C>T	—	—	—	Lower (P = .04)	Lower (P = .0006)	—
c.−384	A>G	—	Trend higher (P = .08)	—	Lower (P = .05)	Trend lower (P = .10)	NS
c.67	G>A	NS	Higher (P = .002)	Trend (P = .08)	NS	NS	NS
IVS1+229	A>T	—	Trend higher (P = .09)	—	—	NS	Trend higher (P = .06)
IVS2+199	G>A	—	NS	—	—	Lower (P = .009)	—
IVS2+216	T>C	—	NS	—	—	NS	—
c.705	A>G	—	—	—	—	NS	—
g.4438	A>C	—	—	—	—	NS	Higher (P = .04)

n, Number of cases/controls in case-control studies and number of informative families in family-based studies.
*Direction of association (higher/lower) refers to mean IgE levels among carriers of minor allele (heterozygotes and homozygotes) compared with major allele homozygotes.

with asthma with mild to moderate disease (of whom 88% were atopic) and excludes children with severe disease, whereas other study populations are primarily (or exclusively) adult and did not specifically exclude subjects with severe asthma. Given that our principal findings of association are with serum total IgE levels—an intermediate phenotype correlated with airways responsiveness and asthma severity[42] highly influenced by age[43]—these differences may be important. With regard to differences in underlying genetic architecture, the comparisons of allele and haplotype frequencies presented indicate that the eotaxin locus is highly variable across ethnic groups. It is therefore possible that the differences in SNP association across study populations are a result of actual ethnic differences in the frequency of functional variants or haplotypes. Alternatively, subtle population substructure becomes a major concern with such differences in genetic architecture. Our family-based design excludes the latter possibility as a cause of spurious association, but could explain why our results differ from those from the other studies, if any of them exhibited substructure. Although we cannot comment directly on whether the Korean or Japanese studies manifest substructure, the US study of the c.67 variant used a cohort of mixed ethnicity, with marked differences in ethnic composition between the asthma cases (87% white, 11% African American) and normal controls (66% white, 20% African American).[27] As we demonstrated, both IgE levels and CCL11 haplotype structure differ across ethnic groups, 2 necessary requirements for population substructure to influence genetic association.

A common feature across studies is the lack of association between CCL11 polymorphisms and asthma susceptibility. In 5 of 6 populations, no evidence of association was observed with eotaxin variants. The association observed in the sixth population (the CAMP African American subjects) between SNP −384A>G and asthma has not previously been reported, although it has only been tested in 2 other cohorts. Additional testing of this variant, particularly in larger African American cohorts, is warranted before the importance of this finding can be asserted. The current data suggest that eotaxin is not likely an asthma susceptibility locus per se, but rather influences disease severity.

In summary, we provide further support for a relationship between eotaxin polymorphisms and serum total IgE levels among children with mild to moderate asthma in both white and African American cohorts. Although the precise mechanism underlying this association remains unclear, these findings are supported by observational and experimental studies implicating eotaxin in the migration and activation of T_H2 lymphocytes. In lymphocytes, CCR3 receptor expression is restricted to subpopulations of $CD4^+$ lymphocytes expressing T_H2-related cytokines (IL-4 and IL-5),[17] and direct stimulation of these cells by eotaxin increases intracellular calcium, promoting cellular activation, migration, and adhesion.[17,19] Eotaxin also appears to augment allergen-induced expression of IL-4 in a murine model of allergic asthma.[18] It is therefore possible that the genetic associations with IgE levels relate to differences in eotaxin's effects on lymphocyte function, perhaps through altered expression.[44] Alternatively, it is possible that eotaxin influences B-cell–mediated IgE production more indirectly, by activating eosinophils which subsequently signal T and B lymphocytes. As reviewed by Zimmerman et al,[45] there is growing evidence that communications between lymphocytes and eosinophils is not unidirectional (ie, T-cell–mediated

activation of eosinophils). Indeed, eosinophil-mediated stimulation and potentiation of lymphocytes, both by eosinophil-derived cytokines (including IL-4, IL-13, and IL-18)[46] and by eosinophil-mediated antigen presentation,[47] has been observed. Recent data also suggests that IL-9 (produced by eosinophils)[48] potentiates IgE production by cultured B cells,[49] inhibits T_H2-lymphocyte apoptosis,[50] and also induces expression of the high-affinity IgE receptor[51] (see review[52]). Although it is unclear though which of these mechanisms eotaxin most likely affects IgE production, functional studies exploring the relationships between these possible mechanisms and eotaxin genetic polymorphism are warranted.

We thank all families for their enthusiastic participation in the CAMP Genetics Ancillary Study. We also thank Jody Senter Sylvia, Mel Hernandez, Maura Regan, and Salvatore Mazza for their assistance with sample management and genotyping. We also acknowledge the CAMP investigators and research team, supported by the National Heart, Lung, and Blood Institute, for collection of CAMP Genetic Ancillary Study data. All work on data collected from the CAMP Genetic Ancillary Study was conducted at the Channing Laboratory of the Brigham and Women's Hospital under appropriate CAMP policies and human subjects protections.

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