

A comprehensive evaluation of *IL4* variants in ethnically diverse populations: Association of total serum IgE levels and asthma in white subjects

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Background: The role of variation in the *IL4* gene in asthma and allergy susceptibility is controversial. This cytokine is important in IgE isotype switching and the regulation of allergic inflammation; however, published studies have not delineated the specific role of variation in this gene in allergic disorders.

Objective: We sought to identify single nucleotide polymorphisms (SNPs) in *IL4* and to evaluate the association of SNPs and haplotypes with asthma and allergic phenotypes (total serum IgE) in white, African American, and Hispanic asthmatic populations.

Methods: Sixteen individuals were resequenced, and 19 SNPs were identified; 2 novel and 17 SNPs were previously reported. Eleven of the SNPs were used to evaluate association in the 3 groups.

Results: Nine polymorphisms were associated with total serum IgE levels in white subjects ($.0012 \leq P \leq .034$), and 5 of these were also associated with asthma in this population ($.010 \leq P \leq .031$). Three common haplotypes were observed, and all were associated with either high or low serum IgE levels in white subjects ($.00008 \leq P \leq .004$). Inspection of the haplotypes revealed that 3017 G/T in intron 2 was the only SNP concordant with serum IgE levels (G allele with lower levels and T allele with higher levels).

Conclusions: After a comprehensive genetic evaluation, our data suggest that the 3017 G/T variant or the haplotype it identifies influences *IL4*'s ability to modulate total serum IgE levels. Inconsistencies with previously reported *IL4* associations might be due to population differences in allele frequencies, the extent of linkage disequilibrium with this SNP or haplotype, or both. (J Allergy Clin Immunol 2004;114:80-7.)

Key words: Asthma, IgE, *IL4*, single nucleotide polymorphism, haplotypes, association

Abbreviations used

BHR: bronchial hyperresponsiveness
IL4RA: *IL4* receptor α
PPAR: Peroxisome proliferator-activated receptor
SNP: Single nucleotide polymorphisms

Asthma is a common respiratory disease caused by acute and chronic bronchial inflammation that results in variable airway obstruction. Clinical disease characteristics closely associated with the asthma phenotype include bronchial hyperresponsiveness (BHR) and increased total serum IgE levels,^{1,2} both of which have a strong genetic component.³⁻⁶ Several potential asthma susceptibility genes for these phenotypes have been identified by using positional cloning approaches.⁷⁻⁹ Furthermore, gene-environment interactions might result in presymptomatic asthma states in susceptible individuals who are characterized by atopy and BHR.^{1,10,11}

Family studies have demonstrated that an asthma susceptibility gene, an atopy susceptibility gene, or both maps to a region on chromosome 5q31-q33 in Dutch,^{5,6,12} Amish,¹³ US,^{14,15} Hutterite,¹⁶ and British^{17,18} populations. A cluster of proinflammatory cytokine genes important in immune regulation resides within the region of linkage, specifically *IL4* and *IL13*, and have been genetically and functionally implicated in the pathogenesis of asthma and atopy. Polymorphisms in *IL4* have been associated with increased total serum IgE levels,^{19,20} asthma,^{21,22} and pulmonary function²² in asthmatic patients in some populations but not in others.^{23,24} Polymorphisms in *IL13* have also been associated with an increased risk for allergic asthma,²⁵ increased total serum IgE levels,²⁶⁻²⁸ asthma,^{29,30} and BHR.²⁸

IL-4 and IL-13 are involved in many of the same immune functions that include promoting IgE isotype switching in human B cells, promoting growth and differentiation of human B cells and monocytes, inhibiting monocyte production of proinflammatory cytokines, and increasing endothelial cell surface expression of vascular cell adhesion molecule 1.³¹ Furthermore, the IL-4 receptor α subunit (*IL4RA*) is a component of both the IL-4 and IL-13 receptor complexes.³² Polymorphisms in *IL4RA* have

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been associated with atopic asthma,³³ changes in total serum IgE levels,^{34,35} and atopic dermatitis.³⁶ Because of the close proximity of the genes encoding IL-13 and IL-4, in addition to the shared *IL4RA* subunit, it is likely that polymorphisms in *IL4* might also contribute to the complex regulation of atopic, asthmatic, or both phenotypes.

In the present study we identified 19 sequence variants (2 novel) in the *IL4* gene. Association studies with 11 of these polymorphisms were performed in US white, African American, and US Hispanic asthmatic case-control populations. Our US white families, which were part of the Collaborative Study for the Genetics of Asthma, have previously shown evidence for linkage to chromosome 5q31 for asthma.¹⁵ Subsequent haplotype analysis was performed in the 3 ethnic populations with asthma to investigate whether a specific haplotype better explained variation in the outcomes than individual SNPs.

METHODS

Study populations

Unrelated subjects meeting the following criteria for asthma were recruited: (1) age of 6 years or greater; (2) either BHR, defined as a methacholine PC₂₀ of 25 mg/mL or less, or reversibility, defined as an increase in FEV₁ of 15% or more after inhalation of a bronchodilator; (3) at least 2 symptoms consistent with asthma (cough, wheeze, and shortness of breath); (4) less than 3 pack-years of cigarette exposure; and (5) a physician's diagnosis of asthma with no conflicting pulmonary diagnoses. To qualify as a control subject, there could be no history of asthma and no first-degree relative with a history of asthma.³⁷ Three ethnic groups were studied, and both parents and grandparents must have been of that ethnicity to be assigned to a specific ethnic group. White and African American subjects were recruited from the University of Maryland (Baltimore, Md) and Wake Forest University School of Medicine (Winston-Salem, NC), whereas the Hispanic population was recruited at the University of New Mexico (Albuquerque, NM).

All subjects were evaluated by using standard protocols, and baseline spirometry was performed according to American Thoracic Society standards.³⁸ Methacholine was administered by using doubling concentrations (0.15 to 10.0, then 25 mg/mL) that were inhaled at 5-minute intervals through a nebulizer attached to a solenoid trigger activated during inspiration for 0.6 seconds for each of 5 breaths.^{39,40} Subjects with a PC₂₀ of 25 mg/mL or less were considered to demonstrate BHR.³⁷

Bronchodilator reversibility was assessed as a 15% or greater improvement in FEV₁ after the administration of albuterol with a metered-dose inhaler or a handheld nebulizer, with the repetition of spirometry 15 minutes after this treatment. The standard American Thoracic Society respiratory questionnaire was modified to include questions about the frequency, severity, and duration of symptoms of both asthma and allergy and to identify the presence of conflicting diagnoses.³⁷ This study was approved by the Institutional Review Boards at Wake Forest University School of Medicine, the University of Maryland, and the University of New Mexico. All participants provided written informed consent.

DNA resequencing of *IL4*

To identify single nucleotide polymorphisms (SNPs) within *IL4*, we performed cycle sequencing of overlapping PCR-amplified DNA fragments from 16 white individuals of European descent. Collectively, the overlapping contiguous fragments span 1.6 kb of

TABLE I. Nomenclature of SNPs

SNP ID	Nucleotide position in AF395008	Location with respect to +1 (nucleotide 12427)*	Location with respect to ATG (nucleotide 12492)*
PR -589 C/T	11903	-524	-589
Ex 1 +33 C/T	12459	33	-33
IVS2 -2681 G/T	15508	3082	3017
IVS2 -2227 C/G	15962	3536	3471
IVS2 -2107 G/C	16082	3656	3591
IVS2 -1617 G/A	16572	4146	4081
IVS2 -1523 G/A	16666	4240	4175
IVS2 -1520 G/C	16669	4243	4178
IVS2 -1443 A/C	16746	4320	4255
IVS2 -1379 T/C	16810	4384	4319
IVS2 -1297 A/G	16892	4466	4401
IVS2 -574 G/A	17615	5189	5124
IVS3 +813 T/C	19178	6752	6687
IVS3 +972 C/A	19337	6911	6846
IVS3 +1410 G/A	19775	7349	7284
IVS3 +1411 G/T	19776	7350	7285
IVS3 -234 A/G	20727	8301	8236
IVS3 -46 A/G	20915	8489	8424
IVS3 -9 C/A	20952	8526	8461

Nineteen SNPs were identified by resequencing and were initially named (SNP ID) in reference to the exons: -, SNP's upstream position from the following exon; +, SNP's downstream position from the previous exon. SNPs are referred to in this article by their location with respect to ATG.

*Reference sequence AF395008.

the 5'-flanking region of the gene, all 4 exons, and most intronic regions. The only portion of the gene not completely resequenced was approximately 300 bp of intron 2, which contains a microsatellite repeat. PCR primer pairs were designed by using Primer Express, version 1.0 (Applied Biosystems, Foster City, Calif).

Amplification products were single-strand sequenced by using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). After cycle sequencing, the products were ethanol precipitated and analyzed with an ABI PRISM 3700 DNA Analyzer (Applied Biosystems). The resulting chromatograms were aligned and viewed with Sequencher software (Gene Codes Corp, Ann Arbor, Mich). The GenBank accession number for the reference genomic sequence used for *IL4* alignments is AF395008. A detailed nomenclature table has been provided to eliminate any confusion about the location of the SNPs (Table I).

Genotyping of *IL4* polymorphisms

PCR and primer extension reactions were performed by using the MassARRAY genotyping system (Sequenom Inc, San Diego, Calif). Fragment sizes were determined by means of mass spectrometry (Bruker Daltonics, Billerica, Mass), and the genotypes were viewed and analyzed with SpectroTyper software (Sequenom).

Genetic analysis

Logistic regression models were used to test for an association between the asthma phenotype and individual SNPs. These models were performed with PROC LOGISTIC in SAS statistical software (SAS Institute, Cary, NC). Linear regression models (PROC GLM) were used to test for an association between mean log IgE levels and individual SNPs. The IgE measurement was transformed by taking the logarithm after adding the value 1 to obtain an approximately

TABLE II. Characteristics of study subjects

	White		African American		Hispanic	
	Patients (n = 233)	Control subjects (n = 245)	Patients (n = 168)	Control subjects (n = 269)	Patients (n = 116)	Control subjects (n = 130)
Age $\pm$ SD (y)	23.9 $\pm$ 13.1	32.8 $\pm$ 11.0	22.3 $\pm$ 12.7	32.5 $\pm$ 11.3	17.4 $\pm$ 11.7	36.8 $\pm$ 12.3
Male sex (%)	40	40	46	45	47	32
Log IgE (geometric mean) $\pm$ SD (IU/mL)	2.14 (138) $\pm$ 0.7	1.23 (16.9) $\pm$ 0.8	2.31 (204) $\pm$ 0.6	1.82 (66.1) $\pm$ 0.6	2.34 (218) $\pm$ 0.7	1.51 (32.3) $\pm$ 0.7
≥ 1 positive skin test response (%)	90.1	—	91.7	—	85.1	—
No. of positive skin test responses, mean $\pm$ SD	4.0 $\pm$ 3.4	—	3.6 $\pm$ 3.7	—	7.2 $\pm$ 4.9	—
Percent predicted FEV ₁ $\pm$ SD (race adjusted)*	82.6 $\pm$ 16.4	—	75.5 $\pm$ 20.6	—	95.2 $\pm$ 15.9	—
FEV ₁ /FVC $\pm$ SD	0.75 $\pm$ 0.1	—	0.74 $\pm$ 0.1	—	0.80 $\pm$ 0.1	—
Patients qualifying by BHR (%)	94.4	—	73.8	—	91.4	—
PC ₂₀ methacholine $\pm$ SD (mg/mL)	3.6 $\pm$ 5.8	—	3.5 $\pm$ 5.0	—	3.9 $\pm$ 5.9	—
Patients qualifying by reversibility (%)	5.6	—	26.2	—	8.6	—

*Percent predicted FEV₁ values for African-American subjects are race adjusted (Lester et al, 2001).

normal distribution, which is an assumption for linear regression analysis. Genotypes were first tested for general association (no mode of inheritance assumption) by using a 2-degree-of-freedom likelihood ratio test for asthma and a 2-degree-of-freedom F test for log IgE. If this was found to be statistically significant, or if there was an a priori hypothesis for a certain inheritance mode, further testing of the SNP effects were performed by using models that assumed specific modes of inheritance. For dominant and recessive genetic transmission models, a single indicator variable was used (eg, I_{dom} is an indicator variable taking the value 1 if an individual has genotype aA or AA and 0 otherwise). A variable taking on the values -1 for genotype aa, 0 for genotype aA, and 1 for genotype AA was used to test for additive genetic effects. Results are reported for best mode of inheritance only.

Tests for association between haplotype and both asthma affection status and continuously distributed IgE levels were performed by using a score test developed by Schaid et al,⁴¹ as implemented in the computer program HAPLO.SCORE (<http://www.mayo.edu/statgen>). HAPLO.SCORE properly weights all possible combinations of haplotype pairs within an individual by using available genotype data and expectation maximization-derived haplotype frequency estimates rather than underestimating the variance by assigning a most likely pair of haplotypes to an individual. This program allows for tests of association for both qualitative and quantitative traits. We tested for association between differences in haplotype frequencies first as a general test of association and second as tests of specific haplotypes versus all others.

A positive (negative) score was indicative of higher (lower) levels of IgE. For the qualitative trait asthma, a positive (negative) score suggests that the haplotype in question is associated with increased (decreased) odds of being asthmatic. The computer program Dandelion was used to generate the frequencies of haplotypes in patients and control subjects on the basis of the asthma phenotype.⁴² Both HAPLO.SCORE and Dandelion use the expectation maximization algorithm to estimate the haplotype frequencies from a pool of

individuals. Linkage disequilibrium (LD) studies were performed by using SNPAnalyzer, a Web-based version of SNPAnalysis.⁴³

RESULTS

Baseline characteristics of the patients and control subjects in each population used for this study are reported in Table II. The asthmatic subjects were either hyperresponsive to methacholine or demonstrated reversibility and had normal or near-normal spirometry, and the majority showed evidence of atopy (increased IgE levels and positive skin test responses). The control subjects were older, permitting a longer period for an asthma diagnosis to be made, were well matched for sex, and had lower total serum IgE levels.

Sequencing of *IL4* identified 19 SNPs in this locus; 2 novel (4175 G/A, 4319 T/C) and 17 previously identified (Fig 1). Twelve of the 19 SNPs were genotyped in the 3 asthma populations; however, only 11 were analyzed for associations with asthma or related phenotypes. We discovered that the 6687 T/C polymorphism lies within a 70-bp repeat (also observed by Kabesch et al⁴⁴), resulting in the presence of an apparent T allele for all genotypes; therefore this SNP was removed from the analysis. The *IL4* SNP genotypes were consistent with Hardy-Weinberg equilibrium ($P > .05$) in all populations except for the 8236 A/G variant in the African American control subjects ($P = .0002$). Examination of the D' values suggested a high degree of LD across these 11 markers and a single haplotype block. The frequencies of the 11 SNPs for all populations studied are shown in Fig 1.

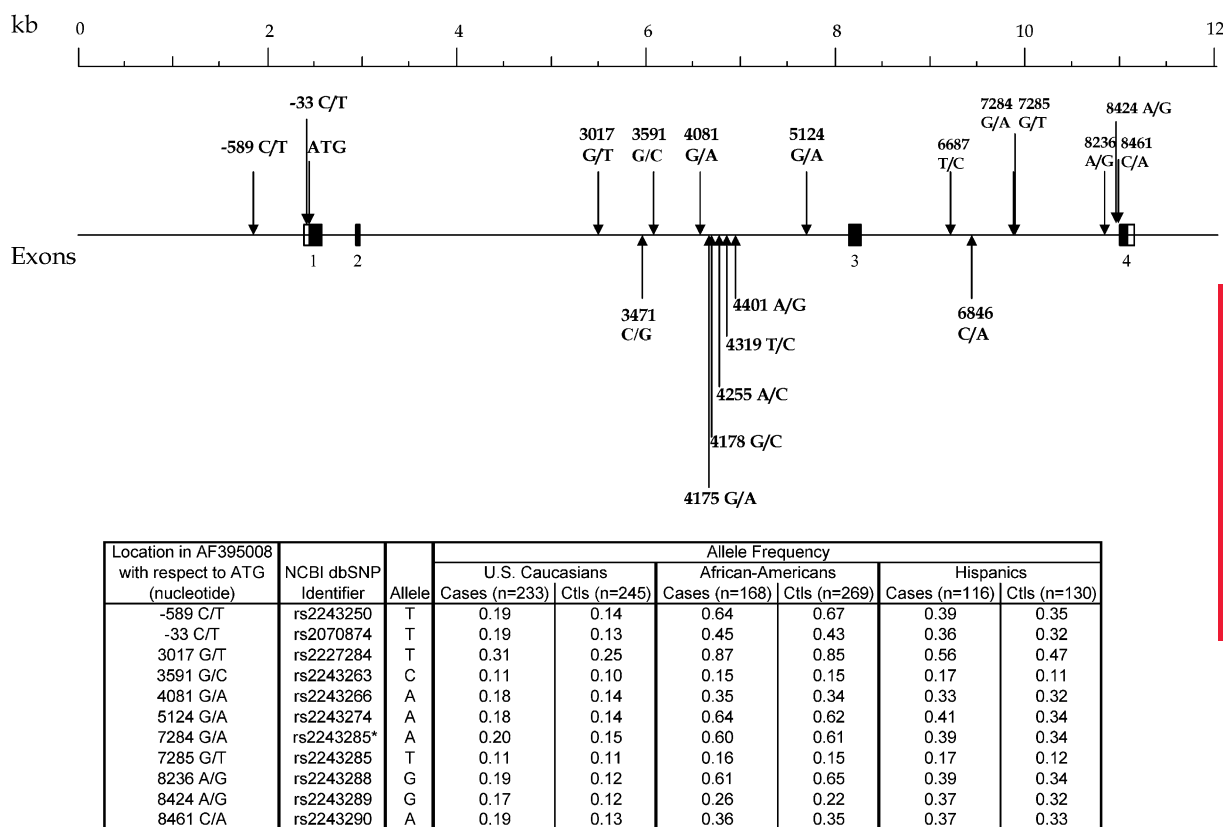


FIG 1. Genomic structure of *IL4* and allele frequencies for the 11 SNPs genotyped. The boxes represent the exons, and the filled blocks represent the coding region. The translation start site is indicated by ATG. The SNPs genotyped are located above the line representing the genomic sequence, and allele frequencies for the minor alleles of the SNPs analyzed are shown. *This SNP did not have a unique rs number; it is adjacent to rs2243285.

There was a significant association with variation in total serum IgE levels in the white population for 9 polymorphisms ($.0003 \leq P \leq .006$), which is consistent with the additive model; tests for additive effects were done post hoc after general tests of association were found to be statistically significant. Five of these 9 SNPs were also associated with asthma in this population (recessive model, $.010 \leq P \leq .031$; Table III). SNP 3017 G/T was associated with total serum IgE levels in both African American ($P = .042$, recessive model) and Hispanic ($P = .013$, dominant model) subjects and also with asthma in the Hispanic subjects ($P = .049$, dominant model). Additionally, SNP 8461 C/A was associated with total serum IgE levels in the Hispanic population ($P = .016$). BHR (PC_{20} as a continuous variable) was also evaluated in the case of each ethnic group; however, no association was observed with this phenotype.

Ten haplotypes across the *IL4* locus were found with at least 1% frequency in one or more of the 3 ethnic groups. These 10 haplotypes accounted for 97% or more of the genetic variation in each group; however, the frequency of the haplotypes varied across the populations (Table IV). In white subjects only 3 common haplotypes were observed, and all were significantly associated with total serum IgE

levels. Haplotype 1 was associated with decreased levels (haplo.score = -3.90 , $P = .00008$), whereas haplotypes 2 and 8 were both associated with increased IgE levels (haplo.score = 3.02 and 2.90 , respectively; $P = .003$ and $.004$, respectively). In African American subjects haplotype 5 was associated with asthma ($P = .022$), but this haplotype only occurred in 1% of the total African American population. Although no significant associations were found with any of the haplotypes and either total serum IgE levels or asthma in the smaller Hispanic population, individuals with haplotype 8 had higher total serum IgE levels (haplo.score = 1.42), whereas individuals with haplotype 1 had lower total serum IgE levels (haplo.score = -0.85), which is consistent with the observations in white subjects. Inspection of the 3 common haplotypes in the white population revealed that only one SNP (3017 G/T) was concordant with levels of total serum IgE. For this SNP, the G allele occurred with lower total serum IgE levels and the T allele with higher levels.

Furthermore, by locating these haplotypes in the University of Washington's Program in Genomic Applications database,⁴⁵ we identified 3 additional SNPs that appear to be in LD with 3017 G/T in white subjects.

TABLE III. Association of *IL4* SNPs with total serum IgE levels and asthma in white subjects*

SNP	Log IgE					Asthma			
	Genotype	n	mean $\pm$ SD	P value	Kabesch et al ⁴⁴	Genotype	n	P value	Kabesch et al ⁴⁴
-589	CC	266	1.67 $\pm$ 0.79	.0061	.01‡	CC	293	.031	.003†
	CT	107	1.89 $\pm$ 0.78			CT	117		.048‡
	TT	7	2.13 $\pm$ 0.75			TT	8		.039§
-33	CC	282	1.69 $\pm$ 0.79	.0013	.02‡	CC	312	.03	.019†
	CT	114	1.90 $\pm$ 0.78			CT	125		.044‡
	TT	9	2.36 $\pm$ 0.83			TT	9		
3017	GG	205	1.69 $\pm$ 0.76	.0008	Not done	GG	227	.023	Not done
	GT	167	1.83 $\pm$ 0.79			GT	183		
	TT	29	2.21 $\pm$ 0.76			TT	32		
4081	GG	276	1.72 $\pm$ 0.79	.01	Not done	GG	297	NS	Not done
	GA	110	1.86 $\pm$ 0.78			GA	119		
	AA	9	2.36 $\pm$ 0.83			AA	9		
5124	GG	255	1.72 $\pm$ 0.76	.0011	Not done	GG	272	NS	Not done
	GA	98	1.93 $\pm$ 0.78			GA	109		
	AA	10	2.35 $\pm$ 0.89			AA	10		
VE6523	—	—	—	Not done	.02‡	—	—	Not done	.012†, .03‡
7284	GG	274	1.71 $\pm$ 0.78	.0034	Not done	GG	292	NS	Not done
	GA	114	1.89 $\pm$ 0.79			GA	127		
	AA	11	2.29 $\pm$ 0.87			AA	11		
8236	AA	263	1.72 $\pm$ 0.78	.0003	Not done	AA	287	.017	Not done
	AG	87	2.01 $\pm$ 0.73			AG	99		
	GG	13	2.21 $\pm$ 0.84			GG	13		
8424	AA	269	1.72 $\pm$ 0.78	.008	Not done	AA	289	NS	Not done
	AG	91	1.92 $\pm$ 0.78			AG	99		
	GG	9	2.25 $\pm$ 0.99			GG	10		
8461	CC	282	1.69 $\pm$ 0.79	.0005	Not done	CC	312	.01	Not done
	CA	106	1.92 $\pm$ 0.79			CA	117		
	AA	11	2.35 $\pm$ 0.75			AA	11		

NS, Not significant; VE, variable element.

*All data shown, except for Kabesch data, are for US white subjects. In our study *P* values shown for IgE represent the significance for the additive genetic model, whereas *P* values shown for asthma represent the significance for the recessive genetic model.

†Whole population.

‡Individuals with positive skin test responses only.

§Individuals with negative skin test responses only.

TABLE IV. Association of haplotypes with total serum IgE levels in US white subjects

Haplotype ID	Haplotype	White			African American, Freq (N = 842)	Hispanic, Freq (N = 426)
		Freq (N = 840)	Score	P value		
1	CCGCGGGGGAAC	0.74	-3.90	.00008	0.16	0.50
2	CCCTCGGGTAAC	0.10	3.02	.003	0.13	0.14
3	CCTCGGGTAAC					
4	CCTGGAAGGAC				0.04	
5	CCTGGAGTAAC				0.01†	
6	TCTGGAAGGAC				0.23	0.02
7	TTTGAAGGAA				0.07	
8	TTTGAAGGGA	0.13	2.90	.004	0.25	0.31
9	TTTGAAGGGA				0.01	
10	TTTGGGGGAAC				0.09	

Haplotypes were analyzed for association with total serum IgE levels and asthma in all 3 populations. The program Haplo.Score was used to generate the haplotypes and their *P* values (both overall and haplotype specific) for both phenotypes.

N, Number of haplotypes.

†Only one haplotype was associated with asthma, and this association was observed in the African American population.

These 3 SNPs are located at nucleotide positions 15962 (intron 2), 22574 (intron 3), and 22590 (intron 3) in the AF395008 reference sequence. For SNP 15962, the G allele is always on haplotype 1 (associated with lower total

serum IgE levels), and the C allele is found on haplotypes 2 and 3 (associated with higher total serum IgE levels), whereas for SNPs 22574 and 22590, the A allele is always on haplotype 1 (associated with lower total serum IgE

levels), and the G allele is found on haplotypes 2 and 3 (associated with higher total serum IgE levels).

DISCUSSION

Asthma is an inflammatory airways disease that is characterized by BHR and variable airway obstruction. The asthma phenotype is associated with atopic phenotypes that include increased total serum IgE levels. It is well established that genetic factors have an important role in the pathogenesis of asthma and allergic diseases.^{11,30,46,47} Because IL-4 is an important cytokine involved in allergic inflammation and IgE isotype switching on chromosome 5q31, a linked region in multiple asthma populations, it represents an excellent biologic candidate for asthma and allergic phenotypes. In the present study *IL4* was resequenced for the identification of polymorphisms and then examined for associations within 3 different ethnic populations. Nineteen SNPs were detected in the *IL4* locus, 2 novel and 17 previously identified. SNPs and haplotypes were primarily associated with variation in total serum IgE levels and asthma in white subjects. Evaluation of the 3 common haplotypes in the white population revealed that the 3017 G/T SNP was the only polymorphism concordant with levels of total serum IgE. Therefore this SNP, or the haplotype that it identifies, appears to be directly responsible for the associations observed with IgE and asthma. We also attempted to study interactions between *IL4*, *IL13*, and *IL4RA* but were limited by the small number of individuals in the risk group.

The 3017 G/T SNP of intron 2 might independently be involved in the regulation of *IL4* transcription because it resides in a putative transcription factor binding site for peroxisome proliferator-activated receptor alpha (PPAR α); the T allele resides within the core consensus sequence for PPAR α (ATAG), whereas the G allele does not. PPAR activators have been shown to inhibit the activation of *IL2*, *IL6*, *IL8*, and *TNFA*,⁴⁸ which could result in a shift of T cells toward a T_H2 profile. Although this hypothesis is inconsistent with a study that measured IL-4 levels in human CD4⁺ T cells stimulated with a PPAR α activator,⁴⁹ the authors in that study did not determine which allele was present at the 3017 locus of *IL4*. In our study the frequencies for the G allele in the 3 populations were 0.75, 0.15, and 0.53 (white, African American, and Hispanic, respectively). Therefore assuming Hardy-Weinberg equilibrium, we would expect 56% of white subjects, 2% of African American subjects, and 28% of Hispanic subjects to be homozygous for the G allele and hence would not possess a functional PPAR α binding site on either chromosome.

Previous studies evaluating variation in *IL4* have examined selected SNPs in a variety of populations for association with asthma or a related phenotype. The primary SNP evaluated was the -589 C/T promoter polymorphism (sometimes referred to as -590), which was first examined by Rosenwasser et al in 1995.¹⁹ The

T allele of this SNP was found to be associated with (1) increased total serum IgE levels and increased *IL4* transcription in white subjects,¹⁹ (2) development of asthma in Japanese children,²¹ (3) FEV₁ in white asthmatic subjects,²² and (4) asthma and increased levels of total serum IgE in German children with positive skin test responses.⁴⁴ Other investigators, however, were not able to replicate association of this SNP with variation in total serum IgE levels.²¹⁻²⁴ Additionally, the T allele of the -33 C/T polymorphism in exon 1 (sometimes referred to as +33) was weakly associated with total serum IgE levels in a Japanese population.²⁰

The previous discrepancies reported in the literature concerning the association of the -589 C/T SNP with total serum IgE levels might be due to variation in allele frequencies and in the extent of LD between these SNPs in different populations. We performed LD studies for the 11 SNPs analyzed, and pairwise comparisons of D'⁵⁰ are located in the Online Repository (Table E1, see Online Repository at www.mosby.com/jaci). These studies show that the pairwise D' observed between -589 C/T and 3017 G/T is 0.95. Our analysis also revealed that the risk alleles for both -589 (T) and -33 (T) were on one of 2 haplotypes with the risk allele for 3017 (T; Table IV).

A recent publication by Kabesch et al⁴⁴ screened for polymorphisms by denaturing HPLC in DNA samples from 2 populations of German schoolchildren ages 9 to 11 years. Children were considered to have an "asthma" phenotype if they had a physician's diagnosis of asthma, recurrent spastic bronchitis, or recurrent asthmatic bronchitis, perhaps reflecting a broader phenotypic definition of asthma. Control subjects were those individuals who did not meet the criteria for asthma. This group reported association of -589, -33, and a 70-bp repeat in intron 3 with asthma and variation of total serum IgE levels in individuals with positive skin test responses (Table III). In their study the 3017 G/T SNP (referred to as G2979T) was not found to be associated with asthma or variation in total serum IgE levels, which is in contrast to our results in white subjects. This group also did not observe the strong LD between C-589T and G2979T (3017) that we found.

The overall discrepancies between our results and those of Kabesch et al⁴⁴ could be due to several factors. In the Kabesch study the group of phenotypes differs from the definition used in the present study adapted by the National Heart, Lung, and Blood Institute Collaborative Study for the Genetics of Asthma, in which a diagnosis of asthma was based on a confirmed physician diagnosis of asthma, the presence of BHR to methacholine (or bronchodilator reversibility of FEV₁), and documentation of recurrent asthma symptoms.³⁷ In our study allergic characteristics were assessed for all subjects with asthma but were not part of the asthma definition. Our more strict standardized asthma definition could partially account for the differences observed between these 2 studies. Additionally, the overall mean IgE value in the whole Kabesch population is lower than that in our population, as expected, because only 26% of the Kabesch population had positive skin test responses.

It is possible that the 3017 G/T SNP might not be causative in nature but is in LD with a different susceptibility locus in this gene. This point is supported by the discovery that 3 other SNPs, identified by the University of Washington Program in Genomic Applications,⁴⁵ appear to be in LD with the 3017 G/T SNP. It is unclear whether one of these SNPs is the primary susceptibility locus or whether the haplotype of the 4 SNPs concordant for total serum IgE levels (or some combination of these variants) is responsible for the strong associations that were observed.

This study provides strong evidence for the association of *IL4* polymorphisms and haplotypes with total serum IgE levels and potentially asthma and other allergy-related phenotypes. Interestingly, we only observed consistent association with total serum IgE levels (and asthma) in our white subjects and not in our African American or Hispanic asthmatic populations. Although all 3 populations were ascertained by means of the same criteria, there are inherent differences between the ethnic groups,^{37,51} which might account in part for the lack of association in the African American or Hispanic subjects.

Using comprehensive genetic approaches, we have dissected the *IL4* cytokine gene and suggest that the 3017 G/T intron 2 SNP (or the haplotype which it identifies) influences regulatory control of serum IgE levels. This thorough approach is crucial to our understanding of the genetic basis of asthma and atopy. Furthermore, our results are a necessary precursor for further investigation of gene-gene and gene-environment interactions, as well as functional studies of the regulatory elements, that will provide a more complete understanding of the role of *IL4* in the pathogenesis of asthma and allergic disease.

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