Ethnicity-specific Gene–Gene Interaction between IL-13 and IL-4R α among African Americans with Asthma

Natalie C. Battle^{1,2*}, Shweta Choudhry^{1,2*}, Hui-Ju Tsai^{1,2}, Celeste Eng^{1,2}, Gunjan Kumar^{1,2}, Kenneth B. Beckman³, Mariam Naqvi^{1,2}, Kelley Meade³, H. George Watson⁴, Michael LeNoir⁵, and Esteban González Burchard^{1,2}, on behalf of the SAGE (Study of African Americans, Asthma, Genes, and Environments) Investigators

¹University of California, San Francisco, San Francisco, California; ²Lung Biology Center, UCSF Mission Bay Campus, San Francisco, California;

³Children's Hospital Oakland Research Institute (CHORI), Oakland, California; ⁴The James A. Watson Wellness Center, Oakland, California; and ⁵Bay Area Pediatrics, Oakland, California

Rationale: Genes in the interleukin (IL)-4/IL-13/IL-4R α pathway have been shown to be associated with asthma and related phenotypes in some populations, but not in others. Furthermore, interaction between these genes has been shown to affect asthma in white and Chinese populations.

Objectives: To determine whether there are IL-4/IL-13 and IL-4R α gene–gene interactions that are associated with asthma in African Americans.

Methods: Eighteen single-nucleotide polymorphisms (SNPs) in IL-4, IL-13, and IL-4R α genes were genotyped in 264 African Americans with asthma and 176 healthy control subjects. We tested the SNPs for genetic associations and gene–gene interactions with asthma, baseline lung function, bronchodilator drug response, and total serum IgE levels.

Measurements and Main Results: We identified 94 SNPs in IL-4, IL-13, and IL-4R α genes by directly sequencing these genes in 24 African-American subjects with asthma. Seventeen SNPs were analyzed for association with asthma and related phenotypes. We found no evidence of association in the IL-4 gene. One SNP in the IL-13 gene (A–646G, rs2069743) and two SNPs in the IL-4R α gene (A+4679G, rs1805010, and C+22656T, rs1805015) showed association with lung function (both baseline and post-bronchodilator). Although the association between individual SNPs and asthma-related phenotypes differed from previous studies performed in white and Chinese populations, significant gene–gene interaction was found between the IL-13 (A–646G) and IL-4R α (A+4679G) SNPs for baseline lung function among African-American subjects with asthma.

Conclusions: Gene–gene interaction between the IL-13 and IL-4R α genes may play an important role in asthma among African Americans.

Keywords: asthma; African Americans; gene–gene interaction

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

IL-4, IL-13, and IL-4R α have been associated with asthma-related phenotypes in other populations, but have not been thoroughly studied in African Americans.

What This Study Adds to the Field

Gene–gene interaction between the IL-13 and IL-4R α genes may play an important role in asthma among African Americans.

Asthma is a common yet complex disease caused by a variety of genetic and environmental factors. Symptoms for asthma include wheezing, coughing, bronchial hyperresponsiveness, elevated total serum IgE levels, and allergy. Interleukin (IL)-4 and IL-13 are pleiotropic, proinflammatory cytokines produced by activated T cells as part of an immune response to allergen exposure. The genes for IL-4 and IL-13 lie in a cytokine cluster on chromosome 5q31, a locus previously linked to several asthma phenotypes (1–4). The IL-4 and IL-13 cytokines share many structural and functional similarities, as well as a common receptor component, IL-4R α , located on chromosome 16p11. IL-4 plays important roles in the differentiation of T cells, eosinophilic inflammation, and isotype switching in B cells from IgM to IgE (4–8). IL-13 is a Th2 cytokine found to be overexpressed in the lungs of patients with asthma (4) and in murine models (1).

Investigations of the 5q31 locus have resulted in the discovery of significant associations between genetic variants in IL-4 and IL-13 genes and asthma or asthma-related phenotypes in some populations, but not in others (5, 9–13). We have previously identified an association between the IL-4 C–589T allele and asthma severity in whites but not in African Americans (9). Similarly, Basehore and colleagues reported nine SNPs in the IL-4 gene that were significantly associated with asthma or total serum IgE in whites (5). Only one of these SNPs, an intronic SNP (G+3017T), was marginally associated with IgE levels in African Americans and Hispanics (5). However, this SNP was not associated with asthma or IgE levels in other populations (5, 6, 9, 10, 12).

Because asthma is a complex disease, it may be useful to examine gene–gene interactions, particularly between the ligands IL-4 and IL-13 and their shared receptor component, IL-4R α . Howard and coworkers studied the effects of polymorphisms in IL-13 and IL-4R α genes in a white Dutch population (11, 14). They found associations between asthma-related phenotypes and polymorphisms in both the IL-13 and IL-4R α genes,

(Received in original form July 20, 2006; accepted in final form February 15, 2007)

Supported by the National Institutes of Health (K23 HL04464, HL078885, HL07185, GM61390), the American Lung Association of California, RWJ Amos Medical Faculty Development Award, NCMHD Health Disparities Scholar, Extramural Clinical Research Loan Repayment Program for Individuals from Disadvantaged Backgrounds, 2001–2003 (E.G.B.), the American Thoracic Society “Breakthrough Opportunities in Lung Disease” (BOLD) Award and Tobacco-Related Disease Research Program New Investigator Award (15KT-0008) (S.C.), NCMHD grant 1P60MD000222-01 (K.B.B.), UCSF–Children's Hospital of Oakland Pediatric Clinical Research Center (M01 RR01271), Oakland, California, the Sandler Center for Basic Research in Asthma, and the Sandler Family Supporting Foundation.

* These authors contributed equally to this article.

Correspondence and requests for reprints should be addressed to Esteban González Burchard, M.D., M.P.H., University of California, San Francisco, San Francisco, CA 94143-2911. E-mail: esteban@sfgf.ucsf.edu

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 175, pp 881–887, 2007

Originally Published in Press as DOI: 10.1164/rccm.200607-992OC on February 15, 2007
Internet address: www.atsjournals.org

as well as evidence of gene–gene interaction between IL-13 C–1112T and IL-4R α C+22656T (S478P) SNPs as a contributor to asthma susceptibility (11, 14). In another study, Chan and associates reported significant gene–gene interactions between the IL-13 R130Q and IL-4R α Ile50Val (A+4679G) polymorphisms for asthma risk in a Chinese population (15).

Although African Americans experience higher rates of asthma prevalence and mortality than whites (16), few genetic association studies for asthma have focused specifically on the roles of the IL-4, IL-13, and IL-4R α genes in this population (5, 10, 17). Of these studies, none have examined the effects on asthma of interactions between these genes. This is important because populations may be influenced by ethnicity- or race-specific genetic or environmental modifiers, which could influence genetic effects (18). Our study attempts to determine whether there are gene–gene interactions between genetic variants in the IL-4, IL-13, or IL-4R α genes that influence asthma or related phenotypes in African Americans, as have been described previously in whites (14).

METHODS

Study Participants

Recruitment and patient characteristics are described elsewhere and in detail in the online supplement (19). Briefly, as part of the Study of African Americans, Asthma, Genes, and Environments (SAGE), investigators recruited cases and control subjects from community clinics within San Francisco and Oakland, California. Ethnicity was self-reported, and subjects were only enrolled if both biological parents and all grandparents were of African-American ethnicity. Local institutional review boards and clinics approved the study, and age-appropriate written consent was obtained from all study participants. Table 1 lists clinical characteristics of patients with asthma and healthy control subjects.

Asthma and Medical Questionnaire and Spirometry

All subjects with asthma completed a modified version of the 1987 American Thoracic Society Division of Lung Disease Epidemiology

Questionnaire to collect information on asthma and allergy symptoms (20). Results from spirometric tests were expressed as a percentage of the predicted normal value, as calculated by age- and race-adjusted Hankinson equations (21). FEV₁ was used as a quantitative measure of baseline lung function (pre-FEV₁). FEV₁ was measured again after the administration of albuterol (post-FEV₁). Bronchodilator responsiveness (Δ FEV₁) was reported as the percentage of change in baseline FEV₁ after the administration of albuterol. Pulmonary function tests are described further in the online supplement. Total plasma IgE for subjects with asthma was measured in duplicate using Uni-Cap technology (Pharmacia, Kalamazoo, MI).

Single-Nucleotide Polymorphism Discovery

Single-nucleotide polymorphism (SNP) discovery for the IL-4, IL-13, and IL-4R α genes was performed using our SNP discovery panel of 24 unrelated African-American subjects with asthma. All exons, exon–intron boundaries, and the 1-kb promoter region upstream of IL-4, IL-13, and IL-4R α genes were sequenced. Sequencing was performed using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and the ABI Prism 3700 sequencer (Applied Biosystems).

Genotyping

A total of 94 SNPs were identified in IL-4, IL-13, and IL-4R α genes. SNPs were selected for genotyping based on functional significance, minor allele frequency > 5% in our population, linkage disequilibrium (LD) patterns, and association with asthma and related phenotypes in previous studies. Eighteen SNPs in IL-4, IL-13, and IL-4R α genes were selected for genotyping. Schematic diagrams for IL-4, IL-13 and IL-4R α genes with the genotyped SNPs are provided in Figure 1. Additional SNP details are provided in Table 2. Genotyping methods and conditions are described in detail in the online supplement.

Population Stratification

To test for confounding due to population stratification, we genotyped a subset of the samples (176 cases and 176 controls) using 31 Ancestry Informative Markers. As previously described, we did not find evidence of genetic confounding due to population stratification for asthma disease status or asthma-related phenotypes (19). Thus, individual genetic admixture was not included as a covariate in subsequent analyses.

TABLE 1. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE 264 SUBJECTS WITH ASTHMA AND 176 CONTROL SUBJECTS IN THE SAGE STUDY

	Cases (n = 264)	Control Subjects (n = 176)	p Value
Age, yr (range)	19.4 (7.3 to 40.9)	29.9 (8.2 to 41.2)	< 0.01
Sex, % male	40.20	38.10	0.651
BMI, kg/m ² (range)	26.9 (12.8 to 51.5)	N/A	
Total serum IgE, IU/ml (range)	286.9 (2 to 4,033)	N/A	
Log ₁₀ IgE (range)	2.0 (0.3 to 3.6)	N/A	
SES, %*			0.825
Low	39.30	44.40	
Moderate	37.80	31.40	
Middle	17.30	20.70	
Upper	5.50	3.60	
Admixture proportion, %†			0.972
African proportion	80.7 $\pm$ 8.5	80.7 $\pm$ 9.2	
European proportion	19.3 $\pm$ 8.5	19.3 $\pm$ 9.2	
Regular β_2 -agonist use, %	52.50	N/A	
On steroids, %	54.40	N/A	
Severity, % moderate–severe	66.70	N/A	
Baseline lung function			
FEV ₁	92.1 (40.5 to 144.8)	N/A	
Bronchodilator responsiveness			
Δ FEV ₁	9.6 (–12.4 to 73.7)	N/A	

Definition of abbreviations: BMI = body mass index; N/A = not available; SAGE = Study of African Americans, Asthma, Genes, and Environments; SES = socioeconomic status.

* SES was assigned using Federal Financial Institutions Examination Council's geocoding/mapping system, which classifies subjects into 1 of 4 U.S. census 2000 tract income levels: low, moderate, middle, and upper.

† Admixture proportions are expressed as mean and 1 SD.

Figure 1. Schematic diagrams of (A) IL-4, (B) IL-13, and (C) IL-4R α genes with the genotyped single-nucleotide polymorphisms.

Statistical Analysis

Statistical analyses are described in detail in the online supplement. SNPs were analyzed for Hardy-Weinberg equilibrium (HWE) in control subjects using the χ^2 goodness-of-fit test. Pairwise LD was estimated separately for cases and control subjects using the program LD Plotter (22). Haplotypes were reconstructed using the program PHASE version 2.1 (23).

The difference in allele frequency between subjects with asthma and control subjects was tested using χ^2 analysis. Tests for association with asthma disease status among cases and control subjects were performed using logistic regression analysis, adjusting for covariates. Tests for association with pre-FEV₁, post-FEV₁, Δ FEV₁, and total serum IgE among cases only were performed using linear regression. An additive model was used for all regression analyses. IgE levels were transformed to their log values before analysis for normalization.

To test for the effects of gene–gene interaction, we used the model described by Howard and colleagues (14). SNPs with the strongest evidence for association from our analysis of IL-4 and IL-13 genes were paired with SNPs most strongly associated in IL-4R α . These SNP pairs were examined for association with asthma, pre-FEV₁, post-FEV₁, Δ FEV₁, and log₁₀ IgE. All regression analyses were performed using the statistical software package Stata/SE 9.0 (Stata Corp., College Station, TX). Analyses were not adjusted for multiple testing because we were attempting to replicate previous findings of association between SNPs in these genes and asthma-related phenotypes.

RESULTS

A total of 264 African-American subjects with asthma and 176 healthy control subjects were included in this analysis. Table 1 shows the demographic and clinical characteristics of subjects

TABLE 2. SINGLE-NUCLEOTIDE POLYMORPHISMS GENOTYPED IN IL-4, IL-13, AND IL-4R α GENES

Gene	SNP	SNP Location	Amino Acid Change	dbSNP rs#	Nucleotide Change	No. Controls with Genotype	No. Cases with Genotype	MAF Controls	MAF Cases	p Value, χ^2	p Value, Logistic Regression
IL-4	C–589T	Promoter		rs2243250	T/C	175	255	0.33 (C)	0.33 (C)	0.317	0.856
	C–33T	5'UTR		rs2070874	C/T	176	261	0.43 (T)	0.43 (T)	0.935	0.791
	A+45G	Exon 1	Leu15Leu	rs2243251	A/G	162	261	0.15 (G)	0.17 (G)	0.433	0.337
	C+8427A	Intron 3		rs2243290	C/A	173	256	0.35 (A)	0.34 (A)	0.730	0.849
	A–1313G	Promoter		rs2069739	A/G	173	261	0.33 (G)	0.29 (G)	0.411	0.422
IL-13	C–1112T	Promoter		rs1800925	C/T	173	263	0.42 (T)	0.40 (T)	0.857	0.557
	A–646G	Promoter		rs2069743	A/G	174	264	0.21 (G)	0.17 (G)	0.363	0.257
	C+515A	Intron 1		rs2066960	C/A	172	258	0.22 (A)	0.21 (A)	0.935	0.972
	G+542C	Intron 1		rs1295687	G/C	173	262	0.26 (C)	0.24 (C)	0.609	0.440
	C+749T	Intron 1		rs2069744	C/T	174	260	0.26 (T)	0.24 (T)	0.734	0.218
	A+1923G	Intron 3		rs1295686	A/G	170	261	0.35 (G)	0.34 (G)	0.165	0.931
	C+2044T	Exon 4	Arg144Gln	rs20541	C/T	174	261	0.18 (T)	0.19 (T)	0.702	0.937
	T+2580G	3'UTR		rs848	G/T	173	260	0.49 (T)	0.50 (T)	0.964	0.491
	A+4679G	Exon 5	Val75Ile	rs1805010	A/G	174	262	0.43 (G)	0.46 (G)	0.449	0.219
	A+22348C	Exon 11	Glu400Ala	rs1805011	C/A	173	260	0.43 (A)	0.44 (A)	0.594	0.840
IL-4R α	C+22448T	Exon 11	Leu433Leu	rs2234900	C/T	174	261	0.44 (T)	0.44 (T)	N/A*	N/A*
	C+22656T	Exon 11	Ser503Pro	rs1805015	T/C	171	263	0.38 (C)	0.36 (C)	0.148	0.479
	G+23403T	Exon 11	Ser752Ala	rs1805016	G/T	173	259	0.35 (T)	0.35 (T)	0.798	0.561

Definition of abbreviations: MAF = minor allele frequency; SNP = single-nucleotide polymorphism; UTR = untranslated region.

Case-control genetic association between SNPs and asthma disease status was tested using χ^2 analysis and logistic regression after adjusting for covariates.

* SNP C+22448T was found to be out of Hardy-Weinberg equilibrium in control subjects and was excluded from further analyses.

with asthma and control subjects analyzed in the study. Cases and control subjects were well matched with respect to gender, sex proportion, and average socioeconomic status. Control subjects were older than cases on average ($p < 0.01$). This was purposely done to ensure that control subjects would not develop asthma at an older age.

Table 2 shows the number of cases and control subjects with genotype information for each SNP. For each SNP, the genotype success rate was greater than 96%. All SNPs were tested for HWE using the goodness-of-fit test among control subjects. One SNP was found to be out of HWE with a p value ≤ 0.01 : C+22448T in IL-4R α (p value = 0.004). This SNP was excluded from further analyses. We did not find any significant differences in allele frequency between subjects with asthma and control subjects for the remaining 17 SNPs tested using χ^2 test or logistic regression analysis (Table 2).

Pairwise LD was computed between 17 SNPs using the r^2 statistic. The LD patterns were computed separately for cases and control subjects and also for cases and control subjects combined (see Table E1 of the online supplement). Overall LD was low with one exception: C-33T and C+8427A of the IL-4 gene had an r^2 value of 0.69. In the IL-4R α gene, linkage between A+4679G in exon 5 and the other three SNPs in exon 11 was very low ($r^2 < 0.006$). No significant LD was observed between SNPs on different genes. This suggests that polymorphisms in the three genes are not transmitted together. There was no significant difference in LD pattern between cases and control subjects.

Association Analysis for Asthma-related Phenotypes

Of the 17 SNPs tested, 2 were significantly associated with asthma-related phenotypes. The IL-13 A-646G allele was associated with post-FEV₁ ($p = 0.009$) and Δ FEV₁ ($p = 0.019$) (Table 3). Subjects with the IL-13 -646G allele had lower post-FEV₁ and lower bronchodilator drug responsiveness compared with those

subjects with the A allele. This SNP also had a trend of association with pre-FEV₁ ($p = 0.064$). The IL-4R α C+22656T allele was associated with post-FEV₁ ($p = 0.049$). This SNP had a trend for association with pre-FEV₁ ($p = 0.066$). In addition, there was a trend for association between the IL-4R α A+4679G allele and pre-FEV₁ ($p = 0.064$) and post-FEV₁ ($p = 0.092$). No SNPs in the IL-4 gene were significantly associated with any of the five phenotypes tested (Table 3).

Haplotype Association Analysis

Separate haplotypes were reconstructed for each of the three genes. There were 8, 196, and 14 haplotypes observed for IL-4, IL-13, and IL-4R α genes, respectively. In addition, 5, 13, and 13 haplotypes observed for the IL-4, IL-13, and IL-4R α genes, respectively, had frequencies of greater than 2%. No significant associations were found between haplotypes for IL-4, IL-13, or IL-4R α and asthma disease status, pre-FEV₁, post-FEV₁, Δ FEV₁, or log IgE levels (results not shown) except for a borderline association between the major IL-4 haplotype pair (TTAA/CCAC) and higher risk for asthma ($p = 0.03$).

Gene-Gene Interaction Analysis

In testing for the effects of gene-gene interaction between the IL-4/IL-13 genes and the IL-4R α gene on asthma in our population, we followed the method described previously (14). Individual SNPs that were significant or that approached significance for association with asthma-related phenotypes were selected from each gene for gene-gene interaction analysis. These SNPs were A-646G in the IL-13 gene, and A+4679G and C+22656T in the IL-4R α gene.

Results from gene-gene interaction analysis are shown in Table 4. The IL-13 A-646G and the IL-4R α A+4679G SNPs interacted significantly for pre-FEV₁ ($p = 0.042$) and a trend was observed for log IgE levels ($p = 0.09$). No other significant associations were found.

DISCUSSION

Genetic variants in IL-4, IL-13, and IL-4R α genes have been linked to asthma and related phenotypes in various populations. In this study, we analyzed the effects of genetic variants and haplotypes in these genes in an African-American asthmatic cohort. We also examined the effects of gene-gene interaction between SNPs that showed an association with asthma or asthma-related phenotypes. One genetic variant in the IL-13 gene was significantly associated with post-FEV₁ and bronchodilator responsiveness. Another variant in the IL-4R α gene was associated with post-FEV₁. Two SNPs, which were modestly correlated with baseline lung function independently of each other, interacted to significantly affect this phenotype. Although our results from the interaction analysis were consistent with our results from the single SNP analysis, neither SNP reached statistical significance in association with pre-FEV₁ alone. Both polymorphisms may have modestly contributed to baseline lung function individually, but the effect was more pronounced when the SNPs interacted in our cohort.

Our results show that genetic variants in IL-13 and IL-4R α genes may interact to affect baseline lung function in African-American subjects with asthma. Interaction between genetic variants in these genes has previously been shown to influence asthma susceptibility in several populations. In a study by Chan and colleagues, the IL-4R α (A+4679G) allele was shown to interact with the IL-13 (R130Q) allele to influence asthma risk in a Chinese cohort (15). Similarly, Howard and associates' report on Dutch subjects with asthma demonstrated an interaction between the IL-13 (C-1112T) and IL-4R α (C+22656T) alleles

TABLE 3. ASSOCIATION ANALYSES OF IL-4, IL-13, AND IL-4R α GENOTYPES WITH LUNG FUNCTION, RESPONSE TO ALBUTEROL, AND IgE LEVELS IN SUBJECTS WITH ASTHMA

Gene	SNP	Reference Genotype [†]	p Value*			
			Pre-FEV ₁	Post-FEV ₁	Δ FEV ₁	Log ₁₀ IgE
IL-4	C-589T	TT	(+) 0.983	(-) 0.898	(+) 0.905	(-) 0.917
	C-33T	CC	(+) 0.307	(+) 0.552	(-) 0.287	(+) 0.681
	A+45G	AA	(-) 0.866	(+) 0.854	(+) 0.589	(-) 0.694
	C+8427A	CC	(+) 0.339	(+) 0.603	(-) 0.392	(+) 0.389
IL-13	A-1313G	AA	(-) 0.809	(-) 0.724	(-) 0.641	(+) 0.318
	C-1112T	CC	(+) 0.242	(+) 0.260	(-) 0.857	(-) 0.513
	A-646G	AA	(+) 0.064	(+) 0.009	(+) 0.019	(+) 0.817
	C+515A	CC	(-) 0.415	(-) 0.486	(+) 0.640	(-) 0.310
	G+542C	GG	(+) 0.946	(+) 0.975	(-) 0.983	(-) 0.541
	C+749T	CC	(+) 0.711	(+) 0.746	(+) 0.811	(+) 0.702
	A+1923G	AA	(+) 0.859	(-) 0.890	(-) 0.890	(+) 0.691
	C+2044T	CC	(-) 0.337	(-) 0.700	(+) 0.567	(-) 0.551
	T+2580G	GG	(+) 0.745	(+) 0.796	(+) 0.864	(-) 0.927
	IL-4R α A+4679G	AA	(+) 0.069	(+) 0.092	(-) 0.885	(+) 0.308
IL-4R α	A+22348C	CC	(+) 0.230	(+) 0.337	(-) 0.988	(+) 0.511
	C+22656T	TT	(-) 0.066	(-) 0.049	(-) 0.299	(-) 0.768
	G+23403T	GG	(-) 0.783	(-) 0.842	(-) 0.706	(+) 0.628

Definition of abbreviation: SNP = single-nucleotide polymorphism.

The analyses were performed using linear regression and adjusted for covariates.

* Positive (+) direction of association means that the allele is associated with a higher value of the trait; negative (-) direction of association means that the allele is associated with a lower value of the trait.

[†] Reference genotype is the homozygote genotype for the major allele.

TABLE 4. p VALUES FOR GENE–GENE INTERACTION ANALYSIS*

IL-13 SNP	IL-4R α SNP	Reference Genotype Pair [†]	Asthma	Pre-FEV ₁	Post-FEV ₁	Δ FEV ₁	Log ₁₀ IgE
A–646G	A+4679G	AA/AA	(+) 0.112	(+) 0.042	(+) 0.188	(+) 0.619	(+) 0.090
A–646G	C+22656T	AA/TT	(+) 0.830	(–) 0.663	(–) 0.688	(+) 0.847	(–) 0.130

Definition of abbreviation: SNP = single-nucleotide polymorphism.

* Positive (+) direction of association means that the genotype is associated with a higher value of the trait; negative (–) direction of association means that the genotype is associated with a lower value of the trait.

[†] Reference genotype pair consists of the major homozygote genotypes.

for asthma susceptibility (14). Although the SNPs that interacted in our cohort differed from those of Chan and colleagues or Howard and coworkers, our results suggest that interaction between IL-13 and IL-4R α genes may play a role in lung function in our population as well. It has been suggested that replication of complex genetic associations may be more informative on a genome-wide level as opposed to the individual SNP level, especially among different populations (24). To our knowledge, this study is the first to test the effects of gene–gene interaction between IL-4, IL-13, and IL-4R α genes and asthma in an African-American population.

Gene–gene interaction between IL-4 and IL-4R α and asthma has been demonstrated in several populations (25, 26). Because no significant or moderately significant associations were found for IL-4 SNPs in our population, we did not test for interaction between this gene and IL-4R α . It is still possible, however, that there was an interaction between these two genes, but the effects were not detectable via single SNP analysis. Although we may have been underpowered due to sample size, our sample size of 264 cases and 176 control subjects is comparable to other genetic association studies for these genes. Finally, the interaction with IL-4R α could be with alternative SNPs in the IL-4 gene, which were not analyzed in our study.

Although our results were globally consistent with previous studies of gene–gene interactions, our results were not entirely consistent with studies of individual SNPs. For example, polymorphisms in the IL-4 gene, in particular the C–589T and C–33T alleles, have been linked to asthma and related phenotypes in some studies (5, 6, 9, 10, 12), but not in others (26, 27). One or both of these promoter SNPs has been linked to asthma, total serum IgE levels, or allergy in white (5, 6, 10, 12) and Japanese (28) populations. In contrast, we did not find any associations between IL-4 SNPs and asthma (pre- or post-FEV₁), drug responsiveness, or total serum IgE. However, our results were fairly consistent with Basehore and colleagues' study of 168 African-American subjects with asthma and 269 African-American control subjects (5). Basehore and coworkers genotyped 11 IL-4 SNPs (including C–589T, C–33T, and A+8427A) in white, African-American, and Hispanic populations; 9 of the 11 SNPs were significantly associated with an asthma-related phenotype in whites. However, only one of these SNPs (G+3017T), which was not genotyped in our population, was associated with an asthma-related phenotype in African Americans or Hispanics. Likewise, Donfack and colleagues (10) found associations between C–589T and/or C–33T and asthma-related phenotypes in Europeans and Hutterites, but not in African Americans. We have previously reported an association between the IL-4 C–589T allele and baseline FEV₁ in whites but not in African Americans (9). These studies may suggest that, although polymorphisms in the IL-4 gene may be significant contributors to asthma in whites, their effects may be attenuated in African Americans. Alternatively, SNPs in the IL-4 gene may not them-

selves be biological contributors to asthma but may be in LD with other SNPs that are biological contributors to asthma in whites, but not in African Americans. Furthermore, Basehore and colleagues noted that IL-4 G+3017T SNP was in LD with at least three other SNPs.

In contrast to our results for the IL-4 gene, we found evidence of association between a promoter SNP (A–646G) in the IL-13 gene and post-FEV₁ and drug response, and a trend for association with baseline lung function. SNPs in the promoter region of the IL-13 gene have been associated with various asthma-related phenotypes in other studies. Donfack and colleagues described an association between a two-SNP haplotype containing IL-13 (C–1112T) and IL-4 (G+3017T), and allergic sensitization to molds in an African-American population (10). This result confirmed the results of a study by van der pouw Kraan and colleagues, who found an association between IL-13 (C–1112T) and allergic asthma in another population (referred to as –1055 in van der pouw Kraan and colleagues' study) (13).

It has been shown that soluble IL-4R protein levels are found in higher concentrations in patients with asthma than in healthy control subjects, lending support to a possible role in the pathogenesis of asthma (29). Genetic variants in IL-4R α have been examined several times in association studies for asthma (14, 29) and other related phenotypes (6, 30), although findings were not consistent (31). In our population, two SNPs in the IL-4R α gene (A+4679G and C+22656T) showed a trend of association with pre-FEV₁. Patients with asthma with the minor allele at IL-4R α (A+4679G) or the major allele at IL-4R α (C+22656T) had lower baseline lung function.

We have previously demonstrated that genetic and pharmacogenetic associations for asthma vary among different racial and ethnic groups (18, 19). This has been confirmed for other diseases as well (18). For example, a meta-analysis has shown that the impact of apolipoprotein (APO)-E4 on Alzheimer's disease varies by race (32). Although there are many potential explanations for these observations, one possibility is that there are unique racial and ethnicity-specific modifiers (genetic and environmental) that attenuate or accentuate genetic risk factors.

In this study, we were able to replicate findings of gene–gene interaction between IL-13 and IL-4R α and asthma-related phenotypes, which has been shown in other populations. However, we were unable to replicate previous single SNP associations with these phenotypes. One possible explanation is that genetic associations may be affected by differing LD patterns between ethnic groups (e.g., Africans typically have less LD when compared with other populations). The differences in LD between racial groups can be exploited to identify putative disease-causing genetic variants. Specifically, examination of SNP associations across multiple ethnic groups may reveal precisely which SNPs are consistently observed to be associated in the different populations, regardless of LD pattern. For example, in cloning the phenylthiocarbamide (PTC) bitter-taste gene, three different

amino acid substitutions were observed to be associated with taste blindness (33). Although there was strong to complete LD among these SNPs in most populations, it was also observed that studies of Africans could disentangle the effects of individual SNPs because of reduced LD in this group. Similar such examples have been provided in the association study of adult macular degeneration (34, 35). Such findings underscore the importance of studying multiple ethnic and racial groups in genetic association studies.

Conflict of Interest Statement: N.C.B. has no financial relationship with a commercial entity that has an interest in the subject of this manuscript. S.C. has no financial relationship with a commercial entity that has an interest in the subject of this manuscript. H.T. has no financial relationship with commercial entity that has an interest in the subject of this manuscript. C.E. has no financial relationship with a commercial entity that has an interest in the subject of this manuscript. G.K. has no financial relationship with a commercial entity that has an interest in the subject of this manuscript. K.B. has no financial relationship with a commercial entity that has an interest in the subject of this manuscript. M.N. has no financial relationship with a commercial entity that has an interest in the subject of this manuscript. K.M. has no financial relationship with a commercial entity that has an interest in the subject of this manuscript. H.G.W. has no financial relationship with a commercial entity that has an interest in the subject of this manuscript. M.L. has been reimbursed as a consultant on a community grant dealing with asthma in the African American community; he has received \$155,000 from GlaxoSmithKline directly over 3 years plus a grant. E.G.B. has no financial relationship with a commercial entity that has an interest in the subject of this manuscript.

References

1. Grunig G, Warnock M, Wakil AE, Venkayya R, Brombacher F, Rennick DM, Sheppard D, Mohrs M, Donaldson DD, Locksley RM, *et al.* Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1998;282:2261–2263.
2. Pillai SG, Chiano MN, White NJ, Speer M, Barnes KC, Carlsen K, Gerritsen J, Helms P, Lenney W, Silverman M, *et al.* A genome-wide search for linkage to asthma phenotypes in the genetics of asthma international network families: evidence for a major susceptibility locus on chromosome 2p. *Eur J Hum Genet* 2006;14:307–316.
3. Postma DS, Meyers DA, Jongepier H, Howard TD, Koppelman GH, Bleecker ER. Genomewide screen for pulmonary function in 200 families ascertained for asthma. *Am J Respir Crit Care Med* 2005;172:446–452.
4. Wills-Karp M. Interleukin-13 in asthma pathogenesis. *Immunol Rev* 2004;202:175–190.
5. Basehore MJ, Howard TD, Lange LA, Moore WC, Hawkins GA, Marshik PL, Harkins MS, Meyers DA, Bleecker ER. A comprehensive evaluation of IL4 variants in ethnically diverse populations: association of total serum IgE levels and asthma in white subjects. *J Allergy Clin Immunol* 2004;114:80–87.
6. Isidoro-Garcia M, Davila I, Laffond E, Moreno E, Lorente F, Gonzalez-Sarmiento R. Interleukin-4 (IL4) and interleukin-4 receptor (IL4RA) polymorphisms in asthma: a case control study. *Clin Mol Allergy* 2005;3:15.
7. Padilla J, Daley E, Chow A, Robinson K, Parthasarathi K, McKenzie AN, Tschernig T, Kurup VP, Donaldson DD, Grunig G. IL-13 regulates the immune response to inhaled antigens. *J Immunol* 2005;174:8097–8105.
8. Woitsch B, Carr D, Stachel D, Schmid I, Weiland SK, Fritzsche C, von Mutius E, Kabisch M. A comprehensive analysis of interleukin-4 receptor polymorphisms and their association with atopy and IgE regulation in childhood. *Int Arch Allergy Immunol* 2004;135:319–324.
9. Burchard EG, Silverman EK, Rosenwasser LJ, Borish L, Yandava C, Pillari A, Weiss ST, Hasday J, Lilly CM, Ford JG, *et al.* Association between a sequence variant in the IL-4 gene promoter and FEV₁ in asthma. *Am J Respir Crit Care Med* 1999;160:919–922.
10. Donfack J, Schneider DH, Tan Z, Kurz T, Dubchak I, Frazer A, Ober C. Variation in conserved non-coding sequences on chromosome 5q and susceptibility to asthma and atopy. *Respir Res* 2005;6:145.
11. Howard TD, Whittaker PA, Zaiman AL, Koppelman GH, Xu J, Hanley MT, Meyers DA, Postma DS, Bleecker ER. Identification and association of polymorphisms in the interleukin-13 gene with asthma and atopy in a Dutch population. *Am J Respir Cell Mol Biol* 2001;25:377–384.
12. Kabisch M, Tzotcheva I, Carr D, Hoffer C, Weiland SK, Fritzsche C, von Mutius E, Martinez FD. A complete screening of the IL4 gene: novel polymorphisms and their association with asthma and IgE in childhood. *J Allergy Clin Immunol* 2003;112:893–898.
13. van der Pouw Kraan TCTM, van Veen A, Boeijs LCM, van Tuyl SAP, de Groot ER, Stapel SO, Bakker A, Verweij CL, Aarden LA, van der Zee JS. An IL-13 promoter polymorphism associated with increased risk of allergic asthma. *Genes Immun* 1999;1:61–65.
14. Howard TD, Koppelman GH, Xu J, Zheng SL, Postma DS, Meyers DA, Bleecker ER. Gene-gene interaction in asthma: IL4RA and IL13 in a Dutch population with asthma. *Am J Hum Genet* 2002;70:230–236.
15. Chan IH, Leung TF, Tang NL, Li CY, Sung YM, Wong GW, Wong CK, Lam CW. Gene-gene interactions for asthma and plasma total IgE concentration in Chinese children. *J Allergy Clin Immunol* 2006;117:127–133.
16. Akinbami LJ, Schoendorf KC. Trends in childhood asthma: prevalence, health care utilization, and mortality. *Pediatrics* 2002;110(2 Pt 1):315–322.
17. Ober C, Leavitt SA, Tsalenko A, Howard TD, Hoki DM, Daniel R, Newman DL, Wu X, Parry R, Lester LA, *et al.* Variation in the interleukin-4 receptor alpha gene confers susceptibility to asthma and atopy in ethnically diverse populations. *Am J Hum Genet* 2000;66:517–526.
18. Burchard EG, Ziv E, Coyle N, Gomez SL, Tang H, Karter AJ, Mountain JL, Perez-Stable EJ, Sheppard D, Risch N. The importance of race and ethnic background in biomedical research and clinical practice. *N Engl J Med* 2003;348:1170–1175.
19. Tsai HJ, Shaikh N, Kho JY, Battle N, Naqvi M, Navarro D, Matallana H, Lilly CM, Eng CS, Kumar G, *et al.* Admixture-matched case-control study: a practical approach for genetic association studies in admixed populations. *Hum Genet* 2006;118:626–639.
20. American Thoracic Society. Standardization of spirometry: 1994 update. *Am J Respir Crit Care Med* 1995;152:1107–1136.
21. Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. *Am J Respir Crit Care Med* 1999;159:179–187.
22. Innate Immunity PGA; NHLBI Program for Genomic Applications. LDPlotter Tool, 2006. Available from: <http://www.innateimmunity.net/> (accessed March 8, 2007).
23. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 2001;68:978–989.
24. Neale BM, Sham PC. The future of association studies: gene-based analysis and replication. *Am J Hum Genet* 2004;75:353–362.
25. Chan A, Newman DL, Shon AM, Schneider DH, Kuldane S, Ober C. Variation in the type I interferon gene cluster on 9p21 influences susceptibility to asthma and atopy. *Genes Immun* 2006;7:169–178.
26. Lee SG, Kim BS, Kim JH, Lee SY, Choi SO, Shim JY, Hong TJ, Hong SJ. Gene-gene interaction between interleukin-4 and interleukin-4 receptor alpha in Korean children with asthma. *Clin Exp Allergy* 2004;34:1202–1208.
27. Park BL, Kim LH, Choi YH, Lee JH, Rhim T, Lee YM, Uh ST, Park HS, Choi BW, Hong SJ, *et al.* Interleukin 3 (IL3) polymorphisms associated with decreased risk of asthma and atopy. *J Hum Genet* 2004;49:517–527.
28. Suzuki I, Hizawa N, Yamaguchi E, Kawakami Y. Association between a C+33T polymorphism in the IL-4 promoter region and total serum IgE levels. *Clin Exp Allergy* 2000;30:1746–1749.
29. Hytonen AM, Lowhagen O, Arvidsson M, Balder B, Bjork AL, Lindgren S, Hahn-Zoric M, Hanson LA, Padyukov L. Haplotypes of the interleukin-4 receptor alpha chain gene associate with susceptibility to and severity of atopic asthma. *Clin Exp Allergy* 2004;34:1570–1575.
30. Kruse S, Forster J, Kuehr J, Deichmann KA. Characterization of the membrane-bound and a soluble form of human IL-4 receptor alpha produced by alternative splicing. *Int Immunol* 1999;11:1965–1970.
31. Noguchi E, Nukaga-Nishio Y, Jian Z, Yokouchi Y, Kamioka M, Yamakawa-Kobayashi K, Hamaguchi H, Matsui A, Shibasaki M, Arinami T. Haplotypes of the 5' region of the IL-4 gene and SNPs in the intergene sequence between the IL-4 and IL-13 genes are associated with atopic asthma. *Hum Immunol* 2001;62:1251–1257.
32. Choudhry S, Avila PC, Nazario S, Ung N, Kho J, Rodriguez-Santana JR, Casal J, Tsai HJ, Torres A, Ziv E, *et al.* CD14 tobacco gene-environmental interaction modifies asthma severity and immunoglobulin E levels in Latinos with asthma. *Am J Respir Crit Care Med* 2005;172(2):173–182.
33. Choudhry S, Ung N, Avila PC, Ziv E, Nazario S, Casal J, Torres A, Gorman JD, Salari K, Rodriguez-Santana JR, *et al.* Pharmacogenetic differences in response to albuterol between Puerto Ricans and Mexicans with asthma. *Am J Respir Crit Care Med* 2005;278(16):563–570.

34. Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, Myers RH, Pericak-Vance MA, Risch N, van Duijn CM. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease: a meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA* 1997;278:1349–1356.
35. Kim UK, Jorgenson E, Coon H, Leppert M, Risch N, Drayna D. Positional cloning of the human quantitative trait locus underlying taste sensitivity to phenylthiocarbamide. *Science* 2003;299:1221–1225.
36. Haines JL, Hauser MA, Schmidt S, Scott WK, Olson LM, Gallins P, Spencer KL, Kwan SY, Nouredine M, Gilbert JR, *et al.* Complement factor H variant increases the risk of age-related macular degeneration. *Science* 2005;308:419–421.
37. Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, Haynes C, Henning AK, SanGiovanni JP, Mane SM, Mayne ST, *et al.* Complement factor H polymorphism in age-related macular degeneration. *Science* 2005;308:385–389.
38. Chen X, Kwok PY. Homogeneous genotyping assays for single nucleotide polymorphisms with fluorescence resonance energy transfer detection. *Genet Anal* 1999;14:157–163.
39. Devlin B, Risch N. A comparison of linkage disequilibrium measures for fine-scale mapping. *Genomics* 1995;29:311–322.