Chapter 10 | Association of IL4Rα polmorphisms with atopy and asthma and gene-gene interaction with IL13 in an asthmatic Dutch population

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ABSTRACT

Asthma is a common respiratory disease that is characterized by variable airways obstruction caused by acute and chronic bronchial inflammation. Clinical findings in asthma include bronchial hyperresponsiveness and allergic responses, demonstrated by elevated total serum IgE levels and positive skin tests to common allergens. These closely associated clinical phenotypes have been shown to have a strong genetic component. Binding of IL4 to the IL4 receptor (IL4R) induces the initial response for Th2 polarization. IL4 and IL13 are both produced by Th2 cells and are capable of inducing isotype class-switching of B-cells to produce IgE after allergen exposure. These cytokines also share a common receptor component, IL4Rα, which is a potential biological candidate gene for asthma and atopy. We have investigated five IL4Rα single-nucleotide polymorphisms in a well-characterized population of Dutch families ascertained through a proband with asthma that was initially studied 25 to 35 years ago. Using the probands and their spouses from this population in a case-control study design, we observed significant associations of atopy and asthma related phenotypes with several IL4Rα of the polymorphisms genotyped within the gene. The most significant association was observed with S478P, which was associated with high IgE levels ($p = 0.0007$). In addition, a significant gene-gene interaction was detected between the S478P variation in IL4Rα (significantly associated with high IgE levels) and the –1111 promoter variation in IL13 (significantly associated with hyperresponsiveness). Individuals with the risk genotype for both of these genes were at almost five times higher risk for the development of asthma compared to individuals with both non-risk genotypes ($p = 0.0004$). These data suggest that variations in IL4Rα contribute to elevated total serum IgE levels, and interaction between IL4Rα and IL13 markedly increases an individual's susceptibility to asthma in this Dutch population.

INTRODUCTION

Asthma is an increasingly common inflammatory airways disease characterized by bronchial hyperresponsiveness (BHR) and reversible, intermittent airways obstruction. Multiple studies have demonstrated a strong genetic component to this disease.[1,2] Specifically, BHR and atopic responses such as elevated total serum IgE levels, typical findings in asthma, have been used to identify regions of the genome that may contain genes that play a role in the pathogenesis of asthma and allergy.[3-6]

The interleukin 4 receptor (IL4R) is a key component in the induction of the Th2 phenotype and IgE production. Binding of IL4 to the IL4R initiates B-cell switching from IgG to IgE production after allergen exposure. Antigen-presenting cells stimulate production and secretion of IL4 from T-cells, leading to a Th2 cell phenotype and the subsequent switch to IgE synthesis.[7] A further role of IL4 in the pathogenesis of asthma has been indicated from actively sensitized IL4 knockout mice. Neither specific IgE induction nor bronchial hyperresponsiveness were detected in these mice[8,9] suggesting a critical role for the IL4/IL4R pathway in these phenotypes. The pleiotrophic effects of IL4 are mediated through the IL4R, which is comprised of the high affinity α subunit, and either the common γ subunit or the IL13 receptor α subunit. The IL13 receptor consists of one IL4Rα subunit and either a low-affinity IL13Rα1[10] or a high-affinity IL13Rα2 subunit.[11] The complete receptor for IL4 is composed of one IL4Rα subunit and an IL4Rγ subunit. Therefore, it is possible that different polymorphisms in these receptors, as well as in the IL4 and IL13 cytokines, contribute to the complex regulation of atopy or asthma phenotypes. IL13 also contributes to the maintenance of the Th2 profile that leads to elevated baseline IgE levels. In fact, murine models have demonstrated the critical nature of IL13 independent of IL4.[12]

Since increased levels of total serum IgE have been strongly correlated with asthma and BHR[4,13,14], and mouse models suggest that IL4 and IL13 may modulate atopy and asthma related phenotypes[15], IL4, IL13, and IL4Rα are excellent candidate genes for these conditions. At least thirteen polymorphisms have been reported within the IL4Rα gene.[16-18] The I50V, S478P, and Q551R variants have been associated with a higher risk of atopy[17,19], atopic asthma[20], and variation in IgE levels.[17] In addition, specific alleles of these variants were shown to modulate the activity of IL4Rα.[19,21] In a recent study by Ober and coworkers[18], eight polymorphisms in IL4Rα (seven in exon 12) were studied in both inbred and outbred populations. Significant evidence for an association between several of these variants and the resulting haplotypes were observed for asthma and atopy. We eva luated five polymorphisms (four in exon 12) within IL4Rα in a Dutch po pulation ascertained through a proband with asthma to determine the importance of these variants for susceptibility and expression of asthma and

atopy in this population. Given the biological role of IL4Rα, our primary hypothesis was to test for differences in total serum IgE levels between IL4Rα genotypes, and then to investigate associated phenotypes. Since we have previously found a significant association between IL13 polymorphisms and BHR (Howard et al., submitted) and between IL4Rα and total serum IgE levels in the current investigation, we performed a gene-gene interaction analysis for these two candidate genes and asthma susceptibility.

MATERIALS AND METHODS

Population

This population has been described in detail previously.[3,22] Ascertainment was based on a formal diagnosis of asthma in a clinical setting. The probands with asthma were originally characterized between 1962-1975. Between 1990 and 1998, 200 probands with asthma (together with their spouses, children and available grandchildren) were restudied. Briefly, all individuals underwent spirometry, reversibility to 800 mg albuterol, and bronchial responsiveness testing to histamine using a 30 second inhalation protocol previously described.[22;23] For atopy, adult subjects had intracutaneous skin testing with 16 common aeroallergens and total serum IgE le vels were measured. In the first 92 families, total serum IgE levels were measured by solid-phase immunoassay (Pharmacia Diagnostics, Uppsala, Sweden). Duplicate measurements were made and the mean for each subject was used. If the duplicate samples differed by more than five percent the test was repeated. In the second set of 108 families, total IgE levels were measured by enzyme linked fluorescence assay (Mini Vidas, Biomerieux, Inc.). Although entire families were ascertained for linkage studies, the probands and spouses represent an appropriate cohort for this case-control association study. Probands and spouses have a similar age, removing the confounding effect of age on changes in BHR, IgE and atopy. This study was approved by the Medical Ethics Committee at the University of Groningen. All subjects gave written informed consent.

Molecular Methods

DNA was isolated using standard techniques. For the I50V, E375A, and C406R polymorphisms, PCR was carried out using standard conditions and an annealing temperature of 60°C (I50V), and 68°C (E375A, C406R), and the previously described primers for I50V[20], and 5′-CAGCATGGTGC-CCAGTGGAG-3′ and 5′-CTTGGGAACTCATCCCAGGGC -3′ for E375A and C406R. The E375A and C406R polymorphisms were contained within the same 334 bp PCR product and digested with the restriction enzymes Cac8I (E375A) or Tsp45I (C406R) to distinguish the alleles. The I50V PCR products were digested with the restriction enzyme MslII.

The S478P and Q551R polymorphisms were genotyped using a novel variation of allele-specific PCR that utilizes fluorescent dye and automated

sequencer technology (FAS-PCR).[24] Allele-specific primers were designed for both polymorphisms using the known IL4R sequence (GenBank accession no. X52425) and previously published data.[17,19] Two allele-specific forward primers were designed with different fluorescent labels and an addition of two nucleotides to one. The S478-specific primer was (tet) 5′-TGCTTACCGCAGCTTCAGCAACT-3′ and the P478-specific primer was (fam) 5′-CTTACCGCAGCTTCAGCAACC-3′. The common reverse primer was 5′-TTTCTGGCTCAGGTTGGGGC-3′. The forward primer specific for the Q551 allele was (tet) 5′-GGCCCCCACCAGTGGCTATCA-3′ and the primer specific for the R551 allele was (fam) 5′-CCCCCACCAGTGGCTATCG-3′. The same reverse primer, 5′-CCAGTCCAAAGGTGAACAAGGGG-3′, was used to detect each of the allele-specific products. One-fourth of the Q551 specific primer was used to compensate for the increased intensity of this PCR amplified product. Fragments were separated and analyzed with ABI 377 DNA Sequencers. The IL13 -1111 promoter SNP was also genotyped using a PCR-RFLP assay using the primers 5′-ATGCCTTGTGAGGAGGGTCAC-3′ and 5′-CCAGTCTCTGCAGGATCAACC-3′. PCR products were digested with NheI (New England Biolabs) and the alleles resolved by electrophoresis on a 2% agarose gel.

Genetic Analysis

Analysis was performed for the following phenotypes: total serum IgE levels, skin test responsiveness to common allergens, asthma, and BHR. As described previously, all of the probands fit published criteria for a diagnosis of asthma.[22] Total serum IgE was analyzed as a qualitative and quantitative trait. As a qualitative trait, cases were defined as having total serum IgE > 100 IU/ml; this value best distinguished them from those not affected after examining the overall frequency distribution.[4,5] As a quantitative trait, IgE was logarithm-transformed to approximate a normal distribution. Differences between groups were tested with ANOVA, t-test, and multiple regression. Individuals were considered responsive to an allergen skin test if one or more test showed a mean wheal diameter of > 5mm. For BHR, cases were defined as original probands and spouses with a PC20≤ 32 mg/ml histamine. The control group for both BHR-positive and asthma cases was comprised of BHR-negative spouses (PC20 > 32 mg/ml). Each of the biallelic polymorphisms was analyzed by comparing differences of genotype frequencies between cases and controls. Chi-square tests assu ming a dominant model were performed, due to the small number of homozygotes for the rare allele. For the interaction analysis, the individuals with the non-risk genotypes at each gene polymorphism, based on this and previous results in our study population, were compared with individuals carrying either, or both, risk genotypes.

The linkage disequilibrium test between pairs of SNPs was based on an exact test assuming multinominal probability of the multi-locus genotype, conditional on the single-locus genotype.[25] A Monte Carlo simulation was

used to assess the significance, by permuting the single-locus genotypes among individuals in the sample to simulate the null distribution. The empirical p-values of the LD for each pair of SNPs were based on 10,000 replicate samples.

Table 1. Clinical characteristics of Dutch proband/spouse population*

	Probands	Spouses
Sex, M:F	124:76	76:125
Age, mean	52.1	51.0
SD	8.4	9.2
IgE		
Total IgE, IU (geometric mean)	93.0	26.2
% ≥ 100 IU/ml	72.5	15.4
Skintests		
% with ≥1 positive skintest	81.9	31.0
FEV_1		
% Predicted pre-medication (mean)	69.6	98.4
% Predicted post-medication (mean)	82.4	103.9
Reversibility		
%, ≥ 15% (baseline)	59.4	6.5
%, ≥ 9% (predicted)	62.9	18.9
Airway obstruction		
% FEV_1/VC ≤ 70% and FEV_1 ≤ 75%	51.0	3.0
BHR †		
PC_{20} ≤ 32 mg/ml, %	88.2	25.6

* Total sample population consisted of 200 probands and 201 spouses. Different numbers for the SNPs in the following tables are due to missing genotype data.
† Thirty probands were not retested due to an FEV_1 that was too low to be tested safely (FEV_1 ≤ 40% predicted).

RESULTS

Clinical characteristics of population

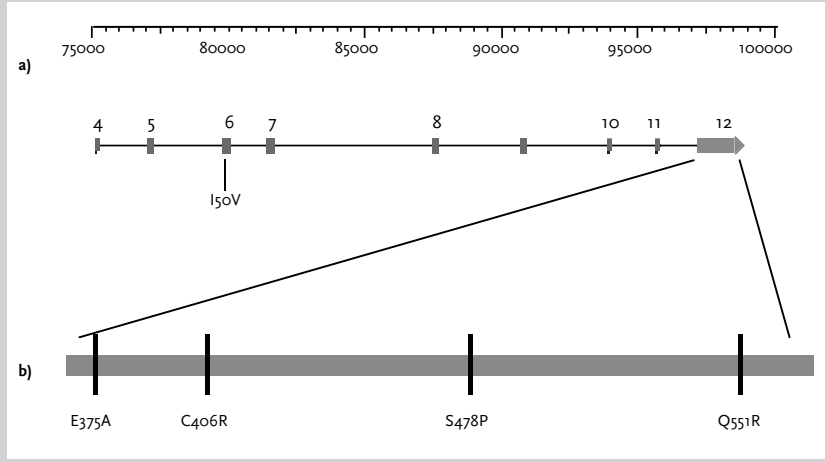
As illustrated in Table 1, the probands and spouses are of similar ages (mean = 52.1 and 51.0 years, respectively). All spouses are included in the table but only BHR-negative spouses (PC_{20} > 32 mg/ml histamine) were used for comparison with the probands for the asthma and BHR phenotypes. All probands and BHR-positive spouses were included in the BHR-positive group for the BHR comparison (with BHR-negative spouses). All

probands were BHR-positive at the time of initial testing. Only 170 of 200 probands were retested, since 30 had an FEV_1 that was too low to be retes ted safely (FEV_1 < 40% predicted); 11.1% of those retested were no longer BHR-positive. A high proportion of the probands (42.9%) were very hyper-responsive to histamine (PC_{20} < 2 mg/ml). Although the probands were not selected for atopy, 81.9% had > 1 positive skin test compared with 31.0% of the spouses.

Association analysis of IL4Rα polymorphisms

The IL4Rα gene contains 12 exons and spans a genomic distance of approximately 51 kb.[17] All of the polymorphisms studied in this report, except I50V, fall within exon 12 of the gene and are therefore within a 528 bp interval (Figure 1). The I50V polymorphism encodes the extracellular portion of the receptor molecule and is located approximately 20 kb upstream from this region.

Figure 1 Genomic structure of the IL4Rα gene.



Genomic structure of the IL4Rα gene. a) The top line represents the genomic sequence of the IL4Rα region of chromosome 16. The bottom line indicates the locations of the exons (boxes) and the introns of IL4Rα aligned to the BAC sequence. b) Four SNPs examined in this association study from exon 12 of IL4Rα.

The probands and spouses from the 200 Dutch families were analyzed with the four exon 12 polymorphisms. Since there was no evidence of association with this SNP and any of the tested phenotypes, the I50V polymorphism was only genotyped in an initial set of 109 probands and 111 spouses. The allele frequencies from this Dutch population were V50 = 0.47, A375 = 0.12, R406 = 0.12, P478 = 0.16, and R551 = 0.20. Each polymorphism was in Hardy-Weinberg equilibrium for the entire population evaluated in this study.

Table 2. Association between Log(IgE) levels and IL4Rα Polymorphisms in Dutch Families.

Polymorphisms	N	Geometric Mean (IU/ml)	Log (Mean ± SD)	P-value*
I50V				
II	65	34.7	1.54 ± 0.66	
IV and VV	155	53.7	1.73 ± 0.71	0.07
E375A				
EE	258	56.2	1.75 ± 0.74	
EA and AA	75	34.7	1.54 ± 0.63	0.02
C406R				
CC	259	55.0	1.74 ± 0.74	
CR and RR	74	32.4	1.51 ± 0.65	0.01
S478P				
SS	204	64.6	1.81 ± 0.72	
SP and PP	81	31.6	1.50 ± 0.6	0.0007
Q551R				
QQ	226	53.7	1.73 ± 0.73	
QR and RR	113	38.0	1.58 ± 0.69	0.06

* Adjusted for age and sex, and comparing the genotypes '1/1' vs. '1/2 and 2/2'. Chi-square tests were performed using log (IgE) values, since the geometric means were not normally distributed.

A significant association was observed between E375A, C406R, and S478P and increased levels of total serum IgE ($p = 0.0007$-0.02; Table 2). S478P was also associated with one or more positive skin tests ($p = 0.03$; Table 3). In each case the common allele (E375, C406, and S478) was associated with the phenotype. Because of the observed relationship between BHR and elevated total IgE levels[13,14], BHR and asthma were also examined with each of the polymorphisms (Table 3). S478P was the only variation associated with this phenotype ($p = 0.02$ - 0.04; Table 3). More of the BHR-positive indivi duals ($PC20 \leq 32$ mg/ml) were homozygous for the S478 allele than BHR-negative individuals.

Linkage disequilibrium between polymorphisms

Since the four polymorphisms in exon 12 of IL4Rα were only 528 bp apart, we tested for linkage disequilibrium (LD) between the SNPs. In this homogenous population, significant LD between E375A, C406R, S478P, and Q551R was observed ($p<10^{-5}$). The most common haplotype was E375, C406, S478, and Q551. In contrast, LD was not observed between these four polymorphisms and the I50V polymorphism. Due to the degree of LD between the four exon 12 SNPs, construction of haplotypes did not improve the evidence for association with the S478P polymorphism individually (data not shown).

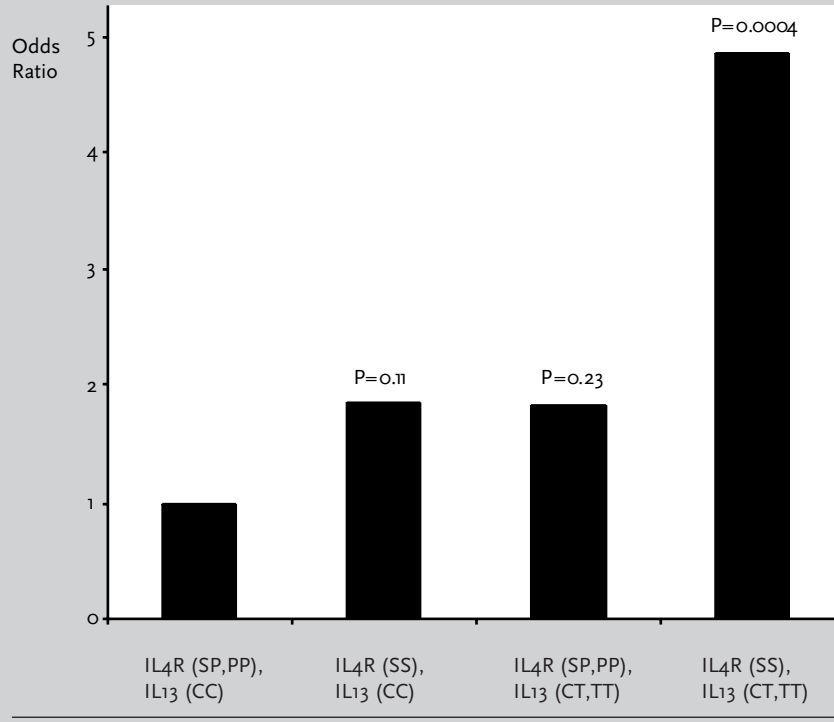
Table 3. Association between asthma and allergy phenotypes and IL4R α Polymorphisms in Dutch families

	Asthma (%)		BHR (%)		Skin Test (%)	
SNPs	Probands	BHR-neg. spouses	$PC_{20} \leq 32$mg/ml	$PC_{20} > 32$mg/ml	≥1	0
I50V	*n=109*	*n=87*	*n=126*	*n=94*	*n=116*	*n=105*
II	0.28	0.29	0.29	0.30	0.26	0.33
IV	0.49	0.48	0.46	0.49	0.48	0.47
VV	0.24	0.23	0.25	0.21	0.26	0.20
	p = 0.85		p = 0.79		p = 0.16	
E375A	*n=150*	*n=112*	*n=178*	*n=124*	*n=166*	*n=137*
EE	0.81	0.71	0.81	0.73	0.82	0.72
EA	0.16	0.28	0.16	0.26	0.16	0.26
AA	0.03	0.01	0.03	0.01	0.02	0.01
	p = 0.06		p = 0.16		p = 0.09	
C406R	*n=148*	*n=108*	*n=175*	*n=120*	*n=162*	*n=134*
CC	0.82	0.71	0.82	0.73	0.82	0.73
CR	0.16	0.28	0.16	0.26	0.16	0.25
RR	0.03	0.01	0.02	0.01	0.02	0.01
	p = 0.05		p = 0.09		**p = 0.05**	

Table 3. Association between asthma and allergy phenotypes and IL4R αPolymorphisms in Dutch families (continued)

S478P	*n=144*		*n=103*	*n=171*		*n=114*	*n=160*		*n=126*
SS	0.77		0.63	0.75		0.67	0.77		0.65
SP	0.20		0.33	0.22		0.30	0.20		0.31
PP	0.03		0.04	0.03		0.04	0.03		0.04
		p = 0.02			**p = 0.04**			**p = 0.03**	
Q551R	*n=151*		*n=114*	*n=181*		*n=127*	*n=169*		*n=140*
QQ	0.68		0.64	0.68		0.65	0.69		0.63
QR	0.26		0.32	0.25		0.31	0.25		0.31
RR	0.05		0.04	0.07		0.04	0.05		0.06
		p = 0.48			p = 0.64			p = 0.2	

Figure 2 Interaction of IL4Rα and IL13 Genotypes.



Bars indicate the odds ratios between the different combinations of genotypes for IL4Rα (S478P) and IL13 (-1111 C/T). The non-risk genotype for each gene was used as the reference odds ratio.

Interaction of IL4Rα and IL13 genotypes

In addition to single SNP and haplotype analysis, an interaction analysis was performed. Because of the biological relationship of IL4Rα and IL13, analysis was performed to determine if individuals with the risk genotypes for both genes were at an increased risk of developing asthma. The most significantly associated SNPs for IL4Rα (S478P with total serum IgE levels) and IL13 (–1111 C/T with BHR; Howard et al., submitted) were examined for potential gene-gene interaction. Each SNP individually was significantly associated with the asthma phenotype. An even greater interaction effect was observed in individuals with the risk genotype for both IL4Rα and IL13 (OR = 4.87, p = 0.0004). This effect was most notable in those individuals homozygous for the common allele for IL4Rα (recessive effect) and in those individuals with the rare allele for IL13 (dominant effect), consistent with the results for the individual SNPs (Table 4, Figure 2). A similar analysis was performed examining total serum IgE levels. In this case, the interaction between the two genes was significant, but less than the effect of IL4Rα S478P alone.

Table 4. Interaction of IL4Rα (S478P) and IL13 (-1111 C/T) Genes on Asthma

		Number of individuals				
IL4Rα	**IL13**	**Case**	**Control**	**OR**	**95% C.I**	**P-values**
(SP, PP)	(CC)	16	22	1		
(SS)	(CC)	62	46	1.85	0.88-3.92	0.11
(SP, PP)	(CT, TT)	16	12	1.83	0.68-4.92	0.23
(SS)	(CT, TT)	46	13	4.87	2.00-11.86	0.0004

DISCUSSION

We have observed significant association between IL4Rα SNPs and atopic phenotypes, most significantly, total serum IgE levels. In addition, gene-gene interaction was detected between IL4Rα and IL13, based on one polymorphism in each gene. Specific alleles of IL4Rα have been associated with atopy and asthma phenotypes and increased gene activity in previous stu dies. We have evaluated five polymorphisms in the IL4Rα gene in a well-characterized Dutch population ascertained through a proband with asthma for association with susceptibility to asthma and atopy and associated phenotypes. Analysis of three polymorphisms within exon 12 of IL4Rα revealed an association with increased total serum IgE levels and several phenotypes associated with asthma. No association was observed with I50V, the only polymorphism examined in the extracellular-coding portion of the gene. We have also identified a significant gene-gene interaction between IL4Rα and IL13, which has been previously shown to be highly significantly associated with the expression of hyperresponsiveness. Individuals with the risk genotype for both genes are at almost a five-fold increase of developing asthma compared to individuals with both non-risk genotypes.

Asthma is an inflammatory airways disease characterized by bronchial hyperresponsiveness and variable airways obstruction. Atopic traits such as elevated total serum IgE levels and positive skin responses are closely associated with this condition and may predict the development of symptomatic asthma.[13,14,26] Interleukin 4 and its receptor play key roles in the regulation of IgE levels and are therefore excellent candidate genes for the development or expression of atopic phenotypes associated with asthma. In addition to the most significant association between IL4Rα and higher total serum IgE levels, we also observed an increased prevalence of the homozygous S478 genotype in BHR-positive individuals (p=0.04). This observation is probably due to the strong correlation between asthma and atopy related phenotypes described by others[13], and also noted in this group of Dutch families (Table 1). The mean total serum IgE in the probands from this cohort was 93.0 IU/ml compared to 26.2 IU/ml in their spouses.

Further evidence for the importance of IL4R in atopic disorders and asthma comes from recent therapeutic reports on the use of soluble IL4R in these conditions.[27,28] These studies show that an IL4R antagonist can effect immunoglobulin synthesis and, in early clinical trials, improve respiratory function and asthma control. Our results, in addition to others identifying IL4Rα as a key component of atopy or asthma-related phenotypes, suggest that screening of this gene may identify those individuals at risk of developing asthma due to the IL4/IL13 pathway. This may lead to better treatment efficacy using appropriate therapeutic interventions, including soluble IL4Rα. In addition, it will be of interest to determine whether IL4Rα polymorphisms affect the response to an IL4R antagonist, as has been shown with other pharmacogenetic relationships.[29,30]

There is a large degree of variability in the results of association studies with IL4Rα polymorphisms. These differences can only be addressed by examining multiple polymorphisms within the gene, as was performed in this and some other studies.[17,18] For instance, two groups have reported an increase in IgE levels and association with an atopic phenotype with two different polymorphisms.[19;20] These reports also provided functional data to corroborate the observed phenotype in their patient population. A potential reason for differences in these previous studies is that only one polymorphism was analyzed; thus, the genotype for the remaining polymorphisms was unknown. For example, if the "true" susceptibility allele causing increased total serum IgE levels is the E375 allele, this allele may have been in linkage disequilibrium with the R551 allele in the original study.[19] Likewise, this allele may have been in disequilibrium with the I50 allele in the study in the Japanese population.[20] Unless the genotype at several of the polymorphic sites is known within the IL4Rα gene and a ha plotype is constructed, it may be difficult to determine which polymorphism is responsible for a given phenotype and a subsequent increased or decreased expression of an allergic phenotype.

This report did not show evidence that the I50V and the Q551R are the primary polymorphisms responsible for allergy susceptibility. Instead, our data suggest that the polymorphism associated with asthma and atopic phenotypes is either the E375A, C406R, S478P variation, a combination of these loci, or an additional yet unknown variant in linkage disequilibrium with these polymorphisms. Because of the complete linkage disequilibrium between the exon 12 polymorphisms in this population, it is impossible to determine which, if any, is the true susceptibility polymorphism without functional data. Alternatively, association studies in a population where LD does not exist between these polymorphisms may help to define the single variant, or the combination of variants that contribute to susceptibility. It is possible that a specific haplotype induces conformational changes that together increase the overall effect.[17] The data presented in this report are consistent with the recent study reporting association of the P478 and R551 haplotype with lower IgE levels.[17]

The evidence provided in this report and in previous studies suggests that IL4Rα may represent a susceptibility gene for asthma and atopy. This locus may also be responsible for modulating asthma severity. In an association study of 149 asthma patients and controls, a significant association was observed with the Q551R polymorphism and FEV_1 (percent predicted) when stratified by mild (FEV_1 >80%), moderate (FEV_1 60%-80%), or severe (FEV_1 <60%).[31] Of the severe asthmatics, 52.6% were homozygous for the R551 allele, compared with 10.5% of the mild asthmatics (p = 0.015).[31] We did not observe this association in our data with the Q551R polymorphism.

Because of the biological interaction between IL4Rα and IL13 in the development of atopy, we have assessed whether polymorphisms in these genes have an interactive effect on expression of atopy and asthma. Indeed, we have identified a significant interaction between polymorphisms in diffe rent genes controlling asthma, i.e., IL4Rα and IL13. Individuals with the risk genotype for the SNPs examined in each gene were almost five times more likely to develop asthma compared to individuals with both non-risk genotypes (Table 4, Figure 2). This effect was much more significant than the effect of either gene alone. While we also observed a significant interaction between IL4Rα and IL13 controlling total serum IgE levels (p = 0.005), this effect was much less than the association of the S478P polymorphism alone with total serum IgE levels (Table 2). This observation suggests a potential etiology, at least in some individuals, for asthma susceptibility. We have previously demonstrated that variants in IL13 contribute significantly to BHR susceptibility (p = 0.007; Howard et al, submitted), but not to total serum IgE levels. In this study, we report a significant association of variants in IL4Rα with elevated total serum IgE levels and only borderline significance with BHR and asthma. More importantly, individuals with the risk genotypes for both of these genes are much more susceptible to asthma, which is a composite of both elevated total serum IgE levels and BHR. Individually, variants of IL4Rα and IL13 may lead to susceptibility of elevated total serum IgE levels and BHR, respectively. Combined, however, they lead to a marked increased risk of developing asthma.
This observation suggests that there is a genetic, as well as biological, interaction between the IL4Rα and IL13 gene products. The IL13 promoter polymorphism most likely effects transcriptional regulation of the gene, whereas the S478P variation of IL4Rα has been suggested to alter the conformation of the receptor protein and possibly modify downstream signaling.[17] Increased amounts of IL13 cytokine may enhance the effect of the altered IL4R receptor complex, intensifying the downstream response. Functional assays are necessary to support this hypothesis.

Asthma and allergy are conditions with complex immunologic, physiologic, and inflammatory etiologies. This is supported by biologic studies in animal models and human studies as well as the results of numerous genome screens conducted in various populations. These genome screens

revealed that different chromosomal loci contribute to asthma or allergy phenotypes in different ethnic groups.[e.g.4,32-36] It is apparent that IL4Rα contributes to atopic phenotypes in at least some populations based on this and previous studies.[18-21] In addition, SNPs in IL13 have been associa ted with increased risk of allergic asthma[37], higher total serum IgE levels[38] [39] and asthma[40] (Howard et al., submitted). Functional studies examining the individual roles of IL4 and IL13 gene products as well as their biologic interactions and the resulting downstream responses will provide valuable insight into the overall mechanisms that cause susceptibility to asthma and atopy. In summary, this study supports observations in other populations suggesting that polymorphisms in IL4Rα are associated with asthma and atopy related phenotypes. Increased total serum IgE levels were signi ficantly associated with three of the five polymorphisms tested within the gene. An additional observation was a significant interaction between polymorphisms in IL4Rα and IL13 that contributes to asthma susceptibility.

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