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Report

Gene-Gene Interaction in Asthma: *IL4RA* and *IL13* in a Dutch Population with Asthma

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Asthma is a common respiratory disease that is characterized by variable airways obstruction caused by acute and chronic bronchial inflammation; associated phenotypes include bronchial hyperresponsiveness (BHR), elevated total serum immunoglobulin E (IgE) levels, and skin tests positive to common allergens. Binding of interleukin-13 (IL13) or interleukin-4 (IL4) to the IL4 receptor (IL4R) induces the initial response for Th2 lymphocyte polarization. Both IL13 and IL4 are 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. We have investigated five *IL4RA* single-nucleotide polymorphisms in a population of Dutch families ascertained through a proband with asthma. By considering the probands and their spouses as an unrelated sample, we observed significant associations of atopy and asthma-related phenotypes with several *IL4RA* polymorphisms, including S478P and total serum IgE levels ($P = .0007$). A significant gene-gene interaction between S478P in *IL4RA* and the -1111 promoter variation in *IL13,* previously shown to be associated with BHR ($P = .003$), was detected. Individuals with the risk genotype for both genes were at almost five times greater risk for the development of asthma compared to individuals with both nonrisk genotypes ($P = .0004$). These data suggest that variations in *IL4RA* contribute to elevated total serum IgE levels, and interaction between *IL4RA* and *IL13* markedly increases an individual's susceptibility to asthma.

Asthma is characterized by bronchial hyperresponsiveness (BHR) and variable airways obstruction. Atopic traits such as elevated total serum immunoglobulin E (IgE) levels and positive skin responses are closely associated with this disorder and may predict the development of symptomatic asthma (Burrows et al. 1989, 1995; Sears et al. 1991). The interleukin-4 (IL4) receptor (IL4R [MIM 147781]), on chromosome 16p, is a key component in the induction of the Th2 lymphocyte phenotype and IgE production. A further role that IL4 plays in the pathogenesis of asthma has been indicated from actively sensitized *Il4* knockout mice. Neither specific IgE induction nor BHR was detected in these mice (Brusselle et al. 1994, 1995), suggesting a critical role for the IL4/IL4R pathway in these phenotypes. Interleukin-13 (IL13 [MIM 147683]), on chromosome 5q, also contributes to the maintenance of the Th2 lymphocyte profile that leads to elevated baseline IgE levels, and murine models have demonstrated the critical nature of IL13 independent of IL4 (Grunig et al. 1998). The pleiotropic effects of IL13 and IL4 are mediated through the IL4R, which is composed of the high-affinity α subunit and either the common γ subunit or the IL13 receptor (IL13R) α subunit. The IL13R is composed of one IL4Rα subunit and either a low-affinity IL13Rα1 (Aman et al. 1996) or a high-affinity IL13Rα2 subunit (Gauchat et al. 1997). The complete IL4R is composed of an IL4Rα subunit and an IL4Rγ subunit. Therefore, it is possible that different polymorphisms in these receptors, as well as in the IL13 and IL4 cytokines, contribute to the complex regulation of atopy or asthma phenotypes.

At least 16 single-nucleotide polymorphisms (SNPs) in the *IL4RA* gene have been reported (Deichmann et al. 1997; Hershey et al. 1997; Mitsuyasu et al. 1998; Kruse et al. 1999*b;* Ober et al. 2000; Hackstein et al. 2001). The I50V, S478P, and Q551R variants have been asso-

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ciated with a greater risk for atopy (Hershey et al. 1997; Kruse et al. 1999*b*), a greater risk for atopic asthma (Mitsuyasu et al. 1998), and variation in IgE levels (Kruse et al. 1999*b*). In addition, specific variants were shown to modulate the activity of *IL4RA* (Hershey et al. 1997; Deichmann et al. 1998) and the levels of soluble IL4RA (Hackstein et al. 2001). Eight polymorphisms in *IL4RA* (seven in exon 12) were studied in inbred and ethnically diverse outbred populations, and significant evidence for an association between several of these variants, as well as the resulting haplotypes, and asthma and atopy was obtained (Ober et al. 2000). We evaluated five polymorphisms (four in exon 12) in *IL4RA* in a Dutch population that was ascertained through a proband with asthma, to determine the importance of these variants in regard to susceptibility to and expression of asthma and atopy in this population. On the basis of the biological role of IL4R, our primary hypothesis was that differences in total serum IgE levels may be due to different *IL4RA* genotypes.

Considering the biological interaction between IL13 and IL4R, we also sought to examine the potential gene-gene interaction between *IL13* and *IL4RA*. We previously reported that the −1111 C/T variant of *IL13* contributes significantly to BHR and asthma susceptibility ($P = .003$ and $P = .005$, respectively) but not to total serum IgE levels (Howard et al. 2001). *IL4RA* has previously been associated with numerous atopic conditions. Since asthma is a disease characterized by BHR and allergic responses, we hypothesized that individuals with the risk genotypes for both of these genes would have a greater risk for asthma.

Probands with clinical asthma were originally characterized between 1962 and 1975 (Panhuysen et al. 1998; Xu et al. 2000). Between 1990 and 1998, 200 probands, their spouses, children and available grandchildren were studied. All individuals underwent spirometry, reversibility to 800 μg albuterol, and bronchial responsiveness testing to histamine (Panhuysen et al. 1998). Skin testing was performed with 16 common aeroallergens (Panhuysen et al. 1998), and total serum IgE levels were measured (Xu et al. 2000). The probands and spouses represent an appropriate unrelated sample for association studies and were similar in age, which removes the possible confounding effect that age has on changes in BHR, in IgE, and in atopy. This study was approved by the Medical Ethics Committees of the University Hospital, Groningen, and Wake Forest University School of Medicine. All case subjects gave written informed consent.

The probands and spouses were of similar ages (mean ages were 52.1 years and 51.0 years, respectively) (table 1). All probands were BHR positive at the time of initial testing; 11.1% of those retested were no longer BHR positive. Approximately 43% of the probands had high levels of BHR ($PC_{20} \leq 2$ mg/ml). Although the probands were not selected for atopy, 81.9%, compared to 31.0% of the spouses, had at least one positive skin test.

Table 1

Clinical Characteristics of Probands and Spouses in a Dutch Population

Characteristic	Probands	Spouses
Sex (M:F)	124:76	76:125
Age (mean ± SD)	52.1 ± 8.4	51.0 ± 9.2
Total IgE (geometric mean [IU])	93.0	26.2
⩾1 Positive skin test (%)	81.9	31.0
FEV_1, predicted premedication (% [mean])	69.6	98.4
Reversibility, ⩾15% (% [baseline])	59.4	6.5
BHR[a], PC_{20} ⩽32 mg/ml (%)	88.2	25.6

NOTE.—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.

[a] Thirty probands were not retested owing to an FEV_1 that was too low ($FEV_1 \leq 40\%$ predicted) to be tested safely.

IL4RA contains 12 exons and spans a genomic distance of ~51 kb (Kruse et al. 1999*a*). We chose to evaluate five polymorphisms that previously had been shown to be associated with asthma- or allergy-related phenotypes. All of these polymorphisms, except I50V, are within a 528-bp interval in exon 12. The I50V polymorphism encodes the extracellular portion of the receptor molecule and is located ~20 kb upstream from this region.

For the I50V, E375A, and C406R polymorphisms, PCR was performed using standard conditions and by the primers described elsewhere (Mitsuyasu et al. 1998), for I50V, or the primers 5′-CAGCATGGTGCCCAGTGGAG-3′ and 5′-CTTGGGAACTCATCCCAGGGC-3′, for E375A and C406R. The E375A and C406R polymorphisms were contained within the same 334-bp PCR product and were digested with the restriction enzymes *Cac*8I (E375A) or *Tsp*45I (C406R), to distinguish the alleles. The I50V PCR products were digested with the restriction enzyme *Msl*II. The S478P and Q551R polymorphisms were genotyped using a variation of allele-specific PCR that utilizes fluorescent dye and automated sequencer technology called "fluorescent allele-specific PCR" (Howard et al. 1999). Allele-specific primers were designed for both polymorphisms by use of the known *IL4RA* sequence (GenBank accession number X52425) and previously published data (Hershey et al. 1997; Kruse et al. 1999*b*). Two allele-specific forward primers were designed with different fluorescent labels and an addition of two nucleotides to one. The S478-specific primer was (tetrachloro-fluorescein and 6-carboxy-fluorescein) 5′-TGCTTACCGCAGCTTCAGCAACT-3′, and the P478-specific primer was (6-carboxy-fluorescein) 5′-CTTACCGCAGCTTCAGCAACC-3′. The common reverse primer was 5′-TTTCTGGCTCAGGTTGG-

GGC-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. Fragments were separated and analyzed by ABI 377 DNA sequencers. The *IL13* −1111 promoter SNP was genotyped using a PCR-RFLP assay by the primers 5′-ATGCCTTGTGAGGAGGGTCAC-3′ and 5′-CCAGTCTCTGCAGGATCAACC-3′. PCR products were digested with *Nhe*I (New England Biolabs), and the alleles were resolved by electrophoresis on a 2% agarose gel.

The allele frequencies for the five *IL4RA* SNPs from this Dutch population were 0.47, for V50; 0.12, for A375; 0.12, for R406; 0.16, for P478; and 0.20, for R551. Each polymorphism was in Hardy-Weinberg equilibrium, and significant ($P < 10^{-5}$) linkage disequilibrium (LD) between E375A, C406R, S478P, and Q551R was observed (Zaykin et al. 1995). LD between these four polymorphisms and the I50V polymorphism was not observed. These results are consistent with those reported by Ober et al. (2000). The most common haplotype was E375, C406, S478, and Q551. Owing to the nearly complete LD between the four exon 12 SNPs, haplotypes did not improve the evidence for individual association with the S478P polymorphism (data not shown).

Analysis was performed first for total serum IgE levels, then for skin-test responsiveness to common allergens, for asthma, and for BHR. Total serum IgE was analyzed as a quantitative trait, after being logarithm transformed, to approximate a normal distribution, and being age and sex adjusted. Differences between groups were tested by ANOVA, *t* test, and multiple-regression analysis. Individuals were considered responsive to an allergen skin test, if one or more test showed a mean wheal diameter ⩾5 mm. For BHR, case subjects were defined as all probands and spouses with a $PC_{20} \leqslant 32$ mg/ml histamine. The control group for both BHR-positive and asthma case subjects was comprised of BHR-negative spouses ($PC_{20} > 32$ mg/ml). Each of the biallelic polymorphisms was analyzed by comparing differences, between case subjects and controls, of genotype frequencies. Assuming a dominant model, because of the small number of homozygotes for the rare allele, χ^2 tests were performed. No corrections were made for multiple comparisons for three reasons: first, because of the biological role that IL4RA plays, our primary hypothesis was that mean IgE levels would differ between genotypes; second, since the phenotypes tested (asthma, BHR, total serum IgE levels, and skin-test response) are strongly associated with each other in this population, the statistical analyses do not represent independent tests; third, we performed tests for association with phenotypes that have been observed by other investigators, both to confirm previous results and to better characterize asthma susceptibility in our population. For the interaction analysis, *P* values were determined by multiple-regression analysis, with adjustments for age and sex.

A significant association between E375A, C406R, and S478P and total serum IgE levels was observed ($P = .0007$–$.02$; table 2). S478P was also associated with skin responsiveness ($P = .03$; table 3). In each instance, the common allele (E375, C406, and S478) was associated with higher total serum IgE levels and at least one positive skin test. S478P was the only variation associated with BHR or asthma ($P = .02$–$.04$; table 3). More of the BHR-positive individuals were homozygous for the S478 allele than BHR-negative individuals.

The SNPs with the strongest evidence for association for *IL4RA* (S478P with total serum IgE levels) and *IL13* (−1111 C/T with BHR; Howard et al. 2001) were examined for potential gene-gene interaction for asthma susceptibility. Individually, each SNP was significantly associated with the asthma phenotype. An interaction effect was observed in individuals with the risk genotypes for both *IL4RA* and *IL13* (odds ratio [OR] = 4.87; $P = .0004$; fig. 1). This effect was most notable in those individuals homozygous for the common allele for *IL4RA* (recessive effect) and in those individuals with the rare allele for *IL13* (dominant effect), consistent with the results for the individual SNPs (fig. 1). A similar analysis examining total serum IgE levels was performed. In this analysis, the interaction between the two genes was significant ($P = .005$) but was similar to the effect of *IL4RA* S478P alone ($P = .0007$).

Table 2

Association between log(IgE) Levels and *IL4RA* Polymorphisms in Dutch Study

Polymorphism	*N*	Geometric Mean (IU/ml)	log(IgE)[a] (Mean ± SD)	*P*
I50V:				.07
II	65	34.7	1.54 ± .66	
IV and VV	155	53.7	1.73 ± .71	
E375A:				.02
EE	258	56.2	1.75 ± .74	
EA and AA	75	34.7	1.54 ± .63	
C406R:				.01
CC	259	55.0	1.74 ± .74	
CR and RR	74	32.4	1.51 ± .65	
S478P:				.0007
SS	204	64.6	1.81 ± .72	
SP and PP	81	31.6	1.50 ± .6	
Q551R:				.06
QQ	226	53.7	1.73 ± .73	
QR and RR	113	38.0	1.58 ± .69	

[a] log(IgE) levels were adjusted for age and sex and were analyzed for the genotypes "1/1" versus "1/2 and 2/2."

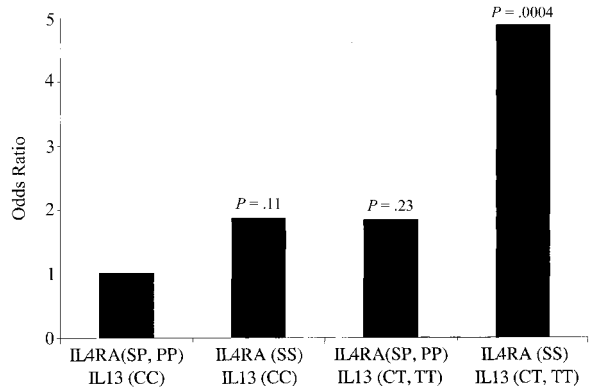
Table 3

Association between Asthma and Atopic Phenotypes and *IL4RA* Polymorphisms in Dutch Study

Polymorphism	Asthma (Frequency)		BHR (Frequency)		No. of Positive Skin Test(s) (Frequency)	
	Probands (n = 109)	BHR-Negative Spouses (n = 87)	$PC_{20} \leq 32$ mg/ml (n = 126)	$PC_{20} > 32$ mg/ml (n = 94)	≥1 (n = 116)	0 (n = 105)
I50V:						
II	.28	.29	.29	.30	.26	.33
IV	.49	.48	.46	.49	.48	.47
VV	.24	.23	.25	.21	.26	.20
	P		P		P	
	.85		.79		.16	
	Probands (n = 150)	BHR-Negative Spouses (n = 112)	$PC_{20} \leq 32$ mg/ml (n = 178)	$PC_{20} > 32$ mg/ml (n = 124)	≥1 (n = 166)	0 (n = 137)
E375A:						
EE	.81	.71	.81	.73	.82	.72
EA	.16	.28	.16	.26	.16	.26
AA	.03	.01	.03	.01	.02	.01
	P		P		P	
	.06		.16		.09	
	Probands (n = 148)	BHR-Negative Spouses (n = 108)	$PC_{20} \leq 32$ mg/ml (n = 175)	$PC_{20} > 32$ mg/ml (n = 120)	≥1 (n = 162)	0 (n = 134)
C406R:						
CC	.82	.71	.82	.73	.82	.73
CR	.16	.28	.16	.26	.16	.25
RR	.03	.01	.02	.01	.02	.01
	P		P		P	
	.05		.09		.05	
	Probands (n = 144)	BHR-Negative Spouses (n = 103)	$PC_{20} \leq 32$ mg/ml (n = 171)	$PC_{20} > 32$ mg/ml (n = 114)	≥1 (n = 160)	0 (n = 126)
S478P:						
SS	.77	.63	.75	.67	.77	.65
SP	.20	.33	.22	.30	.20	.31
PP	.03	.04	.03	.04	.03	.04
	P		P		P	
	.02		.04		.03	
	Probands (n = 151)	BHR-Negative Spouses (n = 114)	$PC_{20} \leq 32$ mg/ml (n = 181)	$PC_{20} > 32$ mg/ml (n = 127)	≥1 (n = 169)	0 (n = 140)
Q551R:						
QQ	.68	.64	.68	.65	.69	.63
QR	.26	.32	.25	.31	.25	.31
RR	.05	.04	.07	.04	.05	.06
	P		P		P	
	.48		.64		.2	

This observation suggests a potential interaction that may be important to the pathogenesis of asthma. We have previously demonstrated that variants in *IL13* contribute significantly to BHR and asthma susceptibility ($P = .003$ and $P = .005$, respectively; Howard et al. 2001) but not to total serum IgE levels. Here, we report a significant association between variants in *IL4RA* and elevated total serum IgE levels and an only-borderline-significant association between variants in *IL4RA* and BHR and asthma. More importantly, individuals with the risk genotypes for both of these genes are much more susceptible to asthma; common features of asthma include both elevated total serum IgE levels and BHR.

These data suggest that there is a genetic, as well as



No. of cases	16	62	16	46
No. of controls	22	46	12	13

Figure 1 Interaction of *IL4RA* and *IL13* genotypes. Bars indicate the ORs between the different combinations of genotypes for *IL4RA* (S478P) and *IL13* (−1111 C/T). The nonrisk genotype for each gene was used as the reference OR.

biological, interaction between the *IL4RA* and *IL13* gene products. The *IL13* promoter polymorphism most likely affects transcriptional regulation of the gene, whereas it has been suggested that the S478P variation of *IL4RA* alters the conformation of the receptor protein and possibly modifies downstream signaling (Kruse et al. 1999*b*). Increased amounts of IL13 cytokine may enhance the effect of the altered IL4R complex, intensifying the downstream response. Functional assays are necessary to support this hypothesis.

Further evidence for the importance of IL4R in atopic disorders and in asthma comes from recent therapeutic reports of the use of soluble IL4R in these conditions (Borish et al. 1999; Henderson et al. 2000). These reports show that an IL4R antagonist can affect immunoglobulin synthesis and, in early clinical trials, can improve respiratory function and asthma control. Our results, in addition to other reports identifying *IL4RA* as a key component of atopy or asthma-related phenotypes, suggest that screening of this gene may identify those individuals at risk for developing asthma, owing to the IL13/IL4/IL4R pathway. This may lead to better treatment efficacy by appropriate therapeutic interventions, including soluble IL4RA. In addition, it will be of interest to determine whether *IL4RA* polymorphisms affect the response to an IL4R antagonist, as has been shown with other pharmacogenetic relationships (Drazen et al. 1999; Drysdale et al. 2000).

There is a large degree of variability in the results of association studies with *IL4RA* polymorphisms. However, if only one or a few polymorphisms were analyzed, it was unknown whether the effect observed was due to LD to the "true" susceptibility allele. We did not observe any evidence that I50V and Q551R are the primary polymorphisms responsible for allergy susceptibility. Instead, our data suggest that the polymorphism associated with asthma and atopic phenotypes is either E375A, C406R, S478P, a combination of these loci, or an additional, as-yet-unknown variant in LD with these polymorphisms (as also concluded in other populations [Ober et al. 2000]). Our results are consistent with a previous study reporting association of the P478 and R551 haplotype with lower IgE levels (Kruse et al. 1999*b*). Because of the strong LD between the exon 12 polymorphisms in this population, it is impossible to determine without functional data which, if any, is the true susceptibility polymorphism. It is possible that a specific haplotype induces conformational changes that together increase the overall effect (Kruse et al. 1999*b*).

Asthma and allergy are conditions with complex immunologic, physiologic, and inflammatory etiologies. It is apparent, on the basis of this and previous studies (Hershey et al. 1997; Deichmann et al. 1998; Mitsuyasu et al. 1998; Ober et al. 2000), that *IL4RA* contributes to atopic phenotypes in at least some populations. In addition, SNPs in *IL13* have been associated with increased risk for allergic asthma (van der Pouw Kraan et al. 1999), higher total serum IgE levels (Graves et al. 2000; Liu et al. 2000) and asthma (Heinzmann et al. 2000; Howard et al. 2001). Functional studies examining the individual roles of *IL13* and *IL4* 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 to atopy.

In summary, this study provides evidence for a significant interaction between *IL4RA* and *IL13* that contributes to asthma susceptibility. In addition to the interaction, our results support observations in other populations suggesting that polymorphisms in *IL4RA* alone are associated with asthma- and atopy-related phenotypes. Increased total serum IgE levels were significantly associated with three of the five polymorphisms in the gene that were tested. In the same manner, additional genes in the same pathway, such as *IL4*, *STAT6*, and *JAK1*, may also contribute to the genetic interaction and account for an additional risk for asthma in specific individuals.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for *IL4RA* sequence [accession number X52425])
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for IL4R [MIM 147781] and IL13 [MIM 147683])

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