

The R576 IL-4 receptor α allele correlates with asthma severity

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Background: Atopic disorders, including asthma, are very prevalent, affecting up to 40% of populations, and their incidence is on the rise. Although environmental factors are important in the development of atopy, there is a strong genetic predisposition. Several genes and chromosomal regions have been linked to atopy and asthma, supporting the polygenic nature of these disorders. IL-4 and IL-13 are T_H2 cytokines with numerous activities that contribute to allergic inflammation and asthma. Both IL-4 and IL-13 use the IL-4 receptor α chain (IL-4R α) as a component of their respective receptor systems. Allelic variants of IL-4R α have been reported, and the R576IL-4R α allele was recently shown to be a risk factor for atopy.

Objective: We sought to determine whether the R576 allele was associated with the prevalence or clinical severity of asthma.

Methods: We developed a rapid, reliable, PCR-based assay to screen individuals for the R576IL-4R α allele and used this assay to genotype prospectively recruited individuals with asthma (n = 149) and control subjects (n = 57).

Results: There was a strong association of R576IL-4R α with the prevalence and clinical severity of asthma. In a prospective cohort, homozygosity for R576 was significantly increased in individuals with asthma (n = 149, *P* = .03; relative risk 8.2) compared with controls (n = 57). Furthermore, 1 or 2 copies R576IL-4R α correlated with asthma severity establishing a genotype-phenotype relationship and suggesting a gene dosage effect.

Conclusions: Thus R576IL-4R α acts as an allergic asthma susceptibility and disease-modifying gene and may serve as a clinically useful marker of asthma severity. (*J Allergy Clin Immunol* 1999;104:1008-14.)

Key words: Asthma, atopic hypersensitivity, genetic predisposition to disease, IL-4, IL-13, bronchial hyperreactivity

Asthma is an inflammatory disorder of the airways. It is the most common chronic disease of childhood and the most frequent reason for pediatric hospital admission,¹

Abbreviations used

- I75: IL-4 receptor α chain allelic variant with isoleucine at position 75 of the polypeptide (numbering including 25 amino acid signal peptide)
- IL-4R α : IL-4 receptor α chain
- ITIM: Immunotyrosine-based inhibitory motif
- Q576: IL-4 receptor α chain allelic variant with glutamine at position 576 of the polypeptide (numbering including 25 amino acid signal peptide)
- R576: IL-4 receptor α chain allelic variant with arginine at position 576 of the polypeptide (numbering including 25 amino acid signal peptide)
- RFLP: Restriction fragment length polymorphism
- SHP-1: SH2 domain containing tyrosine phosphatase-1
- SSCP: Single-strand conformation polymorphism
- V75: IL-4 receptor α chain allelic variant with valine at position 75 of the polypeptide (numbering including 25 amino acid signal peptide)

and its incidence is on the rise.² Although environmental influences are important in the development of asthma, there is a strong genetic predisposition. Children who have one parent with asthma have about a 25% chance for development of asthma, and those whose mother and father both have asthma may have as high as a 50% risk of disease.³ A study of 5864 Norwegians found that the relative risk for development of asthma was 17.9 among twins whose identical co-twin had a positive history of asthma versus only 2.3 for fraternal twins.⁴ In another study of Finnish twins, in families where one of the parents was asthmatic, up to 87% of the susceptibility to asthma was explained by genetic influences.⁵ Among infants and young children who have wheezing with viral infections, a personal history or a family history of allergy is the factor most strongly associated with the development of asthma.⁶ Several candidate genes and loci have been linked to asthma and atopy, including IL-4,^{7,8} IL-4 receptor α chain (IL-4R α),^{9,10} HLA complex,^{11,12} β chain of the high-affinity Fc ϵ receptor,¹³ β_2 -adrenergic receptor,¹⁴ and chromosomal regions including the cytokine cluster on 5q31-33,^{7,15,16} supporting the complex polygenic nature of these diseases. Delineating the genes that contribute to the development of asthma and dissecting the mechanisms by which these genes alter the host response to environmental challenge (antigen, viral, etc) are key steps to furthering our knowledge of the pathogenesis of asthma.

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Several of the atopy susceptibility genes that have been identified regulate the production of IgE or mediate its effector functions. IL-4 is secreted by T_H2 cells and mast cells and, along with IL-13, it is the major cytokine responsible for the induction of IgE synthesis by B cells.^{17,18} Furthermore, IL-4 acts on T_H0 cells and promotes their progression to the T_H2 phenotype,^{19,20} ultimately leading to the secretion of more IL-4 and other T_H2 -derived cytokines,²¹ thereby perpetuating the allergic cascade. IL-4 has numerous other activities that contribute to allergic inflammation, including the induction of adhesion molecules on endothelial cells important for eosinophil migration²² and the induction of CD23²³ and MHC class II.^{24,25} Like IL-4, IL-13 plays a critical role in the development of allergic inflammation. Recently IL-13 has been shown to be a key mediator of asthma independent of IL-4 in mouse models whereby IL-13 blockade prevented allergen-induced airway inflammation.^{26,27} Both IL-4 and IL-13 exert their biologic activities by interacting with their respective cell surface receptors, and both receptor systems share the α chain of the IL-4 receptor (IL-4R α).^{28,29} The central role played by the IL-4R α in regulating IL-4 and IL-13 responses makes it a likely candidate gene for atopy and asthma.

We recently reported the identification of a novel allele of the IL-4R α that confers atopy susceptibility.⁹ This allele contains an arginine in place of a glutamine at position 576 of the protein (numbering including the signal peptide) (R576) and occurs with a frequency of 20% in the general population. Individuals who possess 1 or 2 copies of this allele had a significantly increased (9.3-fold) relative risk toward the atopic phenotype compared with the wild-type variant with glutamine at position 576 (Q576). The Q to R substitution occurs immediately adjacent to a tyrosine residue, Y575, in the cytoplasmic domain of IL-4R α and may alter the efficiency of interactions of Y575 with signaling intermediates such as SH2 domain containing tyrosine phosphatase-1 (SHP-1). The R576 allele strongly segregated with atopy, but its effect on the asthmatic phenotype has not been evaluated. In the current study we have characterized and genotyped a group of nonallergic and allergic adults with asthma and compared them with a sample of control individuals to determine whether the presence of the R576 IL-4R α allele was associated with asthma and whether the presence of the R576 allele influenced the severity of asthma in affected individuals.

METHODS

Subjects

One hundred forty-nine unrelated adult asthmatic patients were prospectively recruited from allergy offices affiliated with the University of Cincinnati Medical Center. Asthma was diagnosed in accordance with the American Thoracic Society criteria by demonstrating a 12% or greater increase in FEV₁ after a bronchodilator or after a 2-week trial of oral corticosteroids.^{30,31} Pulmonary function testing was performed according to the 1994 revised American Thoracic Society guidelines with use of the Pneumedics Dataloop (Norwalk, Conn).³¹ Subjects underwent skin prick testing includ-

ing positive and negative controls and a panel of 14 common environmental antigens indigenous to the Ohio valley (A.L.K. Laboratories, Wallingford, Conn). The patients were instructed to discontinue antihistamines before skin testing in accordance with the published guidelines.³² Patients were divided into allergic and nonallergic groups on the basis of the results of the skin tests. Those with positive reactions (≥ 3 mm wheal with erythema) to 1 or more antigens tested were designated allergic. Baseline FEV₁ values were used to classify the patients into mild, moderate, and severe asthma groups. As outlined in the "Guidelines for the Diagnosis and Management of Asthma, Expert Panel Report 2,"³³ those with a baseline FEV₁ greater than or equal to 80% of predicted were classified as having mild asthma, those with an FEV₁ between 60% and 80% of predicted were classified as having moderate asthma, and those with a baseline FEV₁ of less than 60% of predicted were classified as having severe asthma. There were equal proportions (24%) of smokers (past or current history) in the allergic and nonallergic asthma groups, and there were no significant differences in smoking between the genotypic groups. For the nonallergic, nonasthmatic control group, healthy unrelated volunteers were prospectively recruited from the employee pool of the University of Cincinnati Medical Center and Children's Hospital Medical Center, Cincinnati. Individuals were excluded from this group if they reported a history of allergies, asthma, chronic cough, chronic obstructive pulmonary disease, or smoking. They underwent skin prick testing as outlined above, and those who demonstrated no positive reactions (excluding histamine) were included in the control group. Informed consent was obtained from all participants in these studies. These studies were approved by the Children's Hospital Medical Center Institutional Review Board.

Single-stranded conformational polymorphism analysis

Complementary DNA was synthesized from RNA isolated from EBV-transformed human B cell lines or PBMCs with use of a reverse transcription kit from Promega (Madison, Wis) and analyzed by single-stranded conformational polymorphism analysis (SSCP) for the Q576R IL-4R alleles as previously described⁹ with primers from Integrated DNA Technologies (Coralville, Ia). The nested primers used to amplify nucleotides 1840-2125 of IL-4R α complementary DNA (cDNA) were outer primer pairs GCCCACACTGGAA-GAATTGTCTTAC (sense) and TTTTGGGGGTCTGGCTTGAG (antisense) and inner primer pairs CCGAAATGCTCCAGCATG (sense) and CCAGTCCAAAGGTGAACAAGGGG (antisense).

PCR-based restriction fragment length polymorphism assay for detection of the Q576 and R576 alleles

Genomic DNA isolated from EDTA anticoagulated whole blood with use of the GenomicPrep kit from Pharmacia Biotech (Piscataway, NJ) was analyzed for the presence of the Q576 or R576 alleles by PCR. The sense primer was constructed with a guanine at position 1898 instead of a thymidine. This generated a *Pvu* I restriction site only in the R576 allele that has a guanine at position 1902 in lieu of an adenine. The primers used were sense TCTCGGCCCCACACAGTGGCGATC and antisense GAGGTCTTGGAAG-GCTTATAC. PCR was carried out in a total volume of 50 μ L under the following conditions: 94°C for 20 seconds, 32 cycles of 94°C for 20 seconds, 58°C for 30 seconds, 72°C for 30 seconds, and then 72°C for 5 minutes. After PCR amplification, 10 μ L of the original volume was digested with 5 units of *Pvu* I (New England Biolabs, Beverly, Mass), and the fragments were resolved on a 4% NuSieve gel (FMC Bioproducts). The Q576 and R576 alleles yielded 209 bp and 186 bp bands, respectively.

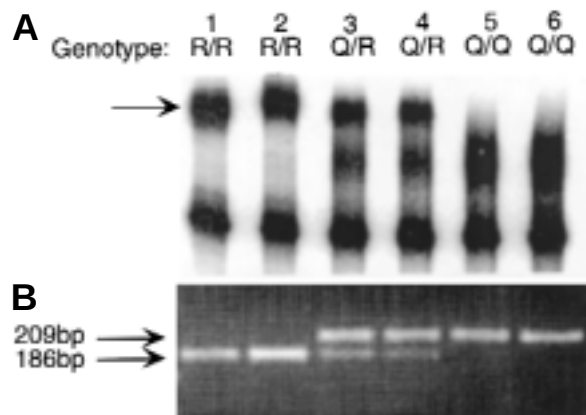


FIG 1. Identification of R576 and Q576 IL-4R α allelic variants. **A**, SSCP analysis of individuals homozygous for R576 (lanes 1 and 2), heterozygous Q576/R576 (lanes 3 and 4), or homozygous for Q576 (lanes 5 and 6). Arrow, Upper band seen only in presence of R576 allele. **B**, Same patients genotyped with PCR-based RFLP assay. R576 and Q576 alleles correspond to 186- and 209-bp bands, respectively.

Statistical analysis

Statistical analyses were performed with the Primer of Biostatistics software version 3.01 for Apple Macintosh.³⁴ To calculate *P* values for associations with the R576 allele, the allele frequencies in the indicated groups were analyzed with use of the chi-square test. The mean FEV₁ values were analyzed by ANOVA and the Bonferroni multiple comparison procedure to determine the significance of the association between R576 and asthma severity. Relative risks were calculated by standard methods.

RESULTS

PCR-based RFLP assay for Q576 and R576 IL-4R α allelic variants

Atopy has been linked to highly polymorphic markers within and flanking the gene IL-4R α gene on chromosome 16,³⁵ and IL-4R α allelic variants have been implicated as atopy susceptibility genes.^{9,10,35} Because atopy is a major risk factor for asthma, we investigated whether the R576 allele was associated with asthma. To screen large numbers of asthmatic patients, a PCR-based RFLP assay that used genomic DNA was developed (Fig 1). The sense primer was constructed with a guanine at position 1898 of the IL-4R α complementary DNA sequence instead of a thymidine. This generated a *Pvu* I restriction enzyme recognition site only in the R576 allele. Digestion of the PCR fragment with *Pvu* I generated a 209-bp band from the Q576 allele and a 186-bp band from the R576 allele (Fig 1, B). This allowed rapid and reliable detection of both the Q576 and R576 allelic variants compared with the SSCP method used previously to distinguish between these alleles.⁹ By SSCP analysis, the R576 allele results in the appearance of an additional upper band not seen in the control subjects. Individuals homozygous for R576 have only this upper band (Fig 1, A, lanes 1 and 2).

TABLE I. Frequency of R576 and Q576 IL-4R α alleles in allergic asthma, nonallergic asthma, and control groups

Population	Q576/ Q576	Q576/ R576	R576/ R576
Asthmatic individuals (n = 149)			
No.	81	49	19
Percent	54	33	13*
Alleles (n = 298)	Q576		R576
No.	211		87†
Allele frequency (%)	71		29
Allergic asthmatics (n = 107)			
No.	57	34	16
Percent	53	32	15‡
Alleles (n = 214)	Q576		R576
No.	148		66§
Allele frequency (%)	69		31
Nonallergic asthmatics (n = 42)			
No.	24	15	3
Percent	57	36	7
Alleles (n = 84)	Q576		R576
No.	63		21
Allele frequency (%)	75		25
Controls (n = 57)			
No.	36	20	1
Percent	63	35	1.7
Alleles (n = 114)	Q576		R576
No.	92		22
Allele frequency (%)	81		19

*Association of homozygosity for R576 and asthma: *P* = .03, chi-square test; relative risk 8.2.

†Association of R576 allele and asthma: *P* = .056, chi-square test.

‡Association of homozygosity for R576 and allergic asthma: *P* = .018, chi-square test; relative risk 9.8.

§Association of R576 allele and atopy: *P* = .034, chi-square test.

||Association of R576 allele and asthma (independent of atopy): *P* = .431, chi-square test.

Homozygosity for R576IL-4R α strongly associates with atopic asthma

To determine the role of the R576 allele in asthma, 149 individuals with asthma were genotyped with use of the assay described above. The results are summarized in Table I. The allele frequencies for the Q576 and R576 alleles in this population (*n* = 149) were 71 (211/298) and 29% (87/298), respectively, compared with 81% (92/114) and 19% (22/114) in the control nonasthmatic, nonallergic group (*n* = 57). There was a weak association between R576 and asthma overall (*P* = .056). However, a strong association was noted between homozygosity for the R576 allele and asthma, with 13% (19/149) of the asthmatic individuals tested being homozygous for R576 compared with only 1.7% (1/57) of the controls (*P* = .03, chi-square test; relative risk 8.2). Thus individuals possessing 2 alleles of R576 have an 8.2-fold increased relative risk toward asthma compared with individuals not homozygous for R576. Because the R576 allele is associated with atopy, the asthma population was subdivided into allergic and nonallergic groups on the basis of skin prick testing to 14 common environmental antigens, to

TABLE II. Effect of R576 and Q576 IL-4R α allelic variants on asthma severity

Asthma group	Q576/Q57 6	Q576/R57 6	R576/R57 6
Mild (FEV ₁ >80%)	22 (27.1%)	10 (20.4%)	2 (10.5%)
Moderate (FEV ₁ 60%-80%)	33 (40.7%)	21 (42.9%)	7 (36.8%)
Severe (FEV ₁ <60%)	26 (32.1%)	18 (36.7%)	10 (52.6%)
Statistical significance for severe vs mild	<i>P</i> = .6	<i>P</i> = .118	<i>P</i> = .015*
Mean FEV ₁ (% predicted)	67.5	66	57.8 (<i>P</i> < .05)*

*Statistically significant.

uncover any effect of the R576 allele independent of atopy. By this criteria, 107 (71.8%) of the 149 asthmatics were found allergic and 42 (28.2%) were nonallergic, which approximates published reports on the prevalence of atopy in asthmatic patients.³⁶⁻³⁸ There was a significant association of the R576 allele with allergic asthma (*P* = .034, chi-square test) as expected given the known association with atopy. Specifically, the allele frequency for R576 was 31% (66/214) in the allergic asthma group compared with 19% (22/114) in the control group. Furthermore, homozygosity for the R576 allele was strongly associated with allergic asthma (*P* = .018, chi-square test; relative risk 9.8). Thus, an individual homozygous for R576 has a 9.8-fold enhanced relative risk toward allergic asthma than does an individual with the genotype Q/Q576 or Q/R576. In the allergic asthma group, 15% (16/107) of individuals were homozygous for R576 compared with only 1.7% (1/57) of controls. There were no significant differences noted in frequencies of the Q576 and R576 alleles in the nonallergic asthma group compared with the control group (25% and 19%, respectively). These data support the association between the R576 allele and atopy and demonstrate a significant association between homozygosity for R576 and asthma but do not reveal a statistically significant association between R576 and asthma independent of allergy (ie, in the nonallergic group). However, the R576 allele frequencies were increased in the allergic and nonallergic asthma groups compared with the controls. Thus it is possible that larger population studies will reveal a stronger association with nonallergic asthma.

R576IL-4R α correlates with asthma severity

Potential relationships between the R576 and Q576 allelic variants and asthma severity were next explored. Baseline FEV₁ values were used to classify the patients into mild, moderate, and severe asthma groups. As outlined in the "Guidelines for the Diagnosis and Management of Asthma, Expert Panel Report 2,"³³ those with a baseline FEV₁ greater than or equal to 80% of predicted were classified as having mild asthma, those with an FEV₁ between 60% and 80% of predicted were classified as having moderate asthma, and those with a baseline FEV₁ less than 60% of predicted were classified as having severe asthma. The mild, moderate, and severe asthma groups were then separated by their genotype at the IL-4R α 576 locus (Fig 2). In the group homozygous for the Q576 allele, there were no significant differences among the rel-

ative numbers of patients in the group with mild, moderate, or severe asthma. However, in the group homozygous for the R576 allele, 52.6% (10/19) had severe asthma and only 10.5% (2/19) had mild asthma (*P* = .015, chi-square test), demonstrating a significant association between asthma severity and homozygosity for the R576 allele. Furthermore, the heterozygote group carrying 1 copy of the R576 allele had 36.7% (18/49) patients with severe asthma, a value that is between that seen in those carrying 2 copies of R576 (52.6%, 10/19) and the Q576 homozygotes (32.1%, 26/81). The proportion of patients with mild asthma in the heterozygote group (20.4%, 10/49) also fell between that observed in the Q/Q576 (27.1%, 22/81) and R/R576 (10.5%, 2/19) groups. The mean baseline FEV₁ values for each group are shown in Table II. A comparison of the mean FEV₁ values among the groups revealed that the group homozygous for R576 had a significantly lower mean (57.8) baseline FEV₁ compared with the group homozygous for the wild-type Q576 allele, mean = 67.5 (*P* < .05). The median baseline FEV₁ values for the R/R576, Q/R576, and Q/Q576 groups displayed the same trend as the mean baseline values (59, 66, and 70, respectively). Thus homozygosity for the R576 allele is associated with a severe asthma phenotype as defined by the baseline FEV₁. The FEV₁/forced vital capacity mean ratios were comparable in all groups (wild type 0.67, heterozygote 0.66, and homozygote 0.66). Interestingly, the mean and median baseline FEV₁ values in the heterozygotes were between the Q576 homozygote and R576 homozygote groups. This is displayed graphically in Fig 2. Thus the presence of the R576 allele correlates with asthma severity.

DISCUSSION

The familial nature of asthma and other atopic diseases is well established. It is increasingly evident that many different genes influence the development of asthma and atopy. Three different classes of disease-associated genes have been described: susceptibility genes that are strongly associated with asthma or atopy and likely play a causative role, disease-modifying genes that are not causative but modify the phenotype of the disease, and drug-modifying genes that alter the response to pharmacologic agents in affected individuals. The current findings demonstrate that the presence of the R576 allele correlates with asthma severity in individuals with asthma and thus acts as a disease-modifier. One or two copies

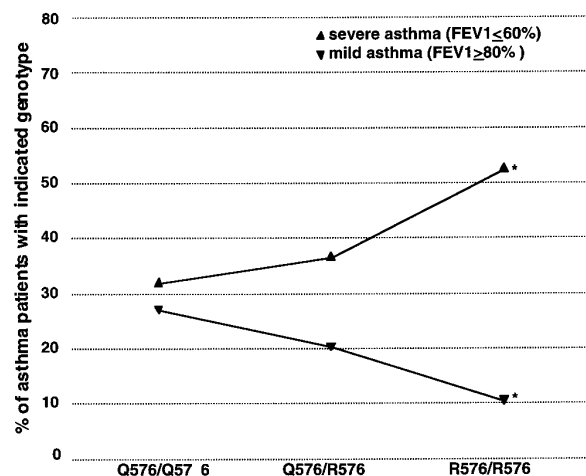


FIG 2. R576IL-4R α allele correlates with asthma severity. Asthmatic individuals were subdivided into groups by their genotypes at IL-4R α 576 locus. Percentage of patients in each group with severe asthma (upward triangle) versus mild asthma (downward triangle) is shown graphically. Asterisk, Statistically significant difference ($P = .015$). Severe (baseline FEV₁ $\leq 60\%$) and mild asthma (baseline FEV₁ $\geq 80\%$) were defined according to "Guidelines for Diagnosis and Management of Asthma, Expert Panel Report 2."³³

of R576 were associated with more severe asthma as defined by baseline FEV₁ compared with an asthmatic group homozygous for the wild-type Q576 allele.

The R576 allele also acts as an atopy and allergic asthma susceptibility gene. The allele frequency for R576 was increased in allergic asthma and in asthma overall compared with control subjects. Furthermore, homozygosity for R576 was significantly associated with asthma and carried an 8.2-fold relative risk toward asthma and a 9.8-fold relative risk for atopic asthma compared with Q576. A gene dosage effect was suggested both in terms of asthma phenotype and prevalence. Two copies of R576 were associated with increased asthma prevalence and increased asthma severity compared with 1 copy. This was illustrated by the heterozygote R/Q576 group, which had intermediate mean and median FEV₁ values compared with the Q/Q576 and R/R576 groups and which contained intermediate proportions of patients with severe and mild asthma compared with the 2 homozygote groups. Large population studies incorporating multiple measures of severity will be needed to confirm the possible gene dosage effect. The observed correlation between R576 and allergic asthma supports the reported association of R576 with atopy.⁹ Our results conflict with those in a recent report that found similar R576 allele frequencies in asthma and control Japanese groups,³⁹ but that study had very few R576 homozygotes and thus was likely underpowered or it was a characteristic of their specific population. Asthma severity was not examined in that study.

To elucidate the role of R576 in asthma independent of atopy, we divided the asthmatic individuals into allergic and nonallergic groups. The nonallergic group had a higher allele frequency for R576 than the controls, but

the association was not statistically significant, indicating that R576 likely does not play a major causative role in nonatopic asthma. The main association of R576 is with atopy, and this may underlie the association with allergic asthma. However, it is possible that in analyses of larger groups a significant correlation with nonatopic asthma will be uncovered. Nonatopic asthma may be different from atopic asthma in terms of its pathogenesis, and thus the genetic influences may be entirely distinct from those of atopic asthma. The role of Q576R in other allergic conditions remains unclear. It is not responsible^{9,40} for the expression of hyper IgE syndrome.

The molecular mechanism underlying the observed association between R576 and asthma severity remains to be determined. We have previously shown that PBMCs derived from individuals carrying the R576 variant have enhanced IL-4 responsiveness compared with Q576.⁹ Two studies examining the functional consequences of the Q576R substitution have not demonstrated any effect on Stat6 activation or CD23 expression.^{39,41} One of these studies³⁹ used Ba/F3 cells for the transfections. These cells have been shown to display IL-4-dependent Stat6 activation independently of Stat6 docking sites in the IL-4R α cytoplasmic domain, which are required for IL-4-dependent Stat6 activation in other cells.⁴² Thus Ba/F3 may not be the optimal cell type to study IL-4 signaling. The other study⁴¹ studied the effect of R576 in the context of the wild-type IL-4R α sequence but did not examine its effect in combination with the other known IL-4R α polymorphisms.⁴³ The functional consequences of Q576R may be evident only in the context of one or more other genetic polymorphisms. Importantly, because IL-4R α is also a component of the IL-13 receptor system, one or more of the IL-4R α polymorphisms may alter IL-13 responses, which may be the molecular mechanism underlying their association with atopy. Studies to address these questions are in progress.

We have previously shown that the R576 allele alters the interactions between Y575 and the SH2 domain containing tyrosine phosphatase, SHP-1, which has recently been shown to down-regulate IL-4 signaling.⁴⁴ IL-4R α contains 5 conserved tyrosine residues in its cytoplasmic domain, one of which, Y713, is part of an ITIM (immunoreceptor tyrosine-based inhibitory motif) recognition sequence⁴⁵ and has been implicated as a docking site for SHP-1.^{46,47} Because SHP-1 contains 2 SH2 domains, Y575 and Y713 may act in a concerted fashion to recruit SHP-1 and lead to the termination of IL-4 signaling. A decrease in the efficiency of the interaction between IL-4R α and SHP-1 resulting from substitution from Q to R adjacent to Y575 could lead to a dysregulation of IL-4 responses. This may be one mechanism underlying the genetic predisposition conferred by R576. If this is the case, transfectants expressing Q576 or R576 will need to be examined for changes in the rate of turnover or dephosphorylation of Stat6 and other signaling intermediates. It will also be important to determine the effects of the IL-4R α polymorphisms on other signaling molecules important in the IL-4 signal transduc-

tion, such as JAK kinases, which have been implicated as SHP-1 substrates.⁴⁸ We are currently addressing these possibilities.

As mentioned above, 5 other polymorphisms of the IL-4R α have been reported,⁴³ and one of these, Ile75, has recently been associated with atopic asthma and enhanced IL-4 signaling^{10,39} in Japanese populations. It is not known whether this polymorphism may also affect asthma severity. A recent study by another Japanese group failed to find an association between Ile75Val and asthma.⁴⁹ It will be important to determine how the IL-4R α polymorphisms interact with each other to determine the functional state of the receptor and the overall risk for asthma. Multiple polymorphisms in specific combinations may be required to alter IL-4 function, and the combinatorial variants may have unique mechanisms that involve different signaling molecules. We currently cannot rule out that the R576 is in linkage disequilibrium with another mutation in the IL-4R α gene or another gene that is actually responsible for the association at a molecular level. However, Q576R is not in linkage disequilibrium with the IL-4R α chain allelic variant with isoleucine at position 75 of the polypeptide (numbering including 25 amino acid signal peptide) (I75) in our population because we found similar I75 allele frequencies in the asthma and control groups (unpublished observations).

It has previously been shown that cells from bronchoalveolar lavage specimens from steroid-resistant asthmatic patients displayed increased IL-4 expression compared with the steroid-sensitive asthmatics, supporting a role for the dysregulation of T_H2/T_H1 cytokines, specifically IL-4, in asthma severity.⁵⁰ A combination of IL-4 and IL-2 were sufficient to sustain reduced glucocorticoid receptor binding affinity in PBMCs from steroid-resistant asthmatic patients.^{51,52} Thus hyperresponsiveness to IL-4 may result in decreased sensitivity to steroids and more-difficult-to-control asthma. Although the mechanisms responsible for the observed associations between R576 and atopy, as well as the correlation of R576 with asthma severity, are unclear, it is important to continue to pursue different possible mechanisms to explain the clinical associations. Because asthma is a complex polygenic disease, individual polymorphisms may have small functional consequences in isolation and may require additional genetic polymorphisms in other genes to alter function significantly. Thus studies of multiple genes will be important to identify potential candidates that may act in conjunction.

Homozygosity for R576 carries an 8.2-fold increased relative risk for asthma and is associated with increased asthma severity. The association of R576 with asthma severity has potential clinical applications. Early recognition of infants at risk for severe asthma by determination of their IL-4R α 576 genotype followed by close medical follow-up and early environmental or pharmacologic intervention may delay, attenuate, or prevent the progression of disease. In adults identified as "at risk" for severe asthma at the time of diagnosis, closer medical follow-up and early institution of therapy may alter their

outcomes. Thus the R576 allele is an atopy and asthma susceptibility gene as well as a disease-modifying gene that may be a useful genetic marker of asthma severity.

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