

TABLE III. Administrative data markers of uncontrolled asthma in patients not in the study during 6 months before and 6 months after recruitment

Parameter	Group	
	Uncontrolled	Controlled
Asthma hospitalization or ED visit*	(n = 547)	(n = 1750)
Before	2.9%	0.9%†
After	1.6%	1.7%
Any oral corticosteroid dispensing*‡	(n = 523)	(n = 1683)
Before	17.8%	10.5%†
After	17.2%	11.7%†
SABA ≥7*‡§	(n = 523)	(n = 1683)
Before	6.7%	2.0%†
After	6.3%	3.5%†

ED, Emergency department; SABA, short-acting β -agonist canisters.

*Only patients with continuous enrollment during the 6 months before and 6 months after study recruitment were included in these analyses.

† $P < .01$ in patients with controlled asthma not in the study versus those with uncontrolled asthma not in the study.

‡Only patients with pharmacy benefits during the 6 months before and 6 months after study recruitment were included in these analyses.

§This SABA cutoff was based on a previously validated β -agonist long-term control scale.⁶

other characteristics of patients who make changes). It is not certain what more would need to be done for a successful intervention, but possibilities include the following: (1) taking into account a history-RAST correlation to determine individual patient relevance of the allergic sensitization; (2) a more tailored environmental control education program based on a patient's specific sensitization and environment; (3) a more individualized environmental control education program, taking into account the patient's total asthma state (severity, control, and treatment); (4) 1 or more in-person sessions to optimize information transfer and bidirectional communication; and (5) home visits.

Further studies will be necessary to determine which of these or other aspects of an environmental control intervention (eg, targeting patients who express a willingness to make changes) would successfully improve asthma outcomes in allergic patients. However, considering the role allergens can play in asthma and the ameliorative effect of environmental control,¹ the economic and human costs of uncontrolled asthma, and the expense and possible side effects of medications, such interventions would certainly be worth pursuing.

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IL-4 receptor α polymorphisms are predictors of a pharmacogenetic response to a novel IL-4/IL-13 antagonist

To the Editor:

Evidence from genomic research, *in vitro* cell signaling, and animal studies strongly implicates the IL-4/IL-13 pathways in the pathogenesis of allergic asthma.¹⁻⁷ However, there are limited data on pharmacologic modulation of these pathways. We performed a descriptive pharmacogenetic analysis using data from an early phase II mechanistic trial of the drug pitrakinra, a novel IL-4/IL-13 dual antagonist, in patients with allergic asthma (ClinicalTrials.gov identifier NCT00535431).⁸ Pitrakinra is similar to the native IL-4 molecule, although specifically designed with 2 amino acid changes (R121D/Y124D), rendering it an effective competitive antagonist of IL-4 receptor α (IL-4R α).⁸ Pitrakinra reduces late-phase responses to inhaled antigen based on phase II clinical studies to date,⁸ and genomic DNA samples were obtained from 29 participants in this clinical trial. In addition, we obtained DNA from 23 cynomolgus monkeys from preclinical antigen-challenge studies, in which monkeys were treated with an IL-4/IL-13 antagonist similar to pitrakinra and with the same mechanism of action.⁹ We hypothesized that single nucleotide polymorphisms (SNPs) in the gene encoding IL-4R α (*IL4RA*) would predict treatment response in the human studies and that there would be a similar effect of *IL4RA* gene variation in nonhuman primates.

The protocol and results of the early phase II trials have been reported previously and are described in brief below.⁸ The primary clinical outcome was change in late-phase antigen response to inhaled allergen challenge in patients with asthma. Acute and late-phase responses were measured at screening and after 4 weeks of twice-daily therapy with nebulized pitrakinra or placebo. After allergen challenge, FEV₁ was measured over a 10-hour period to assess late antigen response (LAR; 4-10 hours). The outcome for this genetic analysis was the ratio of LAR after 4 weeks of treatment with either placebo or pitrakinra compared with baseline levels. Therefore an increased LAR ratio corresponds to a reduction in late-phase FEV₁ response to antigen after treatment or an improvement in lung function relative to baseline measurements.

Pharmacogenetic analysis was performed on genomic DNA from predominantly non-Hispanic white (83%) male (63%) subjects with asthma randomized to placebo (n = 14) or pitrakinra (n = 15) with complete data on the primary outcome. We selected 14 *IL4RA* tagging SNPs and 3 nonsynonymous amino acid coding changes from the HapMap CEU reference

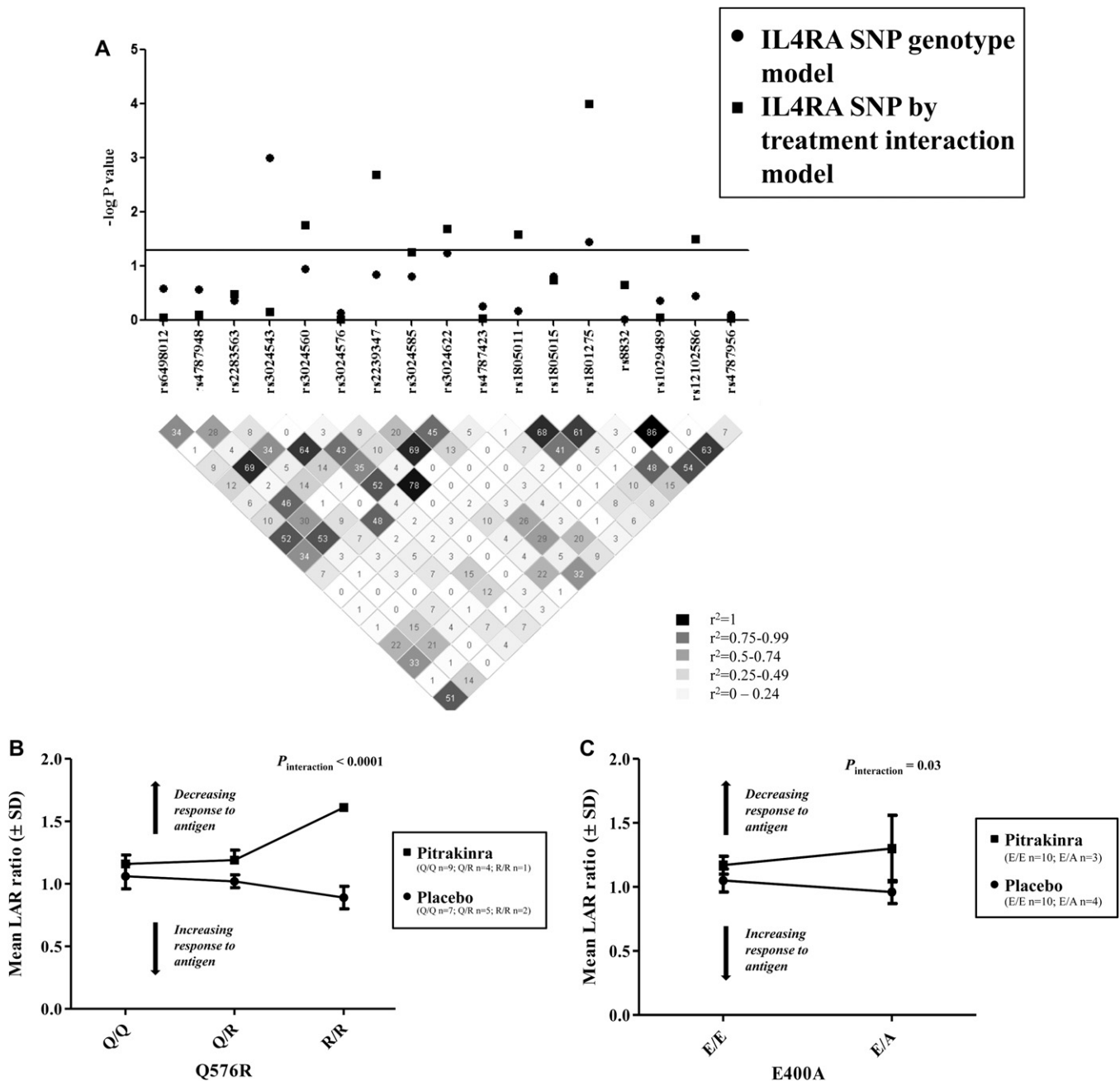


FIG 1. A, Human *IL4RA* SNP linkage disequilibrium structure (r^2) and associations with LAR ratio. Highly correlated SNPs ($r^2 > 0.7$) have similar P values for association. The line indicates significance at a P value of less than .05. The linkage disequilibrium plot was generated in Haploview version 4.1. B, Mean $\pm$ SD LAR ratio by *IL4RA* rs1801275 (Q576R) genotype and treatment group. C, Mean $\pm$ SD LAR ratio by *IL4RA* rs1805011 (E400A) genotype and treatment group.

population for genotyping using the Sequenom MassARRAY iPLEX platform (Sequenom, San Diego, Calif).

We evaluated 17 *IL4RA* SNPs in Hardy-Weinberg equilibrium ($P > .01$) with minor allele frequencies (MAFs) of greater than 5% (Fig 1, A). General linear models with treatment group as a covariate were developed to assess the genetic contribution of each SNP to LAR ratio (genotype model) or with a multiplicative SNP by treatment term to evaluate pharmacogenetic interaction. All statistical analyses were performed in SAS version 9.1

(SAS Institute, Inc, Cary, NC) or Plink version 1.06. We did not correct for multiple comparisons in this exploratory analysis of human and cynomolgus *IL4RA* variation.

Fig 1 shows the linkage disequilibrium structure for the human *IL4RA* SNPs analyzed in this study and SNP associations with LAR ratio. Notably, the nonsynonymous polymorphism rs1801275 (Q576R) was a significant predictor of mean difference in LAR ratio by genotype ($P = .04$) and interaction with treatment ($P < .0001$). As shown in Fig 1, B, 1 subject

TABLE I. Cynomolgus *IL4RA* SNPs significantly associated ($P < .05$) with BHR and eosinophil counts (after treatment with IL-4/IL-13 antagonist compared with control)

Cynomolgus <i>IL4RA</i> polymorphism	Rhesus chromosome 20 location	<i>IL4RA</i> SNP location	Major/minor allele; MAF	BHR, P value	Eosinophil, P value
PR2	25763581	5' Proximal	C/T; 0.26	.03	.61
PR11	Gap	5' Proximal	C/T; 0.02	.14	.05
PR20	25763996	5' Proximal	T/A; 0.02	.14	.05
In1_5	Gap	Intron	G/C; 0.27	.04	.65
In3_2	25794379	Coding Syn Ser	C/T; 0.07	.04	.60
In3_3	25794391	Intron	T/C; 0.08	.02	.44
Ex5_2	25799301	Coding Syn Asn	C/T; 0.26	.03	.61
In5_7	25799454	Intron	C/T; 0.02	.03	.01
In7_2	25803175	Intron	G/C; 0.08	.64	.05
In8_3	25806353	Intron	G/A; 0.02	.14	.05
Ex9_3	25809423	Coding Syn Ile	C/T; 0.34	.04	.74
In10_4	25811349	Intron	G/A; 0.33	.05	.91
In10_5	25811415	Intron	C/T; 0.36	.05	.82

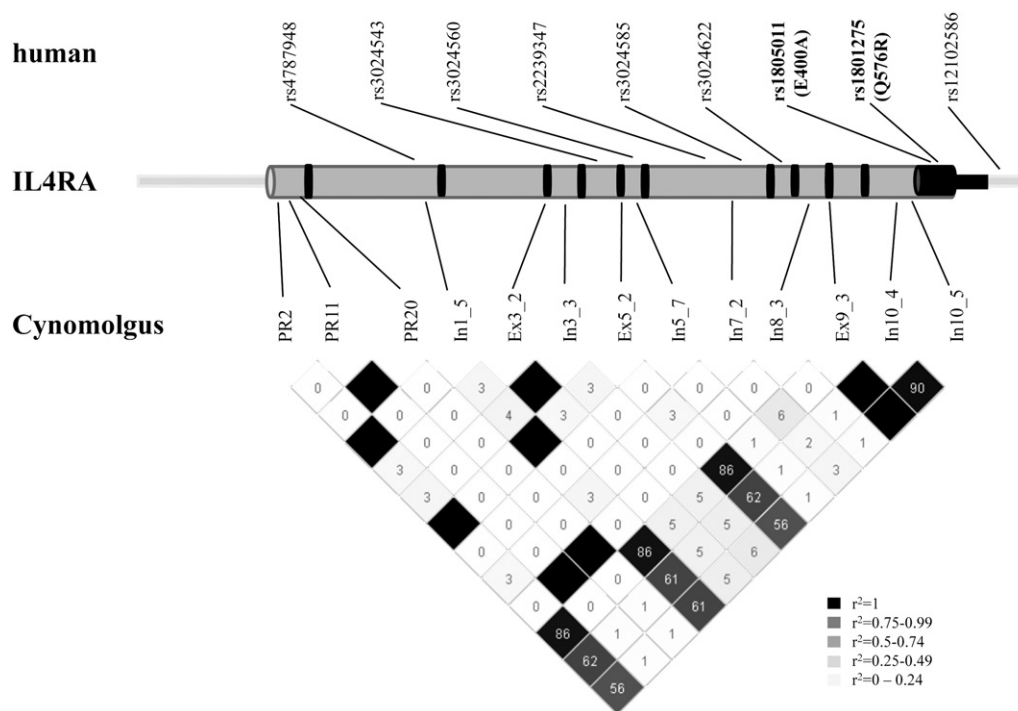


FIG 2. Map of human and cynomolgus monkey *IL4RA* polymorphisms significantly associated with allergen-induced clinical outcomes. *IL4RA* introns and the promoter are depicted in gray; exons and the 3' untranslated region are depicted in black. Genomic information was primarily adapted from the human and rhesus University of California, Santa Cruz, genome browser. The rhesus sequence (the closest publicly available sequence to the cynomolgus monkey) contains gaps, and therefore the map is not to scale. Cynomolgus *IL4RA* SNP linkage disequilibrium plot and SNP correlation (r^2) were generated in Haploview version 4.1.

homozygous for the R/R variant had an increased LAR ratio, indicating improved lung function relative to baseline, in the pitrakinra treated group only. This 1 homozygous subject might be primarily responsible for the significant association with LAR. If Q/R and R/R subjects were analyzed together, there was a borderline significant interaction with treatment ($P = .06$). Similarly, rs1805011 (E400A) E/A heterozygotes treated with pitrakinra had a higher mean LAR ratio than E/E homozygotes (Fig 1, C).

The complementary outcomes in the primate preclinical trials were bronchial hyperresponsiveness (BHR) to methacholine (provocative challenge or PC₁₀₀) and bronchoalveolar lavage eosinophilia in response to *Ascaris* species allergen exposure. An IL-4/IL-13 antagonist similar to pitrakinra or vehicle control was administered in a cross-over design; details are presented in Tomkinson et al.⁹ Briefly, aerosolized *Ascaris suum* solution was delivered through a respirator, and BHR to methacholine was measured

in anesthetized ventilated animals. Bronchoalveolar lavage was performed with saline by using standard methods. We used general linear models with an additive genetic term to evaluate cynomolgus *IL4RA* polymorphisms associated with the log-transformed postalergen IL-4/IL-13 antagonist treatment/control ratio (Table I) for both BHR (median ratio, 2.5; range, 0.4-8.7) and eosinophil counts (median ratio, 0.95; range, 0.15-9.9).

Because the cynomolgus genomic *IL4RA* DNA sequence was not available, we directly sequenced 100 bp surrounding *IL4RA* exons, the proximal promoter, and 3' untranslated regions by using DNA from 23 monkeys. The cynomolgus *IL4RA* genomic and predicted mRNA sequence was compared with that of rhesus *Macaca mulatta* using Sequencher DNA analysis software (Gene Codes, Ann Arbor, Mich) and the University of California, Santa Cruz, genome browser (genome.ucsc.edu) BLAT alignment tool; information is available on request. We identified 110 novel *IL4RA* polymorphisms, predominantly in intronic or promoter regions, as well as 7 synonymous and 9 nonsynonymous coding changes and 6 insertion/deletion polymorphisms. All SNPs tested for association ($n = 83$) had 70% or greater genotyping data and an MAF of 2% or greater and were concordant with Hardy-Weinberg equilibrium. The genomic position and MAF for 13 polymorphisms significantly associated with BHR or eosinophilia are presented in Table I, and the linkage disequilibrium structure is shown in Fig 2. For the majority of significant SNPs, P values for association were generally nominal, and the minor allele was a predictor of increased BHR ratio, which corresponds to decreased hyperresponsiveness in pitrakinra-treated animals compared with control treatment. Only 1 relatively low-frequency SNP (In5_7; MAF = 0.02) was associated with both end points (Table I), although this result was mainly due to 1 (C/T) heterozygote with a higher eosinophil ratio and a lower BHR ratio than (C/C) homozygotes. Although this result is somewhat contradictory, the function of this novel intronic SNP is currently unknown, and the mechanism of SNP modulation of these allergic outcomes might form the basis of future study.

Genetic association with response to allergen-induced clinical outcomes was found across the *IL4RA* gene (Fig 2). There was a great deal of variation in the cynomolgus *IL4RA* gene, and SNPs associated with allergic outcomes were not the same as in human subjects. However, replication for pharmacogenetic studies is challenging, and this analysis provides separate lines of evidence that *IL4RA* variation might modulate the pharmacogenetic response to allergen challenge. Admittedly, the sample sizes for this unique analysis are small. In human subjects the lowest P values were found with amino acid changes at the 3' end of *IL4RA*, which have potential functional implications. The *IL4RA* 576R variant has been shown to synergize with the signal transducer and activator of transcription 6 molecule to enhance expression of IL-4- and IL-13-regulated genes and mediate allergic inflammation in a murine model of asthma.⁷ We speculate that the variant at position 400, as well as that at position 576, might also affect signaling through other intracellular mediators, such as Janus kinase 1.

This analysis suggests that *IL4RA* amino acid variations might predict a therapeutic treatment response to anti-IL-4/IL-13 pathway inhibitors, although larger studies are required to confirm these findings. Pharmacogenetic studies such as these might become increasingly important to predict individual response to invasive and possibly expensive novel asthma therapies.

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The need for standardization in exercise challenge testing for exercise-induced asthma/bronchoconstriction

To the Editor:

Objective measurement of lung function based on FEV₁ before and after exercise is required for valid diagnosis of exercise-induced asthma (EIA) and exercise-induced bronchoconstriction (EIB). Airway drying caused by increased minute ventilation