IL-4 receptor polymorphisms predict reduction in asthma exacerbations during response to an anti–IL-4 receptor α antagonist

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Background: This is the first large pharmacogenetic investigation of the inflammatory IL-4/IL-13 pathway in patients with moderate-to-severe asthma. We analyzed genomic DNA from participants in a 12-week placebo-controlled efficacy trial of pitrakinra (1, 3, or 10 mg twice daily), a novel IL-4/IL-13 pathway antagonist (Clinicaltrials.gov NCT00801853).

Objectives: The primary hypothesis for this analysis is that amino acid changes in the 3′ end of the IL-4 receptor α gene (IL4RA) or closely proximal variants would predict reductions in asthma exacerbations for subjects randomized to pitrakinra therapy.

Methods: Nineteen IL4RA single nucleotide polymorphisms (SNPs) were tested in 407 non-Hispanic white subjects for association with the primary clinical end point of asthma exacerbations and changes in secondary end points for asthma symptom scores.

Results: The most consistent pharmacogenetic associations were observed for the correlated tagging SNPs rs8832 and rs1029489 in the IL4RA 3′ untranslated and proximal regions, respectively. Subjects homozygous for the rs8832 common G allele randomized to pitrakinra (placebo group nonsignificant) had decreased asthma exacerbations and decreased nocturnal awakenings and activities limited by asthma. There was also a significant pitrakinra dose-response relationship (placebo/1 mg/3 mg/10 mg) for exacerbations in subjects homozygous for the common allele in rs1029489 (P = .005) and rs8832 (P = .009) and the intronic SNPs rs3024585, rs3024622, and rs4787956 (P = .03).

Conclusion: This study demonstrates a significant pharmacogenetic interaction between anti–IL-4 receptor α therapy and IL4RA gene variation, identifying an asthma subgroup that is more responsive to therapy with this antagonist. (J Allergy Clin Immunol 2012;130:516-22.)

Key words: Pharmacogenetics, pitrakinra, IL-4 receptor, asthma therapy, IL-4 receptor antagonist

Allergic asthma is characterized by chronic inflammation and, in some subjects, airway remodeling, which is often accompanied by increased eosinophil and CD4+ T H2 cell counts.1,2 Previous human and animal studies have shown that T H2 cytokines are critical mediators in chronic allergic airway inflammation,3 and in murine models T H2-type inflammation can also enhance bronchial hyperresponsiveness.4,5 Because of the important role for this pathway in the pathogenesis of asthma, there is growing interest in asthma therapies based on modulation of T H2 cytokines, including IL-4 and IL-13. However, clinical results for drugs targeting this pathway in adults with asthma were initially disappointing, possibly because of T H2 pathway redundancy or the inclusion of subjects with differential therapeutic responsiveness to modulation of this pathway.6-9 There have been recent trials that show promising results, especially in subgroups of patients. For instance, an antibody blocking IL-13 has been effective at reducing airway symptoms and improving lung function in a subset of subjects with T H2-specific inflammatory asthma.10

Another therapeutic approach for asthma is to target the α subunit of the IL-4 receptor, the signaling component of the heterodimeric receptor complex for both IL-4 and IL-13. Pitrakinra is a recombinant form of IL-4 that blocks IL-4 receptor α complexes and inhibits downstream signaling.11 Detailed pharmacology of pitrakinra is presented in an article by Burmeister et al.11 Previous studies provided evidence that this antagonist targets T H2 inflammation in subjects with allergic asthma and preliminary evidence that pitrakinra therapy decreases severe adverse events requiring β-agonist reliever medications.12 Therefore this current clinical trial testing the efficacy of a novel biologic on clinically relevant asthma outcome was warranted. Our research group previously completed a preliminary pharmaco genetic analysis using data from an earlier phase 2 placebo-controlled trial of pitrakinra13 in patients with allergic asthma (Clinicaltrials.gov NCT00535431).13 In a phase 2a allergy challenge study, we demonstrated that variation in the human IL-4 receptor α gene (IL4RA) was associated with therapeutic responses to pitrakinra treatment.15 The nonsynonymous IL4RA single nucleotide polymorphisms (SNPs) rs1805011 (E400A, also called

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516
E375A) and rs1801275 (Q576R, also called Q551R), as well as several other IL4RA tagging SNPs, were significantly associated with a reduction in FEV₁ that occurs during the late antigen response in the pitrakinra-treated group only.¹³ In a separate line of evidence, we also performed a genetic analysis of cynomolgus monkeys treated with an IL-4/IL-13 antagonist similar to pitrakinra¹⁴ and identified novel cynomolgus IL4RA polymorphisms significantly associated with a therapeutic reduction in bronchial hyperresponsiveness, eosinophilia, or both.¹³

In this phase 2b clinical trial of pitrakinra’s efficacy and safety (Clinicaltrials.gov NCT00801853), participants with moderate-to-severe asthma were evaluated for the primary clinical end point of asthma exacerbations in a double-blind, randomized, placebo-controlled study. Subjects were randomized to pitrakinra or placebo by means of inhalation twice daily for 12 weeks. For the global intent-to-treat study population (n = 534), there was no statistically significant difference between pitrakinra and placebo in the incidence of asthma exacerbations; however, there was a significant reduction in exacerbations (10 mg of pitrakinra compared with placebo, P = .004) in a prespecified subset of subjects (n = 125) with evidence of increased blood eosinophil counts.¹⁵,¹⁶ We performed a pharmacogenetic analysis of IL4RA polymorphisms in 407 non-Hispanic white subjects with available DNA from the global trial to identify potential genetic modifiers of the pitrakinra treatment response.

Although there is limited published information regarding genetic predictors of asthma exacerbations, our group previously reported that the minor alleles of IL4RA E400A and Q576R variants were related to asthma exacerbations and lower lung function.¹⁷ On the basis of our initial pharmacogenetic results and reported IL4RA associations with exacerbations, the primary hypothesis for this analysis is that IL4RA amino acid changes E400A and Q576R or closely proximal genetic variants in the 3’ end of this gene will predict reductions in asthma exacerbations in participants randomized to anti–IL-4 receptor α (IL-4Rα) therapy.

METHODS

Clinical trial population
A pharmacogenetic analysis was performed in a phase 2b clinical trial of pitrakinra, an IL-4Rα antagonist. In this trial 534 participants with moderate-to-severe asthma from the intent-to-treat population were enrolled in a 12-week randomized, placebo-controlled, multicenter (sites in the United States and Europe), double-blind, dose-ranging study (1, 3, or 10 mg twice daily) to test the safety and efficacy of pitrakinra. Subjects were evaluated for the primary end point of incidence of asthma exacerbation and several secondary clinical end points. Patients were stabilized for 4 weeks of run-in on inhaled corticosteroids and long-acting β-agonists (LABAs) and then randomized to pitrakinra or placebo for a 12-week treatment period. LABAs were withdrawn at 4 weeks (study day 28), and inhaled corticosteroid withdrawal began 6 weeks after initiation of blinded treatment (stepped down on study days 42 and 56 and stopped on day 70).¹⁵ Complete participant inclusion and exclusion criteria can be viewed at Clinicaltrials.gov NCT00801853. Briefly, inclusion criteria defined a population of nonsmoking male and female subjects 18 years of age and older who had moderate-to-severe asthma and whose symptoms were not well controlled with current asthma therapy. All subjects had experienced an asthma exacerbation requiring oral corticosteroid treatment at least once within the preceding 2 years. Because the majority of participants reported non-Hispanic white ethnicity, genetic analysis was performed in 407 non-Hispanic white subjects from the intent-to-treat population, who provided genomic DNA and consent to participate in a genetic study. Although DNA was available from a total of 465 subjects, we analyzed non-Hispanic white subjects only because of the small numbers of subjects with other ethnic backgrounds. Characteristics of participants in the global clinical trial and non-Hispanic white subjects analyzed at study entry (after the 4-week run-in period on combination 2250/50 μg fluticasone propionate/salmeterol therapy) are reported in Table 1. The safety profile for pitrakinra based on adverse events and laboratory measurements was satisfactory.¹⁵ All subjects provided informed consent, and this pharmacogenetic analysis was approved by the Wake Forest School of Medicine Institutional Review Board.

Outcome definitions
The primary end point of incidence of asthma exacerbation was defined according to the following criteria: a morning peak flow of at least 30% below baseline for 2 or more consecutive days and 6 or more additional uses of short acting β-agonist reliever medication (1 puff = 1 reliever occasion) in a 24-hour period over baseline levels for 2 or more consecutive days; deterioration of asthma requiring treatment with oral corticosteroids; deterioration of asthma requiring treatment increase of 4 or more times the baseline dose of inhaled corticosteroids; deterioration of asthma requiring hospitalization; or deterioration, in the opinion of the investigator, of a patient’s condition significant enough to be considered an asthma exacerbation. When a subject experienced an asthma exacerbation, he or she had completed the primary end point and was withdrawn from the study.¹⁵ This definition of exacerbation is consistent with other relatively short-duration clinical trials performed by the National Heart, Lung, and Blood Institute’s Asthma Clinical Research Network (www.acrn.org).¹⁸,¹⁹

Additional secondary end points analyzed in this genetic analysis included time (in days) to an asthma exacerbation and changes from baseline in pulmonary function and asthma symptom scores. Pulmonary function, including measurement of FEV₁ percent predicted, forced vital capacity percent predicted, and FEV₁/forced vital capacity ratio, were performed according to standard procedures by using Vitalograph, Inc (Buckinghamshire, United Kingdom), spirometric equipment according to American Thoracic Society guidelines.¹⁸ Asthma symptom scores ranged from 0 to 4 and were reported by the participants in a daily diary. Questions used in this analysis are available in Table E1 in this article’s Online Repository at www.jacionline.org. For all secondary end points, the weekly average change from baseline was evaluated at study completion for participants who finished the study (week 11 for diary symptom scores and visit 8 for in-clinic measurements) or the week before exacerbation or study withdrawal.

Genotyping and quality control methods
Twenty-one tagging and nonsynonymous SNPs in IL4RA were genotyped in 407 non-Hispanic white subjects. SNPs that tagged blocks of correlated IL4RA polymorphisms (r² > 0.8) were selected based on available genomic information (hapmap.org and ncbi.nih.gov/snp) to provide maximum coverage of common polymorphisms in the primarily non-Hispanic white participants who comprised the global clinical trial population. Genomic DNA was isolated and purified by PPD Central Laboratories (Richland Heights, Ky) and then shipped to Wake Forest for genotyping with the Sequenom MassARRAY iPLEX platform (Sequenom, Inc, San Diego, Calif). Standard genotyping quality control procedures were performed by using the publicly available programs Plink version 1.07²¹ and Haploview version 4.2.²² On the basis of the quality control criteria of minor allele frequency (MAF) of greater than 5%, genotyping efficiency of greater than 80%, and consistency with Hardy-Weinberg equilibrium (P > .01, Fisher exact test), 19 IL4RA SNPs were...
TABLE I. Baseline characteristics of the global intent-to-treat pitrakinra clinical trial population and genotyped non-Hispanic white participants analyzed in this study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Non-Hispanic white genotyped subjects (n = 407)</th>
<th>Global intent-to-treat population (n = 534)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (% female)</td>
<td>59</td>
<td>59</td>
</tr>
<tr>
<td>Ethnicity (% non-Hispanic white/African American/Hispanic/other)</td>
<td>—</td>
<td>88/8/3/2</td>
</tr>
<tr>
<td>Recruitment country (% United States/Europe)</td>
<td>41/59</td>
<td>48/52</td>
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<tr>
<td>Age at enrollment (y)</td>
<td>45.1 ± 13.8</td>
<td>45.4 ± 13.7</td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>29.4 ± 6.3</td>
<td>30.0 ± 7.5</td>
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<tr>
<td>Baseline lung function</td>
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<td></td>
</tr>
<tr>
<td>FEV₁ (% predicted)</td>
<td>72.3 ± 11.3</td>
<td>72.9 ± 11.5</td>
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<tr>
<td>FVC (% predicted)</td>
<td>84.4 ± 12.3</td>
<td>85.3 ± 12.6</td>
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<td>69.0 ± 10.3</td>
<td>68.9 ± 10.2</td>
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<tr>
<td>Blood eosinophils (cells/mm³)</td>
<td>265 ± 216</td>
<td>265 ± 210</td>
</tr>
<tr>
<td>Total serum IgE (IU/mL*)</td>
<td>356.5 ± 733</td>
<td>405.3 ± 850</td>
</tr>
<tr>
<td>FENO (ppb)</td>
<td>19.3 ± 15.6</td>
<td>18.9 ± 14.7</td>
</tr>
</tbody>
</table>

FVC: Forced vital capacity.

*One outlier removed.

In genetic association tests. For all SNPs tested, MAFs were consistent across treatment groups. For example, the MAF for rs8832 in the placebo, 1-mg, 3-mg, and 10-mg treatment groups was 0.43, 0.41, 0.48, and 0.42, respectively. A list of *IL4RA* SNPs analyzed is presented in Table E2 in this article’s Online Repository at www.jacionline.org, and a linkage disequilibrium plot and map of these SNPs is included as Fig E1 in this article’s Online Repository at www.jacionline.org.

### Statistical analysis

Pharmacogenetic analyses were performed by using *IL4RA* SNP genotype by exacerbation status contingency tables stratified by treatment status with an additive test for trend. An additive model is consistent with outcome values for heterozygotes intermediate to subjects homozygous for major and minor alleles. We tested the SNP association with the primary clinical outcome: exacerbations in subjects randomized to placebo and all doses of pitrakinra both pooled and each dose separately. To evaluate the pitrakinra dose response (placebo/1 mg/3 mg/10 mg), we developed contingency tables with exacerbation status by treatment assignment stratified by SNP genotypes (heterozygotes intermediate to subjects homozygous for minor allele combined). On the basis of these results, we also performed a subgroup analysis of subjects randomized to the 3- and 10-mg doses of pitrakinra.

Because participants with high blood eosinophil counts responded more effectively to pitrakinra, 5,16 we tested SNP associations with exacerbations stratified by eosinophil counts (using designations of ≥350 cells/mm³ as high and ≤350 cells/mm³ as low). For continuous variables, general linear models were developed to test change from baseline to treatment assignment, adjusting for region and baseline values and stratified by genotype, as described above. We also used Kaplan-Meier plots with a log-rank test (each dose compared with placebo) to evaluate differences in time to exacerbation by treatment assignment stratified by SNP genotype. Statistical analyses were performed with PLINK version 1.07, 21 Haploview version 4.2, 22 and SPSS/PASW version 18 (SPSS, Inc, Chicago, Ill). A 2-sided *P* value of less than .05 was considered statistically significant, and we adjusted for multiple comparisons by using Bonferroni correction based on our primary hypothesis.

### RESULTS

#### Demographics

Demographic and baseline clinical characteristics, including lung function, total serum IgE levels, and fraction of exhaled nitric oxide (FENO) levels, of participants were similar for subjects in the overall intent-to-treat trial population (n = 534) and the genotyped non-Hispanic white subjects (n = 407) analyzed in this study (Table I).

### Asthma exacerbations

In this pitrakinra 2b clinical trial the frequency of asthma exacerbations was 19.9% overall, and there were no significant differences in exacerbations in participants assigned to active pitrakinra doses compared with those assigned to placebo. 15 In 407 genotyped non-Hispanic white subjects, exacerbations occurred in 80 participants (overall, 19.7%), and there were also no statistically significant differences in exacerbations by treatment assignment: 22 (20.6%) exacerbations in the placebo group, 20 (19.6%) exacerbations in the 1-mg group, 24 (23.3%) exacerbations in the 3-mg treatment group, and 14 (14.7%) exacerbations in the 10-mg treatment group. To test for significant differences in exacerbations by genotype, we analyzed *IL4RA* SNP genotype associations with asthma exacerbations stratified by treatment assignment (placebo and all doses of pitrakinra pooled). The frequency of exacerbations in subjects with the common GG genotype in the tagging SNP rs8832 who were receiving active pitrakinra (11%) was significantly reduced compared with that of subjects with the AG (22%) or AA genotypes (25%, *P* = .03), and this SNP was not associated with exacerbations in the placebo group.

Because there was evidence that *IL4RA* tagging SNPs might interact with pitrakinra treatment and identify a therapeutically responsive subgroup, we then investigated a pitrakinra dose-response relationship (placebo/1 mg/3 mg/10 mg). The correlated SNPs (*r² = 0.75*) rs8832 (Fig 1, A) and rs1029489 (Fig 1, B) showed a similar dose-response relationship for subjects with the common GG genotype for either SNP (*P* = .005–.009). When individual doses of pitrakinra were compared with placebo within the rs8832GG genotype, there was a significant decrease in exacerbations at the 10-mg dose (*P* = .03), corresponding to a 22% overall reduction and an 88% relative reduction. Significant dose-response trends for subjects homozygous for the major allele were also observed for rs3024530, rs3024622, and rs4787956 (*P* = .03, data not shown). There were also no significant differences in baseline demographic or clinical characteristics between subjects with the rs8832GG genotype and subjects with AG/AA genotypes, which would confound these therapeutic associations with asthma exacerbations (see Table E3 in this article’s Online Repository at www.jacionline.org).

In a subgroup analysis of participants randomized to 3- and 10-mg doses of pitrakinra only, subjects homozygous for the common alleles in 6 *IL4RA* SNPs were significantly less likely to have an asthma exacerbation than subjects with the minor allele, including rs8832 and rs3024530, which had the lowest *P* values (Fig 2, *P* = .007). Inversely, participants with the rs1110470 minor allele were less likely to experience exacerbations compared with subjects with the common allele (Fig 2). These SNPs were not associated with exacerbations in the placebo group. Additionally, a lower *P* value for association with asthma exacerbations in the combined 3- and 10-mg treatment groups was observed for 4 haplotypes that contain the rs8832 G allele (3, 4, 6, and 7 listed in Table II). As indicated by an odds ratio of less than 1, subjects with these haplotypes were less likely to experience exacerbations.
exacerbations and asthma symptom scores. Therefore we ex-
response for secondary end points related to increased time to
eosinophil counts, there was also a favorable pitrakinra treatment
was lower in this group.
were smaller, and therefore our power to detect associations
association with this SNP and asthma exacerbations in subjects with
Il4Ra SNPs. There was a significant dose-response trend for treatment response
to exacerbations in subjects homozygous for the rs8832GG genotype,
for treatment response to exacerbations in subjects homozygous for the rs8832GG genotype (A) and the GG genotype in the correlated SNP rs1029489 (B).

Secondary outcomes
Although there was little overlap between subgroups of
participants with high blood eosinophil counts and the Il4Ra/
rs8832GG genotype, we explored the relationship between
the rs8832 genotype and exacerbations, as stratified by eosinophil
counts. Subjects with the GG genotype receiving active pitrakinra
(all doses combined) in the low-eosinophil-count group only were
less likely to have an exacerbation (11%) than subjects with the
AG (16%) or AA (24%) genotype (P = .04). There was no asso-
ciation with this SNP and asthma exacerbations in subjects with
high blood eosinophil counts, although the number of subjects
were smaller, and therefore our power to detect associations
was lower in this group.
For subjects in the intent-to-treat population with high blood
eosinophil counts, there was also a favorable pitrakinra treatment
response for secondary end points related to increased time to
exacerbations and asthma symptom scores. Therefore we ex-
plor ed these clinical end points in our pharmacogenetic analysis
in all genotyped non-Hispanic white subjects. Kaplan-Meier plots
were developed to test for significant differences in time to asthma
exacerbation by treatment assignment stratified by Il4Ra SNP
genotypes. As shown in Fig 3, for subjects with the rs8832GG
genotype, there were significant differences in time to asthma
exacerbation for the subjects randomized to 10 mg of pitrakinra
compared with those receiving placebo (P = .02, log-rank test).
Similar results were noted for subjects homozygous for the com-
mon alleles in rs1029489 (P = .005, placebo vs 10-mg log-rank
and rs4787956 (P = .02).
We also investigated IL4Ra variation and changes in asthma
symptoms. There was a dose-dependent reduction in nocturnal
awakenings in subjects with the rs8832GG (Fig 4, A) and
rs3024622CC (Fig 4, B) genotypes. Furthermore, activities
limited by asthma remained similar to baseline or improved in sub-
jets randomized to pitrakinra with the rs8832GG (P = .009),
rs1029489GG (P = .01), and rs4787956AA (P = .01) genotypes.
Our primary hypothesis was that IL4Ra amino acid changes or
closely proximal SNPs at the 3’ end of this gene would interact
with responses to pitrakinra therapy (including rs1805011,
rs1801275, rs8832, rs1029489, rs12102586, and rs4797956; see
Fig E1). For our primary dose-response relationship analysis,
rs8832 (adjusted P = .05) and rs1029489 (adjusted P = .03)
were associated with a therapeutic response to this intervention
after this correction.

Discussion
This is the first large pharmacogenetic analysis of the Th2-
related IL-4/IL-13 inflammatory receptor in subjects with
moderate-to-severe asthma. In this relatively short (12-week)
trial, LABAs were tapered 4 weeks after initiation of blinded
IL-4Rα antagonist or placebo treatment, and inhaled corticoste-
roids were tapered and withdrawn subsequently. Although there
was no overall significant difference between active IL-4Rα ther-
apy and placebo,5,16 therapeutic efficacy was observed by IL4Ra
genotype in this pharmacogenetic analysis. Consistent with our
hypothesis that IL4Ra variants were associated with a specific re-
duction in asthma exacerbations in subjects randomized to anti-
IL-4Rα receptor antagonist, the most consistent pharmacogenetic
association was observed for the tagging SNP rs8832. Non-
Hispanic white subjects homozygous for the rs8832GG common
allele randomized to active pitrakinra experienced fewer exacer-
bations (P = .03), nocturnal awakenings (P = .04), and asthma
limitations in activities (P = .009) compared with subjects with
the rs8832 A minor allele. More importantly, there was a signifi-
cant dose-response relationship to this IL-4Rα inhibitor for
asthma exacerbations in subjects with the rs8832GG genotype,
which was also observed for the correlated SNP rs1029489
(which remained significant after adjustment for multiple test-
ing). A significant clinical response was observed for 4 haplotypes
that contained the rs8832 common G allele associated with de-
creased exacerbations (P = .0003-.0006) in subjects randomized
to 3 and 10 mg of pitrakinra. These results provide evidence that
IL-4Rα variation modulates therapeutic responses in asthmatic
subjects and identifies a subgroup of subjects with moderate-to-
severe persistent asthma who respond with reduced asthma exac-
terations and symptoms. On the basis of extrapolation from the
survival curve in Fig 3, we speculate that there might be a long-
term positive effect for subjects with the rs8832GG genotype
receiving active therapy compared with those receiving placebo.
This analysis is based on an earlier pharmacogenetic analysis
that we performed on IL-4Rα inhibition with pitrakinra to reduce
late-phase allergen responses.15 In this prior smaller allergen
challenge study of 28 subjects, we identified 2 nonsynonymous
variants in IL4Ra (E400A and Q576R) and several other tagging
SNPs that were associated with a reduction in delayed allergen responses. In addition, we supplemented these preliminary pharmacogenetic responses with an analysis of late-phase allergen responses in nonhuman primates, showing that IL-4Rα inhibition reduces bronchial hyperresponsiveness and eosinophilia after antigen airway challenge.13 In this current analysis E400A and Q576R polymorphisms were not associated with asthma exacerbations and treatment responses, possibly because of small numbers of subjects in the previous analysis or differences in study design and primary outcome. However, in this pharmacogenetic analysis significant results were noted for the tagging SNPs rs2239347 and rs3024622, which were statistically associated with reduced late-phase antigen response and therapeutic pitrakinra responses previously13 and were also predictors of reduction in exacerbations in subjects randomized to 3- and 10-mg doses of pitrakinra in this study. The most important pharmacogenetic associations were observed for the correlated SNPs rs8832 and rs1029489 (r² = 0.75), which were not associated with a therapeutic pitrakinra response in our previous small antigen challenge studies.13 Although differences in the 2 studies can be caused by different therapeutic end points, our primary hypothesis that IL4RA variation identifies a subset of asthmatic subjects more responsive to IL-4Rα inhibition was confirmed.

The pharmacologic mechanism for modulation of pitrakinra by rs8832 and rs1029489 is unknown because these tagging SNPs have no known function and reside in the IL4RA 3’ untranslated region and the proximal gene region, respectively (see Fig E1). Additionally, because IL4RA SNPs significantly associated with clinical outcomes were common in the non-Hispanic white population (approximately 10% to 45% MAFs) and were chosen to “tag” correlated IL4RA variants, these SNPs might only be markers for a different functional variant, and further in-depth sequencing of the IL4RA 3’ region might provide additional information regarding the functional mechanism of IL4RA interaction with pitrakinra. We speculate that genetic variants in the 3’ untranslated region of the receptor might affect RNA stability or processing that could potentially interact to modulate Tfh2 inflammation. However, an important concept of this analysis is that it identifies a subset of subjects with severe persistent asthma who are responsive (33% of subjects with the rs8832GG

**TABLE II.** IL4RA 5-SNP sliding window haplotypes and association with asthma exacerbations in non-Hispanic white subjects randomized to 3 and 10 mg of pitrakinra treatment

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>rs3024585</th>
<th>rs3024622</th>
<th>rs4787423 (E400A)</th>
<th>rs1801275 (Q576R)</th>
<th>rs8832</th>
<th>rs1029489</th>
<th>rs12102586</th>
<th>rs4787956</th>
<th>Frequency</th>
<th>Odds ratio</th>
<th>P value*</th>
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<tbody>
<tr>
<td>1</td>
<td>G</td>
<td>C</td>
<td>T</td>
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<td>0.38</td>
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</table>

Selected IL4RA haplotypes from the 3’ end and the proximal gene region with frequencies of greater than 10% are shown, with P values and odds ratios for association with asthma exacerbations. An odds ratio of greater than 1 indicates a haplotype is associated with increased exacerbations.

*P values adjusted for geographic region in logistic regression models.
genotype) to anti–IL-4Rα therapy and also shows that the other 67% were not responsive. Without this genetic biomarker, these responsive subjects are not easily identified. Other approaches that include use of blood biomarkers, such as eosinophil counts, characterize a somewhat more responsive subset that is not as specific as these pharmacogenetic findings. For example, subjects with the rs8832GG genotype had a greater relative reduction in asthma exacerbations at the 10-mg level compared with placebo than the group with high blood eosinophil counts.\textsuperscript{15,16} This genetic variant might potentially represent an excellent predictive genetic marker for therapeutic responses to anti–IL-4Rα receptor intervention. However, the predictive value of variation in IL4RA needs to be further replicated in additional studies with either pitrakinra or other biologic therapies that target IL-4, IL-13, or their receptor, perhaps by using a genotyped stratified trial design.\textsuperscript{23,24}

These pharmacogenetic approaches could lead to targeted therapies in this pathway. A similar method has been recently proposed for anti–IL-13 treatment and predictive responses in asthmatic patients with higher serum periostin levels or higher levels of FENO.\textsuperscript{10} These biomarkers identify a subset of asthmatic subjects more responsive to an IL-13 antibody.\textsuperscript{17} Periostin, FENO, and other biomarkers also require replication and could potentially be used in conjunction with pharmacogenetic analysis.

In this important pharmacogenetic study, we were able to take advantage of comprehensive pharmacologic and phenotype information to evaluate the therapeutic interaction between IL4RA variants and different doses of pitrakinra, an IL-4 receptor antagonist. These results provide novel evidence that in non-Hispanic white subjects with moderate-to-severe asthma with a common genotype, the rs8832GG genotype treated with this anti–IL-4Rα inhibitor demonstrates a significant dose-dependent reduction in asthma exacerbations and improved outcomes for several other asthma-related clinical end points. Thus these results represent one of the first examples of a pharmacogenetic effect based on receptor variation that identifies a subset of subjects with severe asthma who respond to a new biologic intervention. These analyses provide a basis for understanding therapeutic heterogeneity in asthmatic subjects and are an important step toward
paradigms that are based in individualized or stratified treatments in subjects with asthma and other common disorders.

We thank Carla Martin, Shelly Smith, and Siqun (Lilly) Zheng at the Wake Forest Center for Genomics and Personalized Medicine Research for genotyping assistance.

Clinical implications: Pharmacogenetic analysis of the IL4RA gene identified a subgroup of asthmatic subjects by genotype who were more responsive to therapy with an IL-4/IL-13 pathway antagonist.

REFERENCES
FIG E1. Map of *IL4RA* variants genotyped in this study and the linkage disequilibrium (*r*²) plot (generated in Haploview version 4.2) for genotyped non-Hispanic white subjects. Significant pharmacogenetic associations with asthma exacerbations are noted.
### TABLE E1. Asthma symptom score questionnaire

<table>
<thead>
<tr>
<th>Asthma symptoms question</th>
<th>Scores and possible answers</th>
</tr>
</thead>
<tbody>
<tr>
<td>How many times did you awaken because of asthma last night?</td>
<td>0: Did not awaken because of asthma</td>
</tr>
<tr>
<td></td>
<td>1: Awoke once because of asthma</td>
</tr>
<tr>
<td></td>
<td>2: Awoke twice because of asthma</td>
</tr>
<tr>
<td></td>
<td>3: Awoke 3 times because of asthma</td>
</tr>
<tr>
<td></td>
<td>4: Was not able to sleep at all because of asthma</td>
</tr>
<tr>
<td>Were your daily activities affected by your asthma today?</td>
<td>0: No effect of asthma on my daily activities</td>
</tr>
<tr>
<td></td>
<td>1: Activity was normal and only mildly affected by my asthma</td>
</tr>
<tr>
<td></td>
<td>2: Activity was normal but moderately affected by my asthma</td>
</tr>
<tr>
<td></td>
<td>3: Asthma symptoms limited to my activity to a significant degree</td>
</tr>
<tr>
<td></td>
<td>4: Asthma symptoms severely restricted my daily activities</td>
</tr>
<tr>
<td>Morning question: How many puffs of rescue inhaler did you use last night?</td>
<td>Participants numerically reported</td>
</tr>
<tr>
<td>Evening question: How many puffs of rescue inhaler did you use today?</td>
<td>Participants numerically reported</td>
</tr>
</tbody>
</table>

Score changes from baseline were analyzed as secondary end points in this pharmacogenetic analysis.
**TABLE E2.** Tagging and missense *IL4RA* polymorphisms genotyped in non-Hispanic white subjects in all treatment groups in the pitrakinra 2b clinical trial population

<table>
<thead>
<tr>
<th>SNP</th>
<th>Location</th>
<th>Major: minor allele</th>
<th>No. of subjects genotyped</th>
<th>MAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs6498012</td>
<td>Intron</td>
<td>G:C</td>
<td>405</td>
<td>0.39</td>
</tr>
<tr>
<td>rs1110470</td>
<td>Intron</td>
<td>G:A</td>
<td>406</td>
<td>0.48</td>
</tr>
<tr>
<td>rs4787948</td>
<td>Intron</td>
<td>A:G</td>
<td>403</td>
<td>0.31</td>
</tr>
<tr>
<td>rs2283563</td>
<td>Intron</td>
<td>C:T</td>
<td>405</td>
<td>0.31</td>
</tr>
<tr>
<td>rs3024530</td>
<td>Intron</td>
<td>A:G</td>
<td>405</td>
<td>0.45</td>
</tr>
<tr>
<td>rs3024543</td>
<td>Intron</td>
<td>G:A</td>
<td>402</td>
<td>0.13</td>
</tr>
<tr>
<td>rs1805010</td>
<td>Missense (I75V)</td>
<td>A:G</td>
<td>400</td>
<td>0.45</td>
</tr>
<tr>
<td>rs3024560</td>
<td>Intron</td>
<td>T:G</td>
<td>405</td>
<td>0.37</td>
</tr>
<tr>
<td>rs3024576</td>
<td>Intron</td>
<td>G:A</td>
<td>404</td>
<td>0.08</td>
</tr>
<tr>
<td>rs2239347</td>
<td>Intron</td>
<td>A:C</td>
<td>403</td>
<td>0.47</td>
</tr>
<tr>
<td>rs3024585</td>
<td>Intron</td>
<td>G:A</td>
<td>406</td>
<td>0.46</td>
</tr>
<tr>
<td>rs3024622</td>
<td>Intron</td>
<td>C:G</td>
<td>405</td>
<td>0.37</td>
</tr>
<tr>
<td>rs4787423</td>
<td>Intron</td>
<td>T:C</td>
<td>403</td>
<td>0.13</td>
</tr>
<tr>
<td>rs1805011</td>
<td>Missense (E400A)</td>
<td>A:C</td>
<td>406</td>
<td>0.11</td>
</tr>
<tr>
<td>rs1801275</td>
<td>Missense (Q576R)</td>
<td>A:G</td>
<td>406</td>
<td>0.21</td>
</tr>
<tr>
<td>rs8832</td>
<td>3’ Untranslated region</td>
<td>G:A</td>
<td>405</td>
<td>0.44</td>
</tr>
<tr>
<td>rs1029489</td>
<td>3’ Proximal region</td>
<td>G:A</td>
<td>405</td>
<td>0.39</td>
</tr>
<tr>
<td>rs12102586</td>
<td>3’ Proximal region</td>
<td>C:T</td>
<td>396</td>
<td>0.10</td>
</tr>
<tr>
<td>rs4787956</td>
<td>3’ Proximal region</td>
<td>A:G</td>
<td>403</td>
<td>0.34</td>
</tr>
</tbody>
</table>
TABLE E3. Comparison of baseline demographic and clinical characteristics between the rs8832GG and AG/AA genotypes

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Non-Hispanic white subjects with rs8832 GG genotype (n = 134)</th>
<th>Non-Hispanic white subjects with rs8832 AG/AA genotype (n = 271)</th>
<th>P value, t test or χ² test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (% female)</td>
<td>60</td>
<td>58</td>
<td>.68</td>
</tr>
<tr>
<td>Country (% United States/United Kingdom/Poland/Hungary)</td>
<td>36/4/32/28</td>
<td>44/4/30/22</td>
<td>.49</td>
</tr>
<tr>
<td>Age at enrollment (y)</td>
<td>44.8 ± 14.1</td>
<td>45.2 ± 13.6</td>
<td>.81</td>
</tr>
<tr>
<td>Age of asthma onset (y)</td>
<td>29.6 ± 19</td>
<td>27.6 ± 18.5</td>
<td>.31</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>29.3 ± 6.6</td>
<td>29.4 ± 6.2</td>
<td>.85</td>
</tr>
<tr>
<td>Baseline lung function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV₁ (% predicted)</td>
<td>72.4 ± 10.4</td>
<td>72.2 ± 11.8</td>
<td>.87</td>
</tr>
<tr>
<td>FVC (% predicted)</td>
<td>83.3 ± 11.4</td>
<td>84.9 ± 12.7</td>
<td>.22</td>
</tr>
<tr>
<td>FEV₁/FVC ratio</td>
<td>70.0 ± 9.4</td>
<td>68.5 ± 10.8</td>
<td>.17</td>
</tr>
<tr>
<td>Blood eosinophils (cells/mm³)</td>
<td>289.6 ± 239.1</td>
<td>253.5 ± 204.3</td>
<td>.12</td>
</tr>
<tr>
<td>Total serum IgE (IU/mL)*</td>
<td>429.7 ± 683.6</td>
<td>337.8 ± 777.2</td>
<td>.07</td>
</tr>
<tr>
<td>FENO (ppb)</td>
<td>17.6 ± 12.2</td>
<td>20.1 ± 17</td>
<td>.33</td>
</tr>
</tbody>
</table>

FVC, Forced vital capacity.

*One outlier removed; t test performed on log-transformed values.