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IL4RA Polymorphisms are Predictors of a Pharmacogenetic Response to a Novel IL4/IL13 Antagonist

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To the Editor:

Evidence from genomic research, *in vitro* cell signaling, and animal studies strongly implicates the interleukin 4/13 (IL4/13) pathways in the pathogenesis of allergic asthma.[1-7] However, there is limited data on pharmacological 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 IL4/13 dual antagonist, in patients with allergic asthma (Clinicaltrials.gov identifier NCT00535431).[8] Pitrakinra is similar to the native IL4 molecule, though specifically designed with two amino acid changes (R121D/Y124D), rendering it an effective competitive antagonist of interleukin 4 receptor α (IL4Rα).[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 pre-clinical antigen-challenge studies, in which monkeys were treated with an IL4/IL13 antagonist similar to pitrakinra and with the same mechanism of action.[9] We hypothesized that single nucleotide polymorphisms (SNPs) in the IL4Rα gene (IL4RA) would predict treatment response in the human studies and there would be a similar effect of IL4RA gene variation in non-human 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

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response to inhaled allergen challenge in patients with asthma. Acute and late phase responses were measured at screening and after four weeks of twice-daily therapy with nebulized pitrakinra or placebo. After allergen challenge, Forced Expiratory Volume in 1 second (FEV_1) 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 four weeks of treatment with either placebo or pitrakinra compared to baseline levels. Therefore, an increased LAR ratio corresponds to a reduction in late phase FEV_1 response to antigen following 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 single nucleotide polymorphisms (SNPs) and 3 non-synonymous amino acid coding changes from the HapMap CEU reference population for genotyping using the Sequenom MassARRAY iPLEX platform. We evaluated 17 IL4RA SNPs in Hardy-Weinberg equilibrium (HWE; $P > 0.01$) with minor allele frequency (MAF) >5% (Figure 1A). 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 v9.1 (SAS Institute Inc., Cary, NC) or Plink v1.06. We did not correct for multiple comparisons in this exploratory analysis of human and Cynomolgus IL4RA variation.

Figure 1 shows the linkage disequilibrium (LD) structure for the human IL4RA SNPs analyzed in this study and SNP associations with LAR ratio. Notably, the non-synonymous polymorphism rs1801275 (Q576R) was a significant predictor of mean difference in LAR ratio by genotype ($P = 0.04$) and interaction with treatment ($P < 0.0001$). As shown in Figure 1B, one individual homozygous for the R/R variant had an increased LAR ratio, indicating improved lung function relative to baseline, in the pitrakinra-treated group only. However, there was only one individual homozygous for the R/R variant, which may be primarily responsible for the significant association with LAR. If Q/R and R/R individuals were analyzed together, there was a borderline significant interaction with treatment ($P = 0.06$). Similarly, rs1805011 (E400A) E/A heterozygotes treated with pitrakinra had a higher mean LAR ratio than E/E homozygotes (Figure 1C).

The complementary outcomes in the primate pre-clinical trials were bronchial hyperresponsiveness (BHR) to methacholine (provocative challenge or PC_{100}) and bronchoalveolar lavage eosinophilia in response to Ascaris allergen exposure. An IL4/13 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 via respirator and BHR to methacholine was measured in anesthetized, ventilated animals. Bronchoalveolar lavage was performed with saline using standard methods. We used general linear models with an additive genetic term to evaluate Cynomolgus IL4RA polymorphisms associated with the log-transformed post-allergen IL4/13 antagonist treatment to 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).

Since Cynomolgus genomic IL4RA DNA sequence was not available, we directly sequenced 100 base pairs surrounding IL4RA exons, the proximal promoter and 3' untranslated (UTR) regions using DNA from 23 monkeys. Cynomolgus IL4RA genomic and predicted mRNA sequence was compared to Rhesus *Macaca mulatta* using Sequencher DNA analysis software and UCSC 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 seven synonymous, nine non-synonymous coding changes and six insertion/deletion polymorphisms. All SNPs tested for association (n=83) had ≥70% genotyping data, a MAF of ≥ 2% and were concordant with HWE. The genomic position and MAF for 13 polymorphisms significantly associated with BHR or eosinophilia are presented in Table I and the LD structure is shown in Figure 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 to controls. Only one relatively low frequency SNP (In5_7; MAF = 0.02), was associated with both endpoints (Table I), though this result was mainly due to one (C/T) heterozygote with a higher eosinophil ratio and a lower BHR ratio than (C/C) homozygotes. Though 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 may form the basis of future study.

Genetic association with response to allergen-induced clinical outcomes was found across the IL4RA gene (Figure 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 the human. However, replication for pharmacogenetic studies is challenging and this analysis provides separate lines of evidence that IL4RA variation may modulate pharmacogenetic response to allergen challenge. Admittedly, the sample sizes for this unique analysis are small. In humans, 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 IL4 and IL13 regulated genes and mediate allergic inflammation in a mouse model of asthma.[7] We speculate that the variant at position 400 as well as 576 may also affect signaling through other intracellular mediators such as janus kinase 1.

This analysis suggests that IL4RA amino acid variations may predict a therapeutic treatment response to anti-IL4/IL13 pathway inhibitors, though larger studies are required to confirm these findings. Pharmacogenetic studies such as these may become increasingly important to predict individual response to invasive and possibly expensive novel asthma therapies.

Acknowledgments

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Abbreviations

BHR	Bronchial hyperresponsiveness
HWE	Hardy-Weinberg equilibrium
FEV_1	Forced Expiratory Volume in 1 second
IL4	Interleukin 4
IL4RA	Interleukin 4 receptor α gene
IL13	Interleukin 13
LAR	Late antigen response
LD	Linkage disequilibrium
MAF	Minor allele frequency

PC_{100}	Provocative challenge
SD	Standard deviation
SNP	Single nucleotide polymorphism

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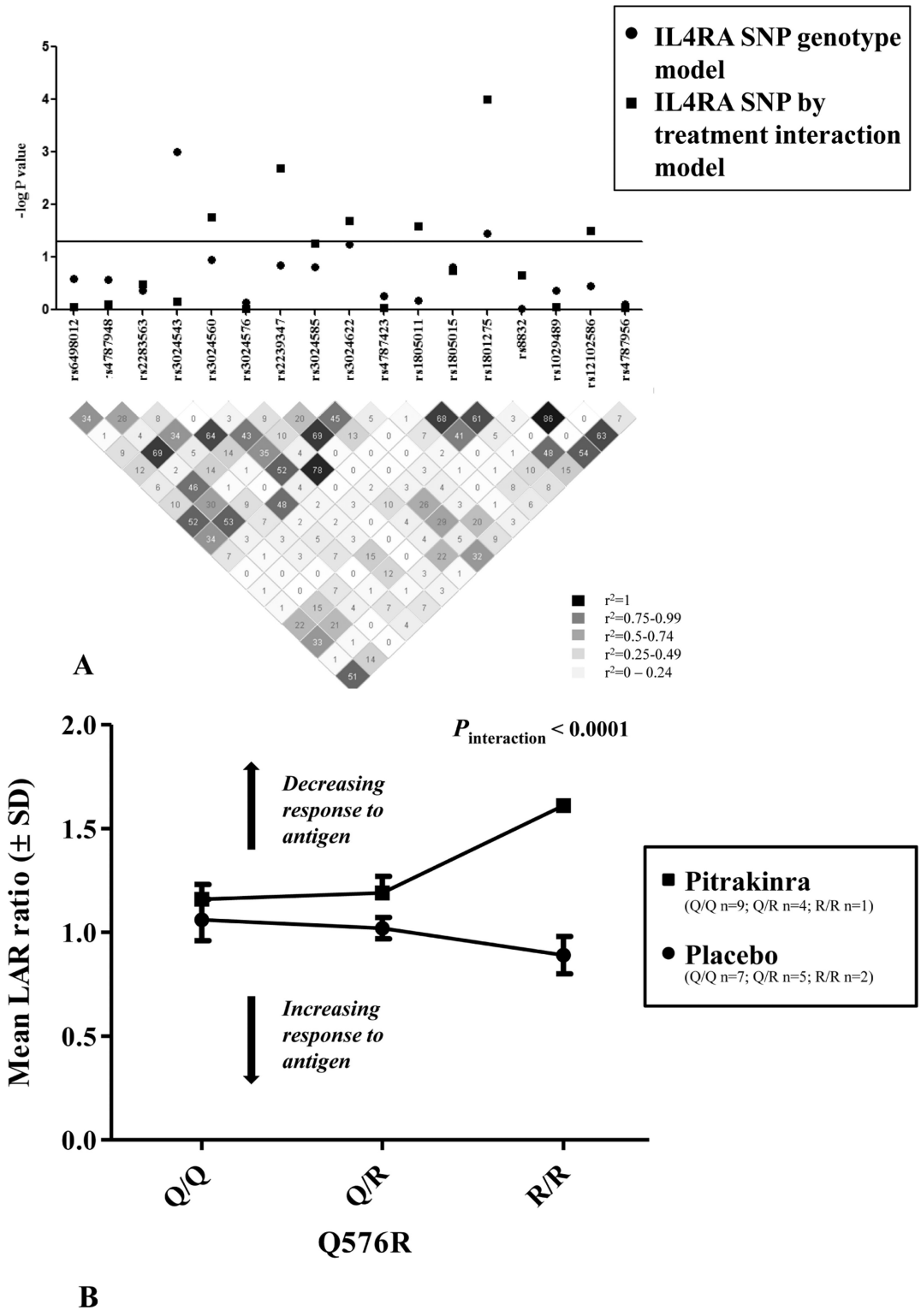
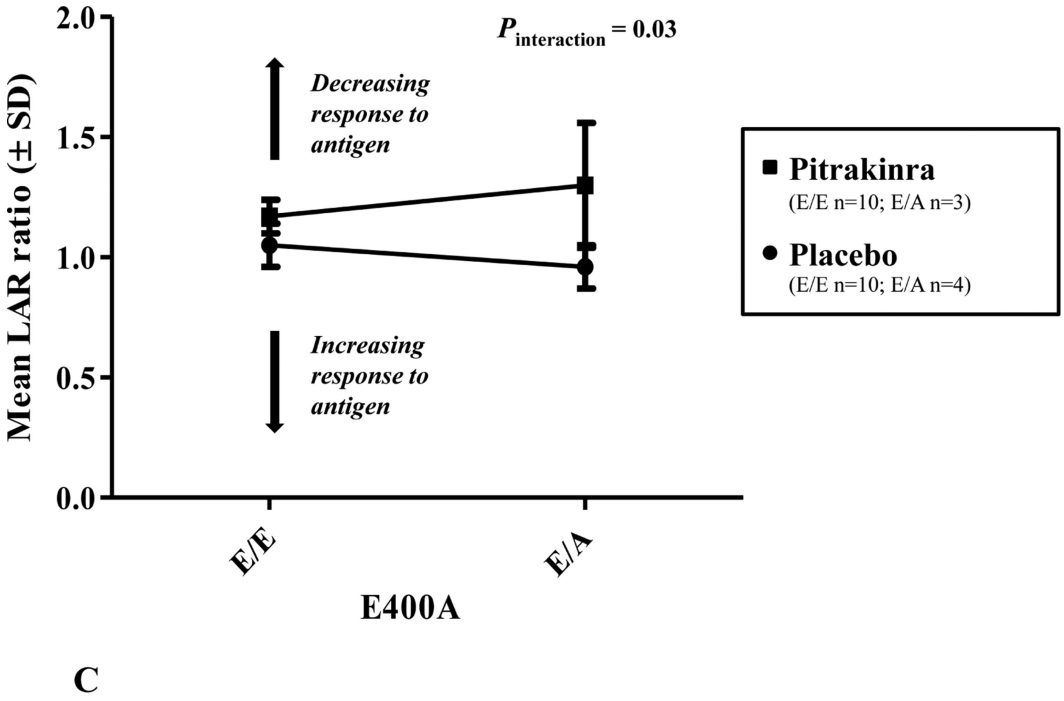
Capsule summary

The IL4RA Q576R polymorphism may predict treatment response to an IL4/IL13 antagonist in patients with asthma. Pharmacogenetic analysis of IL4RA in humans and non-human primates was concordant and supports further studies in larger populations.





C

Figure 1.

A, Human IL4RA SNP LD 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 $P < 0.05$; LD plot generated in Haploview v4.1. **B,** Mean (± standard deviation; SD) LAR ratio by IL4RA rs1801275 (Q576R) genotype and treatment group. **C,** Mean (±SD) LAR ratio by IL4RA rs1805011 (E400A) genotype and treatment group.

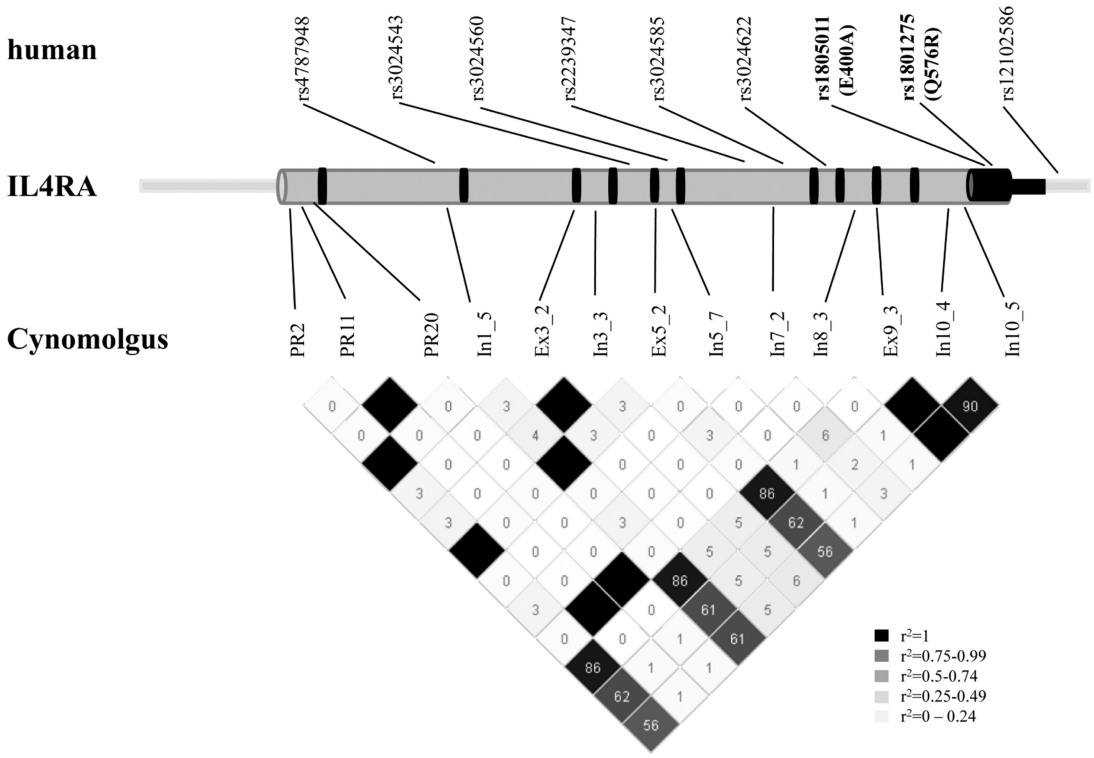


Figure 2.

Map of human and Cynomolgus monkey IL4RA polymorphisms significantly associated with allergen-induced clinical outcomes. IL4RA introns and promoter are depicted in grey; exons and 3' untranslated region in black. Genomic information primarily adapted from the human and Rhesus UCSC genome Browser. Rhesus sequence (the closest publically available sequence to Cynomolgus) contains gaps, therefore map is not to scale. Cyno IL4RA SNP LD plot and SNP correlation (r^2) generated in Haploview v4.1.

Table I

Cynomolgus IL4RA SNPs significantly associated ($P < 0.05$) with BHR and eosinophil counts (post-treatment with IL4/13 antagonist compared to control).

Cynomolgus IL4RA polymorphism	Rhesus chromosome 20 location	IL4RA SNP location	Major/minor allele; minor allele frequency	BHR *P* value	Eosinophil *P* value
PR2	25763581	5' proximal	C/T; 0.26	0.03	0.61
PR11	Gap	5' proximal	C/T; 0.02	0.14	0.05
PR20	25763996	5' proximal	T/A; 0.02	0.14	0.05
In1_5	Gap	intron	G/C; 0.27	0.04	0.65
In3_2	25794379	Coding Syn Ser	C/T; 0.07	0.04	0.60
In3_3	25794391	intron	T/C; 0.08	0.02	0.44
Ex5_2	25799301	Coding Syn Asn	C/T; 0.26	0.03	0.61
In5_7	25799454	intron	C/T; 0.02	0.03	0.01
In7_2	25803175	intron	G/C; 0.08	0.64	0.05
In8_3	25806353	intron	G/A; 0.02	0.14	0.05
Ex9_3	25809423	Coding Syn Ile	C/T; 0.34	0.04	0.74
In10_4	25811349	intron	G/A; 0.33	0.05	0.91
In10_5	25811415	intron	C/T; 0.36	0.05	0.82

BHR, bronchial hyperresponsiveness