Abstract

**Background.** Interleukin 4 (IL-4) and its receptor play important roles in the pathologies of asthma and atopy. The alpha subunit of the IL-4 receptor (IL-4RA) is included in 2 types of receptors which have different modulatory effects on immune responses. This distinct pattern reflects involvement in the immunopathology of both asthma and atopy. A number of studies have proven the association between \(\text{IL4RA}\) gene polymorphisms and asthma and atopy, but it is still an open question whether these variants are functional.

**Objectives.** To analyze the data from \(\text{IL4RA}\) gene expression in PBMC in relation to specific polymorphisms — the most frequently studied I50V and Q551R and the less known C-3223T.

**Material and methods.** The analysis was performed for 36 subjects, both atopic and non-atopic. Real-time polymerase chain reaction (PCR) was used with specific primers for the quantification and genotyping. Delta Ct (\(\Delta\text{Ct}\)) and delta-delta Ct (\(\Delta\Delta\text{Ct}\)) values were used for the relative quantification of \(\text{IL4RA}\) expression in PBMC.

**Results.** We observed no significant differences in the \(\text{IL4RA}\) expression profile between the 3 genotypes. A trend toward higher relative expression was observed for homozygous minor I50V and C-3223T genotypes.

**Conclusions.** We did not find a statistically significant relationship between the genetic polymorphisms and the relative expression of \(\text{IL4RA}\). The effect of genetic polymorphism on \(\text{IL4RA}\) mRNA expression could interfere with other factors, such as environmental stimuli, and should be evaluated in future studies.

**Key words:** gene expression, polymorphism, asthma, atopy, \(\text{IL4RA}\)
Introduction and objectives

Interleukin 4 (IL-4) and its receptor play important roles in the pathologies of asthma and atopy. The alpha subunit of the IL-4 receptor (IL-4RA) is included in 2 types of receptors, which have different modulatory effects on immune responses. Type I is responsive to IL-4 only, and is mainly expressed on myeloid cells. According to the eQTL database, the highest expression of IL4RA (type I) is in whole blood (Genehopper, http://genehopper.ifis.cs.tu-bs.de; GTExPortal, https://gtexportal.org). Type II IL-4R acts as the receptor for both IL-4 and IL-13. It is expressed in bronchial mucosa and a variety of other cells. While type I receptors transmit signals related to immunoglobulin switching and immunoglobulin E (IgE) production, type II plays a role in bronchoconstriction, inflammation and mucus production. This distinct pattern reflects involvement in the immunopathology of both asthma and atopy. Increased IL4RA expression has been found in both conditions.1–3

While IL4RA is expressed by all subtypes of lymphocytes, the majority are expressed on B and Th2 cells, as reported in the expression profiling database. Changes in the mRNA expression of IL4RA mirror changes in the proportion of different T cell subsets.2 The regulation of IL-4RA protein production is complex and not yet fully understood. Furthermore, the process differs across different tissue types. T cells react mainly to stimulation by IL-4, but also react to IL-2. Secretion of the IL-2 cytokine occurs as a natural response to microbial infection and autoimmune phenomena, and together with IL-4 can induce and maintain IL4RA expression by activating STAT5.4 The activation pathway differs for different types of T cells. For instance, naïve CD4+ T cells react by upregulating IL-4RA, which is not antigen-specific. The highly activated T cells respond by downregulating IL-4RA, specifically with high amounts of antigen. This mechanism is part of a homeostasis process aimed at “saving” IL-4 for B cells and IgE production.5 Also, sIL-4R, which is the soluble form of IL-4R, is known to regulate IL-4. It is formed by alternative splicing or proteolytic shedding of the membrane-bound form. The alternation in the concentration of sIL-4R and the expression of mIL4RA could reflect immune tolerance, which occurs in situations such as during the course of immunotherapy.2

There are a number of studies in the literature that suggest an association between IL4RA polymorphisms and asthma and atopy.6–8 Functional changes have been observed for Q551R and I50V, which seem to enhance the response to IL-4 in vitro.9 However, only a few experiments have confirmed this phenomenon (OMIM database, https://www.omim.org). It has been shown that the presence of Q551R could indicate the degree of responsiveness to IL-4RA-antagonist treatment.10 This specific polymorphism has also been found to be associated with the IL-4-related Treg differentiation pathway, a subpopulation of T cells which are crucial for immune tolerance. This dependence is present in the subgroup of asthma patients with the mixed-cellularity Th2/Th17 phenotype.11 Furthermore, upregulation of IL-4RA due to a gain-of-function mutation in the gene with the F709 single nucleotide polymorphism (SNP) results in a failure to produce antigen-specific Treg.12 Apart from the studies mentioned above, there is still the open question of whether variants within IL4R are functional or are only markers for causative variation nearby in the region. Is that specific polymorphism in fact related to the pattern of increased or decreased IL4R expression, thus mediating the risk for allergy and asthma?

Our group previously reported IL4RA expression in relation to atopy status and place of residence. We did find a trend for atopic subjects to have a higher expression and for those living in the countryside to have lower values, though the differences were not statistically significant.13 Herein, we present the results of IL4RA expression analysis in relation to specific polymorphisms within the gene – most of them well-known from association studies, I50V and Q551R, and the less-studied C-3223T.

Material and methods

The methods for relative gene expression using real-time quantitative polymerase chain reaction RT-qPCR) and the characteristics of the study group have already been described in detail.13 We used the expression data from the subjects enrolled in our previous study and performed genotyping for IL4RA polymorphisms. In the current analysis, 36 subjects with available data were enrolled, 18 of whom were atopic. The subjects assigned as controls were otherwise healthy. Atopy was confirmed by the result of a skin prick test (SPT) to common allergens. The SPTs were performed in all atopic and control subjects. Four of the subjects were not assigned to any group due to the lack of conclusive SPT results. Venous blood samples were collected into 2 tubes containing EDTA (Sarstedt AG & Co., Nümbrecht, Germany) for DNA extraction, PBMC isolation and RNA extraction. DNA was extracted from whole-blood samples using the QIAMP kit (Qiagen Inc., Valencia, USA) according to the manufacturer’s instructions. The expression data originated in the experiments using a LightCycler 1.5 and specific hybridization probes. ACTB (β-actin) was used as reference.13 Delta Ct (ΔCT) and delta-delta mean Ct (ΔΔCT) values were used for relative quantification of IL4RA expression in PBMC, and 2−ΔΔCT was used for fold change (FC) estimation in groups according to genotype. Genotyping for specific SNPs, including rs1805010 (I50V), rs1801275 (Q551R) and rs2057768 (C-3223T), was performed using specific Light SNP primers (TIB Molbiol, Berlin, Germany) and the LightCycler 1.5. The PCR conditions were as follows: denaturation – 1 cycle at 95°C for 10 min; cycling – 45 cycles of 95°C
for 10 s, 60°C for 10 s and 72°C for 15 s; melting – 1 cycle at 95°C for 30 s, 40°C for 2 s and a temperature rise to 75°C; cooling – 1 cycle at 40°C for 30 s.

The Fisher’s exact test was performed to compare the frequencies between the specific groups assigned based on their atopic status or genotype. The exact test was used to determine Hardy–Weinberg equilibrium (HWE) in the controls. The Kruskal–Wallis test and the Mann–Whitney U test were used to compare ΔCT between groups in relation to genotype. The analysis was performed in 3 groups: 1) pulled – all subjects with genotype data, 2) atopic and 3) the controls separately. The statistical tests were done with STATISTICA v. 13.2 software (StatSoft Inc., Tulsa, USA).

This study was approved by the ethical committee of the Wroclaw Medical University (Poland). All participants signed an informed consent form.

Results

The genotype and allele frequencies are presented in Table 1. For all genotypes, we observed no deviation from the HWE in the control group.

While comparing genotype frequencies in the groups related to atopic status, we observed significant differences only for I50V for the recessive model. Carriers of 2 minor alleles were more prone to be atopic. Other SNPs were not significantly associated with atopy; however, for SNP C-3223T we observed a trend for the variant allele to be predominant in the atopic group (Table 1).

There were no significant differences in relative IL4RA gene expression between the 3 genotypes (I50V, Q551R, and C-3223T; Fig. 1) in any of the 3 analyzed groups, with the use of different models. The most clear trend was observed in the pulled group, were there was a 1.39-fold change (2^ΔΔCT) in relative expression for the I50V SNP in the recessive model (GG compared to AA+AG) and a 2.5-fold change for the C-3223T SNP in the recessive model (AA compared to GG+AG). For Q551R, we only included the dominant model, as there was only 1 subject with the GG genotype (AG+GG compared to AA), which showed a 1.09-fold change. The direction of changes in relative gene expression was different in regard to atopy status, which suggests that atopy and the milieu of cytokines associated with it may be an additional determinant of expression (Table 2).

We also analyzed combinations of different genotypes. The most common was the genotype which was homozygous for major alleles of the 3 studied SNPs (25%). There were no significant differences in IL4RA gene expression when these combinations of genotypes were analyzed.

Table 1. Genotype frequencies according to genotype for SNPs I50V, Q675R and C-3223T in relation to atopy status and different models. Fisher’s exact test for significance; p < 0.05 was regarded as statistically significant

| Variable | Total n = 36 (%) | Atopic n = 18 (%) | Control n = 14 (%) | HWE in controls, p-value | χ²/Fisher, p-value |
|----------|-----------------|-----------------|-----------------|--------------------------|-----------------|
| I50V     |                 |                 |                 |                          |                 |
| AA       | 12 (0.33)       | 4 (0.22)        | 7 (0.5)         | 0.75                     | 0.08            |
| AG       | 14 (0.38)       | 7 (0.38)        | 6 (0.42)        |                          |                 |
| GG       | 10 (0.27)       | 7 (0.38)        | 1 (0.06)        |                          |                 |
| AA+AG    | 26 (0.72)       | 11 (0.61)       | 13 (0.92)       | 0.03                     | 0.10            |
| AG+GG    | 24 (0.67)       | 14 (0.77)       | 7 (0.5)         |                          |                 |
| A        | 38 (0.53)       | 15 (0.41)       | 20 (0.71)       | 0.02                     |                 |
| G        | 34 (0.47 – MAF) | 21 (0.58)       | 8 (0.29)        |                          |                 |
| Q551R    |                 |                 |                 |                          |                 |
| AA       | 16 (0.44)       | 12 (0.66)       | 5 (0.35)        | 0.15                     | 0.06            |
| AG       | 19 (0.53)       | 5 (0.27)        | 2 (0.14)        |                          |                 |
| GG       | 1 (0.03)        | 1 (0.05)        | 0              |                          |                 |
| AA+AG    | 35 (0.97)       | 17 (0.94)       | 14 (1.0)        | 0.72                     | 0.12            |
| AG+GG    | 20 (0.56)       | 6 (0.33)        | 9 (0.64)        |                          |                 |
| A        | 51 (0.71)       | 29 (0.8)        | 19 (0.67)       | 0.24                     |                 |
| G        | 21 (0.29 – MAF) | 7 (0.19)        | 9 (0.32)        |                          |                 |
| C-3223T  |                 |                 |                 |                          |                 |
| GG       | 23 (0.64)       | 10 (0.55)       | 10 (0.71)       | 0.6                      | 0.39            |
| AG       | 10 (0.27)       | 5 (0.27)        | 4 (0.28)        |                          |                 |
| AA       | 3 (0.08)        | 3 (0.16)        | 0              |                          |                 |
| GG+AG    | 33 (0.92)       | 15 (0.83)       | 14 (1.0)        | 0.15                     | 0.37            |
| AG+AA    | 13 (0.36)       | 8 (0.44)        | 4 (0.28)        |                          |                 |
| G        | 56 (0.78)       | 25              | 24             | 0.11                     |                 |
| A        | 16 (0.22 – MAF) | 11              | 4              |                          |                 |

MAF – minor allele frequency; HWE – Hardy–Weinberg Equilibrium exact test.
Discussion

In our previous study, we compared the expression profile of individuals in relation to atopy status and place of residence. Although the results were not statistically significant, they may suggest an environment-related regulatory mechanism connected with rural living.\textsuperscript{13} The aim of the current study was to determine whether the most important and well-studied SNPs within the \textit{IL4RA} gene are responsible for the different patterns of gene expression. We observed no significant differences in expression profile according to genotype within the different models. A trend was observed for I50V and C-3223T. Individuals who were homozygous for minor alleles had a higher gene expression of \textit{IL4RA}. This suggests a pattern where \textit{IL4RA} expression is to some extent related to the presence of the SNPs reported as being associated with allergy.

To our knowledge, there are only 2 studies in the literature to which we can refer our results. As in our study, these researchers found no clear relationship between Q551R and the expression profile of \textit{IL4RA} in their 33 subjects.\textsuperscript{3} The trend they observed was different to our findings regarding this SNP, though: minor alleles were rather associated with higher, not lower expression. The eQTL database (GTExPortal) revealed a significant eQTL in whole blood related to the C-3223T genotype. The same was present for I50V, but not for Q551R.\textsuperscript{14} In both cases homozygous minor alleles showed lower expression, which is contrary to our findings. The small body of evidence in this field is striking in light of the fact that biological treatment with IL-4RA antibody is already in use.\textsuperscript{15,16}

There are also 2 studies suggesting an epigenetic effect of the \textit{ILR4A} polymorphism. In the 1\textsuperscript{st} study, out of the 9 SNPs evaluated (including C-3223T), only 1 (rs3024685) showed an interaction with the methylation status of the gene, which could somehow reflect expression.\textsuperscript{17} In the 2\textsuperscript{nd} study, 8 SNPs and 4 CpGs connected to the TH2 pathway were included in the model of interaction with DNA methylation and asthma risk; between them, cg26937798 was revealed to confer such a risk.\textsuperscript{18}

The polymorphisms we choose for this study were previously explored in various contexts, mainly related to asthma and atopy. Both I50V and Q551R were the first SNPs within \textit{IL4RA} to be described in relation to these conditions. The C-3223T SNP was first described by Hackstein in 2001, and has been subsequently investigated in a few studies.\textsuperscript{19} GWA studies (Genome Wide Association Studies) have confirmed the role of all 3 of these polymorphisms.\textsuperscript{20} Both I50V and Q551R appear to have functional outcomes through enhanced IL-4 signaling, as indicated in the OMIM database. In a meta-analysis including 50 studies, the I50V variant was found to be associated with asthma in the dominant model, and Q551R in the recessive model. In addition, I50V has been associated with asthma in Asian populations, and has also been related to pediatric and atopic asthma.\textsuperscript{7} In other studies,
I50V and C-3223T (homozygous), but not Q551R, were found to be related to early-onset asthma. In another study that compared eight SNPs of *IL4RA*, including C-3223T, Q551R, and I50V, only I50V showed a significant association with total IgE. However, in a study that investigated asthma phenotypes in young infants, none of the abovementioned SNPs demonstrated any significant relationships.

Functional experiments were performed for I50V and Q551R. Mice that are homozygous for Q551R present increased inflammation, mucus production, airway hyper-reactivity, eosinophilia, and neutrophilia. The underlying mechanism is possibly associated with redirection of iTreg into Th17. This phenomenon has also been reported in the subgroup of asthma patients who have mixed Th2/Th17 cellularity. This asthma phenotype is characterized by increased severity, steroid resistance and neutrophilia. Two other *IL4RA* SNPs, rs8832 and rs1029489, located within the 3’ untranslated and proximal regions, have been associated with the response to anti-IL-4/IL-13 treatment (IL-4RA competitive antagonist). Individuals with these variants showed reduced asthma exacerbation and better response to treatment in a dose-dependent manner. Both E400A and Q551R were also associated with a reduction in FEV1 (Forced Expiratory Volume in the first second) and an antigen response during the course of treatment.

The promoter polymorphism C-3223T has not been widely studied, even though the location suggests a possible impact on transcription. In our previous study, we found an association between this polymorphism and the level of the soluble form of IL-4R. Another group reported a similar relationship with *IL4RA* haplotypes. TVR (T-3223, V50, R551) subjects were also reported to have lower levels of sIL-4R. The limitation of our study is the small sample size. The possible effect and statistical power could be missed because of that. Nevertheless, the results suggest some relationship which could be further investigated.

All of the abovementioned studies suggest a relationship between the genetic polymorphism of *IL4RA* and the pattern of expression, reflecting the link between genotype and phenotype. Some elements of this puzzle are still missing, which may require more comprehensive analysis that includes gene–gene and gene–environment interaction.

### Table 2. Relative *IL4RA* gene expression in PBMC in the genotype groups.

| Variable | Pulled (n = 36) | p-value | Atopic (n = 18) | p-value | Control (n = 14) | p-value |
|----------|----------------|---------|----------------|---------|-----------------|---------|
| ΔCT AA   | 8.66 ±2.32     |         | 7.70 ±1.67     | 9.06 ±2.75 |
| ΔCT AG+GG| 7.86 ±2.19     | 0.56    | 7.79 ±2.03     | 0.87    | 8.39 ±2.89      | 0.89    |
| ΔCT AA+AG| 8.26 ±2.53     | 0.76    | 7.96 ±2.41     | 1.0     | 8.58 ±2.78      | –       |
| ΔCT GG   | 7.78 ±1.19     |         | 7.48 ±0.65     |         | 10.64           |         |
| FC dominant | 1.74             |         | 0.94           |         | 1.59            |         |
| FC recessive | 1.39             |         | 1.39           |         | –               |         |
| ΔCT AA   | 8.19 ±2.11     |         | 7.78 ±2.22     | 9.31 ±1.65 |
| ΔCT AG+GG| 8.06 ±2.43     | 0.87    | 7.76 ±1.24     | 0.81    | 8.40 ±3.23      | 0.5     |
| ΔCT AA+AG| 8.12 ±2.27     | –       | 7.74 ±1.96     | –       | 8.73 ±2.73      | –       |
| ΔCT GG   | 8.31           |         | 8.31           |         | –               | –       |
| FC dominant | 1.09             |         | 1.01           |         | 1.88            |         |
| ΔCT AA   | 8.07 ±2.22     |         | 7.60 ±1.71     | 8.73 ±2.73 |
| ΔCT AG+AA| 8.22 ±2.34     | 0.89    | 7.98 ±2.23     | 0.76    | 8.71 ±3.15      | 1.0     |
| ΔCT AA+AG| 8.23 ±2.29     | 0.3     | 7.94 ±2.02     | 0.28    | 8.73 ±2.73      | –       |
| ΔCT AA   | 6.91 ±0.90     |         | 6.91 ±0.90     |         | –               | –       |
| FC dominant | 0.9              |         | 0.77           |         | 1.01            |         |
| FC recessive | 2.5              |         | 2.04           |         |                 |         |

FC – fold change (2^-ΔΔCT).

Conclusions

We did not find a relationship between these 3 genetic polymorphisms and the relative expression of *IL4RA*. The effect of genetic polymorphism on *IL4RA* mRNA expression could interfere with other factors, such as environmental stimuli, and should be evaluated in future studies.
References

1. Kotsimbos TC, Ghaffar O, Minshall EM, et al. Expression of the IL-4 receptor alpha-subunit is increased in bronchial biopsy specimens from atopic and nonatopic asthmatic subjects. *J Allergy Clin Immunol*. 1998;102(5):859–866.

2. Nestor CE, Dadfar E, Ernerudh J, et al. Sublingual immunotherapy alters expression of IL-4 and its soluble and membrane-bound receptors. *Allergy*. 2014;69(11):1564–1566. doi:10.1111/all.12505

3. Pascual M, Roa S, García-Sánchez A, et al. Genome-wide expression profiling of B lymphocytes reveals IL4R increase in allergic asthma. *J Allergy Clin Immunol*. 2014;134(4):972–975. doi:10.1016/j.jaci.2014.05.015

4. Liao W, Schones DE, Oh J, et al. Priming for T helper type 2 differentiation by interleukin-2-mediated induction of IL-4 receptor α chain expression. *Nat Immunol*. 2000;9(11):1288–1296. doi:10.1038/ni.1656

5. Perona-Wright G, Mohrs K, Mayer KD, Mohrs M. Differential regulation of IL-4R alpha expression by antigen versus cytokine stimulation characterizes Th2 progression in vivo. *J Immunol*. 2010;184(2):615–623. doi:10.4049/jimmunol.0902408

6. Hesselmar B, Bergin A-M, Park H, et al. Interleukin-4 receptor polymorphisms in asthma and allergy: Relation to different disease phenotypes. *Acta Paediatr*. 2010;99(3):399–403. doi:10.1111/j.1651-2227.2009.01631.x

7. Nie W, Zang Y, Chen J, Xiu Q. Association between interleukin-4 receptor α链 (IL4RA) I50V and Q551R polymorphisms and asthma risk: An update meta-analysis. *J Immunol*. 2014;183(3):1607–1616. doi:10.4049/jimmunol.090210014

8. Hytonen A-M, Lowhagen O, Arvidsson M, et al. Haplotypes of the interleukin-4 receptor alpha-subunit is increased in bronchial biopsy specimens from atopic and nonatopic asthmatic subjects. *J Allergy Clin Immunol*. 2009;18(6):559–565.

9. Sunadome H, Matsumoto H, Petrova G, et al. IL4Ra and ADAM33 as genetic markers in asthma exacerbations and type-2 inflammatory endotype. *Clin Exp Allergy*. 2017;47(8):998–1006. doi:10.1111/cea.12927

10. Ford AQ, Heller NM, Stephenson L, Boothby MR, Keegan AD. An atopy-associated polymorphism in the ectodomain of the IL-4Rα chain (V50) regulates the persistence of STAT6 phosphorylation. *J Immunol*. 2009;183(3):1607–1616. doi:10.4049/jimmunol.0803266

11. Slager RE, Otulana BA, Hawkins GA, et al. IL-4 receptor polymorphisms predict reduction in asthma exacerbations during response to an anti-IL-4 receptor α antagonist. *J Allergy Clin Immunol*. 2012;130(2):516–522.e4.

12. Massoud AH, Charbonnier LM, Lopez D, Pellegrini M, Phiptananukul W, Chatila TA. An asthma-associated IL4R variant exacerbates airway inflammation by promoting conversion of regulatory T cells to Th17-like cells. *Nat Med*. 2016;22(9):1013–1022. doi:10.1038/nm.4147

13. Rivas MN, Burton OT, Wise P, et al. Regulatory T cell reprogramming towards a Th2 cell-like lineage impacts oral tolerance and promotes food allergy. *Immunity*. 2015;42(5):512–523. doi:10.1016/j.immuni.2015.02.004

14. Danielewicz H, Dębińska A, Drabik-Chamerska A, Kalita D, Boznański A. IL4RA gene expression in PBMC with regard to place of living and atopy status. *Adv Clin Exp Med*. 2018;27(2):173–177.

15. GTEx Portal. https://gtexportal.org/home/snp/16_27356203_A_G_b37. Accessed July 13, 2019.

16. Chung KF. Dupilumab: A potential new treatment for severe asthma. *Lancet*. 2016;388(10039):3–4. doi:10.1016/S0140-6736(16)30311-7

17. Beck LA, Thaçi D, Hamilton JD, et al. Dupilumab treatment in adults with moderate-to-severe atopic dermatitis. *N Engl J Med*. 2014;371(2):130–139. doi:10.1056/NEJMoa1314768

18. Soto-Ramirez N, Arshad SH, Holloway JW, et al. The interaction of genetic variants and DNA methylation of the interleukin-4 receptor gene increase the risk of asthma at age 18 years. *Clin Epigenetics*. 2013;5(1):1. doi:10.1186/1868-7083-5-1

19. Zhang H, Tong X, Holloway JW, et al. The interplay of DNA methylation over time with Th2 pathway genetic variants on asthma risk and temporal asthma transition. *Clin Epigenetics*. 2014;6(1):8. doi:10.1186/1868-7083-6-8

20. Hackstein H, Hecker M, Kruse S, et al. A novel polymorphism in the 5′ promoter region of the human interleukin-4 receptor α chain gene is associated with decreased soluble interleukin-4 receptor protein levels. *Immunogenetics*. 2001;53(4):264–269. doi:10.1007/s002510030342

21. Michel S, Liang L, Depner M, et al. Unifying candidate gene and GWAS approaches in asthma. *PLoS One*. 2010;5(2):e13894. doi:10.1371/journal.pone.0013894

22. Hesselmar B, Enlund A-C, Eriksson B, Padyukov L, Hanson LÅ, Aberg N. The heterogeneity of asthma phenotypes in children and young adults. *J Allergy*. 2012;2012:163089. doi:10.1155/2012/163089

23. Maier LM, Howson JNN, Walker N, et al. Association of IL13 with total IgE. Evidence against an inverse association of atopy and diabetes. *J Allergy Clin Immunol*. 2006;117(6):1306–1313. doi:10.1016/j.jaci.2005.12.1354

24. Hoffjan S, Ostrovnaia J, Nicole D, et al. Genetic variation in immunoregulatory pathways and atopic phenotypes in infancy. *J Allergy Clin Immunol*. 2004;113(3):511–518. doi:10.1016/j.jaci.2003.10.044

25. Slager RE, Hawkins GA, Amplesford EF, et al. IL-4 receptor α polymorphisms are predictors of a pharmacogenetic response to a novel IL-4/IL-13 antagonist. *J Allergy Clin Immunol*. 2010;126(4):875–878. doi:10.1016/j.jaci.2010.08.001

26. Danielewicz H, Hurkacz M, Boznański A, Wiela-Hojeńska A, Chamerska-Drabik A. Association of soluble IL-4R serum levels and IL-4Ra chain gene polymorphisms. *Adv Clin Exp Med*. 2009;18(6):539–565.

27. Hytten A-M, Lowhagen O, Arvidsson M, et al. Haplotype profiling of the interleukin-4 receptor alpha chain gene associated with susceptibility to and severity of atopic asthma. *Clin Exp Allergy*. 2004;34(10):1570–1575. doi:10.1111/j.1365-2222.2004.02069.x