

IL4RA gene expression in relation to I50V, Q551R and C-3223T polymorphisms

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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 *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 *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 (Δ Ct) and delta-delta Ct ($\Delta\Delta$ Ct) values were used for the relative quantification of *IL4RA* expression in PBMC.

Results. We observed no significant differences in the *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 *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.

Key words: gene expression, polymorphism, asthma, atopy, *IL4RA*

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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 shading 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 (Δ CT) and delta-delta mean Ct ($\Delta\Delta$ CT) values were used for relative quantification of *IL4RA* expression in PBMC, and $2^{-\Delta\Delta$ CT 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 Wrocław 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, where there was a 1.39-fold change ($2^{-\Delta\Delta CT}$) 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	χ ² /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
AG+GG	24 (0.67)	14 (0.77)	7 (0.5)		0.10
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)	9 (0.64)		
GG	1 (0.03)	1 (0.05)	0		
AA+AG	35 (0.97)	17 (0.94)	14 (1.0)		0.72
AG+GG	20 (0.56)	6 (0.33)	9 (0.64)		0.12
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
AG+AA	13 (0.36)	8 (0.44)	4 (0.28)		0.37
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.

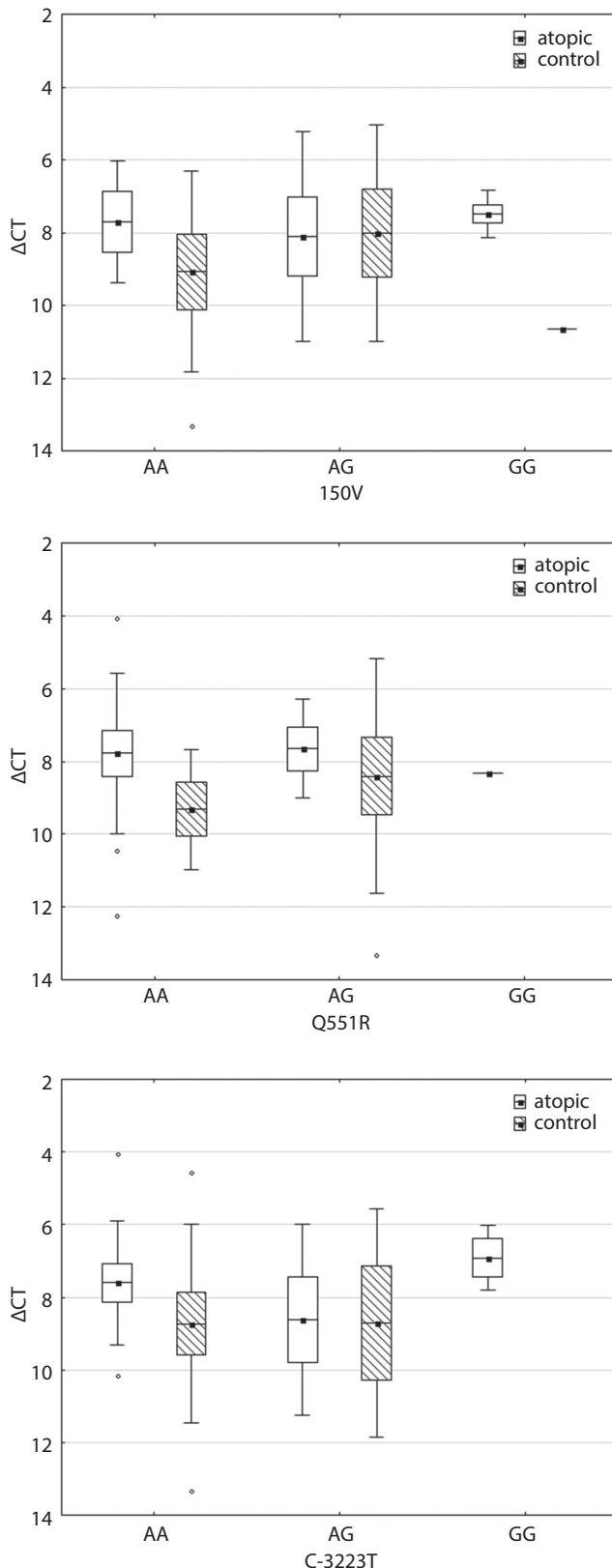


Fig. 1. Relative *IL4RA* gene expression in PBMC in the genotype groups, expressed as ΔCT , where $\Delta CT = Ct\ IL-4RA - Ct\ ACTB$ for each sample. The lower the ΔCT values, the higher the gene expression. The Y-axis has been reversed for better illustration. Data is presented as mean \pm standard error (SE) \pm standard deviation (SD). The differences were not statistically significant according to the Kruskal–Wallis rank test ($p > 0.05$)

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.¹³ The aim of the current study was to determine whether the most important and well-studied SNPs within the *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 *IL4RA*. This suggests a pattern where *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 *IL4RA* in their 33 subjects.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.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.^{15,16}

There are also 2 studies suggesting an epigenetic effect of the *IL4RA* polymorphism. In the 1st 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.¹⁷ In the 2nd 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.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 *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.¹⁹ GWA studies (Genome Wide Association Studies) have confirmed the role of all 3 of these polymorphisms.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.⁷ In other studies,

Table 2. Relative *IL4RA* gene expression in PBMC in the genotype groups. Relative gene expression of *IL4RA* is expressed as ΔCT , where $\Delta CT = Ct\ IL4RA - Ct\ ACTB$ for each sample. The lower the ΔCT values, the higher the gene expression. Data are presented as mean \pm standard error (SE) \pm standard deviation (SD). The differences were not statistically significant according to the Mann–Whitney U test ($p > 0.05$)

Variable	Pulled (n = 36)	p-value	Atopic (n = 18)	p-value	Control (n = 14)	p-value
I50V						
$\Delta CT\ AA$	8.66 \pm 2.32		7.70 \pm 1.67		9.06 \pm 2.75	
$\Delta CT\ AG+GG$	7.86 \pm 2.19	0.56	7.79 \pm 2.03	0.87	8.39 \pm 2.89	0.89
$\Delta CT\ AA+AG$	8.26 \pm 2.53	0.76	7.96 \pm 2.41	1.0	8.58 \pm 2.78	–
$\Delta CT\ GG$	7.78 \pm 1.19		7.48 \pm 0.65		10.64	
FC dominant	1.74		0.94		1.59	
FC recessive	1.39		1.39		–	
Q551R						
$\Delta CT\ AA$	8.19 \pm 2.11		7.78 \pm 2.22		9.31 \pm 1.65	
$\Delta CT\ AG+GG$	8.06 \pm 2.43	0.87	7.76 \pm 1.24	0.81	8.40 \pm 3.23	0.5
$\Delta CT\ AA+AG$	8.12 \pm 2.27	–	7.74 \pm 1.96	–	8.73 \pm 2.73	–
$\Delta CT\ GG$	8.31		8.31		–	
FC dominant	1.09		1.01		1.88	
C-3223T						
$\Delta CT\ GG$	8.07 \pm 2.22		7.60 \pm 1.71		8.73 \pm 2.73	
$\Delta CT\ AG+AA$	8.22 \pm 2.34	0.89	7.98 \pm 2.23	0.76	8.71 \pm 3.15	1.0
$\Delta CT\ GG+AG$	8.23 \pm 2.29	0.3	7.94 \pm 2.02	0.28	8.73 \pm 2.73	–
$\Delta CT\ AA$	6.91 \pm 0.90		6.91 \pm 0.90		–	
FC dominant	0.9		0.77		1.01	
FC recessive	2.5		2.04			

FC – fold change ($2^{\Delta\Delta CT}$).

I50V and C-3223T (homozygous), but not Q551R, were found to be related to early-onset asthma.²¹ In another study that compared eight SNPs of *IL4RA*, including C-3223T, Q551R, and I50V, only I50V showed a significant association with total IgE.²² However, in a study that investigated asthma phenotypes in young infants, none of the abovementioned SNPs demonstrated any significant relationships.²³

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.¹¹ 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.¹⁰ 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.²⁴

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.²⁵ Another group reported a similar relationship with *IL4RA* haplotypes. TVR (T-3223, V50, R5 51) subjects were also reported to have lower levels of sIL-4R.²⁶

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.


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.

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