

Impact of *IL13* Genetic Polymorphism Arg130Gln on Total Serum IgE Levels and IFN- γ Gene Expression

Running title: Genetic Susceptibility to Allergy

Smolkova Bozena^{1,2*}, Tulinska Jana², Palkovicova Murinova Lubica³, Buocikova Verona², Liskova Aurelia², Rausova Katarina³, Kuricova Miroslava², Patayova Henrieta³, Sustrova Maria², Neubauerova Svorcova Eva², Ilavska Silvia², Szabova Michaela², Nemessanyi Tomas², Jahnova Eva², Dusinska Maria⁴, Ciznar Peter⁵, Fuortes Laurence⁶

¹ Cancer Research Institute, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia

^{2,3} Slovak Medical University, Faculty of Medicine², Faculty of Public Health³, Bratislava, Slovakia

⁴ Health Effects Laboratory, Department of Environmental Chemistry, NILU-Norwegian Institute for Air Research, Kjeller, Norway

⁵ 1st Pediatric Department, Comenius University, Faculty of Medicine Bratislava, Slovakia

⁶ University of Iowa, College of Public Health, Iowa City, Iowa, USA

* To whom correspondence should be addressed at: Department of Genetics, Cancer

Research Institute, Biomedical Research Center, Slovak Academy of Sciences,

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Dúbravská cesta 9, Bratislava 845 05, Slovakia. Tel: 02/32295 189; Email:

bozena.smolkova@savba.sk

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Abbreviations:

ANOVA	analysis of variance
ARMS-PCR	amplification refractory mutation system-polymerase chain reaction
Cq	quantification cycle
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
HIV	human immunodeficiency virus
HWE	Hardy–Weinberg equilibrium
IFN- γ	interferon gamma
IgE	immunoglobulin E
IL	interleukin
RFLP	restriction fragment length polymorphism
SNP	single nucleotide polymorphisms
TB	tuberculosis
Th1	type 1 T helper cells
Th2	type 2 T helper cells

Summary

This cross-sectional study was designed to investigate the extent of genetic susceptibility by targeting variants in IL-4/IL-13 signalling pathways leading to atopic disease in early childhood. We evaluated involvement of five single nucleotide polymorphisms *IL4* C-590T, *IL13* C-1055T, *IL13* Arg130Gln, *IL4RA* Ile50Val and *IL4RA* Ser478Pro, in the control of serum total and antigen-specific IgE levels. Furthermore, we analyzed their association with changes in gene expression of five cytokines having key roles in inflammatory and anti-inflammatory immune response (IL-4, IL-13, IFN- γ , IL-8 and IL-10). Total and antigen-specific IgE levels in serum and gene expression of selected cytokines in peripheral blood were measured in 386 children aged 1 to 8 years. TaqMan allelic discrimination, ARMS-PCR and RFLP methods validated by sequencing were used for genotyping. All genotypes for children with total and antigen-specific IgE levels in the normal range were in Hardy-Weinberg equilibrium. Gene expression analyses were carried out using TaqMan Gene Expression Assays. We found elevated total IgE levels in carriers of *IL13*Arg130Gln variant allele (OR 1.84; 95% CI 1.16-2.93). This effect was more apparent for boys (OR 2.31; 95% CI 1.25-4.28). However, no significant association was observed for the other 4 variants examined. We found up-regulation of IFN- γ in children with elevated serum total IgE levels carrying Arg130 allele ($P=0.005$). No differences were found for *IL4*, *IL8* or *IL10* while *IL13* gene expression was under detection limit. *IL13* Arg130Gln genotypes can play a role in genetic susceptibility to allergy via regulation of serum total IgE levels and affecting IFN- γ gene expression.

Introduction

Allergic diseases resulting from Th2 mediated inflammation represent a significant global health problem due to their continual rise in developed countries. Therefore, there is a great interest in detecting biomarkers of susceptibility that can be used to identify risk and prevent adverse health effects through prevention and intervention strategies.

Atopy is an exaggerated immunoglobulin E (IgE)-mediated immune response resulting in development of certain allergic diseases. Among them, the most typical for childhood are atopic dermatitis, allergic rhinitis and asthma. Besides environmental factors, genetic variability is associated with increased susceptibility to this condition. Chromosome 5q.31-33 contains several candidate loci, including genes for Th2 cytokines interleukin-4 (IL-4) and interleukin-13 (IL-13) that are the central mediators of allergic inflammation (1). They exert their biological effects by binding to their functional receptors on target cells having the interleukin-4 receptor alpha chain (IL-4Ra) as a component (2).

Cytokines play a crucial role in atopic diseases by alteration of the balance between Th1 and Th2 immune response triggered by lifestyle factors and environmental exposures. Cytokine profiles measured in peripheral blood may not only reflect cellular functions, but can also indicate cellular composition in the immune system. The expression of inflammatory and anti-inflammatory cytokines IL-4, IL-13, IL-10, IL-8 and IFN- γ , measured in peripheral blood in our study, is mediated by different cell types. Whereas IL-4 and IL-13 are expressed mainly by the Th2 cells (3), IL-10 is produced by various immune cell types including Th1, Th2 and Th17 cell subsets, regulatory T cells,

CD8⁺ T cells and B cells (4). IL-8 chemokine, one of the main mediators of inflammatory response, is produced in macrophages, epithelial cells, endothelial cells and human peripheral blood eosinophils (5). IFN- γ is secreted predominantly by natural killer and natural killer T cells, as well as by CD4 Th1 and CD8 cytotoxic T cells once antigen-specific immunity develops (6). The impact of genetic variation in *IL4* and *IL13* genes on expression of inflammatory and anti-inflammatory cytokines in atopic diseases has not been widely studied (7).

We selected five functional variants in three genes, namely *IL4* variant C-590T (rs2243250), *IL13* variants C-1055T (rs20541) and Arg130Gln (rs1800925) and two *IL4RA* variants Ile50Val (rs1805010) and Ser478Pro (rs1801275) to analyze their impact on serum total and antigen-specific IgE levels and their influence on gene expression of selected inflammatory/anti-inflammatory cytokines IL-4, IL-13, IL-8, IL-10 and IFN- γ . Changes in their expression profiles can play a significant role in Th1/Th2 immune balance and inflammatory response. To ascertain genetic effects on IgE levels that are representative of the general population, we conducted a cross-sectional study, with the aim of evaluating the extent of genetic susceptibility by targeting candidate SNPs in pathways leading to atopic disease in early childhood.

Methods

Subjects

This study involved 418 children from 2 Slovak regions – Bratislava and Stará Ľubovňa. Of these, 386 children aged 1 to 8 years provided biological samples for molecular-genetic analyses between 2002-2009. Characteristics of the study subjects are summarized in Table 1. Children were recruited in cooperation with regional paediatricians. Inclusion criteria for enrolment to the study were: atopic dermatitis, respiratory allergy, chronic urticaria or gastrointestinal problems with suspect food allergy. Exclusion criteria were acute infectious disease, systemic corticosteroid therapy, TB, HIV, and any malignancies. Children were clinically examined and samples of peripheral blood for genotyping and IgE measurements were taken using standardized protocols. The questionnaires focused on life style, family habits and nutritional factors and were administered to parents.

After centrifugation serum was collected and frozen for analyses of total and antigen-specific IgE. Peripheral blood for genotyping was drawn into EDTA anticoagulant tubes and frozen at -20°C. Questionnaires focused on environmental risk factors for allergy development were administered to mothers. None of the children received inhaled or topical corticosteroids. To reduce potential histamine-mediated effects in children receiving antihistamines, ten days washout period preceded blood sampling.

All studies were approved by the Ethics Committee of the Slovak Medical University and parents of participants gave written informed consent. Samples were coded and analyses were performed in a blinded fashion.

Total and specific serum IgE measurement

Total serum IgE levels were measured using ELISA. The age-specific reference intervals based on data from the available literature were defined as follows: < 20 kU/L for age 0–3 years, < 35 kU/L for 3–4 years, < 60 kU/L for 4–7 years, < 110 kU/L for 7–14 years (8, 9). Peak IgE levels greater than the age specific normal ranges listed above were referred as ‘elevated’ IgE.

Specific IgE antibodies were determined by means of CAP-RAST-FEIA (Pharmacia, Uppsala, Sweden) and EUROLINE (EUROIMMUN AG, De) test systems. In the case of CAP-RAST-FEIA, we defined sensitization to the most common specific food (milk, egg white, fish, wheat, soy, nuts) and inhalation allergens (dog dander, cat dander, grass and tree pollen weeds and house dust mite) as a concentration of ≥ 0.35 kU/l of the antigen-specific IgE. The results of the test for specific allergens (test for 16 allergens) of EUROLINE test system were interpreted as 0 - no signs of allergen-specific IgE, + low concentration of allergen-specific IgE, ++ moderate elevation of specific IgE and +++ high concentration of allergen-specific IgE. Allergens tested were: egg white, egg yolk, milk, yeast, wheat flour, rye flour, rice, soya, peanut, hazelnut, almond, apple, kiwi, apricot, tomato, carrot, potato, celery, codfish and crab.

DNA extraction and genotyping

DNA for genotyping was extracted from peripheral blood of 386 children using a modified salting-out method (10). Five putatively functional SNPs located in *IL13*, *IL4* and *IL4RA* genes were genotyped by restriction fragment length polymorphisms (RFLP-PCR), TaqMan allelic discrimination and allele-specific PCR (ARMS-PCR)

(11-16). Genotyping method, primer sequences and PCR conditions for individual SNPs are shown in Table 2.

Genotyping results were validated by direct sequencing at the Department of Molecular Biology, Faculty of Natural Sciences, Comenius University in Bratislava. In the case of agreement of both methods we performed further evaluation. All observed genotype frequencies in children with both total and antigen specific IgE levels in normal range were within Hardy–Weinberg equilibrium (HWE).

RNA isolation and reverse transcription

The PAXgene Blood RNA Isolation System (PreAnalytiX GmbH) was used for isolation of total RNA from the peripheral blood of 207 children. The whole blood was collected into PAXgene Blood RNA tubes and stored at -20°C . Total RNA was isolated from blood samples using the PAXgene Blood RNA Kit, (QIAGEN) including 15 minute DNase treatment at room temperature. RNA concentration and quality were determined by gel electrophoresis and spectrophotometrically at 260/280 nm.

Total RNA was converted into cDNA using TaqMan Reverse Transcription Reagents (Applied Biosystems, USA) with MultiScribe Reverse Transcriptase and Random Hexamers. cDNA was prepared from 2 μg of mRNA. The samples were incubated at 25°C for 10 minutes and 42°C for 61 minutes; the reaction was stopped by heating at 95°C for 5 minutes. The resulting cDNA was diluted into aliquots 5 ng/ μl and stored at -20°C .

Real-time PCR

Real-time PCR reactions were performed using the ABI Prism 7500 Sequence Detection System (Applied Biosystems. USA) and TaqMan Gene Expression Assays (Applied Biosystems. USA): Hs00174086_m1 (*IL10*); Hs00174103_m1 (*IL8*); Hs00174143_m1 (*IFNG*); Hs00174122_m1 (*IL4*); Hs00174379_m1 (*IL13*) and Hs99999905_m1 (*GAPDH*) as internal control. A singleplex reaction mix was prepared according to the manufacturer's instructions. Aliquots of cDNA were amplified in the 20 µl reaction mixture for *IL8*, *IL10* and *IFNG* and in a 50µl reaction mixture for *IL4*. All amplifications were carried out in triplicate. The thermal cycling conditions included an initial activation of DNA polymerase 10 min at 95°C, followed by 40 cycles (95°C for 15 s and 60°C for 1 min.).

Quantification cycle (Cq) values were determined by automated threshold analysis using ABI Prism Sequence Detection Software v1.3.1 (Applied Biosystems. USA) and relative gene expression was calculated using the $2^{-\Delta\Delta C_t}$ method. The results were reported as the n-fold change in cytokine gene expression in the experimental group normalized to the endogenous control GAPDH and relative to the control group (= 1). Data represent Mean $\pm$ SEM of 3 independent experiments.

Statistical analysis

Normality of distribution was tested by Kolmogorov-Smirnoff test. If normally distributed, sample means were tested by Student t-test or analysis of variance (ANOVA) with Bonferroni's or Tamhane's tests for multiple comparisons depending on homogeneity of variance. The nonparametric Mann-Whitney U test was used for not-normally distributed data. All tests were two-tailed, performed at the significance level $\alpha = 0.05$. A likelihood ratio test was applied to assess whether the observed genotypes

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were in HWE. Categorical data were tested by Chi square testing. Multivariate logistic regression was used to test the association between elevated total IgE levels, (categorized by age of children) and analyzed genetic variants after adjusting for sex as a confounder. All relative quantifications were calculated by REST software, RG mode, using pair-wise fixed randomization test (17). The significance of fold change in gene expression between groups was analyzed using the Student t-test (data were normally distributed) applied to the ΔCt values. SPSS 15.0 was used for statistical analysis of the data.

Results

Clinical characteristics of the studied population are shown in Table 1. We found elevated serum total IgE levels in 32.1% children, while elevated antigen-specific IgE levels were present in 32.4%. Elevated total IgE levels were more frequent in boys (40.6% vs. 23.3%; $P < 0.001$), while elevated antigen-specific IgE levels occurred more often in girls (35.4% vs. 29.4%). Among 386 children 220 (57.0%), 107 boys (54.3%) and 113 girls (59.8%) had both, total and antigen-specific IgE levels, in normal range.

Frequencies of *IL4*, *IL13* and *IL4RA* polymorphisms in the studied population are shown in Table 3. They were similar to those published in 1000 Genomes Phase 3 for CEU population (<http://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/>) (HapMap individuals of European descent). Significant differences between children with normal and elevated serum total IgE levels were found only for Arg130Gln variant ($P=0.024$). No association was detected between analyzed SNPs and elevated antigen-specific IgE. Among children with elevated total IgE levels, variant allele (Gln130) was found in higher frequency for both, homozygotes (10.9% vs. 7.0%) and heterozygotes (40.9% vs. 29.5%). This was more apparent in the dominant model, where 51.8% children with elevated serum total IgE levels possess the variant allele in contrast with 36.5% of those with normal IgE levels ($P=0.007$). Children carrying the variant allele had elevated serum total IgE levels. This difference reached significance only for boys ($P=0.005$), who had higher total IgE levels than girls ($P=0.008$) (Fig. 1). Multivariate analysis identified the Gln130 allele as an independent risk factor for elevated total IgE, after controlling for sex as a confounder. The results for boys were more significant ($P=0.008$) if compared to the whole group combined ($P=0.010$) (Table 4). While

presence of the variant allele in the total population studied increased the risk of having elevated total IgE levels 1.84 times (95% CI 1.16 - 2.93), in boys it was 2.31 times (95% CI 1.25 - 4.28).

Analyses of gene expression of the selected cytokines IL-4, IL-13, IFN- γ , IL-10 and IL-8 revealed changed IFN- γ expression profile in the carriers of variant *IL13* Gln130 allele, compared to wild type allele (Arg130) carriers. In girls, carriers of Gln130 allele, expression of IFN- γ was 1.4 time higher than in Arg130 carriers (P=0.040). No difference between genotypes was found in boys. Children with normal total IgE concentrations carrying the variant Gln130 allele had 1.4 times higher expression of IFN- γ compared to those with Arg130 allele (P=0.033). On the other hand, children with the Arg130 allele and elevated total IgE levels had 1.5 times higher IFN- γ gene expression than those with normal total IgE levels (P=0.005) but no difference was found in children with the variant allele (Fig. 2). No differences in the expression of IL-4, IL-10 or IL-8 were found, if stratified by *IL13* Arg130Gln genotypes. Gene expression of IL-13 was under the detection limit in peripheral blood of all children.

Discussion

Our results revealing higher prevalence of elevated total IgE values in boys compared to girls are in agreement with the results published previously for a German pediatric population (9). The authors showed higher total IgE values in boys than in girls at each age, while within the group of atopic children sex differences were not significant (9). In our group of children, prevalence of elevated antigen-specific IgE was more common in girls than in boys. Higher total IgE levels for males were published for different populations and age groups (18, 19).

The influence of *IL4*, *IL13* and *IL4RA* genetic variants on total IgE levels and atopic diseases have been analyzed in different populations with conflicting results (15, 20, 21). Among 5 studied single nucleotide polymorphisms (SNPs) in our study only *IL13* Arg130Gln variant showed statistically significant association with total IgE levels. This association could reflect either direct impact of *IL13* Arg130Gln on IgE phenotype or its tight linkage disequilibrium with a putative susceptibility allele. Total IgE concentrations were reported to be associated with *IL13* variants in two large genome-wide association studies (GWAS) (22, 23). Association of Arg130Gln variant with total IgE levels was found also by other authors (24, 25). The non-synonymous *IL13* Arg130Gln polymorphism results in the non-conservative replacement of arginine 130 with glutamine. By comparing the activity of the variant allele Gln130 to that of the common Arg130 variant on primary effector cells of human allergic inflammation, it was shown that the Gln130 was significantly more active than Arg130 allele (26). According to the authors, increased allergic inflammation in carriers of the Gln130

allele depended on enhanced IL-13-mediated Th2 effector functions rather than increased Th2 differentiation.

This gain of function variant affected also IFN- γ gene expression in peripheral blood of children. Impact of Gln130 allele was more visible when IgE levels were in normal range; therefore we speculate that Gln130 allele-associated elevated IFN- γ gene expression in girls was affected by lower incidence of elevated total IgE in this group.

Together with Th2, simultaneous Th1 responses have been demonstrated during allergic inflammation accompanied by activation of IFN- γ -induced signaling and transcriptional regulation of gene expression by IFN- γ -regulated genes. IFN- γ was shown to play an essential role in reversing allergen-induced airway inflammation and hyperresponsiveness, but it can have dual actions on the latter (27).

Mixed Th1 and Th2 immune response associated with inflammatory diseases mediated by IL-13 and IFN- γ was detected in a mouse model of airway inflammation (28). Similar findings were published by D'Andrea et al., who showed that short-term treatment of peripheral blood mononuclear cells with IL-4 or IL-13 enhances their antigen presenting function and ability to support antigen-dependent IFN- γ production. Their data suggest that IL-4 and IL-13, primary products of the Th2 cells, might play a role in the differentiation and activation of antigen presenting cells that may favor Th1 cell development (29). We hypothesize that the Gln130 allele can be more active in enhancing IFN- γ production than the wild type Arg130 allele. As reported previously, the frequencies of both CD4⁺ and CD8⁺ T cells producing IFN- γ , but not type 2 cytokines, increased in subjects with atopic asthma compared with normal subjects (30). The authors showed that increased production of IFN- γ resulted from the skewing of cytokine production toward type 1 as a result of *in vitro* stimulation with phorbol

myristate acetate (PMA) and ionomycin. To avoid effects of stimulation we analyzed cytokine mRNA expression in peripheral blood of children without stimulation. Therefore low IL-13 expression levels in peripheral blood were not surprising and are in agreement with findings of Cho et al., who investigated frequencies of IFN- γ , IL-4 and IL-5 producing CD4⁺ and CD8⁺ in blood and sputum T cells from subjects with asthma and normal subjects with and without *in vitro* stimulation. They hypothesized that increased cellular activation in allergic asthma leading to increased IFN- γ production is a marker of inflammation or is caused by preferential recruitment of allergen-specific Th2/Tc2 cells to the lung, resulting in reduced type 2 cytokine producing T cells in the peripheral blood (30).

Our results suggest possible interaction between Th1 and Th2 immune response that could be mediated by increased IL-13 activity in the Gln130 genetic variant. Co-expression of IL-13, IL-5 and IFN- γ has recently been seen in asthmatic children, and was suggested to be a result of inter-chromosomal interaction, but this interpretation needs to be further investigated (31, 32).

IL4 and *IL4RA* polymorphisms were not associated with total IgE levels in our study. Negative findings for these polymorphisms could be explained by their indirect effect on allergic disorders rather than impact on total IgE level or they could be modifier genes affecting disease severity (15).

To conclude, *IL13* Arg130Gln variant can play a role in genetic susceptibility to allergy by participating in serum total IgE levels regulation and affecting IFN- γ gene expression.

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Author Contributions

TJ and PML designed involved studies and drafted the manuscript; SM and CO coordinated clinical examination of recruited patients and follow-up; NSE, KM, LA, PH and RK recruited patients and collected patient material; BV performed genotyping; SB realized gene expression experiments and wrote the manuscript; IS, SM, NT and JE

realized measurements of IgE levels; DM and FL revised article critically for important intellectual content. All authors revised and approved the final version of the manuscript.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest to disclose.

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Figure legends

Figure 1. Differences in serum total IgE levels between boys and girls stratified by *IL13*

Arg130Gln genotypes

Values of total IgE are presented as Mean $\pm$ SE

Dominant genetic model: Arg130 allele (GG genotype) versus Gln130 allele (GA+AA genotypes)

Altogether 386 samples were genotyped, 359 of them possess total IgE values and were successfully genotyped for *IL13* Arg130Gln variant

Figure 2. Gene expression of IFN- γ stratified by sex, total IgE values and *IL13*

Arg130Gln genotypes

Dominant genetic model: Arg130 allele (GG genotype), Gln130 allele (GA+AA genotypes)

Gene expression analyses were performed on 207 samples. Results presented herein show 166 samples possessing both *IL13* Arg130Gln genotyping data and gene expression data.

Error bars show SE values

Table 1. Characteristics of the studied population

	All	Boys	Girls
	386 (100%)	197 (51%)	189 (49%)
Age (years $\pm$ SE)	3.7 $\pm$ 0.06	3.7 $\pm$ 0.09	3.7 $\pm$ 0.07
Mean Total IgE (IU/l) $\pm$ SE	61.8 $\pm$ 10.61	67.0 $\pm$ 8.5	56.3 $\pm$ 19.8
Range Total IgE (IU/l)	0.96 – 3577.0	1.4 – 1028.7	0.96 – 3577.0
Prevalence of elevated age related* total IgE values	124 (32.1%)	80 (40.6%)	44 (23.3%)
Prevalence of elevated Antigen-specific IgE [#]	125 (32.4%)	58 (29.4%)	67 (35.4%)
Children with normal total and antigen-specific IgE	220 (57%)	107 (54.3%)	113 (59.8%)

* Age related values of serum total IgE: 0-3 years > 20 IU/l; 3-4 years >35 IU/l; 4-7 years > 60 IU/l; 7-14 years > 110 IU/l

[#] IU/l > 0.35

Table 2. Genotyping methods, primer sequences and annealing conditions

SNP	Method	Primers 5'-3'		Annealing	PCR product/ RE digestion	Reference
IL4 C-590T (rs2243250)	ARMS-PCR	IL4 590C-F IL4 590T-F IL4 com-R HGH1-F HGH1-R	cta aac ttg gga gaa cat tgt <u>C</u> cta aac ttg gga gaa cat tgt <u>T</u> agt aca ggt ggc atc ttg gaa a gcc ttc cca acc att ccc tta tca cgg att tct gtt gtg ttt c	65.5°C 10 cycles, 60°C 20 cycles	156bp <i>IL4</i> 429 bp <i>HGH1</i>	(11)
IL13 C-1055T (rs1800925)	RFLP-PCR	IL13 C 1055T-F IL13 C 1055T-R	gga atc cag cat gcc ttg tga gg gtc gcc ttt tcc tgc tct tcc cGc	58°C	246 bp/ BstUI 223 bp +23 bp	(15)
IL13 Arg130Gln (rs20541)	TaqMan allelic discrimination	Il13 Arg130Gln-F Il-13 Arg130Gln-R 6-FAM-TAMRA VIC-TAMRA	taa agg acc tgc tct tac att taa aga aa cct gtc tct gca aat aat gat gct ttc ga tcg cga ggg ac <u>G</u> gtt caa ctg aaa tcg cga ggg ac <u>A</u> gtt caa ctg aaa	61°C		(16)
IL4RA Ile50Val (rs1805010)	RFLP-PCR	IL4RA Ile50Val - F IL4RA Ile50Val - R	ggc agg tgt gag gag cat cc gcc tcc gtt gtt ctc agg Ta	60°C	273 bp/ RsaI 254 bp +19 bp	(12)
IL4RA Q576R (rs61543625)	RFLP-PCR	IL4RA Q576R – F IL4RA Q576 - R	tcg gcc ccc acc agt ggc tCt cca gtc caa agg tga aca agg gg	60°C	268 bp/ DdeI 248 bp +20 bp	(13, 14)

Underlined letters in primer sequences depicts mismatches, RE= restriction enzyme

Table 3. Frequencies of *IL4*, *IL13* and *IL4RA* polymorphisms in the studied population related to the total IgE levels

SNP	Total IgE [#] Normal n (%)	Total IgE [#] Elevated n (%)	OR	95% CI	P*	MAF
<i>IL13</i> C-1055T(rs1800925)						
CC	138 (58.0)	57 (51.8)	ref.	-		
CT	77 (32.4)	43 (39.1)	1.35	0.81-2.26		
TT	23 (9.6)	10 (9.1)	1.05	0.44-2.50	NS	
T allele	25.8	28.6	1.15	0.79-1.67		0.18
<i>IL13</i> Arg130Gln (rs20541)						
GG	155 (63.5)	53 (48.2)	ref.	-		
GA	72 (29.5)	45 (40.9)	1.83	1.09-3.06		
AA	17 (7.0)	12 (10.9)	2.06	0.86-4.93	0.024	
A allele	21.7	31.4	1.65	1.13-2.39		0.22
<i>IL4</i> C-590T (rs2243250)						
CC	168 (70.9)	77 (70.4)	ref.			
CT	61 (25.7)	27 (24.5)	0.97	0.55-1.69		
TT	8 (3.4)	6 (5.5)	1.64	0.48-5.42	NS	
T allele	16.2	17.7	1.11	0.71-1.73		0.13
<i>IL4RA</i> Ile50Val (rs1805010)						
AA	74 (31.8)	34 (27.4)	ref.			
AG	107 (45.9)	62 (50.0)	1.26	0.73-2.18		
GG	52 (22.3)	28 (22.6)	1.17	0.61-2.26	NS	
G allele	45.2	47.6	1.10	0.80-1.51		0.45
<i>IL4RA</i> Q576R (rs1801275)						
AA	157 (67.4)	78 (63.4)	ref.			
AG	66 (28.3)	43 (35.0)	1.31	0.80-2.16		
GG	10 (4.3)	2 (1.6)	0.40	0.06-2.02	NS	
G allele	18.5	19.1	1.01	0.67-1.52		0.22

* Chi square test for genotype frequencies; MAF minor allele frequency in 1000 genomes Phase 3, CEU population [#] Genotyping was performed on 386 samples. For each variant samples with available IgE

values, genotyped successfully, were included into statistical analysis. Numbers in bold indicate significant results.

Table 4. The risk estimation of the analyzed variables for presence of elevated serum IgE in peripheral blood using multivariate logistic regression

Elevated serum total IgE	Variables	<i>P</i> value	OR	95% CI
All^a	sex	0.002	0.48	0.30 - 0.77
	Gln130 <i>allele</i> , dominant model	0.010	1.84	1.16 - 2.93
Boys^b	Gln130 <i>allele</i> , dominant model	0.008	2.31	1.25 - 4.28

^a-2 Log likelihood = 416.81; R^2 (Cox & Snell) = 0.045; R^2 (Nagelkerke) = 0.063

^{a-b}-2 Log likelihood = 232.35; R^2 (Cox & Snell) = 0.040; R^2 (Nagelkerke) = 0.054

Dominant genetic model: Arg130 allele(GG), Gln130 allele (GA+AA genotypes)



