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Original Research Article

Polymorphisms in the interleukin 4, interleukin 4 receptor and interleukin 13 genes and allergic phenotype: A case control study

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ABSTRACT

Purpose: Interleukin 4 (IL4), interleukin 4 receptor (IL4R) and interleukin 13 (IL13) play a key role in the pathogenesis of allergy and asthma development. IL4 and IL13 strongly influence bronchial hyperreactivity in response to allergen, airway remodeling, airway inflammation and airway smooth muscle proliferation. Both IL4 and IL13 exert biologic effect via interleukin 4 receptor. The aim of this study was to evaluate the impact of the polymorphisms within interleukin 4 (rs2243250, rs2227284), interleukin 4 receptor α chain (rs1805010, rs1805011) and interleukin 13 (rs20541) genes on the incidence of allergic phenotype in Polish pediatric population.

Material/methods: We compared 177 asthmatic pediatric patients with 194 healthy children. Five polymorphisms within *IL4*, *IL13* and *IL4Rα* genes were analyzed. Genotypes of four polymorphisms (rs2243250, rs2227284, rs1805011, rs20541) were assigned by TaqMan SNP Genotyping Assays (Applied Biosystems), whereas rs18050100 polymorphism was established using PCR-RFLP method.

Results: We observed an association of rs1805011 polymorphism of *IL4Rα* gene with allergy ($p = 0.021$), mild asthma ($p = 0.00005$) and atopic dermatitis ($p = 0.0056$). Significant correlation was found between rs20541 in *IL-13* gene and the positive skin prick test results ($p = 0.029$), along with rs2243250 polymorphism with clinical atopy ($p = 0.033$) and rs2227284 with total IgE levels ($p = 0.00047$). No associations were found for rs1805010.

Conclusions: Our results indicate that rs1805011 polymorphism of *IL4Rα* gene seems to influence allergy risk, especially mild asthma and atopic dermatitis predisposition in Polish children. Subgroup analysis of three other SNPs revealed possible influence on allergy development.

1. Introduction

Both interleukin 4 (IL4) and interleukin 13 (IL13) are cytokines produced mainly by T-helper 2 (Th2) cells and they play a crucial role in the pathogenesis of allergic inflammation including allergic asthma [1,2]. Interleukin 4 and interleukin 13 recruit mast cells and eosinophils, induce isotype class-switching of B-cells for IgE synthesis and regulate expression of MHC II, CD23 and VCAM-1 [3–5]. Interleukin 4 is also responsible for regulating differentiation of naive T cells into Th2 subtype.

Interleukin 4 and IL13 strongly influence bronchial hyperreactivity in response to allergen, airway inflammation, mucus hypersecretion and airway remodeling [4,6]. Interleukin 13 also affects goblet cell hyperplasia and epithelial damage.

IL13 and *IL4* genes are clustered in a 160 kb region on the long arm of chromosome 5 [7]. Two subunits compose the IL4 receptor (IL4R): α chain (IL4Rα), which is also present in IL13R, and γ chain, shared among other cytokine receptors [8]. IL4Rα chain gene is located on chromosome 16p [9].

Previous studies have shown that SNPs in the *IL4* promoter may influence the response of mast cells to IgE-mediated signaling [10]. Polymorphism rs2243250 was associated with IgE levels, asthma, allergic rhinitis and atopic dermatitis [11–14]. Other SNPs in the IL4 gene were associated with allergy in different populations. A significant correlation with total IgE levels, and asthma has been also observed for rs2227284 polymorphism

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Table 1
Description of the analyzed population.

	Allergy	Control
Number	177	194
Gender – male	101	94
Gender – female	76	100
Age	11.5 ± 3.6	12.1 ± 33.4
Weight	46 ± 316.7	46 ± 314.5
Height	150 ± 38.8	153 ± 317.0
BMI	20 ± 35.8	19 ± 33.3
exNO	29.7 ± 336.7	16.7 ± 314.3
FVC	91.2 ± 313.9	100.4 ± 311.4
FEV1	85.5 ± 315.0	104.1 ± 38.2
FEV1%/FVC	88.2 ± 312.7	99.9 ± 35.6
Eosinophil count	5.7 ± 3 3.6	–
Total IgE	257.4 ± 3353.3	–

[15,16]. In regard to interleukin 4 receptor α chain gene, two functional polymorphisms were reported: rs1805010, observed to cause up-regulation of IL4Rα response to IL4 [17], and rs1805011, which function remains unknown. The former genetic variant was found to be associated with asthma [2,18] and the latter with asthma [14] and atopic dermatitis [19].

In interleukin 13 gene, a functional SNP contributing to aminoacid substitution R110Q (rs20541) was described to change the binding strength of IL13 to its receptor, causing higher activity of Gln110 variant [20]. Numerous studies showed that this polymorphism correlated significantly with higher total IgE levels, atopic dermatitis and asthma [1,21,22].

The aim of this study was to evaluate genetic association of five polymorphisms within interleukin 4, interleukin 4 receptor α chain and interleukin 13 genes with allergic phenotype in Polish pediatric population.

2. Materials and methods

2.1. Patients and controls

Our study included 371 Polish children of Caucasian origin, aged from 6 to 18 years old (mean 11.84; SD = 3.48); 177 were asthmatic patients, diagnosed at least six months before the inclusion in the study, and 194 healthy children formed a control group. Participants and their legal guardians gave written informed consent. The project received acceptance from the local ethics committee. The description of the patients and controls was presented in Table 1.

Patients were recruited from inpatients of the Department of Pediatric Pulmonology, Allergy and Clinical Immunology, Poznan University of Medical Sciences, living in ethnically homogenous Wielkopolska region. The diagnosis of asthma was based on clinical asthma symptoms and lung function test (bronchodilator responsiveness, exercise-induced hyperresponsiveness), as recommended by Global Initiative for Asthma (GINA).

To be diagnosed with severe asthma the patient had to have symptoms that required daily therapy with high-dose inhaled corticosteroids (>800 mcg budesonide or >500 mcg fluticasone), despite regular treatment with long-acting beta 2 agonists and/or leukotriene antagonist and/or slow releasing theophylline, as well as one or more emergency care visit or oral steroid burst per year.

For the purpose of the study, allergy was defined as follows: current or past symptoms of allergic rhinoconjunctivitis (seasonal or perennial) or atopic dermatitis. Symptomatic patients had to fulfill one of following criteria to confirm the presence of clinical atopy [23]: total immunoglobulin E (IgE) level higher than the upper normal limits for age, measured by a fluoroimmunossay with Pharmacia UniCap 100 System® (Pharmacia, Uppsala, Sweden); positive skin prick test to at least one allergen with the use of AllergoPharma kit (Germany): *Dermatophagoides pteronyssinus*, *Dermatophagoides farina*, cat, dog, feathers, *Alternaria alternata*, *Cladosporium herbarum*, pollen: grass mix, rye, birch, alder, hazel. Skin prick test result was considered as positive if the mean wheal diameter was at least 3 mm greater than negative control. Skin prick testing was performed in all cases, whereas IgE levels were assessed in 87 subjects.

Control group included carefully chosen healthy volunteers from the same geographic region. We assessed medical history of the subjects and performed clinical examination, spirometry and exhaled NO measurement to exclude any allergic disease or asthma.

2.2. Genotyping

Genomic DNA from asthmatic patients was extracted from whole blood using the salting out method, and from control group from saliva using Oragene kit (Genotek). All DNA samples were assessed on Nanodrop 2000 spectrophotometer. Saliva samples had lower purity, although they were within the ranges provided by kit manufacturer. Working solutions of equal concentrations for all DNA samples (20 ng/μl) were prepared for genotyping with PCR-RFLP method.

Polymorphisms were chosen with the use of accessible literature, NCBI database and HapMap. Selection criteria were as follows: functionality, previous clinical associations (both positive and negative findings) and high allele frequency in Caucasian population (MAF > 0.05).

Genotypes of four polymorphisms (rs2243250, rs2227284, rs1805011, rs20541) were assigned using TaqMan SNP Genotyping Assays (Applied Biosystems) on AbiPrism 7900HT system. Genotypes of rs18050100 polymorphism were established by PCR-RFLP. The sequences of the used primers are shown in Table 2. PCR reaction was carried out on Eppendorf Mastercycler with the initial denaturation of 6 min at 95 °C, 35 cycles of 30 s at 95 °C, 30 s at 60 °C and 45 s at 72 °C and additional 10 min at 72 °C at the end. PCR products were then digested overnight at 37 °C with *RsaI* enzyme and resolved in 3% agarose gel.

2.3. Statistical analysis

The Pearson's chi-square test and Fisher's exact test were used to test differences in the genotypic and allelic (respectively)

Table 2
Description of polymorphisms analyzed in this study.

SNP ID	Gene	Location	Substitution	Method	Assay ID/primers
rs2243250	IL4	2 KB upstream	C/T	TaqMan	C__16176216_10
rs2227284	IL4	Intron	T/G (A/C)	TaqMan	C__11818513_1_
rs1805010	IL4Ra	5′ UTR	A/C	RFLP	F: GGCAGGTGTGAGGAGCATCC R:GCCTCCGTTGTTCTCAGGTA
rs1805011	IL4Ra	3′ UTR	A/C	TaqMan	C__8903098_20
rs20541	IL13	Exon 4	C/T	TaqMan	C___2259921_20