

Interleukin 1 β polymorphism and serum level are associated with pediatric asthma

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Abstract

Background and Aim: Interleukin-1 is a pro-inflammatory cytokine found in two forms (α and β). The α form is mainly cell-bound, whereas IL-1 β is primarily secreted by macrophages in response to immune system stimulation. We hypothesized that polymorphic variants of interleukin 1 genes may play a role in childhood asthma risk. The aim of this study was to investigate if IL-1 α and β polymorphism is associated with asthma in a pediatric population and if the genotype affects its serum level.

Methods: The studied population included 310 children aged 6-18 years old (152 with asthma and 158 healthy children). Genotypes were determined with real-time PCR method using TaqMan Genotyping Assays. Serum level was measured with ELISA Set. Statistical analysis was done in Statistica v.12.0. Linkage disequilibrium and haplotype analysis was done in Haploview v. 4.2.

Results: We found that three IL-1 β polymorphisms rs1143634, rs1143633, and rs1143643 were associated with allergic asthma risk ($P = 0.034$; OR = 1.523; $P = 0.024$, OR = 1.477; 0.044, OR = 1.420, respectively). We also found a strong linkage disequilibrium between these polymorphisms and CAC haplotype was associated significantly with asthma risk ($P = 0.023$). For IL-1 α , we did not observe association with asthma. We then analyzed if IL-1 β expression was altered in serum and we found that asthmatic children showed significantly higher IL-1 β levels than healthy controls ($P = 0.047$). No association with asthma was observed for IL-1 α variants.

Conclusions: This study indicates that IL-1 β gene polymorphism may affect allergic asthma risk in children.

KEYWORDS

allergic asthma, gene, pediatric population, polymorphism

1 | INTRODUCTION

IL-1 is a major pro-inflammatory cytokine that is found in two forms: IL-1 α and IL-1 β that show similar structure and functions by binding to

the same receptors with different affinity. IL-1 α remains primarily cell associated and is present in the cytosol and on the plasma membrane of cells.¹ Interleukin-1 β is the main secreted form of IL-1 and originally was identified as a lymphocyte-activating factor that enhances B cells

activation and IgE production.^{2,3} This cytokine is mainly produced by activated macrophages, blood monocytes, dendritic cells, and mediates inflammatory response via various cell activities such as cell proliferation, differentiation, and apoptosis.

The two forms of IL-1 are encoded by different genes located on the long arm of chromosome 2,⁴ a region that showed linkage to asthma and interleukin 1 family is a plausible candidate gene.⁵

IL-1 β is the mainly secreted isoform of interleukin 1 that mediates the development of allergic diseases⁶ and asthma⁷ via differentiating and activating Th2 cells^{8,9} and Th17 cells.¹⁰ This interleukin also plays a role in host defense system in response to pathogens (bacteria, fungi, viruses) as a part of PAMP pathway (pathogen-associated molecular patterns) as well as to biomolecules produced by host upon tissue injury, that is, DAMP (danger-associated molecular patterns) that activate non-infectious inflammatory response.¹¹ In asthma, it was reported that both PAMP and DAMP factors may contribute to asthma exacerbation and increased inflammation in the airways via activation of inflammasome NLRP3 that produces mature IL-1 β .¹² This was recently confirmed by in vivo and ex vivo studies in animal models showing that upon allergen exposure, IL-1 β stimulates Th2 production and enhances eosinophil activation in the site of inflammation in the airways.¹³ This interleukin also increases cytokine secretion (eg, IL-5) that stimulates production of adhesion molecules (such as ICAM-1, VCAM-1) thus inducing inflammation in the airways and enhancing obstruction of the bronchi.¹⁴ Recently, IL-1 β was shown to mediate functional effects of inflammasome by activating resident epithelial cells and controlling epithelial permeability.¹⁵

Taking into account the previous findings, we hypothesized that polymorphic variants of interleukin 1 genes may influence its expression thus increasing asthma risk. The aim of this study was to investigate if IL-1 α and IL-1 β polymorphism is associated with asthma in a pediatric population and if IL-1 serum level correlates with childhood asthma.

2 | METHODS

2.1 | Study design

In this case-control study, 310 children were included. All participants as well as their parents had given written informed consent. Local ethics committee accepted the study (approval no. 954/15). The study was performed in compliance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

2.2 | Patients

The study was performed on a Polish sample of 152 asthmatic patients of Caucasian origin ranging from 6 to 18 years old. Patients were recruited from inpatients treated for asthma in the Department of Pediatric Pulmonology, Allergy and Clinical Immunology of Poznan University of Medical Sciences and in the Outpatient Allergy Clinics from Poznan and Gdansk. Asthma diagnosis was made according to GINA recommendation, based on clinical asthma

symptoms and lung function test at least 6 months before inclusion in the study. Spirometry was performed using a LungTest 1000 (MES) according to ERS/ATS guidelines.¹⁶ Decreased lung function was defined as FEV1% predicted value below the 5th percentile (FEV1 value adjusted for ethnicity, age, gender, height, and weight) as recommended according to ATS/ERS guidelines.¹⁷

Exhaled nitric oxide (exNO) concentration (an indicator of airway inflammation) was assessed by chemiluminescence using NIOX analyzer and following the ATS/ERS recommended procedures.¹⁸ Increased exNO concentration was regarded as >20 ppb (equivalent to nanoliter per liter).

Clinical diagnosis of atopy depended on current or past symptoms of atopic dermatitis, or allergic rhinitis. Atopic background was confirmed with increased total IgE level and positive skin prick test result to at least one aero-allergen (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat, dog, *Alternaria alternata*, *Cladosporium herbarum*; pollen: grass mix, rye, birch pollen, alder, hazel—Allergopharma, Germany). Total serum IgE level was measured by a fluoroimmunoassay with Pharmacia UniCap 100 System[®] (Pharmacia, Uppsala, Sweden) following manufacturer's instruction.

2.3 | Control group

Control group consisted of 158 healthy subjects of Caucasian origin. Control subjects were recruited from the same geographic region and included a group of carefully chosen volunteers aged from 6 to 18 years without current and past asthma and allergy symptoms. Any allergic disease or asthmatic episodes were excluded based on clinical examination, history, lung function test and exhaled NO measurement. Exclusion criteria were asthma and allergy presence or history, other chronic diseases, FeNO over 20 ppb, lung function below the 5th percentile (adjusted for age, gender, height, and weight) in spirometry.

2.4 | Molecular analysis

The SNP selection included the following criteria: functionality (in experimental functional studies), high frequency (MAF > 0.05), or previous association studies for allergic diseases (both positive and negative findings). SNPs chosen include both coding regions as well as non-coding regions (introns, UTRs) that could affect gene regulation and/or expression.

Genomic DNA from asthmatic patients was extracted from whole blood using the salting out method,¹⁹ whereas DNA from control group was obtained from saliva and isolated with the use of Oragene kit (Genotek, Ottawa, Canada). In the study, the yield of DNA from 2 mL of saliva samples ranged from 15 to 300 μ g and the quality measured as A260/280 ratio was between 1.6 and 1.8. Genotypes of three variants in IL-1 α and seven IL-1 β polymorphisms were determined using TaqMan SNP Genotyping Assays (ThermoFisher Scientific, Foster City, CA). The amplification for TaqMan SNP genotyping assay plates was done in AbiPrism 7900HT Sequence Detection System. Data acquisition and analysis were performed using the allelic discrimination analysis module in SDS v2.4 software (ThermoFisher Scientific). The description of the

analyzed SNPs was shown in Table 1. For each reaction plate genomic control DNA samples and non-template controls (water) were included. The control of TaqMan SNP genotyping assay was also performed (15% of randomly chosen samples from both groups) to check for genotyping accuracy and identical genotypes were identified in all repeated samples. The genotyping was performed without knowing the clinical status of the subjects.

2.5 | ELISA

Blood samples for serum were taken from a subgroup of control subjects and asthmatic patients during routine control visit in a stable period of disease. The samples were collected for tubes without anticoagulant and after 1 h samples were centrifuged to obtain serum and frozen at -80°C for further analysis. Concentration of IL-1 β in undiluted serum samples was measured using ELISA Set (MyBioSource, San Diego, CA) according to the manufacturer's protocol.

2.6 | Statistical analysis

Pearson's chi-squared (χ^2) and Fisher's exact tests were used to test differences in the genotypic and allelic (respectively) distribution in case-control analysis. Normality of data distribution was analyzed using Shapiro-Wilk test. Comparison of serum IL-1 β level between cases and control group was done with one-way analysis of variance (ANOVA). The correlation between IL-1 β and selected clinical parameters was done using Pearson's correlation coefficient. All tests were two-tailed. P level below 0.05 was considered significant. Calculations were performed using Statistica version 12.0 software. Power analysis was done in Quanto. Linkage disequilibrium and haplotype analysis as well as concordance with Hardy-Weinberg law was performed in Haploview v.4.2.²⁰ The multiple testing correction was performed (10 000 permutations) when applicable (haplotype analysis).

3 | RESULTS

The clinical description of the population analyzed in this study was shown in Table 2. The patients and control group did not differ significantly in age, but asthmatic patients were more frequently boys and this group showed significantly decreased lung function parameters.

3.1 | Hardy-Weinberg equilibrium test

All studied polymorphisms were tested for concordance with the Hardy-Weinberg equilibrium law (HWE). No deviation was observed for asthma patients and control group ($P > 0.05$) except one polymorphism (rs1143634) in the asthmatic patients.

3.2 | Power analysis

The power to detect an association for analyzed SNPs ranged from 61% to 68% for the studied population.

3.3 | Single marker association results

3.3.1 | IL1 α gene

We observed that genotype distribution and allele frequencies were similar between asthmatic patients and control subjects ($P > 0.05$ for all three polymorphisms) (Table 3). No association was found with asthma severity (data not shown).

3.3.2 | IL1 β gene

We found that three IL-1 β polymorphisms: rs1143634, rs1143633, and rs1143643 were significantly associated with the asthma risk in children (Table 3). We also tested if these polymorphisms are

TABLE 1 The characteristics of analyzed polymorphisms in *IL-1* genes

SNP ID	Substitution	Polymorphism location	Assay ID	MAF
<i>IL-1α</i>				
rs11677416	C/T	3' gene region	Custom assay	T (0.31)
rs17561	C/A	Exon	C__9546471_10	A (0.31)
rs1800587	G/A	5' gene region	C__9546481_20	A (0.31)
<i>IL-1β</i>				
rs1143634	G/A	Synonymous, coding sequence	C__9546517_10	A (0.21)
rs4848306	G/A	Promoter region	C__11725735_10	A (0.46)
rs1143623	C/G	Promoter region	C__1839941_10	G (0.33)
rs16944	A/G	Promoter region	C__1839943_10	A (0.34)
rs1143633	G/A	Intron	C__1839948_10	A (0.39)
rs1143627	G/A	Promoter region	C__1839944_10	G (0.37)
rs1143643	C/T	Synonymous coding sequence	C__1839949_10	T (0.28)

TABLE 2 Clinical characteristics of the analyzed population

	Patients group (n = 152)	Control group (n = 158)	P-value
Gender, male (%)	54%	37%	0.003
Age, years (mean $\pm$ SD)	11.50 $\pm$ 3.55	12.11 $\pm$ 3.41	0.130
Asthma severity		–	
Mild, n (%)	19 (12.5)	–	
Moderate, n (%)	83 (54.6)	–	
Severe, n (%)	50 (32.8)	–	
FEV1/FVC pred (mean $\pm$ SD)	88.8 $\pm$ 10.5	104.35 $\pm$ 8.2	<0.001
FEV1% pred (mean $\pm$ SD)	86.1 $\pm$ 13.8	102.97 $\pm$ 7.1	<0.001
FVC% pred (mean $\pm$ SD)	91.3 $\pm$ 13.4	101.12 $\pm$ 11.9	<0.001
PEF% pred (mean $\pm$ SD)	81.3 $\pm$ 15.8	90.3 $\pm$ 12.9	<0.001
exNO (ppb) (mean $\pm$ SD)	27.9 $\pm$ 33.2	14.8 $\pm$ 13.2	<0.001
Positive SPT (%)	68.2	–	
IgE (IU/mL) (mean $\pm$ SD)	247.7 $\pm$ 294.0	–	

associated with more severe asthma phenotype, however, we did not observe an association with asthma severity (data not shown).

3.4 | Linkage disequilibrium and haplotype analysis

For IL-1 α gene, we found a strong linkage between studied variants ($D' > 0.99$, $r^2 > 0.98$) grouped in one haplotype block (Figure 1A). However, no haplotype was significantly different between patients and control group (Table 4).

For IL-1 β we found a strong linkage disequilibrium between the analyzed polymorphisms that were grouped in two haplotype blocks (Figure 1B). The first block contained three SNPs (rs1143643, rs1143634, rs1143633), and the second consisted of four polymorphisms (rs1143627, rs16944, rs1143623, rs4848306). Haplotype analysis revealed that CAC haplotype from the first block was

significantly more frequent in the asthmatic patients ($P = 0.02$), while TGT haplotype from the same block was found more frequent in the control population ($P = 0.01$). However, the results did not reach statistical significance after correction for multiple testing (permutation testing). The haplotype from the second block (GACG) was also more frequent in the asthmatic group, but showed the lowest frequency from all studied haplotypes. The results were presented in Table 4.

3.5 | Analysis of serum IL1 β levels

We then analyzed if IL-1 β expression was altered in serum and we found that asthmatic children showed significantly higher IL-1 β levels than healthy controls (Figure 2). However, none of the genotypes of analyzed SNPs was associated with IL-1 β level in asthmatic children (data not shown).

TABLE 3 Single marker association results of IL-1 α and 1 β polymorphism between asthmatic patients and control subjects

SNP	Allele	Case, control ratio	P	OR (95%CI)
IL-1 α				
rs11677416	T	0.32, 0.31	0.72	1.06 (0.76-1.48)
rs17561	A	0.32, 0.30	0.65	1.09 (0.79-1.53)
rs1800587	A	0.31, 0.31	0.93	0.99 (0.70-1.39)
IL-1 β				
rs1143643	C	0.68, 0.59	0.03	1.42 (1.02-1.98)
rs1143634	A	0.28, 0.20	0.03	1.52 (1.04-2.23)
rs1143633	C	0.69, 0.59	0.02	1.48 (1.06-2.06)
rs1143627	G	0.33, 0.32	0.78	0.96 (0.69-1.35)
rs16944	A	0.33, 0.33	0.87	1.05 (0.75-1.48)
rs1143623	G	0.29, 0.28	0.86	0.95 (0.66-1.35)
rs4848306	G	0.59, 0.51	0.07	0.73 (0.52-1.01)

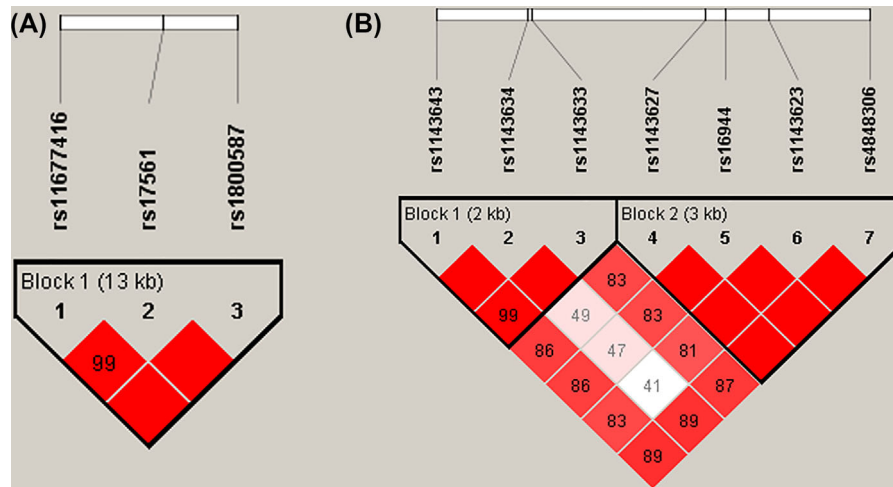


FIGURE 1 Haplotype blocks of analyzed polymorphisms of interleukin 1 genes for (A) *IL-1α* and (B) *IL-1β*

3.6 | *IL1β* level and airway inflammation, lung function, and total IgE

As *IL-1β* activates eosinophils at the site of inflammation and thus may enhance it, we also investigated if airway inflammation marker (increased FeNO) or decreased lung function parameters (spirometry) may correlate with *IL-1β* genotype or level. No association was observed between genotypes of analyzed SNPs and FeNO value and lung function parameters (data not shown). Similarly, serum level of *IL-1β* did not correlate significantly with FeNO and lung function parameters. We also investigated the correlation between the activation of B cells (ie, total IgE level) and serum *IL-1β* level, but we did not find a significant relationship (Supplementary Table S1).

4 | DISCUSSION

The main finding of this study is that variants in *IL-1β* gene, but not *IL-1α*, are associated with asthma in pediatric population and that the association on genetic level is also observed on protein level of *IL-1β*.

Based on the fact that two forms of *IL-1* map to adjacent chromosomal region (chromosome 2, long arm), we tested if both *IL-1α* and *IL-1β* polymorphism may be a susceptibility factor for childhood asthma. For *IL-1α*, we did not observe an association of the analyzed variants with asthma. This is consistent with the previous findings showing lack of association of *IL-1α* gene polymorphism (rs17561) with adult asthma.²¹

The genetic association studies regarding *IL-1β* polymorphism in asthma are limited. The most frequently studied was rs16944 polymorphism that results in A/G substitution in the promoter region. This variant was previously analyzed in the study of adult asthma that reported association of this variant with adult asthma, but only in men with lower IgE level,²² suggesting its association with non-atopic asthma. Consistently, in our study, this variant showed no association with childhood atopic asthma and related phenotypes such as IgE level or FeNO.

Variants showing association with childhood asthma in this study included rs1143634, rs1143643, and rs1143633. The first one was primarily investigated in other chronic inflammatory disorders such as sclerosis multiple²³ and diabetes type 2.²⁴ In our study, we observed that risk allele (A) was significantly more frequent in asthmatic patients. This SNP leads to synonymous substitution in the coding gene region and is located in the site possibly involved in splicing regulation (as predicted by F-SNP database). Moreover, this variant is in complete linkage disequilibrium ($D' = 1.0$, $r^2 = 1.0$) with another SNP (rs1071676) in the same gene, located in the 3'UTR of *IL-1β*, that constitutes the binding site for several miRNAs (among others, miR-30 family) that exert anti-inflammatory and anti-fibrotic effects.^{25,26} The other two associated variants are located in the introns and they may alter transcription factor binding site thus having effect on gene expression (according to F-SNP database). In our study, all three

TABLE 4 Haplotype analysis results of *IL-1α* and *1β* polymorphism between asthmatic patients and control subjects

Haplotype	Frequency	Case, control ratio	P
<i>IL-1α</i>			
CCG	0.68	0.68, 0.69	0.74
TAA	0.31	0.31, 0.30	0.87
<i>IL-1β</i>			
Block 1			
CGC	0.40	0.39, 0.39	0.82
TGT	0.36	0.31, 0.40	0.01 ^a
CAC	0.24	0.27, 0.19	0.02 ^a
Block 2			
AGCA	0.46	0.42, 0.48	0.09
GAGG	0.28	0.28, 0.28	0.96
AGCG	0.22	0.24, 0.19	0.12
GACG	0.04	0.05, 0.03	0.04

^aAfter permutations corrected P-values of 0.08 and 0.12, respectively.

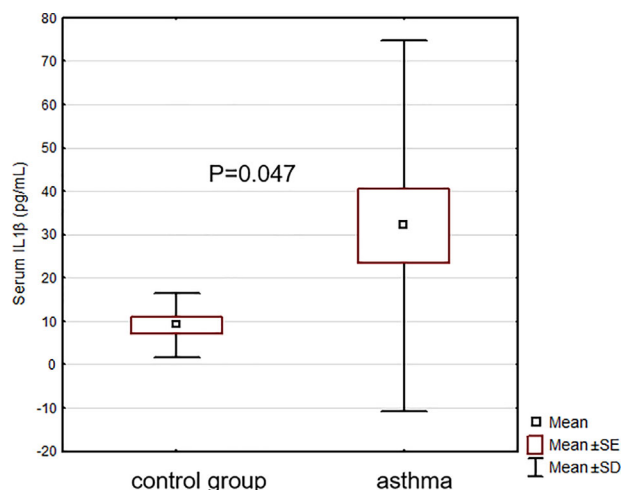


FIGURE 2 Comparison of IL-1 β serum levels between asthmatic and healthy children (one-way ANOVA)

associated variants were in strong linkage disequilibrium ($D' > 0.99$, $r^2 > 0.98$) suggesting that this genomic region may be relevant for asthma development by affecting IL-1 β regulation.

Further, we found increased IL-1 β serum levels in asthmatic children as compared to the healthy control. This is consistent with the findings for the adult atopic asthmatic patients, in particular in the symptomatic period as compared to the COPD patients, that demonstrate airway neutrophilic inflammation, without atopic component.²⁷ It seems that changes in IL-1 β serum levels may reflect inflammatory changes in the allergic airways.

Childhood asthma has distinct etiopathogenesis than adult-onset asthma. The predominant endotype in pediatric population is atopic asthma with underlying eosinophilic inflammation. This type is also observed in asthmatic children with the history of early-life viral infections of the airways²⁸ suggesting that IL-1 β secretion induced in response to PAMP factors may be crucial for asthma development. As a result of airway inflammation in asthma, a secretion of DAMP factors also takes place thus activating inflammasome NLRP3 that is responsible for chronic systemic inflammation.¹²

The role of IL-1 β in allergic airways was confirmed on animal model in the mice immunized with 2,4-TDI (2,4-diisocyanotoluene) and expressing airway hyperreactivity. IL-1 β -neutralizing antibody administered to these mice suppressed eosinophils and lymphocytes recruitment as well as IL-4 secretion in the lung as compared to the animals not treated with this neutralizing antibody.¹⁴ This effect was specific for IL-1 β , but not for IL-1 α -neutralizing antibody. Further studies in vitro and in animal models confirmed that IL1 β is involved in the development of allergic inflammation. IL-1 β mediates IL-17 production, a proangiogenic cytokine that may be responsible for hypertrophy of smooth muscle cells and enhanced asthma symptoms.²⁹ Moreover, allergen administration into the airways of the experimental animals led to enhanced IL-1 β secretion within 4–8 h (early allergic response) which was followed by eosinophils recruitment in the later phase of inflammatory response.³

The main limitation of this study is relatively small sample size of our population, partly related to the inclusion criteria (Caucasian patients of Slavic origin, asthma diagnosed at least 6 months before inclusion in the study, confirmed atopic background). Due to limited sample size, we had moderate power to detect an association (>60%), that is lower than 80% usually appreciated in genetic association studies and could increase the risk of false negatives findings.³⁰ However, the observed genetic differences between asthmatic patients and controls were also found on protein level, suggesting that this gene may be relevant for childhood asthma, although IL-1 β protein level does not seem to be influenced directly by analyzed SNPs, rather by IL-1 β locus in general.

In conclusion, we suggest that IL-1 β gene may constitute a risk factor for childhood atopic asthma via influence on gene regulation leading to altered protein serum level in atopic asthma. Locus for IL-1 α does not seem associated with childhood atopic asthma.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

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