International Journal of Biomedicine 8(3) (2018) 213-216
http://dx.doi.org/10.21103/Article8(3)_OA8

ORIGINAL ARTICLE

Otolaryngology

INTERNATIONAL JOURNAL OF BIOMEDICINE

# Cytokine Gene Polymorphisms in Chronic Adenoiditis

Natalia V. Terskova, PhD, ScD[1]; Natalia A. Shnayder, PhD, ScD[2]; Andrey S. Simbirtsev, PhD, ScD[3]; Sergey G. Vakhrushev, PhD, ScD[1]; Dinara R. Sidorenko, MD[1*]; Natalia V. Platonova, PhD[1]

*[1]V.F. Voino-Yasenetsky Krasnoyarsk State Medical University, Krasnoyarsk, Russia*
*[2]V.M. Bekhterev National Medical Research Centre for Psychiatry and Neurology, St. Petersburg, Russia*
*[3]The State Research Institute of Highly Pure Biopreparations of FMBA, St. Petersburg, Russia*

## Abstract

***The aim*** of our research was to study the multiphase response in a system of pro-inflammatory and anti-inflammatory cytokines due to the additive contribution of homozygous and heterozygous genotypes for the polymorphic allelic variants of the interleukin-1β (*IL-1β*) and interleukin-4 (*IL-4*) genes in patients with chronic adenoiditis.

***Materials and Methods***: The study included 388 children with chronic adenoiditis. Associations between the *IL1B* gene (rs1143634) (C+3954T) SNP and the *IL-4* gene (rs2243250) (C-589T) SNP and the clinical manifestations and clinical outcome of chronic adenoiditis were investigated. Genotyping for the studied SNPs was performed using real-time PCR. The study of genotype-associated cytokine production in accordance with the level of concentration of IL-1β, IL-4 in blood serum with the method of solidphase EIA using horseradish peroxidase as an indicating enzyme was carried out.

***Results****:* The presence of homozygous or heterozygous genotypes of the studied SNPs of the *IL-1β* and *IL-4* genes was characterized with genetically determined cytokine-production forming the phenotypical polymorphism. The conducted research into congenital immunity factors with an assessment of genetically determined cytokine production has revealed 5 options of the cytokine response and their corresponding frequencies. We extrapolated the results on clinical and functional outcomes of chronic adenoiditis, which allowed us to identify non-randomness in the nature of chronic adenoiditis as a multifactorial disease.

***Conclusion:*** The obtained data are evidence of the phenotypic-genetic heterogeneity of chronic adenoiditis. **(International Journal of Biomedicine. 2018;8(3):213-216.)**

**Key Words**: chronic adenoiditis • interleukin-1β • interleukin-4 • single nucleotide polymorphism

## Introduction

The multifactorial character of chronic adenoiditis (CA) is based on the complex interactions of genetic and environmental factors.[1] Non-specificity of clinical manifestations does not allow forecasting the course of the disease. However, there is a preventive approach to the prognosis, favorable or not favorable, that considers the efficacy of conservative therapy and risk of operative intervention.[2,3] The approach studies disorders in the physiologic immune processes in CA in children, which has been convincingly shown on the example of other nosological entities.[4]

***Corresponding author:** Dinara R. Sidorenko, PGS. V. F. Voino-Yasenetsky Krasnoyarsk State Medical University, Krasnoyarsk, Russia. E-mail: sidorenko-dinara@mail.ru*

The aim of our research was to study the multiphase response in a system of pro-inflammatory and anti-inflammatory cytokines due to the additive contribution of homozygous and heterozygous genotypes for the polymorphic allelic variants of the interleukin-1β (*IL-1β*) and interleukin-4 (*IL-4*) genes in CA patients.

## Materials and Methods

The study included 388 children with CA. The CA diagnosis was made on the basis of anamnesis data and clinical, epidemiological, endoscopic, radiological, and immunological data.

The inclusion criteria were the following:

- Children with CA (of both sexes, aged between 3 and 10)

diagnosed by ENT specialist, allergist, and pediatrician
- Residence in Krasnoyarsk city from birth
- Caucasians
- Remission for concomitant diseases
- Lack of care in the previous month
- Patient's ability to perform the procedure protocol

Exclusion criteria were the following:
- Children less than 3 years and over 10 years of age
- Children of a race other than Caucasian
- Exacerbation of concomitant diseases
- Acute respiratory viral infection
- Use of drugs that can affect the results of the study
-Violation of the procedure protocol

Clinical examination included an analysis of the complaints, historical data with exact duration of the disease and causes resulting in the development of CA, including causes of hereditary predisposition. In addition, we estimated the conceptual, subjective, and objective signs: the beginning and duration of the disease, the child's age at the moment the diagnosis was verified, the severity of the course of the disease, frequency and duration of CA manifestations, character of complications, the degree of pharyngeal tonsil hypertrophy, allergic manifestations, the degree of elevation of temperature in CA and/or acute viral respiratory infection, and spread of hypertrophy of cervical lymph nodes.

In the present study, we investigate associations between the *IL-1β* gene *(*rs1143634) (C+3954T) SNP and the *IL-4* gene (rs2243250) (C-589T) SNP and the clinical manifestations and clinical outcome of CA. Molecular-genetic studies were carried out in 317(81.7%) children with CA.

Genotyping for the studied SNPs was performed using real-time PCR followed by amplification with the use of oligonucleotides labeled with fluorescent agents complimentary to the part of PCR-product (*TaqMan* technique) of a sick child's blood sample DNA, with automatic detection. Each experiment contained a negative control in which DNA-matrix for PCR was substituted for distilled water. Amplification was performed in 50 µl volume containing 300 ng of DNA, 0.1µl of primer containing 16-25 pairs of nucleotides (Applied Biosystems, USA). The applied structural designs of primers for describing gene characteristics are presented in accordance with the data of the National Center for Biotechnology Information (rs – reference SNP) according to the protocol attached to the primer[4]:

1) For the *IL-1β* gene (rs1143634) (C+3954T)
CTCCACCTTTCAGAACCTATCTTCTT [C/T]
GACACATGGGATAACGAGGCTTATG
2) For the *IL-4* gene (rs2243250) (C-589T)
AACACCTAAACTTGGGAGAACATTGT [C/T]
CCCCAGTGCTGGGGTAGGAGAGTCT

PCR was performed in the amplifier Rotor-Gene-6000.

We carried out the study of genotype-associated cytokine production in accordance with the level of concentration of IL-1β, IL-4 in blood serum with the method of solidphase EIA using horseradish peroxidase as an indicating enzyme on the base of the certified Regional Laboratory and Diagnostic Center of Immunochemical Methods of Research of Krasnoyarsk cit. For this purpose, we used the certified IL-1β/IL-4 test-systems (BioChemMackDiagnostics, Russia).

Each of the three possible variants of genotypes was associated with production of coded cytokine.

Statistical analysis was performed using IBM SPSS Statistics V22.0 (SPSS Inc., Chi-cago, IL, USA). Baseline characteristics were summarized as frequencies and percentages for categorical variables. Continuous variables were presented as mean(M)±standard error of the mean (SEM) and as median (interquartile range [IQR]). Means of 2 continuous normally distributed variables were compared by independent samples Student's t test. Kruskal - Wallis one-way analysis of variance and Dunn post hoc test with Bonferroni adjustment were nonparametric alternative. Group comparisons with respect to categorical variables are performed using chi-square tests. Three exact probability tests for departure from HWE due to heterozygote excess, heterozygote deficit and omnibus probability test were carried out using GENEPOP (v. 4.7.0) A probability value of $P<0.05$ was considered statistically significant.

The study was carried out as a part of the ENT-department research "Translational otorhinolaryngology" (State registration No. 01201001212). Written informed consent was obtained from the child's parents.

## Results and Discussion

The presence of homozygous or heterozygous genotypes of the studied SNPs of the *IL-1β* and *IL-4* genes was characterized with genetically determined cytokine-production forming the phenotypical polymorphism (Table 1).

***Table1.***

***Associations between the IL-1β gene (C+3954T) SNP and the IL-4 gene (C-589T) SNP and cytokine production in CA patients (n=317)***

| SNP | Genotype | | Concentration of cytokines, pg/ml Me [$Q_{25}$; $Q_{75}$] | $P \leq 0.05$ |
|---|---|---|---|---|
| *IL-1β* gene (C+3954T) | CC | 1 | 199.40 [190.28; 216.43] | $P_{1-2}$; $P_{1-3}$ $P_{2-3}$ |
| | CT | 2 | 158.35 [66.88; 177.99] | |
| | TT | 3 | 129.80 [113.50; 155.74] | |
| *IL-4* gene (C-589T) | CC | 1 | 139.40 [134.16; 149.30] | $P_{1-2}$; $P_{1-3}$ $P_{2-3}$ |
| | CT | 2 | 187.70 [141.10; 200.50] | |
| | TT | 3 | 216.54 [206.40; 247.20] | |

To understand the multiphase response in the system of opposite cytokines in CA, we proceeded from estimating the presence of balance or imbalance of genotype variants. By 'balance' we mean the degree of equilibrium between opposed variants in a healthy subject for adequate functioning of cytokine system.

The most balanced was a heterozygous carrier, which was comprehensively presented in the population taken as a whole and allowed the individuals to adjust to environmental conditions. We found combined carriers of heterozygous genotypes of the *IL-1β* and *IL-4* SNPs in only 6.3±1.4% of cases in 20 CA children (Figure 1 A). This result could relate to

a balanced pro-inflammatory and anti-inflammatory cytokine response within the age reference range, which agreed with the results of laboratory studies of cytokine production.

Slightly more often, we registered disharmonious balance in pro-inflammatory and anti-inflammatory cytokine response, this being named 'high'. It consisted in homozygous genotype (CC)—a highly producing variant of pro-inflammatory cytokine IL-1β, causing elevated gene expression and production of this interleukin—combining with homozygous genotype (TT)—a highly producing variant of anti-inflammatory cytokine IL-4, which was accompanied by elevated gene expression and production of anti-inflammatory IL-4. Herewith, a certain balance between pro-inflammatory and anti-inflammatory responses could be achieved. However, from the point of view of polygenic interaction and mutual influence of other cytokine gene mutations participating in this response, such a balance should be rightly considered disharmonious because it is on the high limit of the reference range of the organism's reactivity. In so doing, a combination of highly producing variants of other pro-inflammatory cytokines, for example, IL-6, IL-8, etc., would cause a quick derangement of unstable disharmonious balance, a sharp increase of pro-inflammatory response, and a recurrent or severe course of CA as well. On the other hand, the same influence on the state of disharmonious genetic balance could be exerted by exogenous environmental factors. Disharmonious balance (high) was seen in 13.6±1.9% of cases in 43 CA children (Figure 1B).

The second kind of disharmonious balance on polymorph allele variants consisted in homozygous genotype (TT)—a low producing variant of cytokine IL-1β, causing down-regulation of gene expression and reducing production of this interleukin—combining with homozygous genotype (CC)—a low-producing variant of anti-inflammatory cytokine IL-4, causing the reduction of gene expression and production of IL-4.

In this case, the balance was also achieved but on the low limit of the reference range of reactivity, and it was disharmonious due to the polygenic influence of other pro-inflammatory and anti-inflammatory cytokines and the influence of exogenous environmental factors. The second variant of disharmonious balance of cytokine response was seen significantly less frequently in comparison with the first variant of disharmonious balance—in 25 children (7.9±1.5 %) (Figure 1C).

Deranged balance with increased pro-inflammatory response and decreased anti-inflammatory response was considered as an imbalance and occurred in the group under study statistically more often, in 35.0±2.7% of cases (n=111). This imbalance consisted in a combination of CC genotype of the *IL-1β* (C+3954T) SNP and CC genotype of the *IL-4* (C-589T) SNP (Figure 1D).

A contraversive variant of imbalance meant a prevalence of anti-inflammatory response and a decrease in pro-inflammatory response. The imbalance consisted in combined carriership of TT genotype of the *IL-1β* (C+3954T) SNP and TT genotype of the IL-4 (C-589T) SNP (Figure 1E).

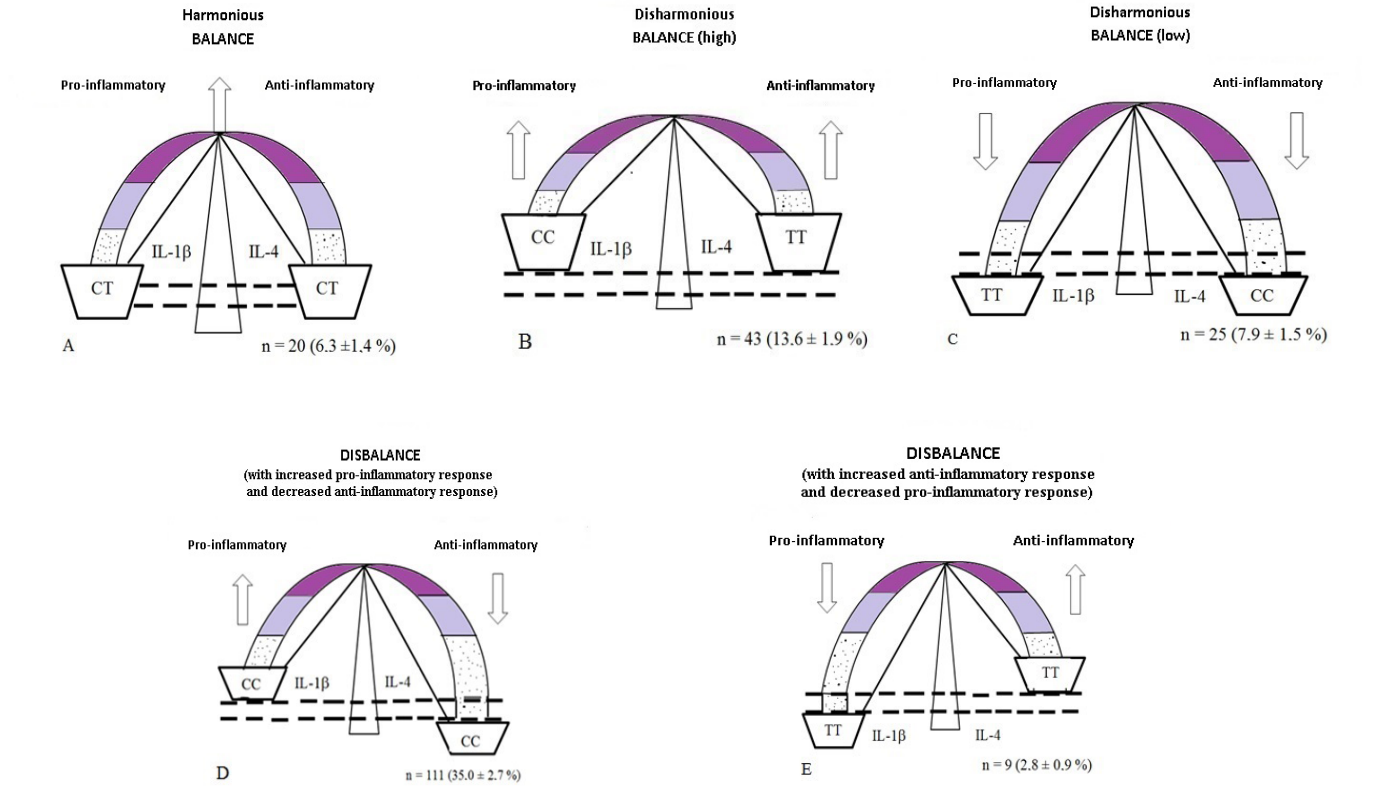


***Fig. 1**. Genetic and phenotypic balance of the state of cytokine response in CA children*

*Risk level: -low risk, -moderate risk, -high risk; dotted line – a reference range of permissible cytokine response (within normal limits)*

This variant was found in 2.8±0.9% of cases (n=9). Overall, the illustration visually demonstrates the genetic heterogeneity of immune cytokine response in CA in children. Also possible are intermediate combinations of homozygous and heterozygous variants of genetic and phenotypic imbalance of status of pro-inflammatory and anti-inflammatory cytokine response in CA. In this way, we should stress that the percentage of children with CA with an inappropriate balance was 93.7%, which was within the confidence interval.

## Conclusion

In conclusion, the conducted research into congenital immunity factors with an assessment of genetically determined cytokine production has revealed 5 options of the cytokine response and their corresponding frequencies. We extrapolated the results on clinical and functional outcomes of chronic adenoiditis, which allowed us to identify non-randomness in the nature of chronic adenoiditis as a multifactorial disease.

Thus, modern advances in molecular and clinical genetics are evidence of the phenotypic-genetic heterogeneity of CA. Thorough analysis of cytokine gene functioning revealed their role and place in the diagnosis of multifactorial disease, in particular, CA in children, and in applied otorhinolaryngology, which previously remained outside the clinical interpretation and integration into existing practice.

## Competing interests

The authors declare that they have no competing interests.

## References

1. Terskova NV, Shnayder NA, Vakhrushev SG. Polymorphism of interleukin-1β gene and susceptibility to chronic adenoiditis development at children of Siberia. Medical and Health Science Journal. 2013;14 (3):150-159.
2. Haukim N, Bidwell JL, Smith AJ, Keen LJ, Gallagher G, Kimberly R, et al. Cytokine gene polymorphism in human disease: on-line databases, supplement 2. Genes Immun. 2002;3(6):313–30.
3. Yu W, Clyne M, Khoury MJ, Gwinn M. Phenopedia and Genopedia: disease-centered and gene-centered views of the evolving knowledge of human genetic associations. Bioinformatics. 2010 Jan 1;26(1):145-6. doi: 10.1093/bioinformatics/btp618.
4. Moore JH. Computational analysis of gene-gene interactions using multifactor dimensionality reduction. Expert Rev Mol Diag. 2004;4(6):795–803.
5. dbSNP (Short Genetic Variations). Reference SNP (refSNP) Cluster Report: rs1143634. NCBI. Available from: https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=1143634