

Characteristics of interleukin-4 gene (C-589T, rs2243250) polymorphism in children with bronchial asthma and allergic rhinitis with isolated or allergic rhinitis-induced comorbid malocclusion

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Purpose. To determine the frequency of interleukin-4 (IL-4) (C-589T, rs2243250) single nucleotide polymorphism in children with bronchial asthma (BA) and allergic rhinitis (AR) and with isolated or allergic rhinitis-induced comorbid malocclusion.

Materials and methods. Single nucleotide polymorphism of the IL-4 (C-589T, rs2243250) gene was analyzed in 170 children aged between 6 and 17 years, 11 months, 29 days. Group I included 89 children with BA; Group II consisted of 31 children with AR; Group III was composed of 27 children with AR and distal occlusion (DO); Group IV comprised 23 children with malocclusion. Genotyping was performed using a commercial “SNP-express RT” kit by real-time polymerase chain reaction method (Applied Biosystems, USA) via TagMan[®]SNP Genotyping Assay on an amplifier CFX96[™] Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., USA).

DNA was isolated using a commercial DNA-express kit (“LLC Research and Production Company LITEKH”). The general, recessive and dominant models of inheritance and the odds ratios with a 95 % confidence interval were used for the analysis. The results analysis was conducted using the Statistica 6.0 RU licensed software package.

Results. IL-4 (C-589T, rs2243250) gene polymorphism in children with allergy and malocclusion living in Zaporizhzhia was analyzed for the first time.

The frequency of C/C – C/T – T/T genotypes registration was 69.66 % – 22.47 % – 7.87 % of cases in children with BA; 58.06 % – 38.71 % – 3.23 % in children with AR; 62.96 % – 29.63 % – 7.40 % in AR with DO. On the contrary, in children with malocclusion, the C/C (34.78 %) and T/T (4.35 %) genotypes were registered less frequently and the C/T genotype (60.87 %) was recorded more often as a genetic feature of the DO phenotype.

Conclusions. The C/C genotype of IL-4 (C-589T, rs2243250) gene was associated with bronchial asthma (OR = 4.31; 95 % CI = 1.63–11.36; P = 0.002) and allergic rhinitis (OR = 4.32 (95 % CI = 1.04–7.81; P = 0.04), in comparison with the fact that the C/T + T/T genotype indicated a predisposition to malocclusion development.

Key words:

genetic polymorphism, interleukin-4, bronchial asthma, allergic rhinitis, orthodontic pathology, children.

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Характеристика поліморфізму гена інтерлейкіну-4 (C-589T, rs2243250) у дітей із бронхіальною астмою, алергічним ринітом і з ортодонтичною патологією, яка ізольована чи коморбідна з алергічним ринітом

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Мета роботи – визначити частоту однонуклеотидного поліморфізму гена інтерлейкіну-4 (IL-4) (C-589T, rs2243250) у дітей із бронхіальною астмою (БА), алергічним ринітом (АР) та з ортодонтичною патологією, що ізольована чи сформована на тлі алергічного риніту.

Матеріали та методи. Дослідження поліморфізму гена IL-4 (C-589T, rs2243250) виконали у 170 дітей віком від 6 до 17 років, 11 місяців, 29 днів. У I групу спостереження увійшли 89 дітей із БА; у II групу – 31 дитина з АР; у III групу – 27 дітей з АР і дистальним прикусом; у IV групу – 23 дитини з ортодонтичною патологією.

Генотипування здійснили методом полімеразної ланцюгової реакції «SNP-експрес-РВ», використовуючи набори реагентів ООО НПФ «Литех», TagMan[®]SNP Genotyping Assay на ампліфікаторі CFX96[™]Real-Time PCR Detection Systems («Bio-Rad Laboratories, Inc.», USA). Для аналізу використовували загальну, рецесивну та домінуючу моделі успадкування та показники «відношення шансів» з 95 % довірчим інтервалом. Результати опрацювали за допомогою ліцензійного пакета програми Statistica 6.0 RU.

Результати. Уперше в дітей із м. Запоріжжя з алергічною та ортодонтичною патологією дослідили поліморфізм гена IL-4 (C-589T, rs2243250). Частота реєстрації генотипів C/C – C/T – T/T становила в дітей із БА 69,66 % – 22,47 % – 7,87 %; з АР – 58,06 % – 38,71 % – 3,23 %; АР із дистальним прикусом – 62,96 % – 29,63 % – 7,40 % випадків. У дітей з ортодонтичною патологією рідше реєстрували генотипи C/C (34,78 %) та T/T (4,35 %), частіше – генотип C/T (60,87 %) як генетичну особливість фенотипу дистального прикусу.

Висновки. Генотип C/C гена IL-4 (C-589T, rs2243250) асоціювався з бронхіальною астмою (OR = 4,31; 95 % CI = 1,63–11,36; p = 0,002) та алергічним ринітом (OR = 4,32; 95 % CI = 1,04–7,81; p = 0,04), а генотипи C/T + T/T свідчили про схильність до розвитку ортодонтичної патології.

Ключові слова:

генетичний поліморфізм, інтерлейкін-4, бронхіальна астма, алергічний риніт, ортодонтична патологія, діти.

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Ключевые слова:

генетический полиморфизм, интерлейкин-4, бронхиальная астма, аллергический ринит, ортодонтическая патология, дети.

Запорожский медицинский журнал. – 2019. – Т. 21, № 6(117). – С. 723–732

Характеристика полиморфизма гена интерлейкина-4 (C-589T, rs2243250) у детей с бронхиальной астмой, аллергическим ринитом и с ортодонтической патологией, изолированной или коморбидной с аллергическим ринитом

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Цель работы – определить частоту однонуклеотидного полиморфизма гена интерлейкина-4 (IL-4) (C-589T, rs2243250) у детей с бронхиальной астмой (БА), аллергическим ринитом (АР) и с ортодонтической патологией, изолированной или сформированной на фоне аллергического ринита.

Материалы и методы. Исследование полиморфизма гена IL-4 (C-589T, rs2243250) проведено у 170 детей в возрасте от 6 до 17 лет, 11 месяцев, 29 дней. В I группу наблюдения включены 89 детей с БА; во II группу – 31 ребенок с АР; в III группу – 27 детей с АР и дистальным прикусом; в IV группу – 23 ребенка с ортодонтической патологией. Генотипирование выполнили методом полимеразной цепной реакции «SNP-экспресс-ПВ» с использованием наборов реагентов ООО НПФ «Литех», TagMan®SNP Genotyping Assay на амплификаторе CFX96™Real-Time PCR Detection Systems («Bio-Rad Laboratories, Inc.», USA). Для анализа использовали общую, рецессивную и доминантную модели наследования и показатели «отношения шансов» с 95 % доверительным интервалом. Анализ результатов проведен при помощи лицензионного пакета программы Statistica 6.0 RU.

Результаты. Впервые у детей г. Запорожья с аллергической и ортодонтической патологией исследован полиморфизм гена IL-4 (C-589T, rs2243250). Частота регистрации генотипов C/C – C/T – T/T составила у детей с БА 69,66 % – 22,47 % – 7,87 %; с АР – 58,06 % – 38,71 % – 3,23 %; АР с дистальным прикусом – 62,96 % – 29,63 % – 7,4 % случаев. У детей с ортодонтической патологией реже регистрировали генотипы C/C (34,78 %) и T/T (4,35 %), чаще – генотип C/T (60,87 %) как генетическую особенность фенотипа дистального прикуса.

Выводы. Генотип C/C гена интерлейкина-4 (C-589T, rs2243250) ассоциировался с бронхиальной астмой (OR = 4,31; 95 % CI = 1,63–11,36; p = 0,002) и аллергическим ринитом (OR = 4,32 (95 % CI = 1,04–7,81; p = 0,04), а генотип C/T + T/T свидетельствовал о предрасположенности к развитию ортодонтической патологии.

Introduction

The morbidity rate of allergic diseases has shown a continuous increase in Europe and in other regions of the world, that currently poses a serious challenge to modern pediatrics and requires further research [1]. Thus, the well-known population-based studies indicate that the frequency of allergic diseases just in teenagers fluctuates from 40.3 % to 71.1 %. The prevalence of bronchial asthma (BA) is 12.9 % and allergic rhinitis (AR) – 32.8 % [2,3]. In Germany, rhinitis was comorbidity of BA in 34.7 % of children, and in Barcelona (Spain), AR was combined with BA in 49.5 % of cases [4,5].

At the same time, in conditions of a high prevalence rate of malocclusion in children and adolescents, various dental anomalies and deformities represent up to 56.13–62.48 %, and in cases of nasopharyngeal diseases, including rhinitis, this figure increases to 86.08 ± 1.59 %. However, the frequency of malocclusion without concomitant pathology is much less and rates to 35.63–45.92 % of cases, which necessitates the study of isolated malocclusion and with AR comorbidity in children [6,7].

Allergic diseases and malocclusion, often combined with AR and coexisted with nasal obstruction or without it, are considered as a multifactorial diseases associated with single nucleotide polymorphism of genes. These diseases are manifested only in interaction between genetic aspects of pathology and environmental factors, a combined effect of which leads to the disease development and phenotypic changes. Nowadays, there is the theory that allergic diseases are caused by a violation of immune system regulation due to activation of CD4+ T helper type 2 lymphocytes and increased secretion of cytokines, including anti-inflammatory interleukin-4 (IL-4), which contributes to IgE synthesis, mast cells and eosinophils activation. As a result, allergic inflammation develops and secretion of other immunoglobulins is activated forming the humoral immune response [8].

The IL-4 gene is located on chromosome 5q31. More than 50 allelic variants of the IL-4 gene polymorphisms have been identified including rs2243250 (S589T) which is the most significant genetic polymorphism observed in the promoter site and characterized by the replacement of cytosine (C) with thymine (T). That is, single nucleotide polymorphism (SNP) in the coding region of the IL-4 receptor (IL-4R) C589T (rs2243250) determines the presence of C versus T at position 589 in the amino acid sequence. It has been studied that the T allele of IL-4 rs2243250 polymorphism can increase binding of nuclear transcription factors to the promoter region of the IL-4 gene, and thus this polymorphism is functionally important. IL-4R transmits signals into the cellular nucleus exerting biological functions and playing an important role in regulating not only IL-4 synthesis, but also estrogens, and influencing bone remodeling [8–10].

This work should confirm or disprove the hypothesis that the IL-4 (C-589T, rs2243250) gene polymorphism, which results in the replacement of C with T at position 589 in the cytoplasmic domain of the mature protein, is associated with allergic diseases and more often recorded in children with BA and / or AR than in children with distal occlusion (DO) without allergic diseases.

Purpose

To determine the frequency of interleukin-4 (IL-4) (C-589T, rs2243250) SNP in children with respiratory forms of allergic diseases (BA and AR) and with isolated or AR-induced comorbid malocclusion.

Materials and methods

SNP of the IL-4 (C-589T, rs2243250) gene was analyzed in 170 children aged between 6 and 17 years, 11 months,

29 days. To confirm or disprove the hypothesis of genetic predisposition and association of the studied gene polymorphism with certain respiratory forms of allergic pathology and to clarify the causes of its comorbidity with AR or isolated malocclusion, 89 children with BA were included in Group 1; Group 2 consisted of 31 children with AR; Group III was composed of 27 children with AR and DO; Group IV comprised 23 children with DO without allergic pathology.

The inclusion criteria for enrolment into the study were diagnosed BA, AR, AR combined with DO, isolated DO; duration of allergic pathology 2 years or more; the absence of chronic pathology in the stage of decompensation. Exclusion criteria were acute infectious diseases; severe concomitant somatic and psychiatric pathology, congenital or acquired heart defects, malignant tumors; refusal of the patient and / or parents to participate in the study. Prior to starting the work, the children and their parents were timely informed of the study purpose, tasks, methods and gave written informed consent to participate in the study.

The IL-4 gene (C-589T, rs2243250) polymorphism was genotyped using a commercial "SNP-express RT" kit by real-time polymerase chain reaction (RT-PCR) according to the manufacturer's instruction (Applied Biosystems, USA). Genomic DNA was extracted from the whole venous blood of children using reagent kits ("LLC Research and Production Company LITEKH"), TagMan®SNP Genotyping Assay on the amplifier CFX96™ Real-Time PCR Detection Systems (Bio-Rad Laboratories, Inc., USA) at the Department of Molecular Genetic Studies of the Educational Medical

and Laboratory Center at the Department of Microbiology, Virology and Immunology, Zaporizhzhia State Medical University, Zaporizhzhia (under the guidance of the Head of the Department of Microbiology, Virology and Immunology – MD, PhD, DSc, Professor O. M. Kamyshnyi). The χ^2 method ($\alpha = 0.05$, $df = 1$) was used to test whether the control sample distribution was in accordance with the Hardy-Weinberg equilibrium. To identify the association between the disease and the IL-4 gene (C-589T, rs2243250) polymorphism, the general, recessive and dominant inheritance models and odds ratio (OR) with a 95 % confidence interval (95 % CI) were used. The data obtained as a result of the study were processed using nonparametric methods of statistical analysis with the Statistica 6.0 RU software package.

Results

In the work presented, we analyzed the distribution of polymorphism genotypes of the IL-4 (S-589T, rs2243250) gene in each study group depending on the nosology, namely, the presence of allergic pathology: BA, AR, comorbid DO induced by nasal obstruction due to AR and isolated malocclusion in the form of DO without allergic diseases.

Thus, in children of Group 1 with BA, the homozygous C/C genotype of the IL-4 (C-589T, rs2243250) gene polymorphism was prevalent and recorded in 69.66 %. Accordingly, the homozygous variant T/T and heterozygous C/T were significantly less frequent, in 7.87 % and 22.47 % of cases, respectively. These data are presented in Fig. 1.

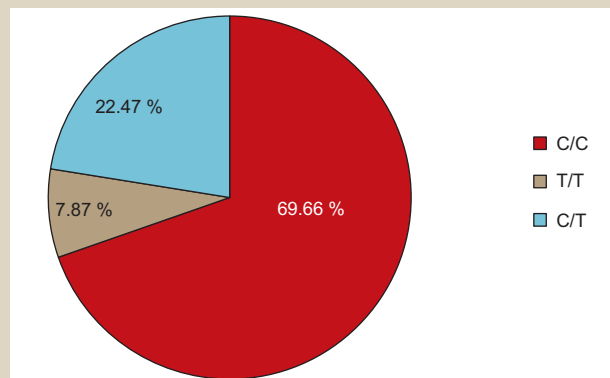


Fig. 1. Distribution of IL-4 (C-589T, rs2243250) gene polymorphism in BA children.

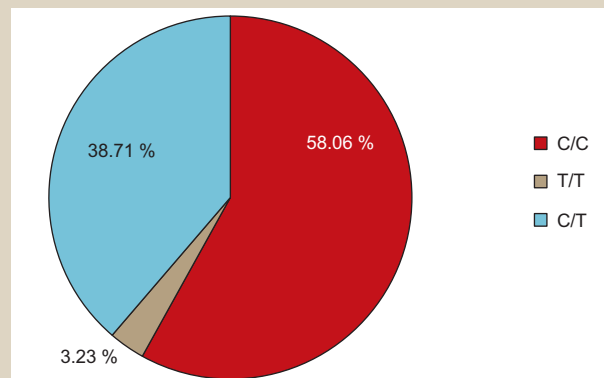


Fig. 2. Distribution of IL-4 (C-589T, rs2243250) gene polymorphism in AR children

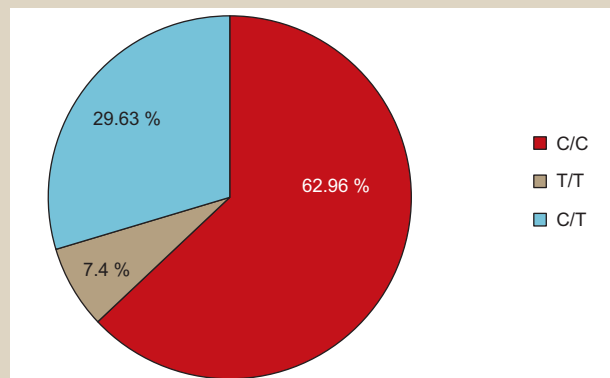


Fig. 3. Distribution of IL-4 (C-589T, rs2243250) gene polymorphism in children with AR and DO.

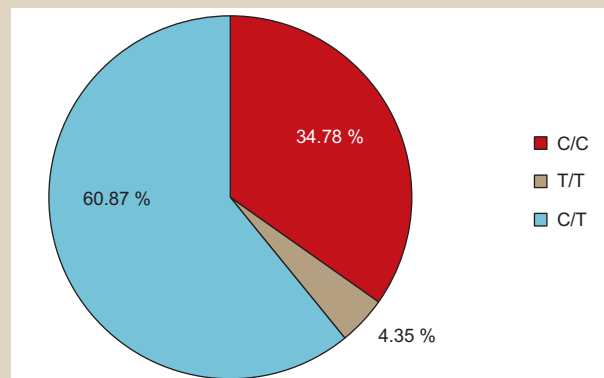


Fig. 4. Distribution of IL-4 (C-589T, rs2243250) gene polymorphism in children with DO.

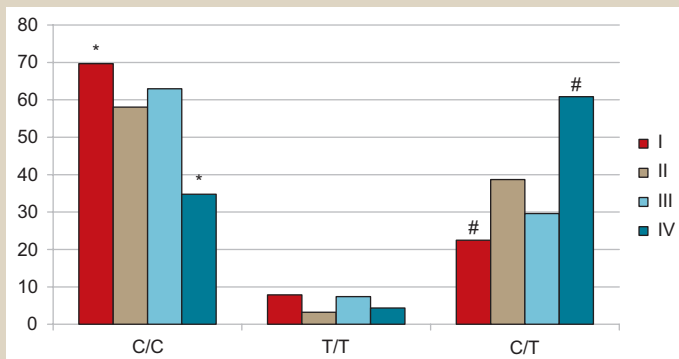


Fig. 5. Distribution of IL-4 (C-589T, rs2243250) gene polymorphism in the examined children.

*: P < 0.05 – the difference is significant between the C/C genotype frequency in Group 1 and 4;
#: P < 0.05 – the difference is significant between the C/T genotype frequency in Group 1 and 4).

Table 1. Distribution of IL-4 (C-589T, rs2243250) gene polymorphism in the examined children according to the general inheritance model (chi-square test, df = 2)

Groups (n)	Genotype C/C	Genotype C/T	Genotype T/T
1 (n = 89)	0.697	0.225	0.079
2 (n = 31)	0.581	0.387	0.032
3 (n = 27)	0.630	0.296	0.074
4 (n = 23)	0.348	0.609	0.043
1–2	$\chi^2 = 0.48, P = 0.18$ OR = 1.66; 95 % CI [0.71–3.86]	OR = 0.46; 95 % CI [0.19–1.10]	OR = 2.56; 95 % CI [0.30–21.69]
1–3	$\chi^2 = 0.58, P = 0.75$ OR = 1.35; 95 % CI [0.55–3.33]	OR = 0.69; 95 % CI [0.26–1.81]	OR = 1.07; 95 % CI [0.21–5.47]
1–4	$\chi^2 = 12.75, P = 0.002$ OR = 4.31; 95 % CI [1.63–11.36]	OR = 0.19; 95 % CI [0.07–0.49]	OR = 1.88; 95 % CI [0.22–16.08]
2–3	$\chi^2 = 0.89, P = 0.64$ OR = 0.81; 95 % CI [0.28–2.35]	OR = 1.50; 95 % CI [0.50–4.50]	OR = 0.42; 95 % CI [0.04–4.87]
2–4	$\chi^2 = 2.88, P = 0.24$ OR = 2.60; 95 % CI [0.85–7.92]	OR = 0.41; 95 % CI [0.13–1.23]	OR = 0.73; 95 % CI [0.04–12.38]
3–4	$\chi^2 = 4.92, P = 0.09$ OR = 3.19; 95 % CI [1.00–10.17]	OR = 0.27; 95 % CI [0.08–0.88]	OR = 1.76; 95 % CI [0.15–20.76]

Table 2. Distribution of IL-4 (C-589T, rs2243250) gene polymorphism in the examined children according to the general inheritance model (chi-square test, df = 2)

Groups (n)	Genotype C/C	Genotype C/T	Genotype T/T
BA (n = 89)	0.697	0.225	0.079
AR (n = 58)	0.603	0.345	0.052
DO (n = 23)	0.348	0.609	0.043
BA – AR	$\chi^2 = 2.70, P = 0.26$ OR = 1.51; 95 % CI [0.75–3.02]	OR = 0.55; 95 % CI [0.26–1.15]	OR = 1.57; 95 % CI [0.39–6.31]
BA – DO	$\chi^2 = 12.75, P = 0.002$ OR = 4.31; 95 % CI [1.63–11.36]	OR = 0.19; 95 % CI [0.07–0.49]	OR = 1.88; 95 % CI [0.22–16.08]
AR – DO	$\chi^2 = 4.78, P = 0.09$ OR = 2.85; 95 % CI [1.04–7.81]	OR = 0.34; 95 % CI [0.12–0.92]	OR = 1.20; 95 % CI [0.12–12.17]

In children of Group 2 with AR, the homozygous C/C genotype also dominated and it was registered in more than half of children (58.06 %). A bit more than one third of these children (38.71 %) showed the heterozygous C/T variant of the IL-4 (C-589T, rs2243250) gene polymorphism, and the homozygous T/T variant was detected only in 3.23 % of cases, as shown in Fig. 2.

In children included in Group 3 with AR and DO as in the previous groups, the C/C and C/T variants of the IL-4 (C-589T, rs2243250) gene polymorphism prevailed representing 62.96 % and 29.63 % of children, respectively. In this case, the T/T genotype was registered only in 7.40 %. These data are shown in Fig. 3.

In children of Group 4 without allergic pathology and only with isolated DO, the homozygous T/T genotype was found only in 4.35 % of children with DO (Fig. 4). Conversely, unlike previous examined groups, the heterozygous C/T genotype was dominant and recorded in 60.87 % of patients only with malocclusion, but significantly more often than in BA children (14/23 vs. 20/89, Yates corrected Chi-square = 10.99, P = 0.0009). The homozygous C/C variant was registered in 34.78 % of patients with malocclusion, significantly less than in BA children (8/23 vs. 62/89, Yates corrected Chi-square = 8.06, P = 0.0045). The comparative characteristic of each genotype frequency in IL-4 (C-589T, rs2243250) gene polymorphism depending on the presence or absence of BA, AR, AR with DO or only malocclusion in the examined children is presented in Fig. 5.

Further, the general inheritance model was used to study a genetic predisposition based on the presence or absence of associations between the IL-4 (C-589T, rs2243250) gene polymorphism and multifactorial allergic diseases development (BA, AR), AR combined with DO or only DO without an allergic pathology after testing the samples to detect the Hardy-Weinberg equilibrium (Table 1).

The data presented above refuted our hypothesis concerning C with T (C-589T) replacement. It can be seen that the C/C genotype was associated with BA development (OR = 4.31; 95 % CI [1.63–11.36], $\chi^2 = 12.75, P = 0.002$ and there was a tendency to C/C genotype predominance in children with AR and comorbid DO unlike in children without allergy but with malocclusion (OR = 3.19, 95 % CI [1.00–10.17], $\chi^2 = 4.92, P = 0.09$).

Taking into account that there were not significant differences in the distribution of the IL-4 (C-589T, rs2243250) gene polymorphism between children in Group 2 and 3 (with AR and AR with DO), therefore these children were grouped together as patients with AR. The analysis of genotypes distribution between groups of children with BA, AR and DO was repeated (Table 2).

However, these data were consistent with the findings that the C/C genotype of the IL-4 (C-589T, rs2243250) gene polymorphism was significantly prevalent in BA children (OR = 4.31; 95 % CI [1.63–11.36], $\chi^2 = 12.75, P = 0.002$ (actual) and in all children with both AR and AR with malocclusion comorbidity (OR = 2.85; 95 % CI [1.04–10.17], $\chi^2 = 4.78, P = 0.09$ (as the tendency) compared to the group of children with DO without allergic pathology).

Therefore, in order to clarify an association between the genotype and allergic pathology or malocclusion development in the examined children, the recessive and dominant inheritance models (Tables 3–6) were also analyzed.

Table 3. Recessive inheritance model of the IL-4 (C-589T, rs2243250) gene polymorphism in children with BA and DO (chi-square test, df = 1)

Genotypes	BA	DO	χ^2	P	OR	
	n = 89	n = 23			value	95 % CI
Genotype C/C	0.697	0.348	9.49	0.002	4.31	1.63–11.36
Genotype C/T+T/T	0.303	0.652			0.23	0.09–0.61

Table 4. Dominant inheritance model of the IL-4 (C-589T, rs2243250) gene polymorphism in children with BA and DO (chi-square test, df = 1)

Genotypes	DO	BA	χ^2	P	OR	
	n = 23	n = 89			value	95 % CI
Genotype C/C	0.348	0.697	9.49	0.002	0.23	0.09–0.61
Genotype C/T+T/T	0.652	0.303			4.31	1.63–11.36

Table 5. Recessive inheritance model of the IL-4 (C-589T, rs2243250) gene polymorphism in children with AR and DO (chi-square test, df = 1)

Genotypes	AR	DO	χ^2	P	OR	
	n = 58	n = 23			value	95 % CI
Genotype C/C	0.603	0.348	4.32	0.04	2.85	1.04–7.81
Genotype C/T+T/T	0.397	0.652			0.35	0.13–0.96

Table 6. Dominant inheritance model of the IL-4 (C-589T, rs2243250) gene polymorphism in children with AR and DO (chi-squared test, df = 1)

Genotypes	DO	AR	χ^2	P	OR	
	n = 23	n = 58			value	95 % CI
C/C genotype	0.348	0.603	4.32	0.04	0.35	0.13–0.96
C/T+T/T genotype	0.652	0.397			2.85	1.04–7.81

While analyzing the study results, it was considered that children with the C/C genotype of the IL-4 (C-589T, rs2243250) gene polymorphism had a relative risk of developing BA at any time of life. In addition, DO predisposition as an isolated malocclusion ($P = 0.002$) with OR = 4.31 (95 % CI = 1.63–11.36) was noted in children with the C/T + T/T genotype.

These data also identified an association between a relatively high risk of developing malocclusion in children ($P = 0.04$) OR = 4.32 (95 % CI = 1.04–7.81) and both the C/C genotype, as a potential marker of AR, and the C/T + T/T genotype of the IL-4 (C-589T, rs2243250) gene polymorphism.

Therefore, summarizing the results of the conducted study, it can be stated that allergic pathology such as BA and AR in children was associated with the C/C genotype (69.7 % and 60.3 %) while malocclusion – with the C/T + T/T genotype (65.2 %) of the IL-4 (C-589T, rs2243250) gene polymorphism. Therefore, it can be assumed that children with the C/C genotype had DO with comorbid AR induced by nasal obstruction of allergic genesis, and malocclusion was formed initially in children with the C/T + T/T genotype.

Discussion

Then, we compared and presented in Fig. 6 our data on the IL-4 (C-589T, rs2243250) gene polymorphism distribution in each study group with known widely varied data of population studies [11].

So, the comparison of genotype frequencies between the examined children of Zaporizhzhia (ZP) and children from Europe (EUR), South Asian (SAS), Admixed Ame-

rican (AMR), East Asian (EAS), Africa (AFR), Colombians from Medellin, Colombia (SLM), Finland (FIN), showed that the C/C – C/T – T/T genotype frequencies in children of Zaporizhzhia with BA (69.66 % – 22.47 % – 7.87 %), AR (58.06 % – 38.71 % – 3.23 %) and AR comorbid with DO (62.96 % – 29.63 % – 7.40 %) were almost matched the frequency in Europe (70.2 % – 26.0 % – 3.8 %) and South Asian (68.1 % – 27.0 % – 4.9 %). At the same time, the C/C – T/T – T/T genotype frequencies in children from Zaporizhzhia with malocclusion (34.78 % – 60.87 % – 4.35 %) partially matched those in Colombians from Medellin, Colombia (39.4 % – 50.0 % – 10.6 %) and Finland (41.4 % – 48.5 % – 10.1 %).

The genotyping performed for all the examined children generally showed that the homozygous C/C genotype, registered in 61.76 % (105/170) cases, was the dominant one. The heterozygous C/T and the homozygous T/T genotypes were less common, in 31.76 % (54/170) and 6.44 % (11/170) of children, respectively. Meanwhile, our data matched the indicators in Europe and South Asian, as the C/C, C/T and T/T genotype frequencies were 70.2 % and 68.1 %, 26.0 % and 27.0 %, 3.8 % and 4.9 % of cases, respectively, in these populations (Fig. 7). A large variability in the incidence rate of C/C–C/T–T/T genotypes of the IL-4 (C-589T, rs2243250) gene polymorphism was noted among the populations of Admixed American (42.1 % – 42.7 % – 15.3 %), East Asian (4.2 % – 35.9 % – 59.9 %), Africa (7.6 % – 38.9 % – 53.6 %) [11].

These data show that hereditary features of immunopathogenesis can be found in different population groups, which are of both theoretical and practical interest for further study.

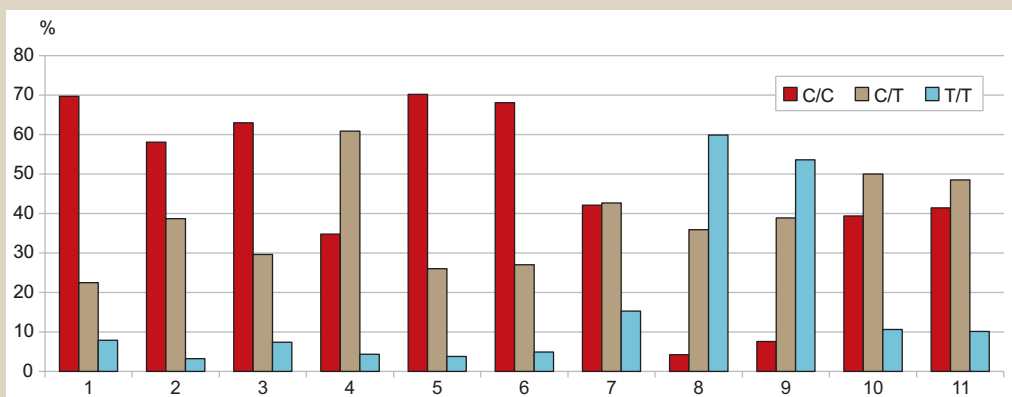


Fig. 6. Genotype frequencies of the IL-4 (C-589T, rs2243250) gene polymorphism in children of different observational groups and population studies, where: 1 – ZP (BA); 2 – ZP (AR); 3 – ZP (AR+DO); 4 – ZP (DO); 5 – EUR; 6 – SAS; 7 – AMR; 8 – EAS; 9 – AFR; 10 – CLM; 11 – FIN.

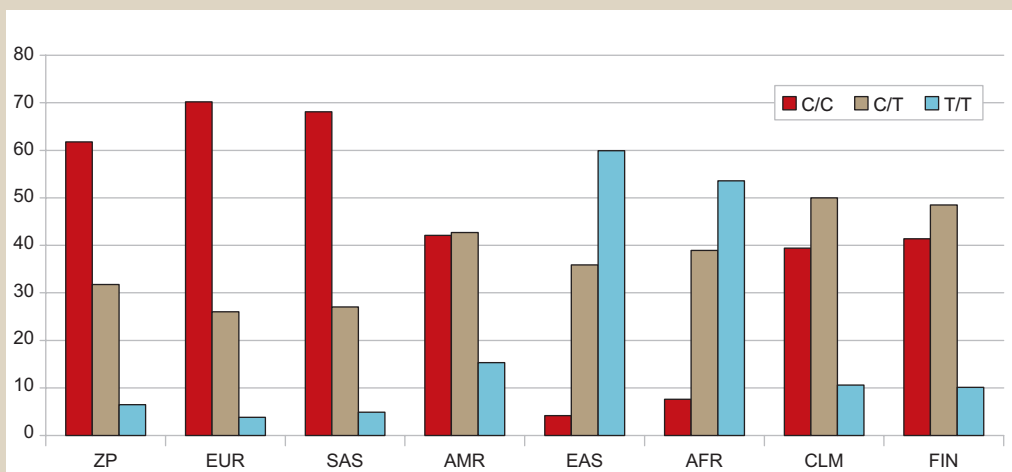


Fig. 7. Frequency of the IL-4 (C-589T, rs2243250) gene polymorphism in all the examined children and in population studies.

It is worth mentioning that we have obtained study results different from our initial hypothesis. Further analysis of information related to this problem has shown all its complexity focusing on issues to study SNP located in the promoter region of the IL-4 gene (C-589T, rs2243250) and determining C versus T presence in different positions of amino acid sequence as well as in relation to other chromosomal regions and segments.

Thus, according to V. B. Ivanova, in children with atopic BA living in Krasnoyarsk, SNP C-590 T of the IL-4 gene (rs2243250) was genotyped. As a result, the homozygous C/C and T/T variants were recorded in 52.38 % and 6.67 % of cases, respectively, while the heterozygous C/T variant was found in 42.38 % of patients, that is, almost two times more frequently than in our study. However, this study also showed that the T/T genotype frequency was 9.2 % in the group of patients with uncontrolled BA and it was higher than in children with controlled BA (3.9 %) [12].

Nevertheless, in the genotypes of other populations, for example, the Mauritian Indian and Chinese Han populations, there was no significant association between C-590 T of the IL-4 gene and BA development as well as no significant differences were observed when comparing these groups based on distribution of polymorphisms IL-4 -590 C/T [13].

At the same time, it is known that in Brazilian adolescents with allergic pathology and sensitization to domestic allergens, the C/T and T/T genotypes of IL-4-590 rs2243250 were recorded in 10.2 % and 42.9 % of cases, respec-

tively, against 43.1 % and 13.8 % in healthy children [2]. Gurieva L. L. also believes that a biological marker for a high risk of developing atopic BA without associated allergic diseases is the T allele of the SNP C-590> T in the IL-4 gene (OR = 1.59; CI 95 % = 1.02–2.48) [14].

But in the studies of Smolnikova M. V. et al., as well as in our study, it was shown that the C/C genotype of IL-4 (rs2243250; rs2070874) can be a genetic marker for risk of developing both controlled (RR 0.26; SE 0.38; P = 0.0008) and uncontrolled (RR 0.3; SE 0.38; P = 0.0018) atopic asthma [15].

The children with infectious-dependent BA were more likely to have the heterozygous C/T genotype of the IL-4 (C-589T) gene constituting 54.05 %. In addition, this genotype was a predictor of severe infectious-dependent BA, resistant to anti-inflammatory therapy [16].

Furthermore, E. A. Khotko and A. D. Taganovych presented a detailed analysis of reference data on the role of gene polymorphism, including the IL-4 gene, responsible for the production of pathogenetically important proteins in immunocompetent cells of patients with chronic obstructive pulmonary diseases [17]. There are also data evidencing a greater susceptibility to the risk of developing chronic obstructive pulmonary diseases in individuals, IL-4 – 589T allele carriers, from the Kazakh population [18].

Under industrial pollution conditions, genotyping of IL-4 – C-589T (rs2243250) SNP among adult patients with occupational BA, asbestosis, and occupational chronic obstructive pulmonary disease revealed that 56.4 % of patients

with occupational asthma had homozygous T/T variant of the IL-4 gene in the presence of concomitant infection of the nasopharynx, neutrophilic inflammation and destructive radiographic changes. This allowed scientists to suggest that the homozygous genotype T/T of the SNP C-589T of the IL-4 gene presence contributes to a long-term circulation of pro-inflammatory cytokines elevated levels. This, in turn, leads not only to destructive processes in the lungs progression, but also to chronic inflammation confirming the association between IL-4 T allele and an inflammatory process activation and maintenance. The association between the T allele of the IL-4 as well as an increased level of systemic inflammatory markers and a higher degree of respiratory failure was also detected in workers exposed to industrial aerosols. Thus, in asbestosis, the presence of T allele IL-4 increased the risk of developing II–III degree respiratory failure by 5.2 times (OR = 5.217, 95 % CI = 1.115–24.407). Therefore, the IL-4 C589T (rs2243250) gene polymorphism identification allowed to identify the risk groups for more severe forms of occupational bronchopulmonary diseases development [19].

There are slightly less studies of the IL-4 gene polymorphism in patients with AR. Thus, when studying SNPs of the IL-4 (rs2243248, rs2243250 and rs2070874) in 98 patients with AR, it was found that C/C genotype in (rs2070874) was significantly correlated with AR development, and SNPs of the IL-4 could change the clinical picture of the disease [20].

In Poland, Barbara Korzycka-Zaborowska with colleagues showed that the (590 C/T) IL-4 gene polymorphism was associated with AR in 15.3 % of patients, compared with 5.8 % of examined controls without AR, $\chi^2 = 4.368$; $P < 0.05$ [21]. In a study of other scientists, the risk of developing AR was associated with the IL-4 gene -590 TT genotype [OR = 1.93, 95 % CI (1.61–2.31), $P = 0.00$] and the TT+TC unlike CC genotype of IL-4 C-33/T polymorphism was significantly associated with allergic diseases [OR = 3.23, 95 % CI (1.13–9.25), $P = 0.03$] [22].

In Pakistan, genotyping for SNP IL-4 C-589T (rs2243250), T + 2979G (rs2227284) and C-33T (rs2070874) was performed in 108 patients with BA and 106 with AR. Although we studied SNP C-589T (rs2243250) of the IL-4 gene, but unlike our results, S. Micheal and colleagues showed that C/C genotype was recorded in 24.1 % of BA patients, C/T – in 58.3 % and T/T – in 17.6 %. Concurrently, in children with allergic rhinitis, the C/C genotype was detected in 17 % of cases; C/T – in 58.5 %; T/T – in 24.55 %. Therefore, SNP C-589T (rs2243250) of the IL-4 was significantly associated with both BA ($\chi^2 = 11.0$; $P = 0.004$) and AR ($\chi^2 = 20.2$; $P < 0.001$). In addition, the polymorphism T+2979G (rs2227284) of the IL-4 gene was also related to genetic risk factors for the developing asthma and AR in Pakistan. But, unlike previous studies, there were no significant differences between the groups of patients with both BA and AR and the control group when studying the SNP C-33T (rs2070874) of the IL-4 gene [23].

We have not found data regarding the polymorphism (C-589T, rs2243250) of the IL-4 gene detection in children with malocclusion in the scientific literature available today. Nevertheless, E. Sh. Hryhorovych studied the molecular genetic basis for periodontitis development in dental patients and showed that the IL-4 gene polymorphism is one

of the predictors of chronic generalized periodontitis, which was associated with a dense mononuclear inflammatory infiltrate, represented by CD4, CD8, CD20 T lymphocytes combined with a large number of CD45RO memory T lymphocytes, CD68 macrophages and intensive neutrophilic infiltration [24].

However, a part of the paradontium is periodontal tissue, which in left untreated dental caries may develop an inflammatory complication such as periodontitis. On the one hand, it independently contributes to various malocclusions that, on the other hand, could be a negative consequence of being edentulous after periodontitis-related dental extraction when myodynamics is disturbed. It was demonstrated that complications of temporary teeth caries were found in more than 2/3 of children and it was the leading cause of defects in the dentition (16.3 ± 3.6 % of cases). Early tooth loss and related defects resulted in various dental anomalies in 61.7 ± 4.8 % of children [25].

The work of A. Z. Isamulaeva et al. covers the influence of genetic polymorphisms on the grade of inflammation, which is a promising direction for early diagnosis, comprehensive assessment of dental status in somatic patients with damage to dental hard tissues, paradontium and oral mucous membranes and the rationale for new therapeutic and preventive measures [26].

Furthermore, modern scientific studies have confirmed that the IL-4 gene polymorphism influence IL-4 and other cytokines production regulating immune responses that directly associated with caries and periodontitis development [27–29].

At the same time, the T allele (OR = 1.2, 95 % CI = 1.02–1.42, $P = 0.03$) and the T/T genotype (OR = 1.68, 95 % CI = 1.05–2.67, $P = 0.03$) of the IL-4 gene (C -590T) were associated with periodontitis in whites, and only the T/T genotype was associated with periodontitis in Caucasians (T/T vs C/T: OR = 1.75, 95 % CI = 1.10–2.78, $P = 0.02$) [30,31]. These data confirm, on the one hand, that the genotype frequencies in studied polymorphism of the IL-4 gene are varied among different populations; on the other hand, it is the basis of a personalized approach to patients and long-term treatment programs implementation.

Of great interest is the wide range of studies devoted to determining the role of SNP (C589T, rs2243250) of the IL-4 gene in disease occurrence.

Thus, the T allele frequency and the homozygous T/T genotype prevailed in children with acute respiratory tract infections (pneumonia, bronchiolitis) living in Egypt (OR = 1.3; 95 % CI = 1.07–1.56, $P < 0.01$ and OR = 2.0; 95% CI = 1.38–2.96, respectively) [32]. In patients with whooping cough, the homozygous T/T genotype (C589T) of the IL-4 gene frequency was higher than in healthy ones, and more than half of patients suffering from pyelonephritis (52.7 %) were carriers of the heterozygous C/T variant of this gene polymorphic region [33,34].

Chronic hepatitis C patients with the homozygous T/T genotype (C-589T) of the IL-4 gene showed more pronounced fibrous changes in the liver than the homozygous C/C genotype carriers ($P < 0.01$) [35]. In patients with ischemic heart disease and coronary atherosclerosis, the C589T frequencies of the IL-4 genotype ($P = 0.04$; OR = 4.09) and 589T alleles ($P = 0.04$; OR = 2.45) were statistically significantly increased [36].

Scientists also took into account the possible risk of developing oncopathology associated with IL-4 genotypes and different sensitivity to chemotherapy for malignant tumors of the female reproductive organs, as well as for breast cancer [37,38].

Comparison of our study results with scientific literature data has proved different effects of the studied mutations of the IL-4 gene on the peculiar features of associative prognostic links with various diseases, which in the long term justifies the need for further research in this area.

Conclusions

1. The polymorphism of the interleukin-4 gene C-589T, rs2243250) has been investigated for the first time in children with respiratory forms of allergy (bronchial asthma and allergic rhinitis) and with isolated or allergic rhinitis-induced comorbid malocclusion living in the city of Zaporizhzhia.

2. The C/C – C/T – T/T genotype frequencies have been calculated in children: with bronchial asthma 69.66 % – 22.47 % – 7.87 %; with allergic rhinitis – 58.06 % – 38.71 % – 3.23 %; allergic rhinitis with distal occlusion – 62.96 % – 29.63 % – 7.40 % of the examined cases, which were matched their frequency in European and South Asian populations.

3. The C/C – C/T – T/T genotypes in children with malocclusion had the following distribution: 34.78 % – 60.87 % – 4.35 % of the examined cases, and the heterozygous C/T genotype prevalence indicated the presence of genetic features of distal occlusion phenotype formation.

4. The inheritance models analysis showed that the C/C genotype of the interleukin-4 gene (C-589T, rs2243250) was associated with bronchial asthma (OR = 4.31; 95 % CI = 1.63–11.36; P = 0.002) and allergic rhinitis (OR = 4.32; 95 % CI = 1.04–7.81; P = 0.04) in comparison with the fact that the C/T + T/T genotype indicated a predisposition to malocclusion development.

Prospects for further researches. In the future, we will focus on the analysis of presented genotypes frequency depending on the severity, stage, clinical and laboratory features of these diseases course.

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