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GENETIC MARKERS OF PREDISPOSITION TO THE DEVELOPMENT OF RECURRENT BRONCHITIS IN CHILDREN WITH LYMPHATIC-HYPOPLASTIC DIATHESIS

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✓ ***Resume***

The analysis of the associative relationship of the gene polymorphism in the IL-4 promoter region in positions (C589T) with the risk of developing RB in 119 children with recurrent bronchitis aged 2 to 7 years and in 110 apparently healthy children of the same age in the Uzbek population.

The results of the study showed statistically significant differences in the frequency distribution of alleles and genotypes of the C589T C>T polymorphic locus of the IL-4 gene in patients with RB. The frequency of the unfavorable allele of the T and T / T genotype was significantly more often determined in patients with RB against the background of lymphotic-hypoplastic diathesis, which indicates a predisposing role of the rs2243250 polymorphism of the IL-4 gene to the development of recurrent bronchitis in LGD children in the Uzbek population. Was carried out.

Key words: recurrent bronchitis, lymphatic-hypoplastic diathesis, gene polymorphisms, cytokines.

ГЕНЕТИЧЕСКИЕ МАРКЁРЫ ПРЕДРАСПОЛОЖЕННОСТИ К РАЗВИТИЮ РЕЦИДИВИРУЮЩИХ БРОНХИТОВ У ДЕТЕЙ С ЛИМФАТИКО-ГИПОПЛАСТИЧЕСКИМ ДИАТЕЗОМ

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✓ ***Резюме***

Проведен анализ ассоциативной связи полиморфизма гена промоторном регионе IL-4 в позициях (C589T) с риском развития РБ у 119 детей в возрасте от 2 до 7 лет и у 110 условно-здоровых детей того же возраста в Узбекской популяции.

Результаты исследования показали статистически значимые различия в распределение частот аллелей и генотипов полиморфного локуса 589 C>T гена IL-4 у больных РБ. Частота неблагоприятного аллеля Т и Т/Т генотипа достоверно чаще определялся у больных РБ на фоне лимфатико-гипопластическим диатезом, что свидетельствуют о предрасполагающей роли полиморфизма rs2243250 гена IL-4 к развитию рецидивирующих бронхитов у детей ЛГД в узбекской популяции.

Ключевые слова: рецидивирующий бронхит, лимфатико-гипопластический диатез, полиморфизм генов, интерлейкин.

ЛИМФАТИКО-ГИПОПЛАСТИК ДИАТЕЗИ БОР БОЛАЛАРДА ҚАЙТАЛАНУВЧИ БРОНХИТ РИВОЖЛАНИШИНИНГ ГЕНЕТИК МОЙИЛЛИК МАРКЕРЛАРИ

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✓ ***Резюме***

Ўзбек популяциясидаги 2 ёшдан 7 ёшгача бўлган қайталанувчи бронхити бор 119 та ва шу ёшдаги 110 та шартли соғлом болаларда IL-4 промотор соҳасидаги ген-полиморфизмнинг (C589T) позициялари ва рецидивланувчи бронхит ривожланиш хавфи бўлган болалар ўртасидаги ассоциатив алоқадорлик таҳлил қилинди.

Тадқиқот натижалари рецидивланувчи бронхити бор беморларида IL-4 генининг аллеллари ва генотиплари 589 C>T полиморфик жойлашувининг тарқалишидаги статистик жиҳатдан муҳим фарқларни кўрсатди. Генотипнинг ноқулай T аллели ва T/T генотиплари частотаси рецидивланувчи бронхити бор беморларда лимфатико-гипопластик диатез фонида анча сезиларли даражада аниқланди, бу ўзбек популяциясида IL-4 генининг rs2243250 полиморфизмининг аниқланиши қайталанувчи бронхит ривожланишига мойиллигини кўрсатади.

Калит сўзлар: қайталанувчи бронхит, лимфатико-гипопластик диатез, ген полиморфизми, интерлейкин.

Relevance

The pathology of the respiratory tract in children is an urgent problem of clinical pediatrics and has medical and social significance [2,4,12]. This is due to the high prevalence of respiratory pathology in the structure of diseases in children. In the Republic of Uzbekistan, according to statistical data, in the structure of general morbidity, respiratory diseases make 24 persons per 100,000 population [2,11,15].

It has been established that the main cause of RB in children is an unfavorable premorbid background [7,9]. These states include constitutional features. Among them, lymphatic-hypoplastic diathesis (LGD) is of significant importance. This is an anomaly of the constitution, caused by insufficiency of the lymphatic system, associated with a reduced function of the thymus gland, dysfunction of the endocrine system, and therefore, these children are intolerable to any infectious diseases, in addition, LGD creates a specific background against which any disease changes its course and clinical picture [8,10].

Despite the fact that the problem of treatment and prevention of bronchitis in children is well covered in the literature, the genetic basis remains poorly studied. In this regard, it is relevant to identify and study genetic markers in children with RB. Based on modern data on the pathogenesis of respiratory tract damage in children, genes for pro- and anti-inflammatory cytokines are candidate genes and are closely related to the development and clinical course of these diseases [1,3,6]. As you know, IL-4, which is a product of CD4 + T-lymphocytes / helpers with the Th2 phenotype, is of particular importance in immune reactions, and acts as an antagonist of Th1-associated cytokines, thereby contributing to the polarization of the immune response in the direction of the humoral type of response [5]. Genetic polymorphism is also characteristic of the IL-4 gene. The IL-4 gene is located on the long arm of chromosome 5 and has 4 exons. An increase in the binding of transcription factors was shown in the presence of the polymorphic T allele for the IL-4 gene rs2243250 [16].

The function of this gene is to encode IL-4, an anti-inflammatory cytokine that activates humoral immunity, controls the proliferation and differentiation of B-cells and T-helper cells, and the production of immunoglobulin E. [10,16].

Despite the numerous studies of cytokine gene polymorphisms, their contribution to the clinical course and to the formation of recurrent bronchitis remains unclear. In addition, the associative relationship of the C589T polymorphism of the IL-4 gene in children of RB against the background of LGD in the Uzbek population has not been studied.

Purpose of the study: To study the role of the C-589T polymorphism of the IL-4 gene in children in the predisposition to the development of recurrent bronchitis against the background of lymphatic-hypoplastic diathesis.

Material and methods

The survey included 119 children aged 2 to 7 years with RB (main group). All patients of the main group were divided into 2 subgroups: subgroup I of 62 children with recurrent bronchitis, subgroup II consisted of 57 patients with RB on the background of LGD. The average age of children was 4.1 ± 0.82 years. The control group consisted of 110 conditionally healthy children of the same age. RB was diagnosed in accordance with the ICD criteria. The diagnosis of LGD was made on the basis of clinical and laboratory studies.

In all patients with RB, with LGD, as well as conditionally healthy children of Uzbek nationality, who made up the control group, PCR genotyping of the C-589T polymorphism of the IL-4 gene was performed in the laboratory of molecular genetics of the Research Institute of Hematology and Blood Transfusion (Tashkent). Blood sampling was carried out on an empty stomach from the cubital vein of the examined children under sterile conditions. DNA isolation from peripheral blood was performed using a standard Ribo-sorb kit (AmpliSens®, Russia).

PCR genotyping of the C-589T polymorphism of the IL-4 gene was carried out using an Applied Biosystems 2720 thermal cycler (USA), using a test kit from Litekh LLC (Moscow) according to the manufacturer's instructions.

The software package "OpenEpi 2009, Version 2.3" for statistical data processing was used as a tool for statistical calculations.

The significance of differences in allele and genotype frequencies was assessed using the 95% confidence interval (CI) for the general frequency value. The strength of association was expressed in terms of relative risk, calculated as odds ratio (OR), giving a 95% confidence interval.

Result and discussion

It was revealed that the frequency distribution of genotypes and alleles of C589T polymorphism of the IL-4 gene in both groups corresponded to the expected Hardy-Weinberg equilibrium law ($p > 0.05$) (Tables 1-2).

Table 1

Expected and observed frequencies of distribution of genotypes of C589T polymorphism of the IL-4 gene by RHV in the general sample of patients

Alleles	**Allele frequency**				
C	0.76				
T	0.24				
Genotypes	**Genotype frequency**		χ^2	**P**	**df**
	$*H_{exp}$	$*H_{obs}$			
C/C	0,58	0,60	0,07	0.2 7	1
C/T	0,36	0,33	0,44		
T/T	0,06	0,08	0,69		
Total	1,00	1,00	1,20		

As can be seen from Table 1, in the group of patients of the general sample, the expected frequency distribution of the homozygous C / C genotype tended to decrease compared to the observed one (0.58 versus 0.60, respectively; $\chi 2 = 0.07$ $p = 0.27$). On the contrary, the observed frequency of the heterozygous C / T genotype is statistically insignificantly higher than the expected one (0.36 versus 0.33, respectively; $\chi 2 = 0.44$ $p = 0.27$). Also, the unfavorable homozygous genotype T / T is insignificantly reduced compared to the expected (0.06 versus 0.08), $\chi 2 = 0.69$ $p = 0.27$, $D = + 0.09$ (tab 3)

Table 2

Expected and observed frequencies of distribution of genotypes of the locus rs2243250 of the IL-4 gene by RHB in the control group

Alleles	**Allele frequency**				
C	0.84				
T	0.16				
Genotypes	**Genotype frequency**		χ^2	**P**	**df**
	$*H_{exp}$	$*H_{obs}$			
C/C	0,70	0,71	0,01	0.46	1
C/T	0,27	0,25	0,15		
T/T	0,03	0,04	0,38		
Total	1,00	1,00	0,54		

In the control sample, the indicators of the observed and expected frequency of the homozygous genotype C / C corresponded to $H_{exp} = 0.70$ versus $H_{obs} = 0.71$, $\chi 2 = 0.01$ $p = 0.46$. The frequency of the heterozygous C / T genotype tended to increase as compared to the expected one ($H_{exp} = 0.27$ and $H_{obs} = 0.25$, respectively; $\chi 2 = 0.15$ $p = 0.46$). As expected, the unfavorable homozygous genotype T / T was insignificantly reduced compared to the expected ($H_{exp} = 0.03$ versus $H_{obs} = 0.04$), $\chi 2 = 0.38$ $p = 0.46$. (tab 2), $D = 0.08$ (tab 3)

Table 3

The difference between the expected and observed frequencies of heterozygosity of the rs2243250 polymorphism locus of the IL-4 gene in the main and control groups

Group	Observed heterozygosity	Expected heterozygosity	D*
Main group	0,33	0,36	0,09
Control	0,25	0,27	0,08

Note: $D=(H_{exp}-H_{obs})/H_{obs}$

D=(0,36-0,333)/0,33=0,09- for the main group

D=(0,27-0,25)/0,25=0,08-for the control group

To assess the associative relationship of the rs2243250 polymorphism of the IL-4 gene with the risk of developing RB, a comparative analysis of the distribution of allele and genotype frequencies in the studied groups of patients and controls was performed. The results of the study in the compared groups are presented in table. 4.

Table 4.

Frequency distribution of alleles and genotypes of the polymorphic locus C-589T of the IL-4 gene in RB patients of the general sample and in healthy children of the control group

№	Group	Allele frequency				Genotype distribution frequency					
		C		T		C/C		C/T		T/T	
		n	%	n	%	n	%	n	%	n	%
1	The main group n=119	181	76,1	57	23,9	71	59,7	39	32,8	9	7,6
2	Control group n=110	184	83,6	36	16,4	78	70,9	28	25,5	4	3,6

As can be seen from the table, the frequency of distribution of alleles C and T of the IL-4 gene in patients with recurrent bronchitis in the general sample was 76.1% and 23.9%, in the control group - 83.6% and 16.4%, respectively (Table . 4).

Statistical processing revealed a significant increase in the frequency of the unfavorable T allele, which showed a significant association with the disease (RR = 1.5; 95% CI: 1.006-2.129, $\chi2 = 4.1$; $p < 0.04$).

Analysis of the distribution of C / C genotypes in the total sample of patients was 59.7%, in the control group it was 70.9%. Indicators of the homozygous genotype C / C tended to decrease compared to the control group (RR = 0.8; 95% CI: 0.696-1.018, $\chi2 = 3.2$; $p < 0.1$), being a marker of a low risk of developing RB. The frequency of heterozygous carriage of the C / T genotype in the total sample of patients was 32.8%, in the control group it was 25.5%. Indicators of heterozygous carriage of the C / T genotype in the general sample of patients tended to increase.

At the same time, the analysis of the frequency distribution of the T / T genotype of the C-589T polymorphism of the IL-4 gene was 2.1 times increased in patients - 7.6% versus 3.6% in the control group (RR = 2.1; 95% CI : 0.659-6.561, $\chi2 = 1.6$; $p < 0.2$).

Table 5.

Frequency distribution of alleles and genotypes of a polymorphic locus C-589T of IL-4 gene in RB patients and the control group

№	Group	Allele frequency				Genotype distribution frequency					
		C		T		C/C		C/T		T/T	
		n	%	n	%	n	%	n	%	n	%
1	Recurrent bronchitis n= 62	102	82,3	22	17,7	43	69,4	16	25,8	3	4,8
2	Control group n=110	184	83,6	36	16,4	78	70,9	28	25,5	4	3,6

As can be seen from Table 5., the distribution of alleles C and T of the IL-4 gene in patients with RB was 82.3% and 17.7% and in the control group - 83.6% and 16.4%, respectively.

Statistical processing revealed an insignificant increase in the frequency of the unfavorable T allele in RB patients, while the relative risk of pathology development was 1.1 with a confidence interval of 95% CI: 0.669-2.129, $\chi 2 = 0.1$; $p < 0.7$.

Analysis of the distribution of C / C genotypes in the group of patients with recurrent bronchitis was 69.4%, in the control group it was 70.9%. Indicators of the homozygous genotype C / C tended to decrease compared to the control group (RR = 1.0; 95% CI: 0.797-1.2, $\chi 2 = 0.05$; $p < 0.1$), being a marker of low risk of development RB. The frequency of heterozygous carriage of the C / T genotype in RB patients and controls did not show statistically significant differences (RR = 1.0; 95% CI: 0.597-1.722, $\chi 2 = 0.003$; $p < 0.96$). However, it should be noted that there is a tendency for the homozygous T / T genotype to increase in RB patients, which was 1.3 times higher than in the control group (RR = 1.3; 95% CI: 0.308-5.754, $\chi 2 = 0.1$; $p < 0,7$).

Table 6.

Frequency distribution of alleles and genotypes of a polymorphic locus C-589T gene IL-4 in RB patients on the background of LGD and in the control group

№	Group	Allele frequency				Genotype distribution frequency					
		C		T		C/C		C/T		T/T	
		n	%	n	%	n	%	n	%	n	%
1	Recurrent bronchitis with LGD n=57	79	69,3	35	30,7	28	49,1	23	40,4	6	10,5
2	Control group n=110	184	83,6	36	16,4	78	70,9	28	25,5	4	3,6

Comparative analysis of the frequency distribution of alleles and genotypes for the C-589T polymorphism of the IL-4 gene in groups of patients with RB against the background of LGD and control showed statistically significant values.

As can be seen from Table 6, there is a significant decrease in the wild C allele, an increase in the frequency of the unfavorable T allele in RB patients with LGD and the risk of developing the disease was 2.3 times ($\chi 2 = 9.22$; $p < 0.002$; RR = 1.9; 95% CI: 1.249-2.817). When comparing the frequencies of genotypes for the groups with RB against the background of LGD and control, showed a statistically significant decrease in the homozygous genotype C / C in patients with RB against the background of LGD ($\chi 2 = 7.69$; $p < 0.01$; RR = 0.7; 95% CI : 0.518-0.926), which indicates the projective function of this genotype in relation to the risk of developing RB. The heterozygous C / T genotype among patients of the main group was more common than in the control group, and the odds ratio showed that the chance of detecting this genotype was 2.0 ($\chi 2 = 3.93$; $p < 0.05$; RR = 1.6; 95 % CI: 1.011-2.484).

Analysis of the frequency distribution of the unfavorable T / T genotype of the C-589T polymorphism of the IL-4 gene in patients of the main group was determined significantly more often ($\chi 2 = 3.17$; $p < 0.1$; RR = 2.9; 95% CI: 0.851-9.845).

Conclusion

Thus, as a result of the study of the polymorphic locus C589T of the IL-4 gene in RB patients against the background of LGD, pronounced differences in the distribution of allele and genotype frequencies between the group of patients and healthy individuals in the Uzbek population were found. In the presence of an unfavorable allele T, the risk of developing RB increases by more than 1.9 times ($\chi^2 = 9.22$; P = 0.002; OR = 2.3; 95% CI 1.327-3.867), which indicates a fairly independent and independent effect of polymorphism C589T gene IL-4 on the risk of developing RB in children. Revealed genetic characteristics of patients, allow to optimize the implementation of therapeutic and prophylactic measures.

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