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The Role of Polymorphism of the VEGF-A-634G/C Growth Factor Gene in Children with Recurrent Laryngotracheitis

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Abstract

Purpose. To study the importance of molecular genetic markers in the formation of recurrent laryngotracheitis in children.
Materials and methods. The basis of our study was 85 patients with recurrent stenosed laryngotracheitis, who were divided into 2 groups, the main group consisted of 55 children and the control group consisted of 30 children of the appropriate age. The study began with an otorhinolaryngological examination, which consisted of endoscopic rhinoscopy, pharyngoscopy, otoscopy and laryngoscopy. DNA samples were isolated from peripheral blood lymphocytes according to a modified technique. The concentration and purity of the isolated DNA were evaluated by measuring the optical density of DNA-containing solutions.
Results. The data obtained indicate that in children with mutant allelic variants of the VEGF-A-634 gene, a disease of the blood system (anemia of varying severity) prevails among background diseases, which requires attention. Since the VEGF-A 634 gene is a vascular endothelial growth factor, which is a growth trigger, with its unfavorable non-functional allele variant, the development of neoplastic angiogenesis is noted, which contributes to an increase in microvascular density. In our opinion, this may play a key role in the mechanism of laryngotracheitis development.
Conclusion. During the examination, it was revealed that the C allele and the hetero-G/C and C/C homozygous genotypes of the Ile 105 Val polymorphism of the VEGF-A-634 gene are significant markers of an increased risk of recurrent laryngotracheitis in children of the Uzbek population.
Keywords: laryngotracheitis, children,gene polymorphism, endothelial growth factor, recurrent

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Роль полиморфизма гена фактора роста VEGF-A-634G/C у детей с рецидивирующим ларинготрахеитом

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Резюме

Цель. Изучить значимость молекулярно-генетических маркеров в формировании рецидивирующих ларинготрахеитов у детей.
Материалы и методы. Основу нашего исследования составили 85 пациентов с рецидивирующим стенозированным ларинготрахеитом, которые были разделены на 2 группы: основную группу составили 55 детей, контрольную – 30 детей соответствующего возраста. Исследование началось с оториноларингологического осмотра, который состоял из эндоскопической риноскопии, фарингоскопии, отоскопии и ларингоскопии. Сбор материала образцов ДНК выделяли из лимфоцитов периферической крови в соответствии с модифицированной методикой. Концентрация и чистота выделенной ДНК оценивались при измерении оптической плотности ДНК-содержащих растворов.
Результаты. Полученные данные свидетельствует о том, что у детей с мутантными аллельными вариантами С гена VEGF-A-634 среди фоновых заболеваний превалирует заболевание системы крови (анемия различной степени тяжести), что требует внимания. Так как ген VEGF-A-634 представляет собой фактор роста эндотелия сосудов, являющийся пусковым сигналом роста, при его неблагоприятном нефункциональном аллельном варианте отмечается развитие неопластического ангиогенеза, что способствует возрастанию плотности микрососудов. Это, на наш взгляд, может играть одну из ключевых ролей в механизме развития ларинготрахеита.
Заключение. При обследовании выявили, что аллель С и гетеро-G/C и C/C – гомозиготные генотипы полиморфизма Ile 105 Val гена VEGF-A-634 являются значимыми маркерами повышенного риска развития рецидивирующего ларинготрахеита у детей узбекской популяции.
Ключевые слова: ларинготрахеит, дети, полиморфизм генов, эндотелиальный фактор роста, рецидивирующий

■ INTRODUCTION

The steady increase in the incidence of laryngotracheitis (LT) among the child population, especially the recurrent course, poses a number of serious questions to clinicians in terms of diagnosis and treatment. Recently, the study of the role of genetic factors in many allergic diseases, including the respiratory tract, has been of particular interest [8, 15]. Laryngotracheitis is a disease mainly of bacterial origin, in which the pathogen and its antigens most often penetrate the vascular walls, and contributes to the development of endothelial shock, causing the development of complicated forms of morbidity [14, 17]. Significantly, the genes of many cytokines are characterized by polymorphism and the presence of isoforms as a property that contributes to a change in the direction of the immune response [18, 20], which leads to the development of a predisposition to various variants of the course of the disease [5, 6, 13]. Among the predictor factors of particular interest are genetic factors with single nucleotide substitutions (SNP), among which is the gene of vascular endothelial factors – VEGF [2, 11]. At the same time, it is important to study the genes that affect the activity of cytokines, which is one of the main tasks in studying the pathogenesis of both initiation and detection of predisposition to the development of morbidity [1, 3]. Lately, in the pathogenesis of a number of allergic respiratory diseases, special attention has been paid to vascular endothelial factors – the VEGF gene [2, 15], which is a powerful multifunctional cytokine [4, 7, 16]. These days, domestic and foreign scientists have indicated a modern concept in the pathogenesis of recurrent laryngotracheitis, which provides for the development of a pathological process as a result of exposure to various factors, both infectious and allergic [9, 10, 12]. At the same time, the study of genes responsible for the production of cytokines involved in the induction of immune disorders is of scientific and practical interest [23, 24].

■ PURPOSE OF THE STUDY

To study allelic variants and the association of genotype polymorphism of the endothelial system gene of the VEGF-A-634 G>C growth factor gene in children with recurrent laryngotracheitis.

■ MATERIALS AND METHODS

85 children aged 2 to 5 years with a diagnosis of recurrent laryngotracheitis were under observation. All the examined children by age made up the children's period (2–11 years old). Among them, girls made up 27 and boys – 58. General clinical, laboratory and molecular genetics and statistical studies were carried out in all children. Patients were consulted by related specialists (infectious disease specialist, allergist, etc.) Among 85 children, the main group consisted of 55 children and the control group consisted of 30 children of the appropriate age.

Molecular genetic examination of biomaterials (DNA) was performed on the basis of the clinic of Geno-Technologies LLC. The object and subject of the study were DNA samples of children, the VEGF gene A-634 G>C. DNA samples were isolated from peripheral blood lymphocytes according to a modified technique. The concentration and purity of the isolated DNA were evaluated by measuring the optical density of DNA-containing solutions at a wavelength of 260 and 280 nm versus THOSE on a NanoDrop 2000 spectrophotometer (USA).

Genotyping of G/C polymorphism, VEGF gene was performed on a real-time PCR amplifier Rotor Gene 6000 Model 65H0-100 (Australia), using the test system of the company «Syntol» Cat. No.-NP_555_100_RG (Russia), according to the manufacturer's instructions. Statistical analysis of the results was carried out using the statistical software package «OpenEpi 2009, Version 2.3». The frequency of variants of alleles and genotypes (f) was calculated by the formula: f=n/2N and f=n/N, where n is the occurrence of the variant (allele and genotype), N is the sample size.

■ RESULTS

The results of molecular genetic studies of the VEGF-A-634 G>C gene are presented in Table 1.

As follows from the table, in the control group of healthy children, the functional allele of the VEGF-A – 634 gene was detected in 98.3% of cases (59/60), and in the main group – in 86.4% of cases (95/110), which was 1 time higher than in the control group. (χ^2=6.52, p<0.01; HR=0.11; 95% CI 0.01–0.83). The nonfunctional mutant allele of the SAFR-A – 634 gene in the control group of healthy children was detected in 1.6% of cases (1/30), whereas in the main group of children with recurrent laryngotracheitis (RLT) was detected in 13.6% of cases (15/110), which was 8.5 times higher than the indicators of the control group (χ^2=6.52; p<0.01; HR=9.32; 95% CI 1.2–72.4).

A comparative assessment of the frequency of occurrence of the mutant variant allele C of the VEGF-A-634 gene among children with PPT was 13.6%, and in the control (1.6%) showed a significant difference in the values of the studied indicator (P<0.01). The obtained result may indicate the connection of the allele «C» polymorphism of the VEGF-A-634 gene, leading to the replacement of C by G at position 634 of the amino acid sequence, with relapse in children with laryngotracheitis. At the same time, the risk of developing recurrent forms of laryngotracheitis in the presence of variant C allele polymorphism in the genome is increased by 8.5 times (HR=8.5).

Analysis of the distribution of genotypic variants of the VEGF-A-634 G>C gene polymorphism in the general inheritance model in the control group of healthy children showed the detectability of functional homozygous variants of G/G in 96.6% of cases (29/30), whereas in patients of the main group this genotype was detected in 78.2% of cases (43/55), which is 1.2 The number of times was lower compared to the control group. (χ^2=5.2; p<0.07; HR=0.12; 95% CI 0.02–1.0). It should be noted that the high population frequency of the functional genotype G/G both in the control group and among children

Table 1
The frequency of distribution of allelic variants and polymorphism of the VEGF-A-634 G>C gene in children with recurrent laryngotracheitis

№	Group	Frequency of alleles				Frequency of genotype distribution					
		G		C		G/G		G/C		C/C	
		n	%	n	%	n	%	n	%	n	%
1.	Main group n=55	95	86,4	15	13,6*	43	78,2	9	16,4*	3	5,5
2.	Control group n=30	59	98,3	1	1,6	29	96,6	1	3,3	–	–

Note: * is the confidence indicator in relation to the control group (P<0.05).

with RLT in the studied sample in combination with a functionally unfavorable genotype may indicate the benefit towards a functionally favorable allele.

Whereas the heterozygous variant of the VEGF-A-634 G>C gene in the control group was detected in 3.3% of cases (1/30), and in the main group – in 16.4% (9/55), which was 4.9 times higher than the indicators of control healthy children (χ^2=5.22; p<0.07; HR=5.67; 95% CI 0.68–47.16) An unfavorable homozygous variant of the VEGF-A-634 G>C gene was detected in 3.6% of cases (2/55) in the group of sick children, and this genotype was absent in the control group of healthy children (χ^2=5.22; p<0.07; HR=4.07; 95% CI 0.2–81.4) (Table 1). However, when calculating the overall inheritance model, statistical analysis showed the absence of significance of these differences (p<0.07).

When analyzing the additive inheritance model according to the Cochran-Armitage test, the association of genotype polymorphism was statistically significant.

The functional genotypes of the G/G gene VEGF-A-634 statistically significantly differed from the indicators of control children (χ^2=4.9; P<0.03; HR=0.12; 95% CI 0.02–1.0). Heterozygous G/C and unfavorable homozygous variant C/C of the VEGF-A-634 gene significantly differed from the indicators of control healthy children, which amounted to χ^2=4.9; P<0.03; HR=5.67; 95% CI 0.68–47.16 and χ^2=4.9; P<0.03; HR=4.07; 95% CI 0.2–81.4 respectively.

The obtained data may indicate that the heterozygous genotype of the polymorphism of the VEGF-A-634 G>C gene is a genetic determinant determining the formation of recurrent laryngotracheitis, and its carrier is a predisposition factor for the development of this pathology, increasing its risk by 6 times (HR=5.67).

Thus, the data of our study showed the connection of the unfavorable variant allele C polymorphism of the VEGF-A-634 G>C gene, leading to the replacement of G by C at position 634 of the amino acid sequence, with the development of a recurrent form of laryngotracheitis. We found that the risk of recurrence in laryngotracheitis in the presence of a variant polymorphism allele in the genome increased by 5.7 times (HR=5.67). The obtained result also indicates that the heterozygous genotype of the VEGF-A-634 gene polymorphism is a genetic determinant determining the formation of a recurrent form of laryngotracheitis, and the carrier of the G/C genotype is a predisposition factor for laryngotracheitis in children with allergic rhinitis.

The reliable association of the functionally unfavorable G allele and the heterozygous G/C genotype of the VEGF-A-634 gene of the studied polymorphism with the pathogenesis of recurrent laryngotracheitis may indicate a high probability of association of this pathology with a homozygous variant genotype. However, confirmation of this hypothesis requires additional studies with a significant increase in the sample size due to the low population prevalence of the functionally unfavorable genotypic variant of the C/C polymorphism of the VEGF-A-634 gene.

It should be noted that the frequency of unfavorable alleles of polymorphic genes in different populations may be different, since each population has its own allele fund. The frequency of occurrence of heterozygous or homozygous genotypes of genes in a population is also a variable value, since they are influenced by a number of dynamic factors that determine the genetic structure of the population [19, 22]. Therefore, an important point in the study of polymorphic genes potentially associated with the development and pathogenesis of diseases is the analysis of the expected and observed

Table 2
Analysis of the observed and expected frequency of genotypes of the polymorphism of the VEGF-A-634 G>C gene in groups of patients with RL (RHB compliance).

Genotypes	Frequency of genotypes		χ^2	P
	Observed	Expected		
G/G	78,2	57,15	0,74	0,18
G/C	16,3	36,9	0,24	
C/C	5,5	5,95	0,019	
Total	100	100	1,7	

frequency of the genotypes of the studied polymorphisms and the correspondence of the frequency distribution to the Hardy-Weinberg equilibrium (XB) [21, 22].

Based on the study of the frequency of alleles, we calculated the theoretically expected (expected, exp.) frequencies of genotypes of the polymorphism of the VEGF-A-634 G>C gene in a conditionally panmictic population and analyzed the correspondence of the actually observed (observed, obs.) frequencies of genotypes to the Hardy-Weinberg law (Table 2).

The results of the analysis of the frequency of distribution of genotypes by RHV of the polymorphism of the VEGF-A-634 G>C gene in the main group of children with RLT showed that the observed frequency of G/G genotypes was 78.2%, heterozygous G/C genotypes – 16.3% and homozygous – C/C – 5.5% – accordingly, while the expected frequency of functional genotypes of the G/G group was 57.15%, which was 1.4 times lower than those observed, while heterozygous variants of G/C were – 36.9%, which was 2.3 times higher than the observed indicators, and the expected homozygous non-functional genotypes of S/S were 5.9%, which was 1.1 times higher than the indicators of the observed genotypes, respectively.

Whereas in the control group, the observed and expected frequency of functional G/G genotypes of the VEGF-A-634 gene was found in 96.7% and 60.6% of cases, respectively, and the heterozygous G/C variant of the observed variant was noted in 3.3% of cases, and the expected one – 34.5% of cases.

A comparative analysis of the expected and observed frequencies of genotypes of this polymorphism revealed the absence of a statistically significant deviation of indicators ($P>0.05$) in all the studied groups, which indicates that the observed proportion of genotypes in the studied samples corresponds to the Hardy-Weinberg equilibrium.

The study of the genetic structure of this marker revealed a relatively high level of expected heterozygosity of the G/C gene VEGF-A-634 in the main group of patients in relation to the control group (36.9% and 34.5%, respectively). The results obtained indicate the possibility of high frequencies of expected heterozygotes, rather than actually calculated heterozygotes.

It should be noted that in the clinical course of the underlying disease, in addition to the etiological factor, an important importance is attached to concomitant pathology. Thus, the results of molecular genetic studies were analyzed taking into account background diseases in children with RLT. Thus, the results of clinical analyses of children showed that in the main group 33 children were diagnosed with anemia of varying severity, which accounted for 60% of cases, allergic rhinitis – in 18 (32.7%) and rickets was detected – in

6 children, which amounted to 10.9% of cases. Taking into account allelic variants of the VEGF-A-634 gene.

Taking into account allelic variants of the VEGF-A-634 gene, anemia was most often noted in children with a favorable allelic variant G – 80%, allergic rhinitis in 23.6%, rickets in 5.5% of cases. Whereas 14.5% (8) of children with unfavorable allelic variants of the VEGF-A-634 gene were often diagnosed with anemia, allergic rhinitis was diagnosed in 9.1% (5 patients), respectively.

Taking into account the correspondence of the observed proportion of genotypes of the polymorphism of the VEGF-A-634 (G/C) gene in the studied samples to the Hardy-Weinberg equilibrium, our study indicates a possible association of the functionally unfavorable allele «C», leading to the replacement of G by C at position 634 of the amino acid sequence, with the risk of recurrent laryngotracheitis. At the same time, the risk of developing laryngotracheitis in children in the presence of a variant allele with r polymorphism in the genome increases the risk by 9 times (HR=9.3). We also showed that the heterozygous genotype of the G/C polymorphism of the VEGF-A-634 gene is a genetic determinant, which is a predisposition factor for the development of this pathology, increasing its risk by 5.6 times (HR=5.7). The data obtained requires close attention from ENT doctors and pediatricians.

■ CONCLUSION

Thus, the C allele and the hetero-G/C and C/C homozygous genotypes of the Ile 105 Val polymorphism of the VEGF-A-634 gene are significant markers of an increased risk of recurrent laryngotracheitis in children of the Uzbek population ($P<0.03$). The G allele and the functionally favorable G/C genotype are reliable protective markers in with respect to the development of pathology ($\chi^2=4.92$; $p<0.03$; HR=5.67; 95% CI 0.68–47.16).

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