**Original Article:**

**Vascular Endothelial Growth Factor: Genetic Aspects in Children with Asthma in the Rostov Region.**

**Authors**

Shkurat TP, Professor, Southern Federal University, Lebedenko AA, Professor, Rostov State Medical University, Mashkina EY, Professor, Southern Federal University, Semernik OE, Assistant, Rostov State Medical University, Dreyzina TK, Southern Federal University, Rostov-on-Don, Russia.

**Address for Correspondence**

Tatiana Shkurat, Professor
Academy of Biology and Biotechnology, Southern Federal University, 194/1, Stachki Avenue, Rostov-on-Don, 344090, Russian Federation.

E-mail: tshkurat@sfedu.ru

**Citation**


**Submitted:** Nov 22, 2016; **Accepted:** Dec 29, 2016; **Published:** Jan 31, 2017

**Abstract:** Vascular endothelial growth factor (VEGF) plays an important role in the pathogenesis of asthma. It activates neoangiogenesis, thus contributing to disruption of the microcirculation in the bronchi and increasing the intensity of exposure to the elements of the inflammatory cascade. So, the goal of this research was to evaluate the role of C-634G VEGFA gene polymorphism in the pathogenesis of childhood asthma. In the analysis of the blood no statistically significant differences has been revealed in the genotype frequencies and allele on investigated polymorphism between the control group of children and the group of children with asthma. The most frequently registered one is homozygous polymorphism of VEGFA gene. The influence of the polymorphic C-634G of VEGFA gene on the concentration of serum IgE is not revealed.

**Key Words:** Asthma, Vascular endothelial growth factor, Gene polymorphism, Children.

**Introduction:**

According to the contemporary definition of Global Initiatives on Asthma (GINA, 2015), “Asthma is a chronic inflammatory disease of the airways, in which many cells and cellular elements are involved. Chronic inflammatory causes the development of bronchial hyperactivity, which leads to the recurrent episodes of wheezing, breathlessness, feeling of fullness in the chest and coughing, particularly at night and in the early morning. These episodes are usually associated with widespread, but variable in their severity of airway obstruction in the lungs, that is often reversible either spontaneously or under treatment procedure.”

Remodeling of the bronchial wall has a negative prognostic value in disease pathogenesis. It is proved there is the association of remodeling processes with the severity of disease and the degree of bronchial obstruction. (1) At the same time there is significantly greater thinking of the basement membrane, hypertrophy of the epithelium and the more intense angiogenesis. (1-5) The process of remodeling has specific phenotypic character, at the same time its genotypic characteristics have yet to decipher. Hypoxia, which is characteristic of asthma, activates the synthesis of vascular endothelial growth factor (VEGF), which is involved into angiogenesis. This process leads to the disruption of the microcirculation and the increasing of the intensity of exposure to the elements of the inflammatory cascade. (6) Vascular endothelial growth factor is one of a family of structurally closely related proteins. VEGF triggers a signaling cascade that ultimately stimulates growth of vessel endothelial cells, their survival and proliferation. Thus, VEGF plays a central role in the process of angiogenesis, stimulating the process of the bronchial wall remodeling, and hence contributing to disease progression. (7)

Probably, the concentration of VEGF in the blood serum is determined by genetic factors. In this regard, the study of the role of polymorphism in children from the Rostov region will determine its significance in the development of chronic diseases, such as asthma.

The aim of the study was to evaluate the contribution of a single nucleotide C-634G (rs201063) of VEGF gene in the formation of predisposition to the development of asthma in children from Rostov region.

**Materials and Methods**

**Inclusion criteria:**

- Patients diagnosed with asthma from the birth living on the territory of the Rostov region
- Age from 6 to 18 years
Exception criteria:
- Patients with comorbidity

The average age of examined patients was 11.1 ± 0.8 years. In assessing polymorphic alleles of the gene in examined patients with asthma, population sample of children of I and II group of health (n=27, the median age - 12 years) - citizens of the Rostov region without comorbidity was used as a control. Patients of primary and control groups were comparable to the gender and age.

The survey was conducted based on the pediatric clinic of the Rostov State Medical University, Rostov-on-Don.

All patients were carried out complex clinical survey, including clinical examination, the collection of an anamnesis assessment of atopic status, study of function of the external breath, the definition of serum IgE and molecular genetic studies. The level of IgE was determined in fresh serum with the help of enzyme linked immunosorbent assay with “VedaLab” (France). Molecular genetic study was conducted at the Department of Genetic of The Southern Federal University.

To isolate DNA the reagent “DNA-express-blood” (Lytech, Russia) was used. Allelic variants C-634G of VEGF gene were studied, using the number of reagent SNP-express (Lytech, Russia). The assay is based on carrying out the amplification reaction with two pairs of allele-specific primers. Separation of amplification products was made by methods of horizontal electrophoresis in 3% agarose gel. The analysis of electrophotograms was performed on trans-illuminator GelDoc (BioRad).

The compliance of allocation of the frequency genotype said to Hardy-Weinberg equilibrium was determined with the help of Hardy-Weinberg equilibrium calculator in the program www.eoge.org/software/Hardy-Weinberg. Evaluation of the differences in the distribution of alleles in the studied groups was performed according to the criterion $\chi^2$. The risk of miscarriage was tried with respect to odds ratio - OR. OR is listed with 95% confidence interval (CI).

**Ethical review**

The study was conducted with the ethical standards set forth in WAME (The World Association of Medical Editors). There was obtained informed written consent for participation in the study from all parents of children and adolescents over the age of 15 years. This informed written consent was approved by the local ethics committee of the Rostov State Medical University.

**Statistical analysis**

Statistical processing of the results of the study was conducted with a set of applied programs of Microsoft Office 2000 Pro for Windows OSR 2 on a computer PC Intel Premium -166 (Microsoft Office 97 Professional, 1997, USA). For statistical analysis was used the computer program STATISTICA v. 6.0. (StatSoft Inc., USA). Statistical data processing included assessment of the conformity of the distribution of genotypes of Hard-Weinberg equilibrium analysis of contingency tables using Fisher’s Exact test, comparing mean values of level distributions of IgE at Wanna-Whitney test and calculator of Spearman correlation coefficient.

**Results**

During the study mild asthma was noted in 23 children (76.6%), moderate asthma in 7 children (23.3%). The average age of onset of the disease was 4.8±0.7 years. For the previous survey year, more than of the surveyed children had attacks of airflow obstruction (63.3%). And most often they were observed at night (86.76%); only in the daytime shortness of breath were only 7 patients (23.3%).

It should be noted that the majority of patients received anti-inflammatory therapy (76.6%) and only quarter of patients (23.3%) didn’t use anti-inflammatory drugs.

It was found that 33.33% among children with asthma are heterozygotes for the C-634G polymorphism of VEGFA gene, homozygous 634GG variant of the polymorphism observed in 60% of cases. At that time, the frequency of homozygous -634G of VEGFA gene is 59.26%, among healthy children, the frequency of heterozygotes is 29.63%. It follows the compliance of genotype frequencies with Hardy-Weinberg equilibrium in all groups of examined patients (the value of $\chi^2$ equal to 0.17 in the group of patients with asthma, while a value of control group is equal to 1.41), so, can be considered representative enough.

It was noticed that the frequencies of alleles and genotypes of the patients don’t have statistically significant differences from the group of healthy children (p>0.05) [Table 1].

**Table 1: The frequency distribution of alleles and genotypes of polymorphism C-634G of VEGFA gene in the surveyed groups of patients**

<table>
<thead>
<tr>
<th>Genotype, allele</th>
<th>Children with asthma n=30</th>
<th>Healthy children n=27</th>
<th>$\chi^2$</th>
<th>P</th>
<th>OR Value 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele 1(C)</td>
<td>0.233</td>
<td>0.259</td>
<td>0.10</td>
<td>0.75</td>
<td>0.87</td>
</tr>
<tr>
<td>Allele 2(G)</td>
<td>0.767</td>
<td>0.741</td>
<td>1.15</td>
<td>0.09</td>
<td>0.57</td>
</tr>
<tr>
<td>C/C</td>
<td>0.067</td>
<td>0.111</td>
<td>0.57</td>
<td>0.38</td>
<td>0.83</td>
</tr>
<tr>
<td>C/G</td>
<td>0.333</td>
<td>0.296</td>
<td>1.19</td>
<td>0.36</td>
<td>0.75</td>
</tr>
<tr>
<td>G/G</td>
<td>0.600</td>
<td>0.593</td>
<td>1.03</td>
<td>0.23</td>
<td>0.87</td>
</tr>
</tbody>
</table>

However, moderate asthma have been reported in C-634G heterozygotes of VEGFA gene more often and the average number of migrated exacerbation over the past year is 3.09±0.41, while in children homozygous for the C-634G allele is 2.8±0.37.

It was established that the distribution of genotype frequencies for the C-634G polymorphism of VEGFA gene in children with asthma and different underlying allergy pathology has no significant differences (p=0.92).

It was noticed that polyvalent sensitization occurs in 634G/G homozygotes of VEGFA gene more often (36.67%) then CC-634G heterozygotes of VEGFA gene (22.2%). It should be noted that VEGF is stimulated by many Pro-angiogenic factors, including epidermal growth factor, basic fibroblastic growth factor, platelet growth factor and interleukin-1B. It was noticed that the reaction to physical activity in 76.67%, most of it was running (83.3%). These patients were homozygous for the C-634G polymorphism of VEGFA gene, while the reaction of the bronchi in CC-634G homozygotes of VEGFA gene was associated with emotional experiences and the changes of weather.

Adverse atopic heredity in 56.67% of cases was combined with carriage of the -634G allele of the polymorphic C-634G locus of VEGFA gene. However, there is no identified significant differences of Ig E values in carriers of different genotypes in the study of the influence of the polymorphic C-634G locus of VEGFA gene.

**Discussion**

Data analysis of single-nucleotide polymorphisms of VEGFA gene and a causal relationship is tentative. It was established the influence of several polymorphisms, in particular, in -2578C/A (rs699947), -1154G/A (rs1570360),634G/C (rs2010963) positions of promoting VEGFA region in gene expression. All alleles 2578G, -1154C and -634C are associated with level of expression of VEGF A. Special attention deserves VEGFA -634CC genotype. This genotype associated with higher serum concentrations of VEGF in healthy people
and increased VEGF production of mononuclear cells, compared with CG and GG genotypes. (10, 11) But at present, population data on the frequencies of the alleles VEGF gene are represented only in a small part of data for ethic groups, living on the territory of the Russian Federation. As it is characterized by the diversity of national composition and climatic and geographical conditions. For this reason the study of genotypic characteristics in children with asthma from the Rostov region causes great practical interest. The data obtained in the study showed that -634G allele is found in 2 times more often among children with asthma from Rostov region in comparison with the data of foreign scientists. (12-14) The most often it’s homozygous -634G/G variant associated with moderate asthma and polyvalent sensitization. The studies have not establish statistically significant influence of polymorphic C-634 G locus of VEGFA gene on the concentration of serum Ig E. But these data dictate the need to continue the study of paired serum concentration of biologically active substances and polymorphisms of this gene in patients with asthma. The presence of certain genotypes at the polymorphic locus of VEGF A gene can influence on the development of the disease. The definition of genetic markers and pathogenetic mechanisms of asthma will help us to predict the course of disease and conduct the possible phenotypic correction of clinical manifestations.

Conclusions:
The homozygous-634G/G polymorphism of VEGFA gene is the most frequently detect variant among children with asthma in the Rostov region. The frequency of alleles and genotypes among children with asthma don’t have statistically significant differences from the group of healthy children. The homozygous -634G/G polymorphism of VEGFA gene is associated with moderate asthma and polyvalent sensitization. The effects of the polymorphic C-634G locus of VEGFA gene on the concentration of serum Ig E are not determined.

Conflict of Interest: None.

Ethics: No ethical issues relate to the present study.

Acknowledgements
This research was supported by the internal grant of the Southern Federal University No. 213.01-2015/003VG.

References