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Genetic Polymorphism A38G of the CC16 Gene in Children with Acute Bronchiolitis

Azimova Kamola Talatovna[1], Garifulina Lilya Maratovna[2]

[1]Basic Doctoral Student of the Department of Pediatrics No. 3 and Medical Genetics of Samarkand State Medical University, Uzbekistan
[2]PhD, Associate Professor, Head of the Department of Pediatrics, Faculty of Medicine, Samarkand State Medical University, Uzbekistan

Abstract DNA samples obtained from 27 children with acute bronchiolitis were examined and similar samples of 23 children with acute pneumonia and 20 conditionally healthy children aged 2 months to 2 years were studied for comparison. The study of genetic polymorphism was carried out using polymerase chain reaction by allelic discrimination with registration of the results using electrophoresis. The protective effect of the A/G genotype of the A38G polymorphism of the CC16 gene has been established, namely, its decrease in the occurrence of acute bronchiolitis in young children. It has been proven that the A/A genotype is associated with severe acute bronchiolitis with the development of severe bronchoobstructive syndrome. The association of the G/G genotype with the development of bacterial complications in children with acute bronchiolitis and with the formation of a burdened allergic anamnesis in this category of patients was revealed. The totality of the analysis of clinical and anamnestic indicators in correlation with genetic factors can provide the basis for building a forecasting model and creating an individual scenario for the development of the disease.

Keywords Acute bronchiolitis, Severity of course, Children, Genetic polymorphism, A38G gene CC16

1. Introduction

Lower respiratory tract infections are a common cause of hospitalization in infants. According to experts, children under 12 months of age with bronchiolitis account for 18% of all pediatrician hospitalizations [1], which poses a great burden for industrialized health systems every winter [2,3]. This condition is caused mainly by viruses such as influenza virus, rhinovirus, respiratory syncytial virus (RSV) and bacterial pathogens that can be transmitted from person to person by airborne droplets and by contact with an infected object [4,5].

The relationship between asthma and bronchiolitis may be bi-directional [6,7], as additional studies have linked viral respiratory infections with the development of asthma [8]. Studies conducted in Spain have shown that children with HBoV bronchiolitis, human metapneumovirus bronchiolitis (hMPV), RSV bronchiolitis are at increased risk of developing asthma at the age of 5-7 years [6,7]. On the other hand, an earlier study in the United States showed that the severity of childhood bronchiolitis is associated with the severity of early asthma [6].

Currently, a number of works have appeared characterizing the active participation of secret globin (CC16), synthesized by cells of the mucous membrane of the airways (Clara cells), in the inflammatory process of the lungs, while its relationship with both the acute inflammatory process and the development of recurrent chronic lung pathology is noted.

The influence of the A38G polymorphism of the CC16 gene in the realization of the recurrent nature of Broncho obstructive syndrome is also possible. In the works of foreign researchers, the relationship of the homozygous position of the mutant allele A of the CC16 gene with the development of bronchial asthma has been traced.

It was revealed that the CC16 level was reduced in Broncho alveolar lavage of asthma patients [10]. The single nucleotide polymorphism A38G (rs3741240), according to the A allele, is associated with a decrease in the transcriptional activity of the gene [11]. It was revealed that the AA genotype is associated with allergies and asthma [11]. It was revealed that patients with the AA genotype had significantly lower serum CC16 levels higher than in patients with the GG and AG genotype [12]. There is reason to suspect that children with bronchial obstruction and having the CC16 38A/38A genotype (and lower serum CC16 levels) have the highest level of local inflammation and bronchoconstriction and, consequently, an increased likelihood of developing bronchial asthma [13].

At the same time, there are no studies clarifying the effect of this gene on the course of the inflammatory process in infectious diseases of the lower respiratory tract, namely acute bronchiolitis, as well as the possible effect on the development of recurrent obstructive pulmonary pathology in this cohort of patients in subsequent years of life.

In this regard, we set a goal: to study the polymorphism

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of the A38G gene in children with acute bronchiolitis and to clarify the possible relationship between the degree of polymorphism and the severity of the disease.

2. Materials and Methods

70 children aged 2 months to 2 years were examined, including 27 children diagnosed with acute bronchiolitis, and for comparison, 23 children diagnosed with acute pneumonia and 20 conditionally healthy children were examined.

The studies were conducted in the period 2021-2022. The observed sample of patients, depending on the severity of their condition, were hospitalized in the department of pulmonology and the intensive care unit of the Samarkand Regional Children's Multidisciplinary Center and in the department of emergency pediatrics and the department of pediatric intensive care of the Samarkand branch of the Republican Scientific Center for Emergency Medical Care. In children with acute bronchiolitis, bronchial obstruction of varying degrees was observed, in children with acute pneumonia, respiratory failure of a mixed type of varying degrees.

To assess the prognosis of the severity of bronchiolitis, a score scale based on clinical and auscultative signs of ESBA was used (J.M. RamosFernandezetall, 2013) [14]. All patients with acute bronchiolitis underwent laboratory and instrumental examination, polymerase chain reaction in real time to detect viral antigen (RSV, adenovirus, rhinovirus, parainfluenza). Reverse transcription and PCR reactions were performed using commercial kits Revert and Amplisens-200 (Russian Federation). Polymerase chain reaction (PCR) of fragments of the CC16 gene was performed using a ready-made reaction mixture from the manufacturer "GenTerra" (Russian Federation).

DNA samples were isolated from the patients' blood by phenol-chloroform extraction [15]. The study of A (38)G polymorphism was carried out using a polymerase chain reaction by allelic discrimination [16], using a system of three primers — two direct allele-specific, differing in 3-terminal nucleotide: direct 1 5'CAgAgACggAACCAgAgACA, direct 2 5'AgAgACggAACCAgAgACg, and a common reverse primer 5'TCCTgAgAgTTCCTAAgTCC. The synthesis of primers was carried out by Algimed, Belarus.

The programmable thermal Cyclerrotorgene 6000 (QIAGEN, Germany) was used for amplification.

The polymerase chain reaction was carried out in a volume of 25 µl in two test tubes. A master mix was added to each tube. Next, direct primer 1 and reverse primer were added to one tube, and direct primer 2 and reverse primer were added to the other. DNA was added in an amount of 1 µl to each tube. Also, water for PCR in each tube. After that, the tubes were placed in a thermal cycler for amplification.

Homozygotes were marked by effective amplification by peak elevation in only one test tube. In heterozygotes, effective amplification occurred in both tubes, which was accompanied by a peak rise in both tubes [17].

To process the data obtained, statistical methods consisted in calculating the frequency of alleles and the frequency of allelic combinations and their correspondence to the Hardy — Weinberg equilibrium according to criterion 2 with the calculated ones, rejecting the null hypothesis when $P < 0,05$ [18].

3. Results and Discussions

In the course of our research, the frequency of occurrence of polymorphism A38G of the CC16 gene in patients with acute bronchiolitis was determined and a group of children with acute pneumonia was studied for comparative characteristics. The blood of conditionally healthy children without the presence of chronic bronchopulmonary pathology was presented as a control.

During the study, the frequency distribution of alleles and genotypes according to the A38G polymorphism of conditionally healthy children of the Uzbek population did not differ from the world literature data and amounted to the frequency of genotype G/G in 45.0% of cases, genotype A/G in 50% of cases, and the lowest percentage of genotype/A in 5% of cases.

Table 1. Distribution of genotypes and allele frequencies of the A38G polymorphism of the CC16 gene in groups of conditionally healthy children with acute bronchiolitis and acute pneumonia

Genotypes	Conditionally healthy childrenn=20	Children with acute pneumonian=23	Children with acute bronchiolitisn=27	P^1	P^2	P^3
G/G	9 (45,0%)	9 (39,1%)	14 (51,8%)	χ^2 =0,072 p=0,790	χ^2 =0,809 p=0,369	χ^2 =0,809 p=0,369
A/G	10 (50%)	12 (52,1%)	7 (25,9%)	χ^2 =0,020 p=0,887	χ^2 =2,884 p=0,090	χ^2 =3,632 p=0,05
A/A	1 (5%)	2 (8,6%)	6(22,2%)	χ^2 =0,225 p=0,636	χ^2 =2,528 p=0,194	χ^2 =1,691 p=0,887
Alleles	n=40	n=46	n=54			
G	28 (%)	30 (%)	35 (%)	χ^2 =0,223 p=0,637	χ^2 =0,280 p=0,598	χ^2 =0,002 p=0,967
A	12 (%)	16 (%)	19 (%)			

Note: P^1 - The significance of differences between control and children with acute pneumonia;
P^2 - The significance of the differences between the control and children with acute bronchiolitis;
P^3 - The significance of differences between children with acute pneumonia and acute bronchiolitis.

To confirm the association of polymorphism of the CC16 gene with the risk of subsequent development of Broncho obstructive diseases in children who suffered from acute bronchiolitis at an early age, a comparison of the frequencies of genotypes of children with acute bronchiolitis occurring with Broncho obstructive syndrome, and acute pneumonia, which is one of the frequent pathologies of the respiratory system of young children, as well as with indicators of conditionally healthy children, was carried out. The observed distribution of genotype frequencies did not differ from the theoretically expected distribution according to the Hardy-Weinberg equation. No significant differences in the frequency of occurrence of alleles and genotypes of the A38G polymorphism of the CC16 gene were found when comparing acute pneumonia with the control group (Table 1), which indicates that no special differences in the genotype of children with acute pneumonia and conditionally healthy children were observed and these genotypes did not contribute to the development of acute pneumonia.

A comprehensive comparison of alleles and genotypes revealed statistically significant differences in the AG genotype in patients with acute bronchiolitis compared with acute pneumonia, which suggests that this genotype is protective and its decrease in acute bronchiolitis contributed to the realization of the disease. At the same time, the highest frequency of the A/A genotype was observed (6 (22%) in this group of children, which was usually a sign of a possible severe obstructive syndrome according to the literature.

Table 2. Distribution of genotypes and allele frequencies of polymorphism A 38 P of the CC 16 gene depending on the severity of acute bronchiolitis on the scale ESBA

Genotypes	Mild to moderate severity N=16	Severe degree N=11	The validity of the difference
G/G	11 (68,75%)	3 (27,2%)	χ^2=4,492 p=0,035
A/G	5 (31,25%)	2 (18,1%)	χ^2=0,580 p=0,447
A/A	0	6(54,5%)	χ^2=11,221 p=0,001
Alleles	n=32	n=22	
G	27 (84,3%)	8 (36,4%)	χ^2=13,177 p=0,001
A	5 (15,7%)	14(63,6%)	

In assessing the severity of acute bronchiolitis, we also used the Acute Bronchiolitis Severity Scale (ESBA), the advantage of this scale over others was the ability to assess the severity of the child's condition from the moment of initial examination, taking into account the respiratory rate and heart rate of the child according to age. According to this scale, the severity of acute bronchiolitis with a score of up to 4 points corresponded to mild severity, 5-8 points of moderate severity and 9-13 points of severe acute bronchiolitis. The total sample of sick children was distributed as follows: children with mild severity made up 11.1% (3), with moderate severity 48.1% (13) and 40.7% (11) of children made up the group with severe acute bronchiolitis. At the same time, the average score in the group with severe severity was 11.34±0.32 points, on average 6.83±0.20 points and 3.40±0.39 points in the group of children with mild acute bronchiolitis, which was statistically lower compared with the average severity and severe acute bronchiolitis ($p<0,001$).

When identifying the ratio of the frequency distribution of alleles and genotypes, it was revealed that in children with severe acute bronchiolitis, the AA genotype was mainly observed, whereas the G/G genotype was found in children with moderate and mild acute bronchiolitis.

The data indicate that the G allele was more common in children with moderate severity of the disease, whereas in children with severe course, the predisposing allele was allele – A, which according to literature data is predisposing to severe obstruction syndrome, which is leading in the clinical pathogenesis of acute bronchiolitis.

Bacterial complications can sometimes complicate the main course of acute bronchiolitis, so complications were diagnosed in 10 (37.0%) of the children observed and manifested by otitis media in 7.4% (2) children and urinary tract infection in 7.4% (2) children, acute enterocolitis in 22.2% (6 children).

When analyzing the distribution of genotypes and alleles of the A38G genaSS16 polymorphism in the general group of patients, it was found that bacterial complications were significantly more common in carriers of the GG genotype. Therefore, the GG genotype can be considered as predisposing to the formation of bacterial complications in acute bronchiolitis. The analysis also revealed that the G allele is a predisposing allele in the development of concomitant bacterial complications in children with acute bronchiolitis.

Table 3. Distribution of genotypes and allele frequencies of the A38G polymorphism of the CC16 gene in children with acute bronchiolitis, depending on the presence of bacterial complications

Genotypes	Bacterial complications:		The validity of the difference
	is available (n=10)	not available (n=17)	
G/G	8 (100%)	6(%)	χ^2=5,040 p=0,025
A/G	1(%)	6 (%)	χ^2=2,098 p=0,148
A/A	1(11,6%)	5(20,2%)	χ^2=1,373 p=0,242
Alleles	n=20	n=34	
G	17 (85%)	18 (53%)	χ^2=5,675 p=0,018
A	3 (15%)	16 (47%)	

Since the polymorphism A38G of the CC16 gene is associated with the risk of developing bronchial asthma, we studied the allergoanamnesis, which was burdened on the mother's side in 14.8% (4), on the father's side in 26% (7), and in 11.1% (3) cases of atopic dermatitis were observed.

As our studies have shown, the presence of concomitant allergic diseases is a prognostically unfavorable factor for the development of bronchial asthma. The association of the

GG genotype with the presence of a burdened personal allergoanamnesis was revealed in the patients with acute bronchiolitis we examined.

Table 4. The distribution of genotypes and allele frequencies of polymorphism A 38 P of the CC 16 gene, depending on the presence of allergic pathology in the anamnesis in children with acute bronchiolitis

Genotypes	Allergoanamnesis:		The validity of the difference
	burdened (n=14)	not burdened (n=13)	
G/G	11 (%)	3 (%)	χ^2 =7,036 p=0,008
A/G	5 (%)	2 (%)	χ^2 =1,451 p=0,229
A/A	4 (%)	2(%)	χ^2 =0,678 p=0,411
Alleles	n=40	n=14	
G	27 (%)	8 (%)	χ^2 =0,195 p=0,660
A	13 (%)	6 (%)	

One of the factors providing anti–inflammatory and immunomodulatory properties of the mucous membranes of the body is secret globin, a protein synthesized in significant quantities by cells of the mucous membrane of the airways, mainly by Clara cells [10].

At the moment, the fact of the influence of the CC16 Clara gene on the occurrence of chronic ENT pathology is known, and this gene is also repeatedly found in works devoted to the study of bronchial asthma. In recent years, mutant alleles of the CC16 gene have been identified, predisposing in a homozygous state to asthma in about 10% of the population [10,12].

The Claracellprotein CC16 gene is responsible for the synthesis of anti-inflammatory protein, which is produced by Clara cells located in the distal parts of the lungs. In some studies, it was found that in people with bronchial asthma, the concentration of the CC16 protein was much lower than in healthy people, and vice versa in others [13].

At the moment, we have studied the polymorphism A38G of the CC16 gene in children with acute bronchiolitis, acute bronchiolitis being a frequent pathology of childhood, in some cases does not go away without a trace, but contributes to the recurrence of Broncho obstructive syndrome, recurrent morbidity, the relationship with the incidence of acute bronchiolitis and the occurrence of bronchial asthma in later life in children has been proven.

We obtained data that are also close to the literature data, while in children of the control group the distribution of genotypes did not differ from the average global data. There were also no statistical differences between the control data and children with acute pneumonia. Pronounced differences were observed between the group of patients with acute pneumonia and acute bronchiolitis: the incidence of homozygous allele combination AA is higher, and heterozygous AG is statistically significantly lower in children with acute bronchiolitis.

The determination of the severity on the ESBA scale and their ratio to the genotypes and alleles A38G of the CC16 gene showed the predominant content of the A/A gene, characterizing the severe course of the disease with severe Broncho obstructive syndrome.

Whereas carriers of the G/G genotype were more likely to have both bacterial complications and an allergic predisposition, which predisposes to a severe course of the disease.

Conclusions:

The protective effect of the A/G genotype of the A38G polymorphism of the CC16 gene has been established, namely, its decrease in the occurrence of acute bronchiolitis in young children.

It has been proven that the A/A genotype is associated with severe acute bronchiolitis with the development of severe Broncho obstructive syndrome.

4. Conclusions

The association of the G/G genotype with the development of bacterial complications in children with acute bronchiolitis and with the formation of a burdened allergic anamnesis in this category of patients was revealed.

The totality of the analysis of clinical and anamnestic indicators in correlation with genetic factors can provide the basis for building a forecasting model and creating an individual scenario for the development of the disease.

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