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EDITOR-IN-CHIEF COLUMN

Dear Colleagues!

This is the 72nd issue of the Yakutsk Medical Journal, which is the final issue of this year. The year 2020 is coming to an end, marked by the pandemic of the new coronavirus infection COVID-19, which has made serious adjustments to all spheres of life of all mankind. Despite all the difficulties, new requirements associated with the strained epidemiological situation, the outgoing year for the Yakutsk Scientific Center for Complex Medical Problems was fruitful, rich in bright, joyful and significant scientific events, new scientific contacts. ‘Yakutsk Medical Journal’ is included into the international citation database Web of Science after two years of testing. There is increasing demand for the journal by authors and readers from different regions of Russia, including Yakutia.

In the current issue you will find scientifically interesting articles by authors from Magadan, Khabarovsk, Yakutsk, Irkutsk, Krasnoyarsk, Ufa, Moscow, Arkhangelsk and Rostov-on-Don. In particular, the heading ‘Original Research’ provides data on the features of the membrane potential of granulocyte mitochondria in children with congenital lung malformations (G.P. Evseeva et al.), The ratio of apoptosis activity and necrosis of neutrophilic granulocytes in peripheral venous blood in practically healthy people living in the North (O.A. Stavinskaya et al.), molecular genetic diagnosis of mutations in the EXT1 and EXT2 genes in patients with multiple exostotic chondrodysplasia in the Republic of Sakha (Yakutia) (A.E. Yakovleva et al.), etc.

In the article by N.A. Barashkov et al. from the heading ‘Diagnostic and Treatment Methods’ results of the diagnostic search for phenotypes corresponding to Pendred's syndrome (sensorineural deafness combined with thyroid disorders) are presented, carried out using instrumental and laboratory methods of research in patients with hearing disorders in the Republic of Buryatia.

The heading "Topical Issue" is devoted to the diagnosis of precancerous and cancerous diseases of gastrointestinal tract (N.N. Gotovtsev et al., Novikova I.A. et al.) and female genital organs (M.P. Kirillina et al., N. A. Petrusenko et al.).

In the heading "Clinical Case" you will get acquainted with three clinical observations of patients with various forms of multisystem atrophy (MCA), modern diagnostic criteria for MCA (A.E. Adamova et al.), a clinical case of Chardzha-Strauss syndrome, first diagnosed in 27 -year-old man with bronchial asthma, peripheral hypereosinophilia and involvement of the gastrointestinal tract (A.S. Asekritova et al.), as well as with a clinical case of a rare variant of ataxia - type 17 spinocerebellar ataxia with expansion of tandem trinucleotide CAGP repeats (TATA-binding protein) (M.A. Varlamova et al.).

I sincerely congratulate our esteemed colleagues-phthisiologists on the 70th anniversary of the foundation of the most important institution for the republic in the fight against tuberculosis, the Yakutsk branch of the Institute of Tuberculosis of the USSR Academy of Medical Sciences (YaBIT), later renamed into the Yakutsk Research Institute of Tuberculosis of the Ministry of Health of the RSFSR and further into the CBI RS (Y) the Scientific center "Phthisiology". And I also congratulate colleagues dentists on the significant date - the 100th anniversary of the Dental Service of the Republic of Sakha (Yakutia), which makes a significant contribution to the health of the population of our republic! Dear colleagues, I congratulate you on the memorable dates and wish you further labor success, recognition in the scientific and medical community!

On the eve of the New Year, I wish our dear authors and readers good health and high spirits, new knowledge and breakthrough researches, new professional achievements and victories! May the work bring you inspiration, all your innermost desires and aspirations come true, all the best and kind will be preserved and multiplied! May the New Year 2021 be successful and productive for all of us! Well-being, harmony and happiness!

To our ‘Yakutsk Medical Journal’ long standing and further development, competitive ability, relevance and high demand among authors and readers!

Chief Editor ***Anna Romanova***

ORIGINAL RESEARCH

F.V. Vinokurova, V.M. Argunova, P.A. Sleptsova, V.M. Nikolaev, E.K. Rumyantsev, T.E. Burtseva, V.G. Chasnyk, M.M. Kostik

PREVALENCE AND STRUCTURE OF JUVENILE IDIOPATHIC ARTHRITIS IN CHILDREN IN THE REPUBLIC OF SAKHA (YAKUTIA)

DOI 10.25789/YMJ.2020.72.01

Juvenile idiopathic arthritis is the most common type of childhood arthritis. The prevalence and structure of this disease has pronounced geographical and ethnic characteristics. One of the factors that determines the prevalence of JIA and its subtypes is the prevalence of the HLAB27 antigen in the population.

Summary: the article presents the results of analysis of the structure and prevalence of juvenile idiopathic arthritis in children in the Republic of Sakha (Yakutia). Differences in the structure of the JIA are determined by different frequencies of the JIA variants. Our study showed that the high prevalence of the HLA B27 antigen affects the structure of JIA in the region.

The purpose of the study is to elucidate the prevalence and structure of JIA in children in the Republic of Sakha (Yakutia).

Methods and objects of research: The retrospective study included 170 sick children with juvenile idiopathic arthritis (ILAR, 2001), hospitalized in the cardio-rheumatology department of the Pediatric Center of the Republic of Sakha (Yakutia) "RB #1-NCM" from 2009 to 2017.

Results: the prevalence of JIA in children under 18 years of age in Yakutia was 64.3 per 100,000 population (0-17 years); the prevalence in children under 14 was 67.2; among teenagers - 47.3 per 100,000 population. Among the JIA cases, the indigenous population accounted for 30.9% of cases, while the Caucasians accounted for 7.4%. The average prevalence of JIA in the Republic of Sakha (Yakutia) is comparable to the all-Russian indicator, but there are significant differences across regions. In 14 districts and the city of Yakutsk, the prevalence of JIA is higher than the nationwide indicator. In 9 districts of Yakutia, no children with JIA were found. In the indigenous population, JIA was more often diagnosed among boys; they were characterized by a later age of JIA onset. In children of the indigenous population of Yakutia, the structure of JIA was significantly dominated by patients with enthesitis-associated JIA. The population of indigenous children was characterized by a rare incidence of systemic arthritis, RF (+) polyarthritis, and psoriatic arthritis. The prevalence of HLA B27 antigen in juvenile idiopathic arthritis was 57%.

Conclusions: the peculiarities of the structure of JIA in Yakutia are due to the high frequency of occurrence of the HLAB27 antigen in the population as a whole and in JIA.

Keywords: juvenile idiopathic arthritis, enthesitis-associated variant of JIA, HLA B27 antigen, frequency.

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Relevance. Juvenile idiopathic arthritis is the most common type of childhood arthritis. [1; 4]. The prevalence of juvenile idiopathic arthritis ranges from 2 to 19 cases per year per 100,000 population, and in recent decades this pathology has been increasing [3]. There are significant ethnic and geographical differences in the prevalence of JIA and its categories, so the prevalence of all juvenile arthritis in different countries ranges from 0.05% to 0.6%. In the Russian Federation, the prevalence of JIA in children under 18 reaches 62.3 per 100 thousand population [2]. The prevalence of JIA in Moscow in 2016 was 52.2 per 100 thousand children (0-14 years old) and 99.8 per 100 thousand adolescents (15-17 years old) [6]. In Bashkortostan, according to 2006 data, the prevalence of JIA is 83.8; high prevalence is in the Kushnarevsky district - 132.3; low - in Ufa - 57.6 cases per 100,000 child population [5].

As a rule, the main differences in the structure of JIA are determined by different frequencies of the oligoarticular variant, enthesitis-associated variant and systemic variants of JIA. Therefore, in Europe and North America, the predominant variant is the oligoarticular variant of JIA, in Japan the systemic variant, and in Asian populations (China, India, South Korea), the enthesitis-associated variant of JIA is predominant. One of the risk factors for the implementation of enthesitis-associated arthritis is the HLAB27 antigen, and its frequency in a healthy population determines the frequency of this subtype of arthritis and the prevalence of JIA itself in the population. Previous studies have shown high frequency of the prevalence of the HLAB27 antigen among the indigenous population of the Republic of Sakha (Yakutia), which could have influenced the structure of JIA in the region.

The purpose of study is to elucidate the prevalence and structure of JIA in children in the Republic of Sakha (Yakutia).

Materials and methods: the retrospective study included data from the case histories of 170 pediatric patients (under 18 years of age) who were treated in the department of cardio-rheumatology Pediatric Center of the Republic of Sakha (Yakutia) "RB #1-NCM" from 2010 to 2019. The diagnosis of JIA was established based on the criteria of ILAR, 1997 [16]. The information was taken from the case histories about the demographic characteristics of patients, the type of

arthritis, the provoking factor, characteristics of arthritis, such as indicators of inflammatory activity, the presence of the HLAB27 antigen, seropositivity for antinuclear and rheumatoid factors, the number of active joints, the presence of comorbid pathology, the therapy and the region of residence the patient. The ethnicity was determined by the parents' self-identification method. The study included children of the indigenous population (Sakha, small indigenous peoples of the North) and Caucasians (Russians, Ukrainians) living in the Republic of Sakha (Yakutia). The prevalence of JIA was calculated using the following formula:

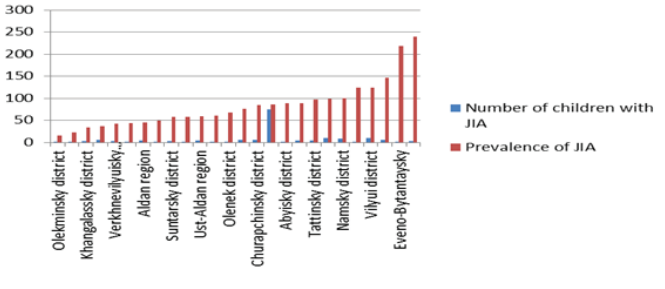
$$\frac{\text{The number of initial visits for diseases, identified in this year and in previous years}}{\text{The average population}} \bullet 100{,}000$$

Ethical examination: this scientific research work has passed the examination on biomedical ethics at the Yakut Science Centre of complex• 100 000. medical problems SB RAMS (extract from the minutes No. 18 of June 16, 2009).

Statistical analysis: The sample size was not pre-calculated. The analysis of the data obtained was carried out using the statistical software packages Statistica v. 10.0 (StatSoft Inc., the USA) and MedCalc (MedCalc Software, Belgium). The description of quantitative indicators is carried out with the indication of the median (25th; 75th percentile). The comparison of quantitative indicators in two independent groups was carried out using the Mann-Whitney test, qualitative indicators - using the Chi-square test ($\chi 2$) or Fisher's exact test if the expected frequency in one of the cells of the table 2 × 2 was <5. Differences or relationships were considered statistically significant at $p < 0.05$.

Results and discussion:

The prevalence of JIA among children under 18 years of age in Yakutia was 64.3 per 100,000 population (0-17 years); among children under 14 the prevalence was 67.2; among adolescents - 47.3 per 100,000 population. Among the JIA cases, the indigenous population accounted for 30.9% of cases, while the Caucasians accounted for 7.4%. The average prevalence of JIA in the Republic of Sakha (Yakutia) is comparable to the all-Russian indicator, but there are significant differences across regions. In 14 districts and the city of Yakutsk, the prevalence of JIA is higher than the nationwide indicator. In 9 regions of Yakutia, no children with JIA were found (figure 1).



Prevalence of JIA in the Republic of Sakha (Yakutia) 100,000 population

Of the features, it should be noted the male predominance in the indigenous population, as well as the later age of arthritis onset. In children of the indigenous people of Yakutia, there was higher prevalence of patients with enthesitis-associated JIA, with a relatively low specific gravity of psoriatic arthritis and rare incidence of systemic arthritis, RF (+) polyarthritis. More than half of the indigenous patients were hereditary tainted by rheumatic diseases, while in Caucasians only a third of patients had a tainted family history. The indigenous children had twice frequent enthesitis compared to Caucasians. When studying the features of articular lesions, it should be noted that children of the indigenous population more often had lesions of the hip and knee joints and sacroiliac joints and relatively less often had the involvement of the small joints of the feet. The detailed description of the patients included in the study is presented in Table 1. Among laboratory tests, children of the indigenous population tended to have a higher degree of laboratory activity, they had higher CRP and platelet counts. There were no significant differences in the frequency of children seropositive for antinuclear and rheumatoid factors.

There was significant difference in the overwhelming number of indigenous children with the HLAB27 antigen. There were no differences in provoking factors between the study groups.

When studying the social status, it was found that the children of the indigenous population more often lived in rural areas and had higher frequency of infection with Mycobacterium tuberculosis.

When studying the features of therapy, it was found that children of the indigenous population more often received sulfasalazine therapy and, less often, methotrexate therapy. The need for GIBD therapy was higher in European children, while more often Caucasian children were 1.5 times more likely to use adalimumab than children of the indigenous population.

Discussion: This study presents data on the prevalence of JIA in the Republic of Sakha (Yakutia). The main differences are associated with higher frequency of the HLAB27 antigen and the associated enthesitis-associated variant of JIA among children of the indigenous population of Yakutia. The influence of HLA on arthritis is associated with such biological intracellular phenomena as endoplasmic reticulum stress (ER) and autophagy. The most important function of the ER is folding of proteins. Disruption of normal folding under the influence of various "triggers" (inflammation, viral and bacterial infections, etc.) and accumulation of altered or incorrectly folded proteins in the EPR lumen with their subsequent aggregation was called “EPR stress”, and the system for controlling the quality of protein folding - UPR (unfolded protein response) [20,13,9]

In arthritis, under the influence of excess IL23 production, the folding of the HLA-B27 heavy chain occurs more slowly than in other HLA alleles, which leads to the formation of misfolding chains [17,13]. During EPR stress, their excessive accumulation occurs, leading to the activation of not only UPR, but also the nuclear factor NF-κB, a key transcriptional regulator of the synthesis of pro-inflammatory cytokines, including IL-17 and TNF-α, which also play an important role in the development of inflammation [18, 19].

According to the results of our studies, it has been shown the prevalence of the HLAB27 antigen in the mid-indigenous population of MS (Y) is 32.9%. The prevalence of the HLA B27 antigen in different populations is different (Table 2).

Enthesitis-associated JIA is the only form associated with male sex. Due to the high rate of enthesitis-associated arthritis among the indigenous population the prevalence of JIA is higher at boys, while there are more girls affected by JIA in most of the known populations. The wide distribution of the HLAB27 antigen is due to the fact that this antigen presents in all JIA subtypes in children of the indigenous people of Yakutia, not revealed in other populations. Thus, the HLAB27 antigen is found in 70% of children with enthesi-

Table1

Demographic characteristics of patients included in the study

Signs	Indigenous. n=144	Caucasians. n=26	p
Gender. male. n (%)	85 (59.0)	11 (42.3)	0.114
Debut age. y.	10.6 (6.0; 13.4)	7.8 (4.6; 11.6)	0.174
JIA subtypes. n (%)			
Oligoarthritis polyarthritis. RF (-) polyarthritis. RF (+) systemic arthritis enthesitis-associated arthritis psoriatic arthritis	36 (25.0) 21 (14.6) 1 (0.7) 4 (2.8) 76 (52.8) 6 (4.2)	5 (19.2) 6 (23.1) 1 (3.9) 2 (7.7) 10 (38.5) 2 (7.7)	0.292
NAJ	4.0 (3.0; 6.0)	3.0 (2.0; 6.0)	0.412
Enthesite. n (%)	36 (25.0)	3/26 (11.5)	0.133
Uveitis. n (%)	16 (11.1)	2/25 (8.0)	0.642
Psoriasis. n (%)	3/143 (2.1)	2/25 (8.0)	0.109
Family anamnesis. n (%)	75/136 (55.2)	8/22 (36.4)	0.102
Laboratory characteristic			
Erythrocyte sedimentation rate. mm / h	26.0 (15.0; 52.0)	23.0 (13.0; 38.0)	0.327
C-reactive protein. mg / l	33.0 (10.0; 58.0)	22.0 (10.0; 26.0)	0.262
Hemoglobin. g / l	116.0 (107.0; 128.0)	119.0 (108.0; 129.0)	0.570
Leukocytes x109 / l	7.7 (5.8; 10.0)	7.2 (6.4; 10.0)	0.787
Platelets x109 / l	339.0 (279.0; 455.0)	310.0 (254.0; 365.0)	0.077
Positivity for HLA B27. n (%)	76/131 (58.0)	8/24 (33.3)	0.026
ANF seropositivity. n (%)	4/46 (8.7)	0/16 (0.0)	0.223
Seropositivity in RF. n (%)	3/141 (2.1)	1/25 (4.0)	0.574
Provoking factor. n (%)			
Not installed Acute intestinal infection Acute respiratory infection Joint injury	36 (25.7) 48 (34.3) 29 (20.7) 17 (19.3)	8 (34.8) 10 (43.5) 2 (8.7) 3 (13.0)	0.393
Social factors. n (%)			
City dwellers	69/142 (48.6)	21/25 (84.0)	0.001
Villager	73/142 (51.4)	4/25 (16.0)	0.001
Infection with mycobacterium tuberculosis	16/141 (11.4)	1/24 (4.2)	0.285
Passive smoking	23/66 (34.9)	7/16 (43.8)	0.507
Therapy. n (%):			
Non-steroidal anti-inflammatory drugs	117/134 (87.3)	21/23 (91.3)	0.588
Sulfasalazine	25 (17.4)	2/25 (8.0)	0.238
Glucocorticosteroids	9/143 (6.3)	1/25 (4.0)	0.655
Methotrexate	97/142 (68.3)	21/24 (87.5)	0.055
Leflunomide	5/143 (3.5)	0/25 (0.0)	0.343
Cyclosporin A	3/144 (2.1)	0/25 (0.0)	0.467
Genetically engineered biological drugs (primary purpose). n (%): Etanercept Adalimumab Tocilizumab Infliximab Golimumab Abatacept	70/144 (48.6) 49 (70.0) 4 (5.7) 9 (12.9) 6 (8.6) 1 (1.4) 1 (1.4)	20/26 (76.9) 14 (70.0) 3 (15.0) 2 (10.0) 1 (5.0) 0 (0.0) 0 (0.0)	0.101
Genetically engineered biological products (second line of therapy). n (%):	8 (5.6)	1 (3.9)	0.720
Adalimumab Golimumab Infliximab Etanercept	4 (50.0) 2 (25.0) 1 (12.5) 1 (12.5)	1 (100.0) 0 (0.0) 0 (0.0) 0 (0.0)	0.936
Time until the first GEBP. y.	0.6 (0.3; 1.4)	1.0 (0.3; 1.9)	0.857

Abbreviations: ANF - antinuclear factor. GEBP - genetically engineered biological preparation. RF - rheumatoid factor.

tis-associated JIA, 44% of children with oligoarthritis, 60% of children with systemic arthritis, 33% of children with psoriatic arthritis, 39% of children with polyarthritis, which makes it impossible to apply the ILAR classification to populations with a high frequency of the HLAB27 antigen, since the HLAB27 antigen is a criterion for excluding several subtypes of JIA, such as systemic arthritis and psoriatic arthritis. The predominance of the HLAB27 antigen among Yakut children with JIA explains the later age of onset, the high incidence of enthesitis, and a family history of arthritis from the HLA B27 group, since this antigen can be transmitted from generation to generation. In the HLAB27 antigen risks of developing ankylosing spondylitis are doubled in a case if there is a relative diagnosed with one of the HLAB27 antigen circle [21]. It is interesting to note that psoriasis and psoriatic arthritis diagnosed as "HLAB27 circle" are very rare in the population of children of the indigenous population of Yakutia, who have high frequency of this antigen. The high prevalence of enthesitis-associated arthritis explains the higher frequency of sulfasalazine use and the lower frequency of methotrexate use in children of the indigenous population of Yakutia, since methotrexate has proven itself better in for the treatment of articular forms of JIA [12]. Among the peculiarities of the use of HIBP, it should be noted that the frequency of adalimumab use in indigenous children is lower, which is associated with a lower frequency of severe uveitis associated with oligoarticular JIA, since uveitis in patients with HLAB27 is more often acute, symptomatic and does not always require aggressive HIBT [17]. The second factor limiting the use of monoclonal antibodies is a higher incidence of infection with Mycobacterium tuberculosis, which increases the risks of reactivation of latent tuberculosis infection into active one under the influence of monoclonal antibodies that bind necrosis factor-a [12,10]. The absence of severe forms of uveitis and the prevalence of infection with Mycobacterium tuberculosis make etanercept the drug of choice in indigenous children with JIA, which is associated with its safe use in children of this group, and its effectiveness has been shown in various forms of JIA [11].

Limitation of the study: the retrospective nature of the study, that caused the insufficiency of information available in the medical records was considered the main drawback of this study. The division of patients according to ethnicity was conditional, especially the group of children of Caucasian origin, who, in fact

Table2

Frequency distribution of HLAB27 in different populations and associated arthritis

Population	HLA B27 frequency (%)	HLA B27 incidence in associated arthritis (%)	link
Koryaks	39.6	38.5	[7]
Eskimos (USA)	39	25	[7]
Russians (Chelyabinsk region)	9.6	22.4	[8]
Bengalis	10.7	49.2	[15]
Chinese	2.4	36.6	[14]

referred to a multinational group. The inability to determine the HLAB27 antigen in all patients led to the fact that this antigen was detected in patients with clinical signs of enthesitis-associated arthritis, which could lead to an artificial increase in the frequency of the HLAB27 antigen among patients with JIA.

Conclusions:

1. The prevalence of JIA in children (0-17 years old) in the Republic of Sakha (Yakutia) is comparable to the all-Russian indicator and amounted to 64.3 per 100,000 population. In 14 districts and the city of Yakutsk, the prevalence of JIA is higher than the nationwide indicator.
2. For the first time in the Republic of Sakha (Yakutia), the structure of juvenile idiopathic arthritis was studied. Enthesitis-associated arthritis is the most common form of juvenile idiopathic arthritis.
3. The HLAB27 antigen associated with juvenile idiopathic arthritis is higher in indigenous children than in Caucasian children.

The work was done within the framework of the research topic of the FGBU "YSC KMP" SB RAMS, "Monitoring the health status of children in the Republic of Sakha (Yakutia)" (state registration number: 0120-128-07-98), the basic part of the state order of the Ministry of Science and Education of the Russian Federation (FSRG-2020-0016) and with the financial support of the RFBR grant (No. 18-05-60035_Arktika).

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ESTIMATION OF THE MITOCHONDRIA MEMBRANE POTENTIAL OF GRANULOCYTES IN CHILDREN WITH CHRONIC INFLAMMATORY DISEASES OF THE LUNGS

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The article presents a study of the features of the mitochondrial membrane potential (MPM) of granulocytes in 72 children with congenital lung malformations (CLM). There was a 5-fold increase in the proportion of cells with reduced granulocyte MPM(ΔΨm) compared to the control group. During periods of exacerbation of the disease, the number of granulocytes with reduced potential increased by 8 times. This was accompanied by an increase in luminol-and lucigenin-dependent chemiluminescence of whole blood phagocytes spontaneous, a significant decrease in the I-lum and I-luc stimulation indices by 30 and 25%. Negative dynamics of the stimulation index indicates the depletion of reserve capabilities of the phagocytic system in children of the study group. There are statistically significant direct correlations between the level of granulocytes with reduced MPM(ΔΨm) and indicators of phagocytic activity of leukocytes.

Key words: children, congenital malformations of the lungs, neutrophilic granulocytes, mitochondrial membrane potential , oxidative metabolism of granulocytes, phagocytic activity.

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Introduction. The pathogenesis of most chronic non-specific lung diseases (CNSLD) is based on a long-term inflammatory process that forms in the structurally altered tissue of the lungs and bronchi due to congenital malformations of the lungs (CLM). One of the key positions in the immune response belongs to phagocytosis, which today is considered not only as a tool of anti-infection immunity, but also as a universal homeostasis effector that responds to numerous signals about destabilization of the internal environment of the body [5]. The constancy of immune homeostasis as a whole depends on the adequate implementation of the physiological functions of neutrophilic granulocytes (NG) [13]. During the inflammatory process in the lungs, when alveolar macrophages do not have time to control invading pathogens, resting neutrophils are attracted to the affected area of the lungs, which are activated and absorb microorganisms through phagocytosis due to a combination of the production of toxic oxygen radicals, proteolytic enzymes and other bactericidal peptides. The degree of neutrophil activation, generation of reactive oxygen species (ROS), and release of granule proteins play a key role in the clearance of microbial pathogens [15]. NG do not live long and in normal conditions, after performing their functions in the inflammatory focus, neutrophils die. Thanks to apoptosis, it is possible to prevent the release of cytotoxic contents of neutrophils into the surrounding tissues and timely eliminate the dying cells through tissue macrophages. Therefore, neutrophil apoptosis can be considered as one of the mechanisms for controlling inflammatory responses. Violations of the mechanisms that regulate apoptosis of immune cells can be a central pathogenetic factor in the initiation and (or) exacerbation of various inflammatory processes and persistence of inflammation [2, 4, 5].

One of the ways to violate the mechanisms of neutrophil apoptosis is to decrease the mitochondrial membrane potential (MMP, ΔΨm)) [10]. It was assumed that neutrophils do not contain at all or contain an insignificant number of mitochondria that do not play an active role in their life, but it was shown that neutrophil mitochondria, despite their limited number, are actually involved in apoptosis [2, 14].

Maintenance of the membrane potential serves as an indicator of the level of metabolic activity of cells and a drop in the value of the mitochondrial membrane potential is one of the main indicators of the initiation of the mitochondrial pathway for triggering apoptosis [6, 11]. During inflammation, the life span of neutrophils increases or neutrophil death is delayed to fight infection and inflammation. However, on the other hand, delayed apoptosis of neutrophils can lead to an exacerbation of the inflammatory process [15]. In this regard, it is relevant to study the mitochondrial membrane potential

of granulocytes in children with CPL as one of the most important mechanisms for the development of an inflammatory response that provides effective antimicrobial protection of the body in CNSLD.

The purpose of this study was to study the features of the mitochondrial membrane potential of granulocyte in children with CLM.

Materials and research methods. The survey of 72 children with CLM in the clinic Institute of Maternity and Childhood Protection. The patients underwent complete clinical examination, morphological test, bronchoscopy/bronchography, and spiral computed tomography. The nosological unit of the defect sections Q32-34 ICD-10 was considered to be the criterion for the inclusion of a case in the study group. There were 38 children (52.8%) in prolonged remission, 34 patients (47.2%) suffered from frequent inflammatory bronchopulmonary diseases. The average age of children was 8.2±0.54 years, of which 32 girls (44.4%) and 40 boys (55.6%). The controls were indicators of 23 healthy children, comparable by sex and age. The study was conducted under the principles of the current revision of the Declaration Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October, 2013) and approved by the Ethics Committee, Research Institute of Mother and Child Health Care.

The mitochondrial membrane potential (ΔΨm) was determined on the BD FACS Calibur cytometer (USA) in the Cell Quest Pro program in heparinized blood using JC-1 dye (5.5 ', 6.6'-tetrachloro-1.1', 3.3 'tetraethylbenzimidazole carbocanine iodide / chloride) (Bector Dikcenson, USA). JC-1 is a cationic dye, the absorption of which by mitochondria is directly related to the size of the mitochondrial membrane potential [7].

The study of luminol-and lucigenin-dependent of whole blood phagocytes was carried out using the method chemiluminescence method (CML) [9], registering the light sum of spontaneous (S-lum, S-luc) and phagocytosis-induced opsonized zymosan CML (S-lunv+zym, S-luc+zym). CML was registered using a luminescent spectrometer PERKIN ELMER LS 50B.

The functional and metabolic activity of neutrophils was evaluated according to the generally accepted method, determining the phagocytic activity of neutrophils and number of phagocytes neutrophils , and the ability of a cell to complete phagocytosis was evaluated in a spontaneous and pyrogenal stimulated nitro-blue tetrazolium (NBT) test.

Statistical calculations were performed using Statistica 10.0 (Statsoft, Inc., USA). Data were tested for normality and are expressed as mean, standard deviation. When evaluating the entire population, mean values (M) and standard deviation (m) were calculated. The statistical significance was evaluated using Student's T-test. The study of the relationship of the determined characteristics was carried out using the Spearman correlation coefficient. The statistical hypotheses test showed the significance level of $p<0.05$.

Results and discussion. The results of the study showed that the development of an acute infectious process in only 44.5% of children with CLM was accompanied by an increase in the number of leukocytes to $7.93\pm0.49*10^9$/l, in 22.2% of children the number of leukocytes did not exceed $5.5*10^9$/l, that is, active inflammatory process in the lungs did not activate the phagocytosis system in them, and it is known that maintaining the corresponding number of neutrophils and neutrophil homeostasis is significant in the conditions of inflammation [15] .

21 patients (29.2%) had a high level (more than 10%) of granulocytes with reduced MPM. In children with CLM (Fig. 1) revealed a 5-fold increase in the proportion of cells with reduced granulocyte MPM (10.9±2.4% and 2.2±0.4%, respectively, $p<0.001$), which may lead to neutropenia [5].

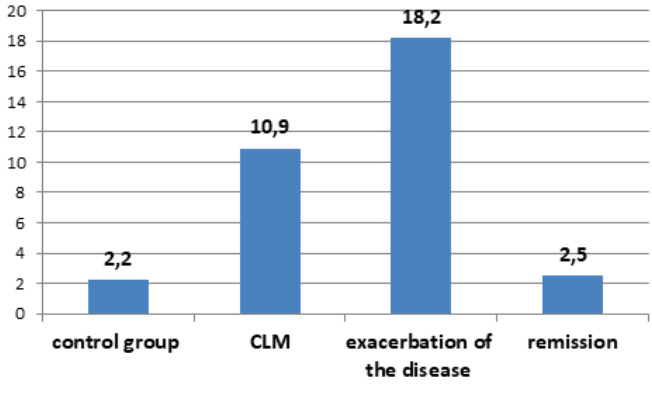
The changes resulted from period of the disease: if during the long-term remission the level of cells with reduced MPM corresponded to the control group (2.5±0.5%), then during periods of relapse of inflammation, the number of granulocytes with reduced potential increased to 18.2±4.2% ($p<0.001$). Moreover, if the exacerbation of the disease was accompanied by clinical symptoms of pneumonia, these changes were more pronounced (22.9±5.2%) than in children, when the exacerbation of the disease proceeded only with impaired bronchial patency (9.7±2.7%, $p <0.05$).

In other words, the number of granulocytes reflecting the processes of initiation of the mitochondrial pathway for triggering apoptosis increases significantly in the group of children with VPR during the inflammatory process in the lungs. Apparently, in the conditions of a chronic inflammatory state, cell-specific apoptosis is stimulated, when inflammatory mediators lead to an increase in the lifetime of neutrophils [12]. With adequate regulation of apoptosis, it supports tissue homeostasis. However, if the mechanisms of cell death are violated, it can lead to immunodeficiency, and an increase in the rate of apoptosis can lead to tissue destruction [1, 15].

According to the authors, mitochondrial dysfunction is accompanied by increased formation of reactive oxygen species [3]. Taking into account the significant changes in the MPM of peripheral granulocytes in children with VPR, we evaluated the functional activity of neutrophils (table 1). Indicators of oxidative metabolism of whole blood phagocytes demonstrated that the values of S-luc (1.1±0.07 Rel. units) and S-lum (1.25±0.09 Rel. units) in children with CPR in the period of exacerbation exceeded similar indicators in children in the period of remission (0.58±0.07 Rel.units, and 0.59±0.07 Rel. units, respectively, $p<0.001$).

The values of the light sum induced by CML zymosan: S-luc+zym (5.45±0.23 Rel. units) and S-lum+zym (6.25±0.22 Rel.units) in children with exacerbation exceeded by 2 times the values of the remission period (3.07±0.11 Rel. units and 4.18±0.15 Rel.units, respectively, $p<0.001$). Both spontaneous and stimulated chemiluminescence of phagocytes was increased in cnzl, but the I-lum and I-luc stimulation indices were significantly reduced in comparison with the control values by 30 and 25%.

The determination of the phagocytic activity of peripheral blood leukocytes is an important diagnostic criterion. The functional state of phagocytic defense factors was characterized by the tension of the redox reactions of neutrophils, the increase in the spontaneous NBT test in patients with exacerbation was significantly increased compared with the



The percentage of granulocytes with reduced membrane potential (%) in children with CLM

Indicators of functional activity of granulocytes in children at different periods of the disease, (M±m)

Indicators	control group	CLM – exacerbation	CLM – remission
Sluc, rel.units	0.21±0.02ΔΔ,##	1.11±0.07**,##	0.58±0.02**,ΔΔ
Sluc+Z, el.units	2.02±0.10ΔΔ,##	5.45±0.23**,##	3.06±0.11**,ΔΔ
K-luc, rel.units	9.42±0.39ΔΔ,##	4.91±0.22**,##	6.05±0.28**,ΔΔ
Slum, rel.units	0.26±0.01ΔΔ,##	1.25±0.09**,##	0.59±0.03**,ΔΔ
Slum+Z, rel.units	2.67±0.11ΔΔ,##	6.25±0.22**,##	4.18±0.15**,ΔΔ
K-lum, rel.units	10.18±0.47ΔΔ,##	5.00±0.24**,##	7.03±0.19**,ΔΔ
NBT sp., st. units	17.87±0.92ΔΔ,#	38.72±2.83**	27.89±4.92*
NBT st., st. units	24.44±1.10ΔΔ,##	42.05±2.48**	34.78±2.80**
Number of phagocytes neutrophils sp., units	11.46±0.95	12.49±1.42#	9.13±0.80Δ
Number of phagocytes neutrophils st, cond. Units	13.03±1.40Δ	9.27±0.58*,#	11.83±1.00*,Δ
Phagocytic activity of neutrophils sp., %	51.16±0.89Δ	34.50±7.33 *,#	53.32±3.71*,Δ
Phagocytic activity of neutrophils st., %	53.55±3.62Δ,##	41.29±4.93*,##	71.93±1.01**,ΔΔ

Note: * - p<0.05 in relation to the "control" group. ** - p<0.001 in relation to the "control" group. Δ - p<0.05 in relation to the group "CLM - exacerbation". ΔΔ - p<0.001 in relation to the group "CLM - exacerbation". # - p<0.05 in relation to the group " CLM remission" ## - p<0.001 in relation to the group " CLM remission"

control (38.72±2.83 standard units and 17.87±0.92 standard units, respectively, p<0.001). However, with additional stimulation of neutrophils, a 2-fold decrease in the coefficient of stimulation of NBT was determined (3.33±0.48 and 6.57±0.09, respectively, p<0.001). According to the pyrogenal-stimulated NBT test, inhibition of oxygen-dependent phagocytic cell metabolism was determined in 50% of children (average values of stimulation indices are lower than 1.0). In addition, in the period of exacerbation of the disease, the lowest indicators of phagocytic activity of cells were determined.

In patients with inflammatory processes in the lungs against the background of CLM, there is suppression of the phagocytic activity of neutrophils to 34.5±7.3%, against 51.2±0.9% in control and 53.3±3.7% in remission (p<0.05), a decrease in the absorption function of neutrophils in stimulated tests. The inhibition of the absorption function of phagocytes in the stimulated test and the absence of prodigiosan-induced response were determined. The average values of number of phagocytes neutrophils in the period of exacerbation were 12.5±1.4 units, in children in remission-9.1±0.8 units (p<0.05), stimulated number of phagocytes neutrophils -9.3±0.6 units and 11.8±1.0 units accordingly (p<0.05), which may cause a violation of the formation of an anti-infectious response and lead to the development of clinical signs of the disease. The correlation analysis revealed statistically significant direct relationships between the level of granulocytes with reduced MPM and the indicators of the phagocytic activity of neutrophils r=0.361 (p<0.05) and number of phagocytes r=0.397 (p<0.05).

Thus, the changes in the level of granulocytes with reduced mitochondrial membrane potential were revealed in children with CLM. This was accompanied by an increase in oxidative metabolism of phagocytes of whole blood in the period of exacerbation that was a reaction to the inflammation, however, the decrease in stimulation index I-lum I-luc, NBT-test, the phagocytic activity of neutrophils points to the depletion of the reserve capacity of the phagocytic system in children from the studied group and may be an important pathogenetic link in diseases of the respiratory system, causing constriction and inflammation of bronchi, destruction of lung parenchyma.

Conclusion. In the children with CLM the disease is accompanied by higher percentage of granulocytes in the peripheral blood with a reduced mitochondrial membrane potential, which indicates the activation of the mitochondrial pathway of apoptosis. The mitochondrial dysfunction of granulocytes affects their functional properties, reduces the intensity of phagocytic reactions, leads to depletion of adaptive reserves of immunocompetent blood cells and can lead to increased susceptibility to viral-bacterial bacterial infection and frequent exacerbations of the disease.

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SMOKING AND ITS INFLUENCE ON THE LEVEL OF ONCOMARKERS IN BLOOD SERUM OF THE POPULATION OF THE REPUBLIC OF SAKHA (YAKUTIA)

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Our results indicate that smoking stimulates the expression of tumor markers in the serum of smokers. The level of tumor markers increases with the increase in smoking history. In the body of smokers, the concentration of tumor markers increases at a young and middle age, rather than in the elderly. The decrease in the indicators of tumor markers in old age is explained by the natural premature dropout of smokers from the population.

Keywords: smoking, tumor markers, cancer-embryonic antigen (CEA), alpha-fetoprotein (AFP), prostate specific antigen (PSA), ovarian tumor marker (CA125).

Introduction. Smoking is a risk factor for many chronic diseases such as chronic obstructive pulmonary disease, hypertension, cardiovascular disease, atherosclerosis, diabetes, cancer and microbial infections (respiratory tract infections, bacterial meningitis), etc. [4, 5]. According to the World Health Organization (WHO), more than 8 million people die annually from tobacco-related diseases, of which more than 7 million are smokers and more than 1.2 million are passive smokers (non-smokers) [27]. Tobacco smoke contains about 4,000 known chemicals; 250 of them are known to be harmful to health and more than 50 cause cancer in humans [23].

There is sufficient evidence of the involvement of smoking in the development of the following cancers: lung [13, 16], oral cavity [7,10], pharynx [18,25], larynx [4,7], esophagus [5, 8], nasal cavity and nasal sinuses [7,18], stomach [5,8], liver [20], kidney [24], cervix [15], etc. Smoking is especially dangerous at a young age, because addiction develops very quickly (cravings, withdrawal symptoms). It has been proven that nicotine contained in tobacco products causes addiction symptoms. Analysis of the sources has shown a directly proportional relationship between the age of onset and the duration of smoking [16]. It should be noted that the World Health Organization and the American Psychiatric Association classify nicotine addiction as a “substance use disorder” [27].

According to various researchers, stopping tobacco use reduces the risk of developing cancer and increases the life expectancy of individuals [13, 14]. Smoking electronic cigarettes, pipes, hookahs and cigars can also cause lung cancer, but the highest risk of developing carcinogenic diseases is caused by cigarette smoking, since it is the most widespread form of tobacco use in the world [16,19]. In developed countries, long-term programs aimed at reducing the number of smokers contribute to a decrease in mortality from tobacco smoking [9,12,15].

According to the sources, researchers have noted a significant increase of tumor markers in the blood serum of patients with cancer: carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), prostate-specific antigen (PSA) and ovarian tumor marker (CA125) [3,26]. An increase in cancer markers among smokers has been noted [26, 28].

The aim of this study is to assess the level of tumor markers in smokers and nonsmokers in the Republic of Sakha (Yakutia), depending on age.

Material and Research Methods. This work was carried out within the framework of the research: "Epidemiological aspects of malignant tumors in the Far North, development of modern methods of early diagnosis and prevention with the usage of highly informative fundamental research methods" in the Department of Adaptation Mechanisms Research, Yakutsk Scientific Center for Complex Medical Problems. We examined 175 residentsof Megino-Khangalass district, aged 22 to 66, of which 83 were

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smokers and 92 were not. The study did not include people suffering from cancer, precancerous conditions and with exacerbation of chronic diseases. The surveyed were divided into age groups according to the classification adopted by the WHO Regional Office for Europe (Kiev, 1963). A questionnaire survey of all subjects was carried out according to a standard questionnaire for assessing the quality of life, modified by the laboratory of medical and social research of the Yakutsk Scientific Center for Complex Medical Problems. The study was approved by the ethics committee (No. 49 dated March 25, 2018).

The study material was blood taken on an empty stomach from the cubital vein. Identification of tumor markers in blood serum: carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), prostate-specific antigen (PSA) and ovarian tumor marker (CA125) was carried out by enzyme-linked immunosorbent assay (ELISA) using test systems (Vector-Best, Russia) , on a Multiskan FC device (Thermo Scientific, USA).

Statistical processing of the obtained data was performed using the IBM SPSS Statistics 19 applied statistical software package The Kolmogorov–Smirnov test was used to analyze the normality of the trait distribution. In cases where the distribution differed from normal, nonparametric statistical methods were used to determine the median of the trait in the groups and its quartile range of 25 and 75%. Differences were considered significant at the achieved level of statistical significance p <0.05.

Results and Discussion. The values of tumor marker levels in the blood serum of residents of the Republic of Sakha (Yakutia) were as follows: CEA - 2.83 (2.15-3.54) ng / ml; AFP - 3.60 (2.08-6.24) IU / ml; PSA - 0.07 (0.03-0.12) ng / ml; CA125 - 3.70 (1.57-6.69) U / ml. The content of tumor markers was within the reference values. The reference values corresponded to: CEA 0-5 ng / ml; AFP 0-10 IU / ml; PSA 0.3-4.0 ng / ml; CA125 0-35 U / ml.

Among smokers, there is a significant increase in the CEA level by 8.60% (Table 1). There are conflicting results in the literature, for example, studies [15] showed a 7.9% decrease in serum PSA levels in smokers and 12.2% in those who quit, compared with never nonsmoking people. In the study [17], on the contrary, an increase in the concentration of PSA in the blood serum of smokers was noted. The average value of the CA125 tumor marker in our group of women who smoked was significantly more by 27,40% in comparison with nonsmokers.

Researchers have found that the concentration of tumor markers depends on age [12,18,22]. The CEA value in young people was significantly lower than in middle and old aged people, by 17,20% and 14,57% respectively. At the same time, the levels of tumor markers AFP, CA125 and PSA tended to decrease depending on age. (Table 2)

In the group of nonsmoking residents of Yakutia, depending on age, the level of CEA significantly increases when comparing the young population with residents of middle and old age by 19.31% and 17.89%, respectively. The CA125 level, on the contrary, decreases when comparing young women with the elderly by 51.17%. The PSA value in men tended to increase depending on age, but we did not find statistically significant differences (Table 3).

According to the questionnaire data, the duration of smoking of young respondents was 15.00 (10.00-20.00) years, of middle aged respondents - 30.00 (24.50-37.00) years and of the elderly - 35.50 (17.5-45.00) years. At the same time, the intensity of smoking (the number of cigarettes per day) was for young people - 10.00 (8.50-15.00) pcs., for middle aged people - 15.00 (10.00-20-00) pcs. and for the elderly - 20 .00 (13.75-28.75) pcs.. Our correlation analysis showed that in all age groups, with an increase in smoking duration, the intensity of smoking increases as well, as evidenced by positive correlation coefficients (young age r = 0.435 (p = 0.05); middle age r = 0.305 (p = 0.05); old age r = 0.441).

The concentration of tumor markers in a group of smokers also depends on age (Table 4). A significant increase in CEA was noted by us in the group of middle-aged people in comparison with young people, which is consistent with the sources [1,11].

When comparing smokers with nonsmokers by age, we noted a significantly high CEA value in middle-aged smokers - 21.2% (p = 0.050). Our results are consistent with those of other studies such

Table1

Concentration of tumor markers in smokers and nonsmokers

Age	CEA(ng/ml)	AFP(ME/ml)	PSA(ng/ml)	CA125(U/ml)
Smokers	3.02 (2.22-3.81)	3.07 (1.94-4.87)	0.07 (0.03-0.12)	5.03 (1.23-8.11)
Nonsmokers	2.76 (2.09-3.40)	3.71 (2.14-5.30)	0.07 (0.05-0.17)	3.65 (1.45-6.30)
p 1-2	0.083	0.041	0.542	0.370

Примечание. В табл.1-4 единицы измерения РЭА, ПСА – нг/мл, АФП – МЕ/мл, СА – ЕД/мл.

Table2

The level of tumor markers depending on age

Age	CEA(ng/ml)	AFP(ME/ml)	PSA(ng/ml)	CA125(U/ml)
Young	2.52 (1.61-3.11)	3.73 (2.20-5.21)	0.08 (0.03-0.06)	4.72 (2.02-9.76)
Middle aged	3.04 (2.42-3.95)	3.32 (1.98-5.37)	0.06 (0.03-0.08)	3.74 (1.59-6.50)
Elderly	2.95 (2.02-3.54)	3.21 (1.80-4.45)	0.07 (0.02-0.13)	2.07 (1.10-4.44)
p 1-2	**0.000**	0.900	0.096	0.101
p 1-3	**0.028**	0.348	0.070	0.128
p 2-3	0.184	0.418	0.385	0.536

Table3

The content of tumor markers in nonsmokers depending on age

Age	CEA(ng/ml)	AFP(ME/ml)	PSA(ng/ml)	CA125(U/ml)
Young	2.34 (1.45-2.97)	4.15 (2.49-5.76)	0.02 (0.01-0.05)	4.24 (2.07-9.08)
Middle aged	2.90 (2.41-3.44)	3.39 (1.96-5.29)	0.06 (0.06-0.08)	3.89 (1.69-6.06)
Elderly	2.85 (2.07-3.61)	3.25 (2.28-5.08)	0.11 (0.07-0.13)	2.07 (0.69-3.65)
p 1-2	**0.004**	0.289	0.032	0.274
p 1-3	**0.017**	0.257	0.164	**0.030**
p 2-3	0.686	0.937	0.221	0.095

as [16] who found that CEA level in blood serum was significantly higher in smokers than in nonsmokers. It should be noted that CEA is known as a nonspecific marker indicating the development of a large list of cancers: pancreatic carcinoma [18], uterine cancer [15], lung cancer [13], breast cancer [14], etc.

The CA125 level tended to increase in the group of young residents, although in the older age groups (middle and elderly) its values were lower compared to nonsmokers. Perhaps the increase in CA125 at a young age is explained by the body's response to the toxic effects of tobacco. At the same time, with an increase in smoking experience, the concentration of CA125 decreases, as evidenced by a negative correlation coefficient (r = -0.191). Our results are consistent with the literature [26], according to the authors, smoking can reduce the concentration of CA125, reducing the level of endogenous estrogen in the body of women. In addition, the level of CA125 may decrease due to the fact that cigarette smoke damages the epithelium of the respiratory tract, which expresses this tumor marker. [6]

of smokers. Our results are consistent with the sources [1,21].

To prevent smoking-related diseases, many researchers suggest quitting tobacco use as early as possible. To combat smoking, developed countries have adopted laws restricting tobacco advertising, establishing age limits on buying and consuming tobacco products and organized special zones for smoking, thanks to the measures taken, mortality from tobacco smoking has reduced [2, 12, 14].

Meanwhile, modern studies have shown that there is a relationship between changes in the activity of the cytochrome P450 enzyme encoded by the CYP2A6 gene and the level of nicotine addiction. The enzyme cytochrome P450 plays a key role in nicotine catabolism; mutations in this gene affect its activity. People with a slower metabolism of nicotine tend to have lower levels of nicotine addiction, and therefore are able to quit using tobacco products relatively more easily [14].

According to some researchers, stopping the consumption of tobacco products normalizes the level of tumor markers, reduces the risk of developing cancer and increases life expectancy [16,17].

Table4

The content of tumor markers in smokers depending on age

Age	CEA(ng/ml)	AFP(ME/ml)	PSA(ng/ml)	CA125(U/ml)
Young	2.60 (2.19-3.26)	3.07 (1.97-4.26)	0.08 (0.03-0.06)	6.90 (1.67-11.50)
Middle aged	3.68 (2.47-4.67)	3.18 (1.94-6.87)	0.06 (0.03-0.08)	2.82 (1.10-6.80)
Elderly	3.17 (1.90-4.05)	3.13 (0.98-5.43)	0.02 (0.01-0.11)	1.82 (1.31-3.82)
p 1-2	**0.005**	0.725	0.481	0.132
p 1-3	0.429	0.070	0.274	0.885
p 2-3	0.293	0.170	0.361	0.487

In nonsmokers, we observed a tendency to an increase in PSA levels depending on age, and in smokers, on the contrary, to a decrease. At a young age, the PSA level in smokers was significantly higher by 4 times (p = 0.021), and in the elderly it was 5.5 times lower. At young and middle ages, we noted positive correlation coefficients with smoking duration and smoking intensity. Moreover, reliable values of the correlation coefficients were noted by us in the groups of young people - smoking duration and CEA content r = 0.337 (p = 0.001); middle aged people - smoking duration and CEA concentration r = 0.385 (p = 0.050), smoking duration and AFP level r = 0.265 (p = 0.050). Smoking duration probably has a greater influence on changes in tumor markers than the number of cigarettes smoked per day in various groups

Thus, our results indicate that smoking stimulates the expression of tumor markers in the blood serum of smokers. The level of tumor markers increases with the increase in smoking duration. In smokers, the concentration of tumor markers increases at a young and middle age rather than in the old age. The decline in tumor markers in the elderly can probably be explained by the natural, premature mortality of smokers in the population.

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CIRCULATING LEVELS OF LEPTIN IN BLOOD OF YOUNG YAKUTS DEPENDING ON BODY MASS INDEX

Currently, adipose tissue is considered an endocrine organ that produces several hormonally active substances – adipokines, including a leptin hormone. This hormone plays a key role in regulating energy metabolism and body weight. Several studies have shown heterogeneity of leptin levels from the ethnic origin and the environment. It is possible that in indigenous populations living in extremely low climatic conditions, leptin levels may affect the development of diseases associated with impaired fat metabolism. In this regard, the purpose of this study is to assess the relationship of leptin circulating in the blood serum with body mass index groups in healthy Yakuts. The present study included 281 Yakuts (186 women and 95 men) with an average age of 19±2 years. The sample was divided into three groups according to the world health organization classification: underweight (n=37), normal (n=215), overweight/obesity (n=29). There were no statistically significant differences in body mass indices between men and women ($p>0.05$). As a result of comparative analysis, it was found that in women and men, leptin levels are statistically higher in individuals with overweight/obese ($p<0.01$), and in individuals with underweight and normal body weight, leptin levels do not statistically differ from each other in both women and men ($p>0.05$). Our results confirm that Yakuts with overweight/obesity body weight have an increased level of leptin against the background of leptin resistance. It is also possible that Yakuts with an underweight to protect the body from low temperatures develop a larger percentage of active brown adipose tissue and the level of leptin in the blood reaches equal values as in Yakuts with normal weight.

Keywords: leptin, underweight, overweight, obesity, adipose tissue, Yakut population.

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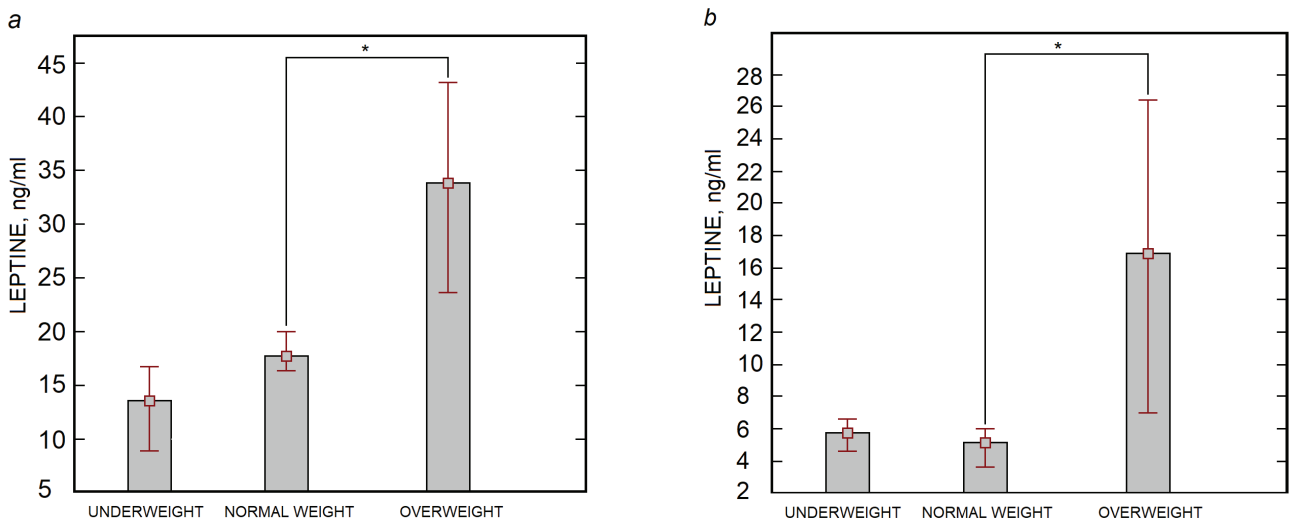
Introduction. Currently, almost one in three people suffer from at least one form of malnutrition: underweight, overweight, obesity, and non-communicable weight-related diseases [30]. Recently, the incidence of overweight, obesity, and type 2 diabetes has been particularly increasing among indigenous people living in circumpolar regions [7]. Thus, in the Republic of Sakha (Yakutia), the prevalence of obesity among Yakuts, Evenks, and evens living in the Olekminsky, Tomponsky, Gorny, and Zhigansky districts was studied. A total of 1,566 people (968 women, 598 men) was examined. As a result of this study, it was found that women (19%) are twice as obese as men (10%) [2, 3, 6]. The same data on the increased prevalence of obesity among women was found for other indigenous peoples of the North, such as the Sami (women 30%, men 23%), the Kven (women 26%, men 18%) [12] and the Inuit (women 25.5%, men 15.8%) [21].

The development of obesity is manifested by excessive accumulation of adipose tissue, which has recently been recognized as an endocrine organ that produces hormonal active substances – adipokines [13]. Leptin was the first adipokine discovered [9] and its main function is to participate in the regulation of energy metabolism and body weight [13]. The maximum amount of leptin is produced by white adipose tissue [18]. Therefore, it is believed that the levels of leptin circulating in the blood are directly proportional to the amount of adipose tissue in the body [24, 26]. Several studies have shown heterogeneity of leptin levels depending on ethnic origin and the environment [16, 17, 27]. It is possible that in indigenous populations living in extremely low climatic conditions, leptin levels may affect the development of diseases associated with impaired fat metabolism. In this regard, this study aims to assess the dependence of serum leptin levels on body mass index (BMI) in healthy Yakuts aged 18 to 30 years.

Materials and Methods. The present study included 281 Yakuts (186 women and 95 men) with an average age of 19.8±1.5 years. All participants were ethnic Yakuts who were healthy at the time of the study and passed a questionnaire indicating their gender, nationality, and age. The sample was made up of individuals who did not complain about their health status and were not registered in a dispensary for chronic diseases. All subjects gave written informed consent to participate in the study and to process their personal data. This work was approved by the local ethical Committee on biomedical ethics at the YNC KMP (Yakutsk, Protocol № 16 of December 13, 2014).

Average BMI values for each group divided by gender

UNDERWEIGHT(n=37)		U-критерий Манна Уитни	NORMAL WEIGHT (n=215)		t-критерий Стьюдента	OVERWEIGHT/OBESITY (n=29)		U-критерий Манна Уитни
WOMEN (n=26)	MEN (n=11)		MEN (n=11))	MEN (n=71)		WOMEN (n=16)	MEN (n=13)	
17.46±0.72	17.39+0.91	U_{test}=138; p>0.05	21.42±1.62	21.96±1.9	p>0.05	27.56±2.88	26.64±1.49	U_{test}=90; p>0.05



Levels of leptin, depending on the categories of BMI: a – female, b – male. Note: * – statistically significant difference

Venous blood for the study was collected in the morning after a 12-hour fast in all participants. Anthropometric indicators (body weight in kilograms, height in centimeters) were determined for all participants using standardized methods. Body mass index (BMI) was calculated by dividing body mass by the square of height. To determine the level of leptin circulating in the blood, an enzyme immunoassay kit "Leptin ELIS Akit"(Diagnostics Biochem Canada Inc., Canada) was used. The leptin concentration in the samples was measured at a wavelength of 450 nm in a VICTORX5 Multimode Plate Reader (Perkin Elmer Inc., USA).

Statistical analysis. The results were analyzed using a computer program for statistical data processing SPSS 18.0 (SPSS: An IBM Company, USA). p≤0.05 were considered statistically significant. All results were expressed as standard deviation (±). Anthropometric and hormonal characteristics between women and men were compared using the student's t-test. The Association of BMI with leptin levels was evaluated using multiple regression analysis.

Results. To assess the dependence of serum leptin levels on BMI, leptin concentrations were determined using enzyme immunoassay and BMI was calculated in a total sample of 281 people. The sample was divided into three groups according to the who classification [14, 29]: underweight (≤18.49 kg/m^2), normal weight (18.5-24.99 kg/m^2), overweight/obesity (≥25 kg/m^2) (Table 1). There were no statistically significant differences in BMI between men and women in the groups (p>0.05).

A comparative analysis of leptin levels was performed in individuals with normal BMI compared to individuals with underweight and overweight/obese (Figure 1). As a result, it was found that in comparison with the norm in both women and men, leptin levels are statistically higher in individuals with overweight/obese (p<0.01). There were no statistically significant differences in leptin levels (p>0.05) between the groups of individuals with a body mass deficit and the norm in both women and men.

Discussion. In the Yakut population, levels of leptin circulating in the blood of individuals over the age of 18 have previously been determined in several studies [5, 8]. In a study by Golderova et al., [5] it was shown that patients with unstable angina had significantly higher leptin levels compared to patients with stable angina. Klimova et al., [8] investigated associations of allelic variants of rs1799883 (*FABP2*) with metabolic syndrome and its components, including leptin, in a population sample of Yakuts. It was shown that the leptin level did not depend on the genotypes of the ALA54THR polymorphism of the *FABP2* gene [8]. In addition, we have recently published data on the dependence of leptin levels in young Yakuts depending on gender and body weight [1].

In this study, we evaluated for the first time the relationship of serum leptin levels with BMI groups (underweight, normal, overweight/obesity) in healthy Yakuts aged 18 to 30 years. We found that the level of leptin in Yakuts with overweight/obesity is significantly higher than in Yakuts with normal weight. It is believed that people suffering from obesity have leptin resistance, an overabundance of leptin against the background of immunity to its action [10, 20]. Thus, our results for the Yakut population are consistent with previous studies on other samples and confirm that overweight/obese individuals also have increased leptin levels against the background of leptin resistance.

Attention is drawn to the fact that in Yakuts with an underweight and normal weight, leptin levels in the blood serum do not statistically differ from each other. Several previous studies have found that people suffering from anorexia nervosa and eating disorders have significantly lower levels of leptin in their blood than healthy people [22, 23, 25, 28]. However, low leptin levels in underweight individuals are thought to signal a potentially

dangerous lack of energy resources to the brain [19]. Thus, in a study by Leonardo et al., [15] it was shown that the indigenous populations of Siberia have increased levels of energy metabolism and basal metabolism to protect the body from low temperatures. In addition, our recent study showed that men from the Northern regions recorded higher levels of leptin (10.03 ng/ml) than men from the southern regions (4.73 ng/ml) (p=0.00001) [27]. Although leptin is secreted mainly by adipocytes of white adipose tissue, in a study by Margetic et al., [18] it was shown that adipocytes of brown adipose tissue can also secrete it. In 2015, in Yakutia [4], for the first time in the world, a case of finding brown adipose tissue in an adult who was exposed to low temperatures most of the time was histologically confirmed. This indicates that people living in cold climates have active brown adipose tissue [4]. In a study by van Marken Lichtenbelt et al., [11] it was shown that in lean people, the number and activity of brown adipose tissue was 4 times higher compared to people who were overweight or obesity. Thus, it is possible that Yakut population with an underweight to protect the body from low temperatures develop a higher percentage of active brown adipose tissue and the level of leptin in the blood reaches the same values as in individuals with normal weight.

Conclusion. 1. In the Yakut population, overweight and obese individuals, both women and men, have higher leptin levels than normal weight individuals.

2. In individuals with low birth weight the levels of leptin in the serum were the same compared to the norm. Perhaps this is due to the protection of the body from low temperatures through the development of a greater number of active brown adipose tissue which secretes leptin additional.

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ASSOCIATION ANALYSIS OF AMINOXIDASE 1 AOC1 AND HISTAMINE-N-METHYLTRANSFERASE HNMT GENE POLYMORPHISMS WITH THE DEVELOPMENT OF ASTHMA IN CHILDREN

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Asthma is a common multifactorial disease characterized by chronic inflammation of the respiratory tract, and respiratory symptoms such as wheezing, shortness of breath (dyspnea), coughing, that vary over time and intensity. The main goal of asthma management is to achieve and maintain clinical control of the disease over a long period of time, considering the safety of therapy and potential adverse reactions. At present, total asthma control is achieved in less than half of patients, and 10-20% of patients show signs of therapeutic resistance to certain groups of drugs. Several studies have revealed that heredity has a significant effect on the individual sensitivity to asthma therapy. In this regard, it is actual to study the genes involved in the metabolism of major groups of drugs used to asthma treatment. Antihistamines are frequently prescribed in the treatment of allergic diseases, it blocks the binding of histamine to its receptors by the mechanism of competitive inhibition, it has antipruritic, antiedema, antispasmodic and local anesthetic effect.

The aim of this work was to analyze associations of polymorphic variants of amine oxidase, copper-containing 1 *AOC1* and histamine N-methyltransferase *HNMT* genes involved in the histamine metabolism with the development of asthma in children living in the Republic of Bashkortostan. **Material and methods.** DNA samples of 430 unrelated individuals aged 2-17 years living in the Republic of Bashkortostan were used as the study material. Genotyping of polymorphic variants is carried out by PCR-RFLP method. **Results.** The associations of rs1049793**CC* genotype and rs1049793**C* allele of the *AOC1* gene with asthma development, with significant decreases in spirometry measures in Russians were revealed. The associations of rs1801105**CT* genotype and rs1801105**T* allele of the *HNMT* gene with significant decrease of MEF25 in asthma patients of Tatar ethnicity were established. **Conclusion.** The results of the study suggest that *AOC1* and *HNMT* polymorphic variants are involved in asthma development.

Keywords: bronchial asthma, polymorphic variant, association, aminoxidase 1 *AOC1* gene, histamine N-methyltransferase *HNMT* gene.

Introduction. Asthma is a severe heterogeneous disease characterized by chronic airway inflammation. The global prevalence of asthma is 1-18% [4]. Despite the rapid development of modern medicine in 10-20% of Russian patient's severe course of asthma with the signs of therapeutic resistance to various groups of drugs are diagnosed [2]. Genetic variability has a significant influence on the patient's sensitivity to the prescribed therapy [5]. Histamine is a biogenic amine that plays an important role in the development of inflammatory processes. The activation of histamine receptors in the lungs leads to bronchospasm and airway obstruction. Histamine is metabolized by two main enzymes, which are histamine-N-methyltransferase (HNMT) and aminoxidase (DAO, AOC1) [7]. Several studies have shown that polymorphic loci in the genes encoding histamine metabolizing enzymes are associated with allergic diseases [1, 7]. **The aim** of this work was to assess the significance of amiloride-sensitive amine oxidase *AOC1* and histamine-N-methyltransferase *HNMT* genetic polymorphisms involved in the histamine metabolism in the prediction of asthma developing in children of different ethnicities living in the Republic of Bashkortostan (RB).

Materials and methods. DNA samples of 430 unrelated individuals aged 2-17 years old from the RB were used in this work. The group of patients included

Table1

Distribution of allele and genotype frequencies of *AOC1* rs1049793 and *HNMT* rs1801105 gene polymorphisms in asthma patients and controls

Polymorphic variant / study group		Genotypes			Alleles		N
		n (%)	n (%)	n (%)	n (%)	n (%)	
AOC1 (rs1049793)		CC	CG	GG	C	G	
Patients	Russians	46 (56.1) p=0.009 OR=2.36 (1.24-4.5)	29 (35.37)	7 (8.54)	121 (73.78) p=0.01 OR=1.86 (1.16-3.01)	43 (26.22) p=0.01 OR=0.54 (0.33-0.87)	82
	Tatars	48 (44.86)	45 (42.06)	14 (13.08)	141 (65.89)	73 (34.11)	107
	Bashkirs	11 (25.0)	24 (54.55)	9 (20.45)	46 (52.27)	42 (47.73)	44
Controls	Russians	26 (35.14)	37 (50.0)	11 (14.86)	89 (60.14)	59 (39.86)	74
	Tatars	35 (43.21)	41 (50.62)	5 (6.17)	111 (68.52)	51 (31.48)	81
	Bashkirs	15 (41.67)	15 (41.67)	6 (16.67)	45 (62.50)	27 (37.5)	36
HNMT (rs1801105)		CC	CT	TT	C	T	
Patients	Russians	66 (78.57)	15 (17.86)	3 (3.57)	147 (87.5)	21 (21.15)	84
	Tatars	76 (72.38)	29 (27.62)	-	181 (86.19)	29 (13.81)	105
	Bashkirs	31 (70.45)	12 (27.27)	1 (2.27)	74 (84.09)	14 (15.91)	44
Controls	Russians	58 (78.38)	13 (17.57)	3 (4.05)	129 (87.16)	19 (12.84)	74
	Tatars	67 (81.71)	15 (18.29)	-	149 (90.85)	15 (9.15)	82
	Bashkirs	30 (83.33)	6 (16.67)	-	66 (91.67)	6 (8.33)	36

Note to tables 1-2. N is the number of individuals; n is the number of groups; allele and genotype frequencies are given in brackets, %; p is the p-value and is shown in the case of statistical significance only (p<0,05); OR is the odds ratio and 95% confidence interval (in brackets).

236 asthma patients (70 girls, 166 boys) of different ethnicities (Russians - 84, Tatars - 108, Bashkirs - 44). All investigated individuals were the patients of the children's division of the Clinic at Bashkir State Medical University of the Russian Ministry of Health (Ufa, Russia) and the Allergology Department of the Republican Children's Clinical Hospital (Ufa, Russia). The evaluation of the external respiration parameters was carried out on a computer spirograph "Erich Jaeger" (Germany). The control group comprised 194 practically healthy individuals (119 girls, 75 boys) of the corresponding ethnicity (Russians - 75, Tatars - 83, Bashkirs - 36) without bronchopulmonary and allergic diseases, with a low level of total immunoglobulin E (0-60 ME/ml). Children from 15 years old and parents of children under 15 years old gave informed consent to participate in the study. The study protocol was approved by the local bioethics committee of the Institute of Biochemistry and Genetics - Subdivision of the Ufa Federal Research Centre of the Russian Academy of Sciences (Protocol no 7 dated February 10, 2011).

Genomic DNA was isolated from the peripheral blood lymphocytes by phenol-chloroform extraction. The analysis of rs1049793 (p.1990C>G, p.His664Asp) polymorphism of the *AOC1* gene and

Table2

Distribution of allele and genotype frequencies of AOC1 rs1049793 and HNMT rs1801105 gene polymorphisms in asthma patients and control groups with different parameters of external respiration function

Polymorphic variant/ study group		Genotypes			Alleles		N
		n (%)	n (%)	n (%)	n (%)	n (%)	
AOC1 (rs1049793)		*CC*	*CG*	*GG*	*C*	*G*	
Asthma patients of Russian ethnicity	FEV1 > 78.1%	9(60)	5(33.33)	1 (6.67)	24(80)	6(20)	15
	FEV1 56.5-78.1%	3	-	4	6	8	7
	FEV1 < 56.5%	18(58.06) p=0.03, OR=2.56. (1.08-6.03)	11(35.48)	2(6.45)	47(75.81) p=0.03, OR=2.08. (1.07-4.05)	15(24.19)	31
	MEF25 > 71.7%	13(65)	6(30)	1(5)	32(80)	8(20)	20
	MEF25 37.7-71.7%	6(54.55)	4(36.36)	1(9.09)	16(72.73)	6(27.27)	11
	MEF25 < 37.7%	18(58.06) p=0.03, OR=2.56. (1.08-6.03)	11(35.48)	2(6.45)	47(75.81) p=0.03, OR=2.08. (1.07-4.05)	15(24.19)	31
Control group of Russian ethnicity		26 (35.14)	37 (50.0)	11 (14.86)	89 (60.14)	59 (39.86)	74
HNMT (rs1801105)		*CC*	*CT*	*TT*	*C*	*T*	
Asthma patients of Tatar ethnicity	MEF25 > 71.7	35(89.74)	4(10.26)	-	74(94.87)	4(5.13)	39
	MEF25 37.7-71.7	6	3	-	15	3	9
	MEF25 < 37.7%	14(51.85) p=0.002, OR=0.24. (0.09-0.62)	13(48.15) p=0.002. OR=4.15, (1.62-10.62)	-	41(75.93) p=0.004, OR=0.32. (0.14-0.72)	13(24.07) p=0.004. OR=3.15, (1.39-7.14)	27
Control group of Tatar ethnicity		67 (81.71)	15 (18.29)	-	149 (90.85)	15 (9.15)	82

rs1801105 (p.314C>T, p.Thr105Ile) polymorphism of the *HNMT* gene was performed by PCR-RFLP. The primer sequences, the amplifiable fragment sizes, and restriction enzyme's names are described earlier [3]. A pairwise comparison of genotype and allele frequencies in asthma patients and in controls was performed by using the χ2 criterion for 2x2 conjugency tables with Yates correction. In the case of statistically significant differences between the compared samples, the odds ratio (Odds Ratio, OR) and the boundaries of 95% confidence interval (CI 95%) were assessed.

Results and discussion. The study of the allele and genotype distributions of the amine oxidase, copper-containing 1 gene *AOC1* rs1049793 and of the histamine-N-methyltransferase gene *HNMT* rs1801105 polymorphic variants in asthma children and healthy individuals of different ethnicities living in RB was conducted (Table 1). The distribution of genotype frequencies in studied polymorphisms corresponded to the Hardy-Weinberg equilibrium (p>0,05).

The *AOC1* gene is located on chromosome 7q36.1 and contains 10 exons. We found statistically significant differences between asthma patients and controls in Russians, based on the analysis of allele frequencies and genotype distributions of rs1049793 polymorphism of the *AOC1* gene. The association of rs1049793**CC* genotype and rs1049793**C* allele of the *AOC1* gene with risk of asthma development in Russians was established (p=0,009, OR=2,36, 95%CI 1,24-4,5 and p=0,01, OR=1,86, 95%CI 1,16-3,01, respectively) (Table 1). A comparative analysis of allele and genotype frequencies of studied polymorphism in asthma patients with different spirometry measures showed that rs1049793**CC* genotype and rs1049793**C* allele of the *AOC1* gene are associated with a significant decrease in the volume of forced expiratory volume in 1 second (FEV1) (p=0,03, OR=2,56, 95%CI 1,08-6,03 and p=0,03, OR=2,08, 95%CI 1,07-4,05) and with a significant decrease in forced expiratory flow during the 25% of forced vital capacity (MEF25) (p=0,03, OR=2,56, 95%CI 1,08-6,03 and p=0,03, OR=2,08, 95%CI 1,07-4,05) (Table 2) in Russians. According to the literature, the association of the rs1049793**CC* genotype of the *AOC1* gene with higher values of maximal response over baseline (Emax) to histamine in asthma children was identified [5]. On the contrary, Szczepankiewicz et al. has not found any associations between polymorphic loci of the *AOC1* gene and asthma [8].

The histamine-N-methyltransferase gene *HNMT* is located at the chromosome region 2q22.1 and contains 9 exons. Analysis of allele and genotype frequencies distribution of the rs1801105 polymorphic variant of the *HNMT* gene did not reveal statistically significant differences between the asthma children and control group from RB (p>0,05) (Table 1). We found a higher frequency of rs1801105**CT* heterozygous genotype and rs1801105**T* allele of the *HNMT* gene in asthma patients of Tatar ethnicity with a significant decrease of FEV25 (48,15% and 24,07%), compared to the corresponding control group of children (18,29%, p=0,002, OR=4,15, 95%CI 1,62-10,62, and 9,15%, p=0,004, OR=3,15, and 95%CI 1,39-7,14) (Table 2). According to literature, an association of the rs1801105**T* allele with asthma in children [8] and with severe allergic rhinitis has been identified [6]. There is no association of the *HNMT* polymorphism rs1801105 with asthma development in Europeans [3].

Conclusion. Thus, an association of rs1049793**CC* genotype and rs1049793**C* allele of the *AOC1* gene with asthma, with a significant decrease in the values of FEV1 and MEF25 in the Russians was established. It was found the association of rs1801105**CT* genotype and rs1801105**T* allele of the *HNMT* gene with reduced MEF25 values in Tatars. The findings of this work demonstrate a certain aspects of asthma molecular pathogenesis, which may be needed to personalize the asthma treatment in the future.

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RATIO OF THE LEVEL OF NECROSIS AND APAPTOSIS OF PERIPHERAL BLOOD NEUTROPHILS

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The purpose of the work is to find out limits and ratio of the activity of apoptosis and necrosis of neutrophil granulocytes in venous peripheral blood in apparently healthy people. **Materials and methods**. 138 people living in the Arkhangelsk region were examined. The survey was conducted in accordance with the norms and rules of biomedical ethics approved by the Helsinki Declaration of the World Medical Association on the ethical principles of medical research (2013). The clinical analysis of peripheral blood was carried out on a Sysmex XS-500i hematological analyzer (Japan). The study of the content of apoptotic cells in the lymph suspension was carried out by the method of double staining with annexin V-FITC (An +/-) and propidium iodide (PI +/-). The results were evaluated on the Epics XL flow cytometer. In blood smears stained according to Romanovsky – Gimze, the neutrogramm structure was detected by microscopy. Serum concentrations of sFas-L, TRAIL, Nt-pro-BNP, cytokines, immunoglobulins were detected by a solid-phase immunoenzyme assay. The content of lymphocyte phenotypes was determined by double peroxidase labeling using monoclonal antibodies. The boundaries of the normal quantitative distribution were determined using the Shapiro-Wilk test. The statistical significance of differences between groups was assessed using Student's parametric t-test and Wilcoxon's test. **Results.** At increase in contents the necrotic neutrophils of AnV+/PI+ statistically authentically grows concentration the apoptotic cells of AnV+/PI- against the background of reduction of number of the circulating neutrophils, monocytes and T-helpers. The concentration of IgE and Nt-pro-BNP increases, but the level of IL-2 decreases. **Discussion.** The higher concentration of Nt-pro-BNP indicates a change in cell membrane activity and osmotic pressure. With increasing number of necrotic neutrophils, there is increase in the migration of all types of white blood cells in the tissue. In addition, the increase in neutrophil necrosis activity in apparently healthy residents of high latitudes creates a risk of autosensitization, which is manifested by increased levels of autoantibodies, including ds DNA. **Conclusion**. In apparently healthy people living in the North, at average, the total number of necrotic neutrophils is 1,98±2,09×10^9 cl/l, apoptotic neutrophils – 7,0±8,25×10^9 cl/l. Activation of neutrophil death by necrosis can become a pathogenetic mechanism for damage to cell membranes (nuclear, lysosomal or cellular), as well as the formation of T-helper immunodeficiencies.

Key words: necrosis and apoptosis of neutrophils, lymphocytes, cytokines, immunoglobulins, apparently healthy people.

Apoptosis and necrosis under physiological conditions, being programmed mechanisms of cell death during organism life, have significant differences [7, 27]. Apoptosis is morphologically manifested by signs of nucleus damage - chromatin agglomeration, karyorhexis and karyolysis with the formation of nuclear-free cells or apoptosis bodies, which are phagocyte by macrophages. Cell death by necrosis is due to cell lysis resulting from impaired membrane permeability, including nuclear, lysosomal, or cellular. Necrosis under physiological conditions is initiated by the inability of the cell to provide active water transfer by osmotic exchange, which requires large energy consumption. Reduced efficiency of self-regulation of cell membrane function leads to alignment of osmotic pressure of cytoplasm and extracellular medium or cytoplasm and lysosomes, or nuclear space and cytoplasm. Membrane damage initiates complete (cytolysis) or partial destruction of cell structures by cell enzymes (autolysis, autophagy). In necrosis, the contents of the cytosol, cellular organelles, including lysosomes, appear in the intercellular medium and are able to initiate reactions of various systems, primarily kallikrein-kinin, complement, fibrinolysis and blood clotting. Cytokines play an important role in the regulation of cell death, but their role is not so unambiguous. Stimulation of neutrophils with IFN-α and IFN-γ leads to a decrease in their apoptosis by tyrosine phosphorylation of STAT1 and STAT3, an increase in the level of mRNA cIAP2, activation of JAK2 [35]. In addition, IFN-γ, acting on the receptors TLR2 and TLR4 inhibits the kinase p38 MAPK [14]. The action of IFN-γ may depend on the number of receptors (IFN-γR2) on the surface of the neutrophil; at their high density, STAT-1 is rapidly activated, the level of IRF-1 is increased, and the programmed death of granulocytes is triggered. With a small amount of IFN-γR2 on the cell membrane, a weaker activation of STAT-1, a slower increase in the content of IRF-1 is detected, and a neutrophil proliferation program is initiated [11]. Biffl W. L. et al. (1996) showed that IL-6 suppresses apoptosis of polymorphonuclear neutrophils through a mechanism involving a platelet activating factor, along with an increase in the level of cytosolic calcium [12]. According to L. Ottonello (2002), IL-6 has an anti-apoptotic effect, reducing the expression of the Bax protein and caspase-3 [33]. However, there is evidence that IL-6, with the participation of IFN-γ, acting through its soluble receptors sIL-6R, causes apoptosis of granulocytes and initiates the secretion of CXCL5, CXCL6 chemokines to attract mononuclear leukocytes [22]. IL-10 prevents programmed death of myeloid progenitor cells, together with growth factors promotes their proliferation. IL-10 increases the tyrosine phosphorylation of the insulin receptor and stimulates the activity of 3-kinase / Akt and p70 S6 [15], thereby enhancing the antiapoptotic effect of ERK1 / 2 molecules [40]. According to other data, granulocytes, pre-treated with lipopolysaccharides, or TNF-α and IFN-γ, interacting with IL-10 are prone to programmed death [24]. When IL-4 acts on neutrophils through the CD132 / IL-4Rα receptors, phagocytosis and cell adhesion of granulocytes are enhanced, and their apoptosis is delayed [19]. It is well known about the proapoptotic activity of TNF-α, which is characterized by an increase in the lev-

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el of IA PI3K enzymes, an increase in the number of reactive oxygen species and caspase-3 within granulocytes [18]. The effect of stimulating apoptosis is directly dependent on the concentration of TNF-α [36]. However, there is information about the antiapoptotic effect of TNF-α on neutrophils [32]. Signaling is via TNFR1 receptors that bind to δ-PKC; the resulting complex promotes the activation of ERK1 / 2 and NFκB, and the suppression of caspase 3 [25]. On the neutrophils themselves, an increase in the amount of membrane pro-TNF is observed [38], the circulation of anti-apoptotic proteins Mcl-1 and BFL-1 [16] is accelerated, which indicates autocrine regulation of the apoptosis process. The relationship between blood levels of brain natriuretic peptide (BNP) and the severity of heart failure is well understood. It is known that in patients with cardiovascular diseases, the concentration of TNF-α in plasma increases, the content of its cellular receptor and the readiness to trigger an apoptotic stimulus increase [21]. At the same time, TNF-α, through the increase in reactive oxygen species, induces nuclear factors of cell death, and it has also been shown that the synthesis of a soluble form of sFas-L is triggered with the participation of metalloproteinases, the activity of which is increased in patients with heart failure [28]. However, the question of the relationship between BNP and cytokines in the activation of cell death, which occurs both in health and in cardiovascular pathology, has not actually been studied. It is especially important to study these phenomena with respect to tissues and cells that provide regulatory and protective responses. Neutrophil granulocytes are the most numerous of the blood leukocytes. In humans, more than 10^{11} neutrophil granulocytes are produced per day, and the turnover is tens of thousands of times less [17, 26]. Therefore, the regulation of neutrophil death is one of the most important processes for maintaining their optimal number and functional activity. In connection with the above, it was interesting to find out the limits and ratio of the activity of apoptosis and necrosis of neutrophil granulocytes in venous peripheral blood in apparently healthy people. The ratio of necrosis to apoptosis at the physiological level has not been apparently studied. At the same time, cell decay type may have different biological significance for differentiation, regeneration and renewal of cells and their level of activation [7]. It can be assumed that the change in the relationship between physiological levels of necrosis and apoptosis may be the cause of the formation of immunodeficiency, autosensibilization, oncopathology, chronic course of the disease and even aging.

Materials and methods. The survey was conducted at 138 apparently healthy people aged 20 to 60 years living in the Arkhangelsk region. The examined persons did not suffer from acute infectious diseases, they showed no signs of autoimmune and lymphoproliferative processes. The survey was conducted in accordance with the norms and rules of biomedical ethics approved by the Helsinki Declaration of the World Medical Association on the ethical principles of medical research (2013).

Clinical analysis of peripheral blood was carried out on a Sysmex XS-500i hematological analyzer (Japan). The study of the content of apoptotic cells in the lymph suspension was carried out by the method of double staining with annexin V-FITC (An +/-) and propidium iodide (PI +/-). The results were assessed by staining or non-staining of cells: living cells - An- / PI-, early apoptosis - An + / PI-, late apoptosis - An + / PI +, necrosis - An- / PI +. The analysis of the results was performed by flow cytometry on an Epics XL flow cytometer (Beckman Coulter, USA); up to 5000 events were studied in each sample. In blood smears stained according to Romanovsky - Giemsa, the nuclear formula of neutrophils (neutrogram) was studied by microscopy (Meiji Techno, Japan). Up to 100 neutrophilic leukocytes were counted, among which cells with 1, 2, 3, 4, 5 and more segments of the nucleus were isolated [9]. To determine the phagocytic activity of neutrophils, 100 μl of latex and 100 μl of blood were mixed in a test tube with heparin, then the tube was incubated in a thermostat at + 37°C for 30 minutes. After the lapse of time, the supernatant fluid was taken, transferred to a glass slide, and a smear was made. The resulting smear was stained according to Romanovsky-Giemsa. We took into account the data on 100 neutrophils, from which the phagocytic activity is calculated - the percentage of phagocytic neutrophils; phagocytic number - the average number of particles captured by one cell [2]. Serum levels of sFas-L, TRAIL, cytokines (TNF-α, IFN-γ, IL-2, IL-4, IL-6, IL-10, IL-17F) were determined by the method of enzyme-linked immunosorbent assay (ELISA), immunoglobulins A, E, M, G (kits from Bender MedSystems, Austria), histamine (DRG, Germany), Nt-pro-BNP (Biomedica, Austria). The reaction was evaluated using a Multiskan MS photometer (Labsystems, Finland) and an Evolis automatic enzyme immunoassay analyzer (Bio-RAD, Germany).The content of lymphocyte phenotypes was examined by double peroxidase labeling using monoclonal antibodies («Sorbent», Russia). The results of the study were processed using the Statistica 6 application package (StatSoft, USA). Type of study retrospective, samples random, one-time. The general population is residents of the north of the European territory of Russia. The boundaries of the normal distribution of quantitative measures were determined using the Shapiro-Wilk test. The mean and standard deviation, median and lower, upper quartiles, were used in the analysis of the results. The validity of differences between groups was assessed using Student's parametric t-test for independent samples and Wilcoxon's nonparametric test. Statistical validity was assigned at $p<0,05$.

Results and discussion. Among the general group of examined people of northerners, persons with a relatively increased and relatively reduced content of necrotic neutrophils AnV+/PI+ and apop-

Content of immunocompetent blood cells in practically healthy northerners depending on the content of dead neutrophils

Indicator, $\times10^9$ cl/l	Necrosis		Apoptosis	
	> 3 % (1) 4.0(3.4-4.9)**	< 1 % (2) 0.8(0.75-0.9)	>8% (3) 14.1(10.5-14.7)*	<3% (4) 1.9(1.3-2.4)
M±m				
Leukocytes	6.9±0.51**[1-2]	9.1±0.58	7.64±0.76*[3-4]	8.78±0.65
Neutrophils	3.58±0.33**[1-2]	5.51±0.44	4.43±0.49*[3-4]	5.4±0.38
Lymphocytes	2.38±0.15	2.87±0.23	2.64±0.27	2.63±0.22
Monocytes	0.26±0.04*[1-2]	0.42±0.04	0.24±0.04*[3-4]	0.36±0.05
Eosinophils	0.21±0.04	0.25±0.04	0.26±0.04	0.23±0.04
ME(Q1-Q3)				
AnV+/PI+	0.17(0.09-0.21)* [1-2]	0.04(0.03-0.05)	0.07(0.05-0.10)	0.06(0.04-0.09)
AnV+/PI-	0.28(0.16-0.46)* [1-2]	0.18(0.1-0.34)	0.5(0.41-0.68)**[3-4]	0.1(0.07-0.14)
% act. phagocytes	56(47-66)	50(45-56)	55(52-58)	55(48-67)

Note. Statistically significant differences: * – $p < 0,05$; ** – $p < 0,01$.

totic neutrophils AnV+/PI- blood were identified according to the results of laser flow cytofluorimetry: AnV+/PI+ >3% (n=35) и <1% (n=40), p=0,0011; AnV+/PI- >8% (n=37) и <3% (n=36), p=0,0001. The median values, upper and lower quartile of the test cells are shown in Table.

It was found that in people with high levels of necrotic cells, the total number of white blood cells due to segmentonuclear neutrophils and monocytes is lower. The phagocytic activity of neutrophils does not decrease. Among the neutrophil granulocyte population, cell content with 2, 3, and 4 nucleus segments is reduced (Fig. 1). Concentrations of stick-nuclear neutrophils and cells with 5 or more nucleus segments did not actually differ. It has long been known that mainly mature neutrophils undergo autolysis, young cells autolysis more slowly [5, 10].

No significant differences in lymphocyte, eosinophil and basophil content have been established. Against the background of an increase in the content of necrotic neutrophil granulocytes, the concentration of apoptotic cells (AnV+/PI-; p<0,001). In the ratio of the activity of apoptosis and neutrophil necrosis, the following patterns were revealed: with an increase in the level of neutrophil necrosis > 3%, the average value of the ratio is 2,93±0,60, in the conditions of a decrease in the level of cellular necrosis < 1%, this ratio increases sharply 7,95±1,76.

With increasing the neutrophil necrosis activity, the absolute content of circulating mature T-lymphocytes (from 0,77±0,06 to 0,62±0,04×10^9 cl/l, p=0,038) due to T-helpers (from 0,69±0,06 to 0,54±0,04×10^9 cl/l, p=0,023). The average total lymphocyte content and the remaining phenotypes CD8+, CD10+, CD16+, CD23+, CD25+, CD71+, HLADR, CD95+ remain virtually unchanged (Fig. 2). The reduction of circulating neutrophils, monocytes and T-helpers against the background of increased levels of necrotized neutrophils in the blood allows us to believe with some certainty that the migration of white blood cells in tissues under these conditions becomes more active. It seems that with the growth of necrotic leukocytes, there is an increase in the migration of all types of leukocytes into the tissue. However, due to the low blood concentrations of some of them (basophils, eosinophils, monocytes) and their predominant presence in tissues, the change in the level of these cells in the periphery is less pronounced.

With increasing the neutrophil necrosis activity, the content of the natriuretic peptide Nt-pro-BNP is higher [112,9(48,7-147,0) and 27,4(17,1-50,4) fmol/ml, respectively; p=0,012], Statistically significant increase of natriuretic peptide concentration indicates change of cell membrane activity and osmotic pressure [3, 20]. Not revealed reactions to increase in activity of necrosis of neutrophils from IgM [3,1(2,7-3,6) and 2,9(2,6-3,3) g/l, p=0,871], IgG [8,8(3,9-10,9) and 7,6(3,7-9,6) g/l, p=0,568], IgA [1,2(1,0-1,5) and 1,3(0,9-1,5) g/l, p=0,932] and also in the maintenance of a histamine [0,96(0,47-1,27) and 0,91(0,61-1,42) ng/ml, p=0,579] and extracellular sFas-L [0,1(0,07-0,25) and 0,07(0,04-0,12) ng/ml, p=0,531].

The cytokine profile of people with the level of granulocytes AnV+/PI+ more than 3% is characterized by falling of concentration of IL-2 from 16,3(2,4-18,2) to 7,8(4,2-12,7) pg/ml, p=0,016, against the background of rather stable maintenance of TNF-α [23,0(19,7-34,1) and 25,8 (17,9-32,0) pg/ml], IFN-γ [19,9(19,6-20,1) and 19,8(19,7-20,0) pg/ml], IL-4 [3,9(2,3-11,7) and 5,6 (2,3-7,9) pg/ml], IL-6 [7,8(5,3-13,5) and 10,3(7,0-13,2) pg/ml], IL-10 [0,34(0,05-0,38) and 0,98 (0,09-2,0) pg/ml], IL-17F [47,8(39,1-53,7) and 49,0(42,5-58,3) pg/ml], p>0,05.

The higher IgE concentrations [from 17,3(9,4-33,3) to 31,1(12,5-59,9) IU/ml; p=0,023] can be explained by the features of Ig of this class. IgE is a typical secretory Ig capable of rapid redistribution to the center of disadvantage effectively neutralizes, binds and reduces concentrations of antigen structures [23, 30]. Moreover, cytolysis with the participation of reagents occurs almost instantly.

The increase in neutrophil necrosis activity in apparently healthy residents of high latitudes creates a risk of autosensitization, which is manifested by increased levels of autoantibodies, including ds DNA [4, 8]. An increase in TRAIL concentrations [from 11,4(4,4-56,6) to 40,6(8,4-60,1) pg/ml, p=0,026] associates with apoptosis activity.

Thus, with raising the death of polymorphonuclear neutrophil granulocytes of more than 3% leads to the decreased content of neutrophils circulating in peripheral venous blood without reducing their phagocytic activity. It is known that more than 60% of circulating neutrophils are potential phagocytes, they interact with the object of phagocytosis, form traps, produce active oxygen species and undergo apoptosis [6, 13]. Secreting neutrophils fully enter the circulating pool, have permeable membranes, produce less active oxygen species, and are practically not involved in apoptosis [1]. Autophagy regulates homeostasis in the endoplasmic reticulum [37]. In turn, the Toso gene inhibits autophagy and apoptosis, and IL-2 STAT5-dependent method suppresses formation of the Toso receptor [31]. Therefore, the reduction in IL-2 content under the conditions of increased necrosis and apoptosis, when the level of neutrophil death increases by more than

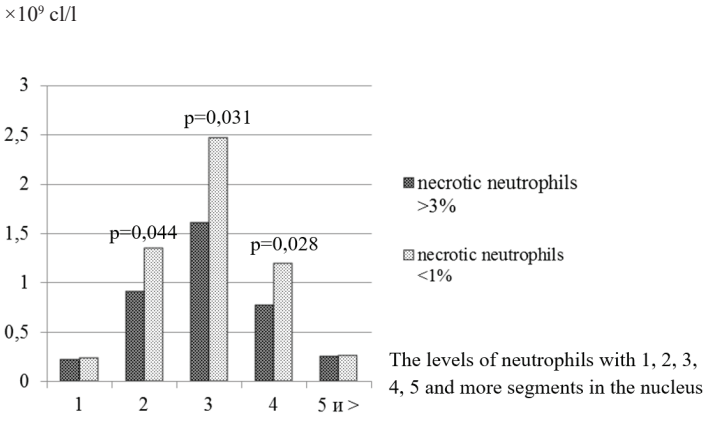

Fig. 1. Neutrogramm of peripheral blood of practically healthy people depending on the content of necrotic neutrophils

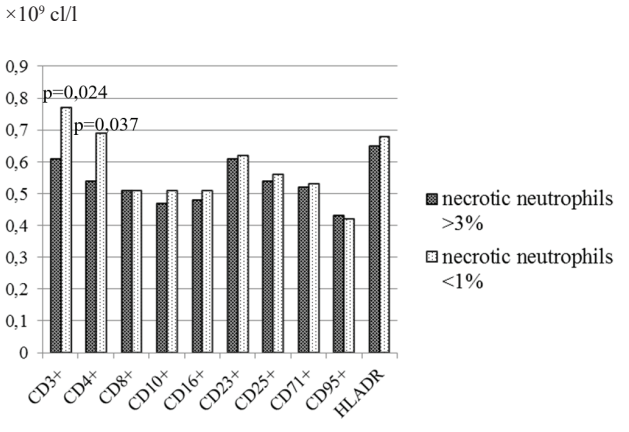

Fig. 2. Content of peripheral blood lymphocyte phenotypes in practically healthy people depending on necrotic neutrophil content

4 times, is not accidental. The ability to intraphagosomal degranulation gives neutrophils, the ability to regulate the functional activity of many immunocompetent cells, including lymphocytes, at autocrine and paracrine levels [34, 39]. Reduction of T-helper content in necrosis-increasing situations may result from increased lymphocyte migration and recycling activity.

Conclusion. In apparently healthy people living in the North, on average, the total number of necrotic neutrophils is 1,98±2,09×10^9 cl/l, apoptotic neutrophils – 7,0±8,25×10^9 cl/l. With increasing neutrophil necrosis > 3% (0,16±0,08×10^9 cl/l), people record an average level of apoptotic neutrophils 0,29±0,21×10^9 cl/l, in the situation of reducing necrosis activity < 1% (0,04±0,03×10^9 cl/l), the number of apoptotic neutrophils is equal to 0,28±0,38×10^9 cl/l. The activation of neutrophil death by necrosis can become a pathogenetic mechanism for damage to cell membranes (nuclear, lysosomal or cellular), as well as the formation of T-helper immune deficiencies.

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EVALUATION OF PARAMETERS OF THE DYNAMIC COMPONENT OF POSTURAL BALANCE IN ELDERLY WOMEN

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The aim of the study was to develop centile tables to assess basic parameters of the dynamic components of the postural balance in women 60-74 years old. Three groups of women aged 60-64 years, 65-69 years, and 70-74 years, with a total number of 186 people, were examined. Evaluation of main indicators of the postural balance was carried out using the computer stabilometric complex "Balance Manager". The presented standards will help to increase the information content of research and objective analysis of the results obtained, as well as to evaluate the effectiveness of the implementation of measures to maintain normal, safe walk or prevention of mobility restriction.

Key words: dynamic component of postural balance, women, elderly age, centile grade.

Introduction. Domestic and international studies have shown that the main component of active longevity is the preservation of autonomy and mobility in the elderly. In order to minimize and prevent the loss of an independent, autonomous existence, specialists working with older people should timely determine the level of risk that a future decrease in mobility can predict [2, 4, 6, 7]. The analysis of walking is a widely used indicator of the effectiveness of the functioning of lower extremities in particular, and the postural balance as a whole [3, 5, 6, 9, 10]. Changing the main parameters of a dynamic component of the postural balance is a clear indicator of the preservation of the dynamic component of the postural balance, since they are the most sensitive to future changes in the functional state of the body of the elderly women. However, in modern domestic literature data there is no information about standards for evaluating the main indicators of the dynamic component of the postural balance in elderly women [1, 8, 11-15]. The purpose of the study was to develop centile tables for evaluating the main parameters of the dynamic components of the postural balance in women 60-74 years old.

Materials and research methods. A cross-sectional study was conducted with the informed consent of participants. The study involved 186 women, aged 60-74 years, who were divided into age groups: 60-64 years old - 61 people (average age - 62.8 ± 1.3 years), 65-69 years old - 63 people (average age - 67.8 ± 1.8 years), 70-74 years - 62 people (average age - 73.1 ± 1.1 years). All women in the course of the study were mobile and did not use additional means of support when walking. The following exclusion criteria were considered: a history of strokes, dementia, being registered in a neuropsychiatric dispensary, traumatic brain injuries, acute and chronic diseases during the exacerbation period, as well as permanent residents in nursing homes.

The evaluation of the main parameters of the dynamic component of the postural balance was carried out using the Balance Manager computer stabilometric complex and included the following tests and parameters: Sit to Stand test (Weight Transfer Time, Rising Index, Sway Velocity), Walk Across (Step Width, Step Length, Speed), Tandem Walk (Step Width, Speed, End Sway), Step - Quick Turn (Time (with left and right legs), Sway (with left and right legs)), Step – Up and Over (Lift Up Index (with left and right legs), Movement Time (with left and right legs), Impact Index (with left and right legs)) [1-3].

Statistical processing of the obtained data was carried out using the application package SPSS 21.0 for Windows. For each of the studied indicators, the distribution of signs on normality was assessed using the Shapiro – Wilk criterion. For each of the studied indicators, arithmetic means (m), standard deviations (s), and values equal to 10, 25, 50, 75, and 90 centiles in each of the age groups were calculated.

Results and discussion. Tables 1-3 show the centile distribution of the main parameters of the dynamic component of the postural balance in women of the studied age groups, namely 60–64 years old, 65–69 years old, 70–74 years old. As a result of the data analysis with increasing age, in all the studied groups there is a uniform offset of median values of

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the dynamic component indicators of the postural balance, demonstrating a decrease in its quality .

For the visual analysis of the data, we compared and evaluated the dynamics of changes in the main indicators of the postural balance in groups of women 60-64 years old and 65-69 years old, and between groups of 65-69 years old and 70-74 years old.

In 'Sit to Stand' test we found that the median of the Weight Transfer Time indicator in the group of women 65-69 years compared to the group of 60-64 years increased in 2.2%, and after that in 6.5% in group 70 -74 years old. The average value of Rising Index in the group of 65-69 years old decreased by 16.7%, and in the group of 70-74 years old - by 4.4%. Median of Sway Velocity increased in 1.4% and in 6.0% with increasing age in the groups.

When analyzing Walk Across test the Step Length decreased with age in 5.7% and in 9.7%, and Speed decreased in 7.9% and in 1.1% in the groups of 65-69 years old and 70-74 years old, respectively. In turn, the Step Width increased with age in 0.8% and in 1.8%, respectively, in the studied groups.

The median values in the performance of the Tandem Walk test showed the greatest shift of decreasing of the postural balance quality. Thus, the average Step Width increased with age in 21.7% and in 8.0%, and the End Sway in 25.8% and in 8.4%, respectively. The median Speed decreased from group to group by 10.0% and 3.8%.

When analyzing the Step - Quick Turn test, the median values of all the studied parameters increased with aging. The Turn Time to the left and right legs increased in 19.8% and in 22.4%, and in 5.9% and in 8.4%, respectively. The Sway during a turn from the left and right legs increased in 7.8% and in 6.9%, and in 5.6% and in 11.1%.

In the Step – Up and Over test we found a decrease in the median of the Lift Up Index from the left and right legs in 2.5% and in 10.3%, and in 1.2% and in 8.2% in the age groups 65-69 and 70- 74 years old, respectively. In terms of Movement Time from the right and left legs, the median values increased from group to group and increased in 6.9% and in 7.8% for the left leg and in 7.8% and in 5.2% for the right leg. The median Impact Index increased with age in 1.7% and in 10.8% for the left leg and in 8.3% and in 12.8% for the right leg.

Earlier studies have shown that in women aged 60-69 years, there is an almost uniform decrease in the indicators of the dynamic component of postural balance: step length, movement speed, quality of the performance of complex coordination and complex motor acts, as well as an increase in step width and final oscillation during tandem walking. At the same time, there is an increase in the number of significant correlations between these indicators, which indicates the preservation of the functional state of the body at a certain level, as a manifestation of compensatory and adaptive reactions. Characteristic of women aged 70-74 years is a sharp decrease in the indicators of the dynamic component of postural balance with a simultaneous decrease in the number of significant correlations, which can be considered as age-related disadaptation changes in the elderly body. It is also noted that the Step/step test is recommended for rapid diagnosis of postural balance disorders, since it is the most sensitive to age-related changes occurring in the elderly body [1-3]. For a more visual representation of the percentile distribution, we have

Table1

Percentile distribution of the main parameters of the dynamic component of the postural balance in women aged 60–64 years

Parameters		Percentile				
Tests		10	25	50	75	90
Sit to Stand	Weight Transfer Time, (s)	0.23	0.31	0.45	0.54	0.70
	Rising Index (cm/s)	11.00	13.00	18.00	22.00	24.00
	Sway Velocity (cm/s)	2.34	2.93	3.60	4.40	4.96
Walk Across	Step Width (cm)	10.51	11.90	13.50	15.00	15.84
	Step Length (cm)	50.60	53.73	58.60	64.90	76.72
	Speed (cm/s)	76.20	80.40	86.00	96.50	105.76
Tandem Walk	Step Width (cm)	5.41	6.17	7.00	7.80	10.02
	Speed (cm/s)	21.52	23.80	29.33	34.10	38.83
	End Sway (cm/s)	3.20	3.70	4.73	5.93	7.38
Step-Quick Turn	Time Left (s)	0.72	0.98	1.31	2.06	2.51
	Time Right (s)	0.79	0.99	1.35	1.65	2.08
	Sway left (cm/s)	14.54	17.80	25.97	34.80	39.60
	Sway Right (cm/s)	16.16	18.30	24.30	30.10	34.82
Step-Up and Over	Lift Up Index Left (cm/s)	29.00	35.00	40.00	50.00	57.80
	Lift up index right (cm/s)	30.76	37.00	43.00	47.00	57.80
	Movement Time Left (s)	1.15	1.22	1.44	1.71	1.84
	Movement Time Right (s)	1.09	1.21	1.42	1.57	1.76
	Impact Index Left (cm/s)	34.00	46.00	59.00	77.00	98.60
	Impact Index Right (cm/s)	35.00	45.00	54.00	69.00	96.00

Table2

Percentile distribution of the main parameters of the dynamic component of the postural balance in women aged 65–69 years

Parameters		Percentile				
Test		10	25	50	75	90
Sit to Stand	Weight Transfer Time, (s)	0.24	0.33	0.46	0.55	0.73
	Rising Index (cm/s)	11.00	12.00	15.00	21.75	29.20
	Sway Velocity (cm/s)	2.67	2.75	3.65	4.44	4.97
Walk Across	Step Width (cm)	8.94	11.99	13.61	15.22	16.25
	Step Length (cm)	43.15	47.18	55.29	62.07	73.62
	Speed (cm/s)	65.01	74.20	79.19	87.85	94.79
Tandem Walk	Step Width (cm)	5.97	6.90	8.52	10.08	12.89
	Speed (cm/s)	18.88	22.03	26.40	34.70	39.55
	End Sway (cm/s)	3.79	4.61	5.95	7.81	9.01
Step-Quick Turn	Time Left (s)	0.91	1.07	1.56	2.22	2.90
	Time Right (s)	0.74	1.05	1.43	1.90	2.61
	Sway left (cm/s)	15.12	21.48	28.00	34.88	41.60
	Sway Right (cm/s)	16.28	19.19	25.65	35.12	37.24
Step-Up and Over	Lift Up Index Left (cm/s)	28.90	33.25	39.00	48.00	55.10
	Lift up index right (cm/s)	28.90	34.50	42.50	46.23	55.10
	Movement Time Left (s)	1.21	1.35	1.54	1.84	2.01
	Movement Time Right (s)	1.20	1.35	1.53	1.66	2.12
	Impact Index Left (cm/s)	38.80	49.50	60.00	79.75	98.20
	Impact Index Right (cm/s)	36.30	47.25	58.50	74.50	98.90

Table3

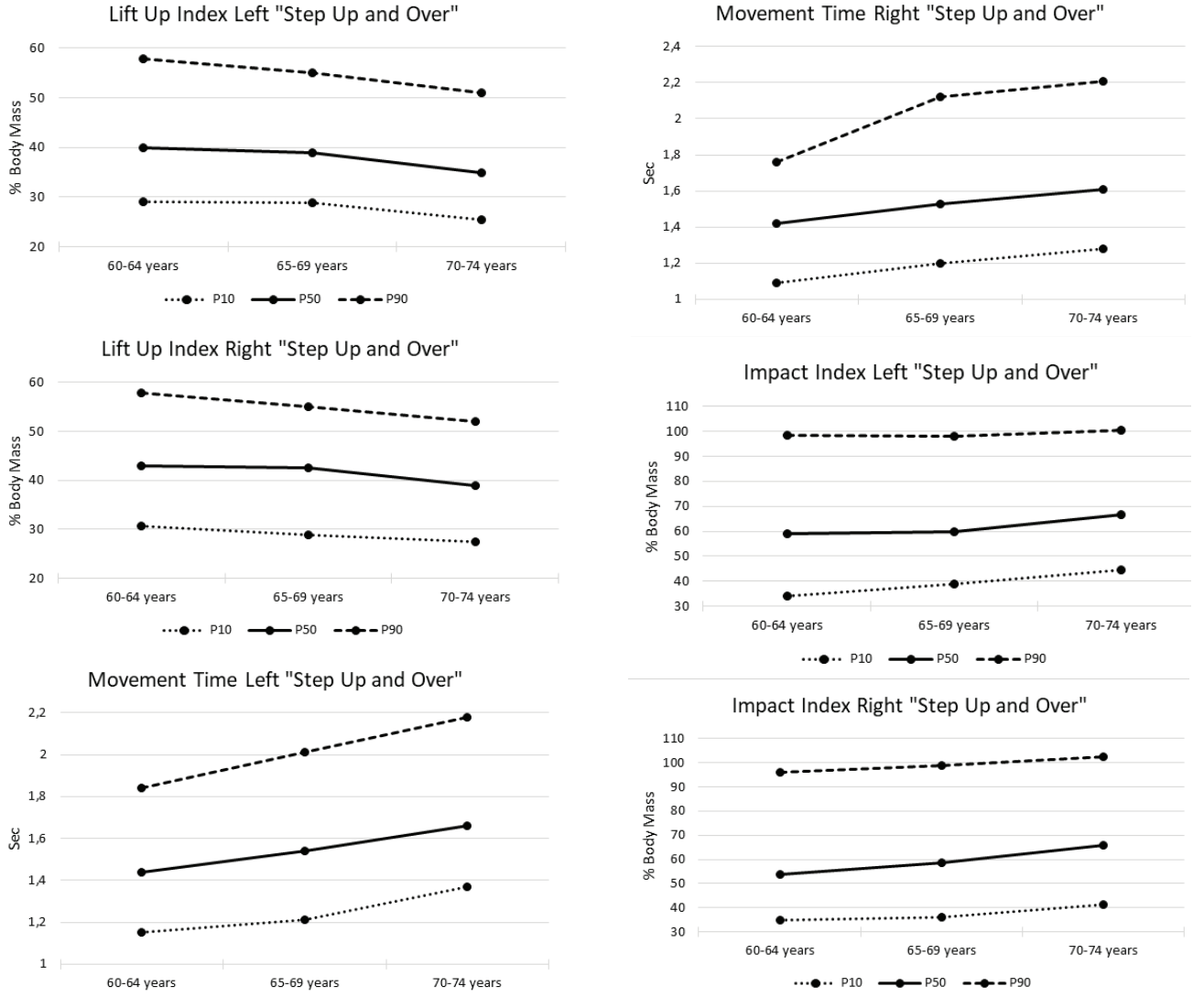
Percentile distribution of the main parameters of the dynamic component of the postural balance in women aged 70-74years

Parameters		Percentile				
Test		10	25	50	75	90
Sit to Stand	Weight Transfer Time, (s)	0.26	0.34	0.49	0.65	0.79
	Rising Index (cm/s)	8.00	11.75	14.34	21.00	29.50
	Sway Velocity (cm/s)	2.25	2.88	3.87	4.53	4.99
Walk Across	Step Width (cm)	8.70	12.23	13.86	15.46	16.87
	Step Length (cm)	40.52	45.32	49.95	57.93	63.90
	Speed (cm/s)	58.57	71.59	78.30	87.23	93.38
Tandem Walk	Step Width (cm)	6.05	7.10	9.20	10.82	15.09
	Speed (cm/s)	17.83	20.13	25.39	35.18	41.05
	End Sway (cm/s)	4.07	5.22	6.45	8.23	9.39
Step-Quick Turn	Time Left (s)	0.95	1.23	1.91	2.71	3.19
	Time Right (s)	0.71	1.22	1.55	2.30	3.09
	Sway left (cm/s)	20.25	24.89	29.95	36.55	46.34
	Sway Right (cm/s)	18.02	21.18	28.50	35.43	38.25
Step-Up and Over	Lift Up Index Left (cm/s)	25.50	30.00	35.00	44.25	51.00
	Lift up index right (cm/s)	27.50	33.75	39.00	45.25	52.00
	Movement Time Left (s)	1.37	1.45	1.66	1.93	2.18
	Movement Time Right (s)	1.28	1.42	1.61	1.89	2.21
	Impact Index Left (cm/s)	44.50	55.00	66.50	86.00	100.50
	Impact Index Right (cm/s)	41.50	55.00	66.00	80.00	102.50

constructed curves of the stabilometric indicators of this particular test, which are presented in figure 1. The choice of centiles was determined by their significance for the diagnosis of disorders in the parameters of physical development of newborns; thus, a stabilometric indicator less than the value of the 10th centile for the corresponding age group is treated as low, and one that exceeds the value of the 90th centile is treated as high .

Such timely evaluation of the dynamic component of the postural balance will undoubtedly prevent serious disorders and, as a result, maintain mobility and promote active longevity of women in elderly age.

Thus, the presented standards, developed on the basis of assessing the state of the dynamic component of the postural balance in older women, will help to improve the quality of research and analysis of the results, as well as help to develop measures to maintain normal,



– Curves of the indicators Lift Up Index, Movement Time and Impact Index with left and right legs of the "Step Up and Over" Test in women 60-74 years old. Note: P-centile.

safe walking or prevent mobility restrictions. Screening by using the developed tables can provide practitioners with a quick and easy way to detect a decrease in the quality of the dynamic component of the postural balance in older women, and to determine the effectiveness of implementing programs to maintain active longevity. However, it should be noted that the presented standards were developed on the basis of a study of a sample of elderly women living in the European North of Russia, using the example of the Arkhangelsk region. As part of a further study, it is necessary to develop similar regulatory data for males and expand the scope of the study to include elderly people living in other regions of Russia.

Conclusion. Thus, with increasing the age, in all the studied groups there is a uniform shift in the median values of the indicators of the dynamic component of the postural balance, which demonstrates the decrease in its quality. The timely assessment of the dynamic component of the postural balance will undoubtedly prevent serious violations of the postural balance, and, as a result, maintain mobility and promote active longevity of women in old age. It is necessary to develop similar regulatory data for males and expand the scope of the study to include elderly people living in other regions of Russia.

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SEARCH FOR MUTATIONS IN THE *EXT1* AND *EXT2* GENES AMONG PATIENTS WITH HEREDITARY MULTIPLE EXOSTOSES IN THE REPUBLIC OF SAKHA (YAKUTIA)

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The article presents the results of the fisrt molecular genetic study in the *EXT1 and EXT2* genes among patients with hereditary multiple exostoses (HME) ant their relatives. A rare nonsense mutation c.751C>T (p.Gln251*) in exon 5 of *EXT2* gene in a heterozygous state was detected. That was the cause of HME among patients of the Yakut ethnic group. At present we are conducting the investigation to search mutations in *EXT1 and EXT2* genes in other families with HME.

Key-words: *EXT1, EXT2,* Hereditary Multiple Exostoses, multiple osteochondromas

Introduction. Hereditary Multiple Exostoses (HME) or multiple osteochondromas (MO) (OMIM #133700, #133701) is a genetically heterogeneous disease with an autosomal dominant mode of inheritance, which accounts for 16.7 to 44% of all benign tumors, tumor-like and dysplastic skeletal lesions [9]. Also, in some cases (from 0.5 to 5%), it is possible to transform individual exostoses into a secondary chondroma or chondrosarcoma [3]. This disease is manifested by the development of 2 or more bone outgrowths on long tubular bones. The number of exostoses can vary significantly with the course of the disease and can range from 15 to 18. In most cases, bone changes are asymptomatic and develop from cartilage, increase in size in the first decade of life, and stop growing during puberty, when the growth plates close [4].

Disease incidence in the world in various populations ranges from 1.3 to 2 per 100 thousand population or 1 per 7000 orthopedic patients, up to 80% are family cases [3]. It was found that in 90% of cases, HME is associated with mutations in the *EXT1* (OMIM # 608177, 8q24.11) and *EXT2* (OMIM # 608210, 11p11.2) genes. Depending on the ethnic group, the frequencies of the pathogenic variants *EXT1* and *EXT2* differ [10].

According to the "Republican Genetic Register of Hereditary and Congenital Pathology", 85 patients with HME from 41 families were registered in the Republic of Sakha (Yakutia). 70 patients of them are from 33 Yakut families, 5 patients are from 1 Evenk, 8 patients are from 5 Russian families and one case is from Tatar and Ukrainian family. The disease was registered in 16 uluses and in Yakutsk from 36 administrative-territorial units of the Republic of Sakha (Yakutia). The prevalence of this disease in the Republic of Sakha (Yakutia) was 8.85 per 100 thousand population. Until now, molecular genetic studies on the search for mutations among patients with HME have not been carried out in Yakutia. In this regard, the aim of this work is to search for mutations in the *EXT1* and *EXT2* genes among patients with HME and their relatives in the Republic of Sakha (Yakutia) using modern molecular genetic methods.

Materials and methods. *The patients.* The material for the molecular genetic study included 55 DNA samples of individuals with a clinically established HME diagnosis and 11 DNA samples of their relatives without clinical manifestations from 31 unrelated families. Of the 55 patients, 28 were males and 27 were females; 45 (81.82%) - Yakuts by ethnic origin, 6 (10.91) - Russians, 2 (3.63) - Evenks, and 1 (1.82%) - Ukrainian and Tatar. Informed consent was obtained from all individuals for this study. The work was approved by the local Committee on biomedical ethics of the Medical Institute of the Ammosov Northeastern Federal University (Yakutsk, Protocol No. 8 of November 11, 2016). All individuals were registered in the Medical and Genetic center of the Republican Hospital №1 RS(Ya) of the National Centre of Medicine.

Molecular genetic analysis. DNA was isolated from whole blood by the standard method of phenol-chloroform extraction [5].

To establish the molecular genetic cause of HME, mass parallel sequencing (MPS) was carried out using the Trusight One Sequencing panel (Illumina, USA), which includes 4800 genes with known clinical significance. The MPS method allows to obtain complete information about the nucleotide sequence of the desired DNA region and includes three main stages: preparation of DNA libraries, sample preparation, and sequencing. Sequencing of the sample was carried out on a high-performance sequencer MiSeq, (Illumina, USA), all stages of sample preparation were carried out according to the manufacturer's instructions of Illumina [1, 11].

To confirm the results, direct Sanger sequencing was carried out on an ABI 3500 genetic analyzer (Life Technologies, USA), using specially designed primers. A complex of basic molecular genetic research methods was used: polymerase chain reaction (PCR), agarose gel electrophoresis, direct Sanger sequencing. For PCR, flanking primers 5'-GACTGGTAAGGAAACACTTAC-3 '(forward), 5'-CATGTCCAGTAAAGAG-

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CAATG-3' (reverse) were used. Primers of the *EXT2* gene were selected using the NCBI / Primer-BLAST program and synthesized at Evrogen Closed Joint Stock Company (Moscow) [8]. The genome assembly number is Genome Reference Consortium Human GRCh38 (GCA_000001405.15). The Bioinformatics program MutationTaster was used to predict the pathogenicity of the found variants (http://www.mutationtaster.org/).

Bioinformatic analysis. The initial analysis of the data obtained as a result of the MPS was carried out directly in the MiSeq system itself. The data were aligned to the reference sequence GRCh37 (hg19). The obtained variants were filtered using the Sophia DDM v4 program (Sophia Genetics, Switzerland). The *EXT2 g*ene transcript: NM_001178083 was selected to annotate the identified variants. The clinical interpretation was carried out in accordance with the Russian guidelines for the interpretation of human DNA sequence data obtained by MPs methods [2]. The following databases were used to verify the results: ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), OMIM (https://www.omim.org), Exome Variant Aggregation Consortium (http://exac.broadinstitute.org/), dbSNP build 153 (https://www.ncbi.nlm.nih.gov/snp/), dbVar (https://www.ncbi.nlm.nih.gov/dbvar/), Exome Variant Server (https://evs.gs.washington.edu/EVS/), Leiden Open Variation Database system (https://databases.lovd.nl/shared/genes/*EXT2*)

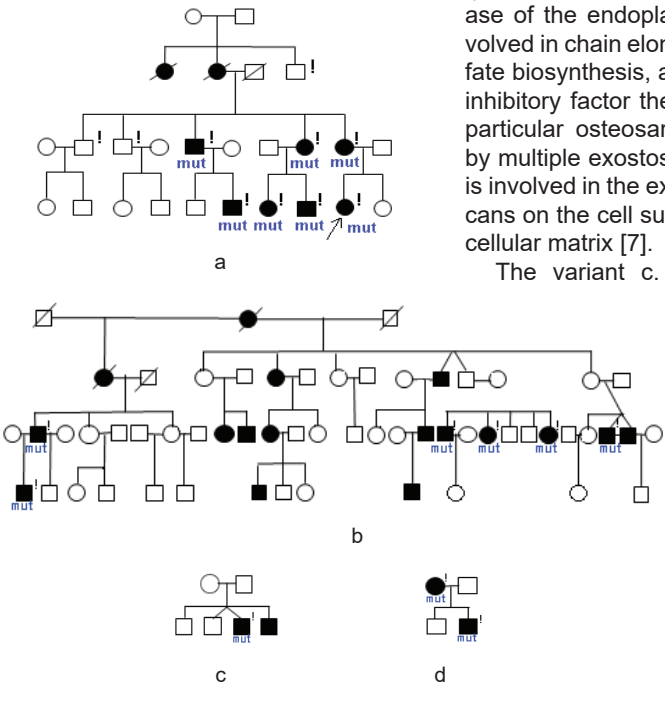
Results and discussions. For the study of mutations, the G's family with the largest number of HME was selected (Fig. 1, A). For the first time the proband addressed to the Medical and Genetic center of the RH1-NCM at the age of 10 with complaints of tumor-like formations in the shoulder area. There were exostoses of large sizes, shortening of the left hand. Then, at the age of 14, the patient had complaints of increased exostosis, curvature of the forearm bones, ulnar deviation of the hands, and restriction of movement in the arms. There were large exostoses, shortening of the left arm. From the age of 14, the patient began to complain of an increase in exostosis, curvature of the forearm bones, ulnar deviation of the hands, and movement limitation in the hands. At the time of the last examination, the proband was 20 years old.

As a result of MPS and the filtration of nucleotide sequence variants, the nonsense mutation c.751C>T (p.Gln251*) in exon 5 of the *EXT2* gene in the heterozygous state was revealed at the proband.

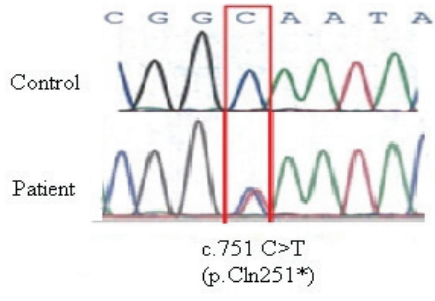
The *EXT2* gene is located on the 11th chromosome at the 11p12-p11 locus, consists of 14 exons and two exons at the alternative splicing site, encodes a type II transmembrane glycosyltransferase of the endoplasmic reticulum, is involved in chain elongation in heparan sulfate biosynthesis, and can also act as an inhibitory factor the growth of tumors, in particular osteosarcomas, accompanied by multiple exostoses. The gene product is involved in the expression of proteoglycans on the cell surface and in the extracellular matrix [7].

The variant c. 751C>T (p.Gln251*) of the *EXT2* gene was detected in the Leiden Open Variation Database system (https://databases.lovd.nl/shared/genes/EXT2). According to the LOVD database, this mutation has been detected in members of two European families. The result is presented with no detailed description of the clinical picture of patients with HME [6, 9]. Further, the direct Sanger sequencing was performed in 54 individuals with HME clinical diagnosis. As a result, 16 (29.09%) of 55 patients with HME had this nonsense mutation c.751C>T (p.Gln251*) in exon 5 of the *EXT2* gene in a heterozygous state (Fig. 2). All 16 patients with this mutation come from Yakut families. The direct Sanger sequencing was also performed on 11 relatives of patients with HME, and the mutation was not detected.

Thus, the diagnosis of HME was confirmed by contemporary methods of the molecular genetic studies in 16 patients from 4 unrelated Yakut families (Fig. 1, B-D).



Picture 1. Pedigrees of families of patients with hereditary multiple exostoses.



Picture 2. Chromatogram fragment of the exon 5 gene *EXT2*
Control – healthy; Patient – patient with hereditary multiple exostoses with nonsense mutation c.751C>T (p.Gln251*)

Conclusion. As a result of the search for mutations in the *EXT1* and *EXT2* genes among 55 patients from 31 unrelated families of different ethnic origin with a clinically diagnosed HME, a rare nonsense mutation c.751C>T (p.Gln251*) in the exon 5 of the *EXT2* was revealed among 16 (29.09%) patients from 4 unrelated Yakut families.

Approaches have been developed for molecular genetic laboratory diagnostics of the identified mutation, which can be used to confirm the diagnosis of HME, predict, and prevent adverse outcomes.

At present, we are conducting the investigation to search for mutations in the *EXT1* and *EXT2* genes in other families with HME.

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DIAGNOSTIC AND TREATMENT METHODS

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ANALYSIS OF THE LEVEL OF THYROID HORMONES IN PATIENTS WITH HEARING DISORDERS IN THE REPUBLIC OF BURYATIA: SEARCH FOR PENDRED SYNDROME PHENOTYPES

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В статье представлены результаты диагностического поиска фенотипов, соответствующих клинической картине синдрома Пендреда (ceThe article presents the results of the diagnostic search of the hereditary autosomal recessive disease Pendred's syndrome (sensorineural deafness combined with thyroid disorders) in patients with hearing disorders in Buryatia using instrumental (threshold tone audiometry) and laboratory methods (ELISA analysis of FT3, FT4 and TSH). Threshold tonal audiometry and analysis of thyroid hormone levels were performed in 164 patients with hearing impairment. The analysis showed that 7.9% (13 out of 164 people) of patients with severe hearing loss and deafness can be assumed to have thyroid disorders (12 people – hypothyroidism, 1 person – hyperthyroidism). Overall, 7.3% of deaf patients with hypothyroidism (12 of 164) were formally consistent with the clinical features characteristic of Pendred's syndrome.

Keywords: Pendred's syndrome, hearing impairment, thyroid-stimulating hormone (TSH), free triiodothyronine (FT3), free thyroxine (FT4), Buryatia.

Summary. The article presents the results of the diagnostic search of the hereditary autosomal recessive disease Pendred's syndrome (sensorineural deafness combined with thyroid disorders) in patients with hearing disorders in Buryatia using instrumental (threshold tone audiometry) and laboratory methods (ELISA analysis of FT3, FT4 and TSH). Threshold tonal audiometry and analysis of thyroid hormone levels were performed in 164 patients with hearing impairment. The analysis showed that 7.9% (13 out of 164 people) of patients with severe hearing loss and deafness can be assumed to have thyroid disorders (12 people – hypothyroidism, 1 person – hyperthyroidism). Overall, 7.3% of deaf patients with hypothyroidism (12 of 164) were formally consistent with the clinical features characteristic of Pendred's syndrome.

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Introduction. Congenital sensorineural deafness or severe hearing loss is registered with an average frequency of 1 per 1000 newborns [11]. About 20-30% of inherited hearing disorders are registered as part of various syndromes [12]. Finding out the etiology and correct diagnosis of syndromic forms of hearing loss and deafness are necessary conditions for proper consultation and choice of treatment tactics for such patients. One of the most common causes of syndromic hearing loss is Pendred's syndrome (OMIM #274600), which is observed in 10% of cases of congenital deafness [16, 20]. The prevalence of Pendred syndrome ranges from 7.5 to 10 cases per 100 000 people [8, 20]. Pendred syndrome is an autosomal recessive disease characterized by a combination of sensorineural hearing loss with or without hypothyroidism [20]. Given its autosomal recessive type of inheritance, the risk of inheritance from heterozygous parents is 25% [5, 20]. It is known that in most cases the syndrome is caused by biallelic mutations in the *SLC26A4* gene that lead to a defect in the pendrin protein [18]. The *SLC26A4* gene is located on chromosome 7 (7q22.3) and is expressed in many organs and tissues, including the inner ear, kidneys, thyroid, and bronchial epithelial cells [18, 20]. The product of the *SLC26A4* gene, the pendrin protein, is a multifunctional anion exchanger that has affinity for chloride, iodide, bicarbonate, and other anions [5, 20].

It should be noted that Pendred's syndrome does not always show abnormalities in the thyroid gland. Usually, they are observed in residents of regions with iodine deficiency. Iodine deficiency of various degrees of severity was detected almost throughout the Russian Federation [1]. In turn, the Republic of Buryatia belongs to the regions with the most tense situation in terms of the severity of natural iodine deficiency [1]. In such regions, there may be high rates of thyroid diseases.

In this regard, the purpose of this work is to analyze the level of thyroid hormones in patients with hearing disorders in the Republic of Buryatia, to search for phenotypes that correspond to the clinical picture of Pendred syndrome.

Materials and methods.

Patients

For this study, ELISA analysis of the level of thyroid hormones including free triiodothyronine (FT3), free thyroxine (FT4) and thyroid-stimulating hormone (TSH) in patients with predominantly congenital pronounced hearing loss of un-

known etiology (n=164) was performed at the clinical and diagnostic laboratory of the N. A. Semashko Republican clinical hospital (Ulan-Ude, Republic of Buryatia). The age of the patients ranged from 18 to 82 years. The average age of women and men is 52.6 and 43.2 years, respectively (table 1). The share of Buryats and Russians by national composition was 47.5% and 46.3%, respectively. The majority of patients had bilateral deafness (70.7%) and early age (0-12 years) manifestations of hearing loss (97.5%). Individuals with a previously established diagnosis of thyroid pathology were not identified.

Threshold tonal audiometry. To determine the type and degree of hearing loss in patients, threshold tonal audiometry was performed using a portable audiometer "MAICO ST 20" (Germany) for air conduction at frequencies 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 kHz and bone conduction at frequencies 0.25, 0.5, 1.0, 4.0 kHz in increments of 5.0 dB. The degree of hearing loss was assessed by the hearing thresholds of the better-hearing ear in the speech frequency range 0.5, 1.0, 2.0, 4.0 kHz according to the international classification, according to which I degree of hearing loss corresponds to 26-40 dB, II degree – 41-55 dB, III degree – 56-70 dB, IV degree – 71-90 dB, deafness >90 dB.

ELISA-analysis of circulating FT3, FT4 and TSH. To determine the concentration of TSH, FT3 and FT4 circulating in the blood, the following enzyme immunoassay kits were used: T3 free-ELISA-BEST (JSC "Vector-best") (sensitivity: 0.5 pmol/ml; measurement range: 0-20 pmol/ml); T4 free-ELISA-BEST (JSC "Vector-best") (sensitivity: 0.5 pmol/ml; measurement range: 0-80 pmol/ml); TTG-ELISA-BEST (JSC "Vector-best") (sensitivity: 0.05 mU/l; measurement range: 0-16 mU/l). The measured concentrations of TSH, FT3 and FT4 were carried out on a microtiter plate reader VICTORX5 Multimode Plate Reader (Perkin Elmer Inc., USA). Reference values of TSH and thyroid hormone levels (FT3, FT4) are given in the explanation to table 2.

Results and discussion. This paper presents for the first time the results of a diagnostic search for phenotypes corresponding to the clinical picture of Pendred syndrome (sensorineural deafness combined with thyroid disorders), conducted using instrumental (threshold tone audiometry) and laboratory methods (ELISA analysis of FT3, FT4 and TSH) in patients with hearing disorders in the Republic of Buryatia. Of the 164 examined patients with hearing impairment, 13 had clinically significant deviations from the reference levels of TSH, FT3, and FT4. 4 patients were diagnosed with manifest hypothyroidism, 8 with subclinical hypothyroidism, and one patient with subclinical hyperthyroidism (table 2, fig. 1). The Type and degree of hearing loss in 13 patients with clinically significant abnormalities in thyroid hormone levels are shown in table 2.

Table1

Characteristics of a sample of hearing impaired patients from the Republic of Buryatia

Characteristics of the sample	n	%
Gender		
Female	96	58.5
Male	68	41.5
Nationality		
Buryat	78	47.6
Russians	76	46.3
Other nationality*	10	6.1
Place of residence		
Republic of Buryatia	112	68.3
Chita region	10	6.1
Irkutsk region	7	4.3
Other region	35	21.3
Degree of hearing loss		
Bilateral deafness	116	70.7
Grade IV bilateral sensorineural hearing loss	12	7.3
Sanseverina bilateral hearing loss of II-III degree	6	3.7
Bilateral, conductive hearing loss of II-III degree	1	0.6
Sensorineural hearing loss of II, III, IV degrees on the right/left, deafness on the right/left	26	15.9
Mixed hearing loss	3	1.8
Manifestation age of deafness/hearing loss		
0 - 12 years	160	97.6
18 – 30 years	2	1.2
Unknown	2	1.2
Heredity		
Unencumbered	123	75.0
Burdened	39	23.8
Unknown (orphan)	2	1.2
Total	164	100.0
Middle age		
Female	52.6 years	
Male	43.2 years	

Note: * Mongols, Evenks, Nanais, Uzbeks, Chuvash and individuals with mixed ethnical origin.

The decreased TSH with normal levels of FT3 and FT4 was observed in one patient (Russian, 82 years old), which corresponds to the preliminary diagnosis of subclinical hyperthyroidism. For Pendred's syndrome, hyperthyroidism is usually not characteristic. It is possible that the hyperthyroid state in this patient is associated with concomitant age-related changes and chronic diseases.

For the clinical picture of Pendred's syndrome, the state of hypothyroidism is more specific. So, in 4 patients, we observed an increased level of TSH, with a reduced FT4 and the normal level of FT3, which corresponds to the preliminary diagnosis-manifest (explicit) hypothyroidism. By ethnicity, these patients were Buryats, female, middle-aged and elderly. There was no information about the previously established diagnosis associated with thyroid pathology in the anamnesis.

Table2

Types and degrees of hearing loss in 13 patients with clinically significant abnormalities in thyroid hormone levels

№	Patient ID	Gender	Age	Nationality	Type / degree of hearing loss	Age onset of hearing loss	Heredity	TSH (mU/l)*	FT3 (pmol/ml)**	FT4 (pmol/ml)***
Overt hypothyroidism										
1	2119	female	57	Buryat	Bilateral deafness	0	burdened	**8.16**	5.17	**10.84**
2	2131	female	69	Buryat	Grade IV bilateral mixed hearing loss	30	-	**8.5**	4.82	**11.4**
3	2190	female	53	Buryat	Bilateral deafness	0	burdened	**5.52**	4.25	**10.49**
4	2240	female	56	Buryat	Bilateral deafness	0	-	**15.42**	3.46	**9.19**
Subclinical hypothyrosis										
5	2152	female	42	Buryat	Grade II sensorineural hearing loss on the right, deafness on the left	0	-	**5.12**	5.4	13.71
6	2178	female	40	Russian	Bilateral deafness	0	burdened	**4.85**	6.72	15.21
7	2199	female	75	Buryat	Bilateral deafness	unknown	-	**4.86**	4.6	13.9
8	2203	female	65	Russian	Grade III sensorineural hearing loss on the left, deafness on the right	2	-	**13.64**	4.36	12.22
9	2116	male	69	Russian	Grade IV bilateral sensorineural hearing loss	4	-	**4.68**	4.3	15.14
10	2187	male	38	Buryat	Bilateral deafness	0	-	**4.72**	5.23	17.97
11	2238	male	23	Buryat	sensenvral hearing loss of II-III degree	3	-	**8.11**	6.01	14.02
12	2241	male	23	Buryat	CAE ,Bilateral, conductive hearing loss of II degree on the right, III degree on the left	0	burdened	**5.61**	5.1	15.1
Subclinical hyperthyroidism										
13	2193	male	82	Russian	Bilateral deafness	3	-	**0.239**	5.09	14.12

Note: * - reference value of TSH level-0.24-4.3 mU/l; * * - FT3-3.1-6.8 pmol / ml; * * * - FT4-12-22 pmol/ml. Bold text indicates deviations from the reference values, dash - heredity is not burdened, CAE - Congenital Artesia of Ears

Subclinical hypothyroidism was detected in 8 patients (5 Buryats, 3 Russians). When divided by age, 5 patients belonged to the young age group, 3 patients to the elderly age group. The clinical characteristics of patients are presented in table 2. Thus, the proportion of cases of hypothyroidism among patients with hearing disorders in Buryatia corresponding to the clinical picture of Pendred's syndrome was 7.3% (67.7% - subclinical hypothyroidism, 33.3% - manifest hypothyroidism) (Fig. 1).

As a rule, patients with Pendred syndrome have a violation of iodine organification in the thyroid tissues, mediated by a violation of the normal conformation of the pendrin protein (anion exchanger) on the apical membrane of thyrocytes, which should transport iodine from the thyrocyte to the follicle. Apical iodine outflow is stimulated by TSH [17]. Under conditions of sufficient dietary iodide intake, most people with Pendred syndrome are clinically and biochemically euthyroid (thyroid hormone levels are in reference values) [4, 10, 12, 19], however, if dietary iodide is not sufficient, patients with Pendred syndrome may experience subclinical or manifest hypothyroidism [2, 9]. For example, patients with documented biallelic mutations in the *SLC26A4* gene from countries with high iodine intake, such as Japan and Korea are always euthyroid [3, 6, 13]. Currently, goiter is not considered a permanent feature in Pendred syndrome, since it is present in 60-80% of patients [14, 18]. It is possible that the apparent and subclinical hypothyroidism in 12 deaf patients is mediated by age or insufficient iodine intake, however, it is possible that the cause of hypothyroidism in these patients may be a defect in the pendrin protein, which disrupts the organization of iodine in thyroid tissues and causes Pendred syndrome. For this group of patients requires further studies using computed tomography of the temporal bone (syndrome-specific anomalies of the inner ear according to the type EVA / Mondini), perchlorate test (to determine the defect iodides organification of the thyroid gland) and molecular genetic studies to search for variants katatelnyh responsible for the development of Pendred syndrome (*SLC26A4* gene).

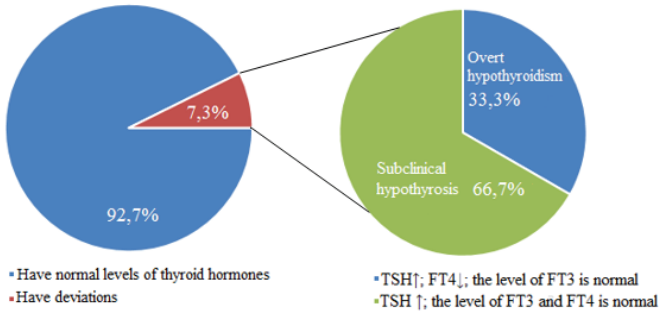

Figure 1. The proportion of cases of hypothyroidism among patients with hearing loss in the Republic of Buryatia (phenotypes of Pendred's syndrome).
Note: TSH – thyroid-stimulating hormone, FT3 – free triiodothyronine, FT4 – free thyroxine, ↑ - level above the reference values, ↓ - level below the reference values.

CONCLUSIONS

1. Analysis of the level of thyroid hormones in patients with hearing disorders from the Republic of Buryatia showed that 7.9% (13 out of 164) of patients can be assumed to have a deviation of the normal functioning of the thyroid gland;

2. Overall, 7.3% of deaf patients with hypothyroidism (12 out of 164) formally corresponded to the clinical features of Pendred's syndrome.

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IMPORTNACE OF PREOPERATIVE DIAGNOSIS OF ABERRANT RIGHT SUBCLAVIAN ARTERY IN PARATHYROID SURGERY: THE CLINICAL OBSERVATION

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Aberrant right subclavian artery (arteria lusoria) is an abnormality in the development of the aortic arch and its branches, which is often associated with the right non-recurrent laryngeal nerve. The presence of this anatomical variant increases the risk of intraoperative damage to the laryngeal nerve to 12.9% (in the classic version, the risk is 1-2%). **The purpose of this article** is to show a clinical observation of the preoperative diagnosis of arteria lusoria when planning surgical intervention for a patient with benign parathyroid disease. **Presentation of the case**. This clinical case presents a 53-year-old male patient with primary hyperparathyroidism. During an additional preoperative examination (MRI) in order to search for the localization of parathyroid adenoma and to exclude multiple disease of the parathyroid glands, an aberrant right subclavian artery was detected. To clarify the vascular architectonics of the branches of the aortic arch, multispiral computed (MSCT) angiography of the brachycephalic arteries was performed. The study showed that the first branch of the aortic arch is the common mouth of the carotid arteries, the second is the left subclavian artery and the third is the right subclavian artery (type H according to the Adachi – Williams classification). The latter goes from left to right in an oblique-lateral direction behind the esophagus, deforming its lumen along the posterior wall. A vascular anomaly was an accidental find that played a significant role in planning the progress of the operation. As a result of the preoperative assessment of the patient's anatomical features, the intraoperative trauma and the search time for the non-recurrent laryngeal nerve and parathyroid adenoma were minimal. **Discussion and conclusions.** We consider it necessary to use all possible methods for imaging the parathyroid glands, including MSCT angiography, MRI in order to exclude the possibility of multiple disease when planning surgical intervention for a patient with primary hyperparathyroidism. In this clinical case, this approach made it possible to diagnose an aberrant right subclavian artery in the preoperative period and suggest an association with an non-recurrent right laryngeal nerve.

Keywords: aberrant right subclavian artery, arteria lusoria, non-recurrent right laryngeal nerve, primary hyperparathyroidism, parathyroidectomy.

Introduction. Aberrant right subclavian artery is the most common anomaly in the development of the aortic arch and its branches [3]. According to autopsy studies, the prevalence of this anomaly ranges from 0.16 - 4.4% in the general population [12,14].

The first description of the aberrant right subclavian artery was given by Hanuld in 1735 [18]. D. Bayford in 1761 published an observation by a patient with long-term dysphagia, which led to her death. At autopsy, the author found an abnormal discharge of the right subclavian artery compressing the esophagus [5]. It was D. Bayford who called this anomaly “arteria lusoria” and the most common symptom is “dysphagia lusoria” from the Latin phrase “lusus naturae”, which literally translates as “freak of nature”. According to the Adachi – Williams classification, there are 4 types of arteria lusoria: type G — the right subclavian artery departs from the distal part of the aortic arch with the last branch. The remaining branches of the aortic arch depart unchanged; type CG - the right subclavian artery departs, as in type G, the left vertebral artery - from the aortic arch; type H - the right subclavian artery departs, as in type G, the common right and left carotid arteries depart as a single trunk; type N - is a reflection of type G [4.15]. In addition, 3 variants of the location of this anomaly are distinguished depending on the relationship to the trachea and esophagus: behind the esophagus (80%), between the esophagus and trachea (15%) and before the trachea (5%) [10].

Most often, the aberrant subclavian artery is combined with developmental abnormalities such as Commerl's diverticulum (saccular aneurysmal expansion in the area of the mouth of the left subclavian artery), the common mouth of the carotid arteries, Fallot tetrad, interventricular septal defect, atresia of the pulmonary trunk, pulmonary arteries [2]. From the point of view of the endocrine surgeon, it is extremely important to know about the combination of arteria lusoria with the right non-recurrent laryngeal nerve (NRLN). G. Stedman in 1823 for the first time reported the right of NRLN, which was accompanied by an anomaly in the branching of the branches of the aortic arch [16]. NRLN is a rare anatomical variant, in which the nerve departs from the cervical region of the vagus nerve and enters the larynx, without forming a loop under the subclavian artery in the mediastinum [8]. The prevalence of NRLN in the population is 0.3-0.8% on the right, 0.004% on the left [11] and is closely related to vascular abnormalities of the aortic arch [17]. The presence of this anatomical variant increases the risk of intraoperative damage to the laryngeal nerve to 12.9% (in the classic version, the risk is 1-2%) [17].

The main aim of this article is to show a clinical observation of the preoperative diagnosis of arteria lusoria when planning surgical intervention for a patient with primary hyperparathyroidism.

Presentation of case.

A 53-year-old patient turned to a local neurologist with complaints of anxiety in 2019. During the examination, an el-

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evated serum level of total calcium was detected (02.15.2019) - 2.66 mmol / l (reference values: 2.10-2.60 mmol / l) and parathyroid hormone (02.15.2019) - 73.68 pg / ml (reference values: 15.00-65.00 pg / ml). According to the ultrasound examination (ultrasound) of the thyroid gland (03/07/2019): a hypoechoic homogeneous formation 1.2 × 0.9 × 0.9 cm in size was determined from the posterior contour of the right thyroid gland. To determine further treatment tactics, he was referred to an endocrine surgeon at the Irkutsk Regional Clinical Hospital (IRCH).

In 2020, it was examined on the basis of the IRCH. In the patient's biochemical blood analysis (02.26.2020), the serum level of total calcium was increased - 2.69 mmol / l (reference values 2.1-2.6 mmol / l), ionized calcium - 1.5 mmol / l (reference values 1.15-1.27 mmol / l), parathyroid hormone - 78.4 pg / ml (reference values 15.0 - 68.3 pg / ml). The daily urinary calcium excretion was 12.24 mmol / day (reference values 2.5-6.25 mmol / day). On scintigraphy, an increase in the functional activity of the parathyroid glands was not significantly established. The minimum T-score according to osteodensitometry was -1 in the neck of the left femur. Ultrasound scanning of the kidneys revealed the presence of microliths in both kidneys. In order to clarify the localization of the thyroid adenoma, magnetic resonance imaging (MRI) of the neck was performed with intravenous contrast (03.03.2020), on which the right upper thyroid gland, enlarged to 1.5x1.0x1.0 cm, was located at the lower pole of the right lobe of the thyroid gland (Thyroid gland) (Fig. 1).

In addition, it was found that the right subclavian artery is located behind the esophagus and departs directly from the aortic arch. To clarify the vascular architectonics of the branches of the aortic arch, multispiral computed (MSCT) angiography of the brachycephalic arteries was performed. The study showed that the first branch of the aortic arch is the common mouth of the carotid arteries, the second is the left subclavian artery and the third is the right subclavian artery (type H according to the Adachi – Williams classification) (Fig. 2a).

The latter one goes from left to right in an oblique-lateral direction behind the esophagus, deforming its lumen along the posterior wall (Fig. 2b).

According to a preoperative study, the patient was scheduled for surgery in the amount of cervicotomy, right upper parathyroid parathyroidectomy, a biopsy of the right lower thyroid with intraoperative monitoring of parathyroid hormone.

The operation took place on 03.04.2020. According to the standard method, a cervicotomy and access to the thyroid gland were performed. The patient had a peculiarity of vascular architectonics: an additional artery located deeper than the common carotid artery. Non-recurrent right lower laryngeal nerve extending from the vagus nerve in the neck, at the level of the upper pole of the thyroid gland, was also found (Fig. 3).

An encapsulated right upper parathyroid gland 1.5 x 1.0 x 1.0 cm dark brown in color was found dorsally with respect to the right non-recurrent laryngeal nerve at the level of the middle third of the right thyroid gland, encapsulated. Ventrally with respect to the right non-recurrent laryngeal nerve, at the level of the lower third of the right thyroid lobe, caudal to the lower pole of the right lobe of the thyroid gland, the left lower parathyroid gland of 0.6x0.3x0.2 cm of gray-yellow color was not visually changed. The mobilization and removal of the right upper thyroid and a 1/3 biopsy of the right lower thyroid for histological control were performed. The dynamics of the level of intraoperative monitoring of intact PTH was as follows: before the skin incision - 129 pg / ml; at the time of mobilization of the right upper parathyroid gland - 192 pg / ml; 10 minutes after removal of the right upper parathyroid gland - 36.3 pg / ml. The intraoperative test according to the Miami criterion is positive.

According to a

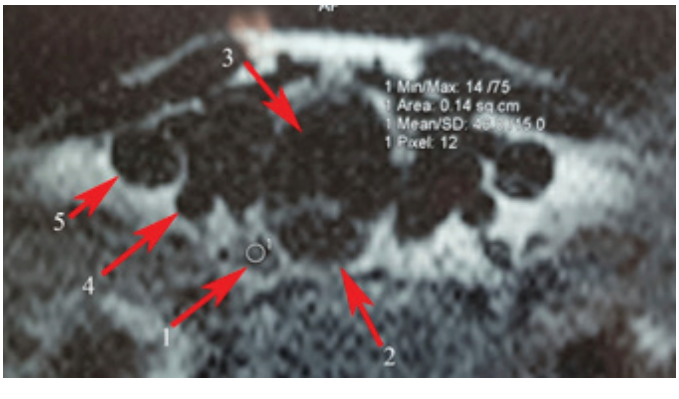
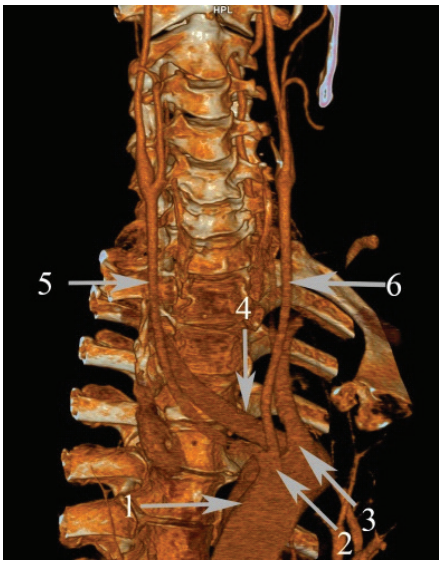
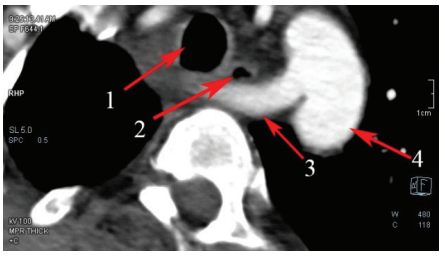


Fig. 1. MRI-image. MRI of the neck with intravenous contrast. Arrows indicate anatomical structures: 1 - the right upper parathyroid gland; 2 - the esophagus; 3 - trachea; 4- right common carotid artery



a



b

Fig. 2a. MSCT-image. 3D reconstruction, frontal projection. Angiography of the aortic arch and its branches. Arrows indicate vascular structures: 1 - aortic arch; 2 - a common trunk of the common carotid arteries; 3 - the left subclavian artery; 4- aberrant right subclavian artery; 5 - right common carotid artery; 6 - the left common carotid artery.

Fig. 2b. MSCT-image. Axial projection. Angiography of the aortic arch and its branches. Arrows indicate anatomical structures: 1 - lumen of the trachea; 2 - the lumen of the esophagus; 3 - aberrant right subclavian artery; 4- aortic arch

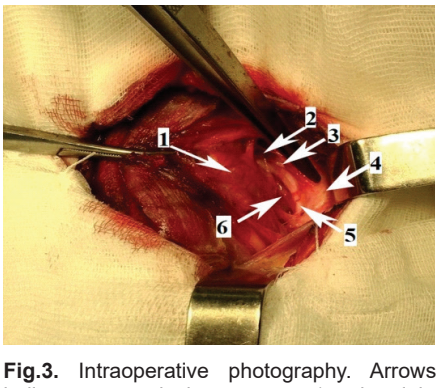


Fig.3. Intraoperative photography. Arrows indicate anatomical structures: 1 - the right lobe of the thyroid gland; 2- lower edge of adenoma of the right upper parathyroid gland; 3 - the right non-recurrent laryngeal nerve; 4- right common carotid artery; 5 - the right vagus nerve; 6 - upper edge of adenoma of the right upper parathyroid artery

histological study, the right upper parathyroid gland is represented by an adenoma from the dark main cells. She had her own capsule and a portion of unchanged tissue of the parathyroid gland from the main light cells on the periphery (Fig. 4). On biopsy sections, 1/3 of the right lower parathyroid gland had normal tissue structure.

In the postoperative period, laryngoscopy was performed - normal mobility of the vocal folds was established. On the 1st day after surgery, the level of PTH was 32.6 pg / ml. The total blood calcium was determined on 05.03.2020 2.26 mmol / l (albumin 48 g / l), 06.03.2020 - 2.54 mmol / l (albumin 44 g / l). On day 2, drainage was removed. Sutures were removed on day 7, healing by first intention. Discharged under the supervision of an outpatient surgeon and endocrinologist.

Discussion. According to the 2017 meta-analysis, the prevalence of NRLN on the right is 0.7%, and in 86% this anatomical feature is associated with an aberrant subclavian artery [9]. Another study reported a combination of NRLN with arteria lusoria in 97.7% of cases [6]. A high percentage of combination of NRLN with an aberrant right subclavian artery entails a high risk of intraoperative damage to the laryngeal nerve. Options for preoperative diagnosis of NRLN were considered. Ultrasound proved to be a simple, non-invasive, cost-effective method for diagnosing NRLN with a sensitivity of 99-100% and a specificity of 41-100% [7]. MSCT is an indirect method for the diagnosis of NRLN by detecting an aberrant right subclavian artery [13]. In most cases, the association of NRLN with the aberrant subclavian artery is established retrospectively in the postoperative period after targeted detection of an abnormality of the laryngeal nerve during surgery [1]. In the presented clinical case, diagnostic imaging methods (MRI, MSCT) were used to search for adenomas and exclude the multiple nature of the thyroid lesion (due to the negative result of scintigraphy). It should be noted that MRI was performed before MSCT for technical reasons, and not because of the preference of this method. A vascular anomaly was an accidental find that played a significant role in planning the progress of the operation. As a result of the preoperative assessment of the patient's anatomical features (Fig. 5), the intraoperative trauma and the time of searching for NRLN and parathyroid gland were minimal.

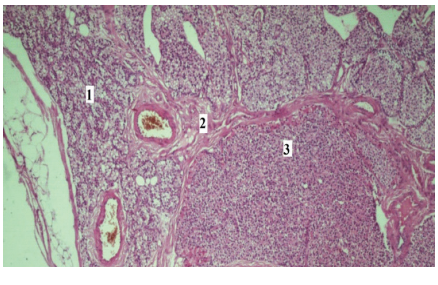


Fig.4. Microphotography of operational material. Hematoxylin-eosinoma staining. Magnification 10x0.25. Tissue of the right upper parathyroid gland. A section of unchanged tissue with light main cells (1), a section of connective tissue capsule (2), a section of adenoma tissue from the main dark cells (3).

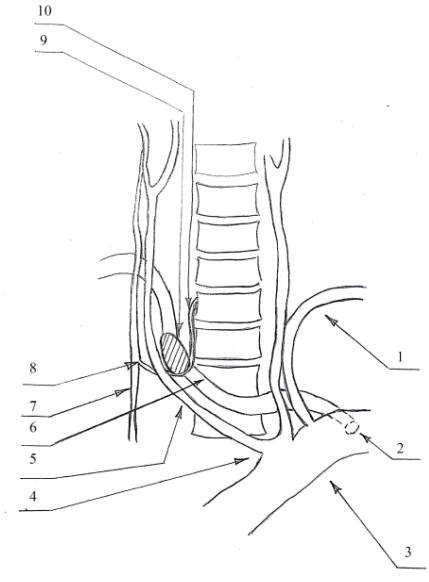


Fig.5. Schematic representation of the vascular architectonics of the branches of the aortic arch in relation to the right upper parathyroid gland and the right irreversible laryngeal nerve. Arrows indicate anatomical structures: 1 - left subclavian artery; 2- the mouth of the aberrant right subclavian artery; 3- aortic arch; 4- common mouth of the common carotid arteries; 5- left common carotid artery; 6- aberrant right subclavian artery; 7 - the right vagus nerve; 8.10 - the right non-recurrent laryngeal nerve; 9 - the right upper parathyroid gland.

Conclusion. The high frequency of association of the aberrant right subclavian artery with non-recurrent laryngeal nerve and the high risk of its intraoperative damage explains the high significance of this anomaly in parathyroid surgery. Given the low incidence of this anatomical variant in the population, a routine preoperative search is not economically feasible. When planning surgical intervention for primary hyperparathyroidism, we consider it necessary to use all possible methods for imaging the parathyroid glands, including MSCT angiography, MRI in order to exclude the possibility of multiple lesions. In this clinical case, this approach made it possible to diagnose an aberrant right subclavian artery in the preoperative period and suggest an association with non-recurrent right laryngeal nerve. The suggestion was confirmed intraoperatively and helped to avoid trauma to the laryngeal nerve.

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TREATMENT OF CONGENITAL FOOT DEFORMITY IN CHILDREN

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Foot deformities in children with no proper correction are often accompanied by pain, functional changes and high risk of developing disability, which determines the high social significance of the nosology. The aim of this study was to evaluate the effectiveness of treatment of children's foot deformities in outpatient and inpatient settings. In the period from 2015 to 2020 109 children were examined and treated at the clinical base of the Moscow City Children's Clinical Hospital named after N.F. Filatov. The results of the study showed that in all children after the comprehensive assessment and the combination of conservative and operative correction techniques, there was complete elimination of congenital foot deformity. To achieve the complete and effective res equino-varus correction, the Ponseti procedure must be carefully followed. The early detection and correction of foot deformity is effective.

Keywords. clubfoot, adducted foot, vertical ram, congenital foot deformity, Ponseti procedure, pes equino-varus, metatarsus varus, vertical talus, pes varus, pes planovalgus, pes cavus.us.

Introduction. Pathology of the foot of congenital etiology is represented by such nosologies as Pes equino-varus (clubfoot), metatarsus varus (reduced foot), vertical talus (vertical RAM), pes varus (varus foot), pes planovalgus (flat foot), pes cavus (hollow foot). According to ICD-10 code Q66.5. The epidemiology of pes equino-varus is 1 per 1000 newborns [4], while vertical talus and metatarsus varus are quite rare [3, 8].

These nosologies are accompanied by a pronounced pain syndrome, functional changes in foot, which forces the patient to use orthopedic shoes. In the absence of proper surgical correction, the risk of disability is high. Functional disorders affect the patient's quality of life and determine the high social significance of these nosologies [5].

To date, there are a number of classifications of congenital foot pathology. According to Zatsepin-Bohm, there are two clinical forms of Pes equino-varus: typical and atypical [6]. Based on the literature available to us, the typical type of deformation accounts for 80% of cases. This type of deformity lends itself well to such treatment methods as bandaging and plaster casting.

There are also three types of soft tissue component involvement - soft tissue and bone (rigid). Belonging to a particular type of pathology is distinguished by the possibility and effectiveness of a conservative method of treatment. A number of soft-tissue types of deformations are described in the literature as the most common [1].

The aim of the study was to improve the results of treatment of pes equino-varus using the Ponseti procedure, as well as vertical talus correction by Dobbs in children in combination with massage, physiotherapy and physical therapy.

Materials and methods. In the period from 2015 to 2020, a double prospective cohort study was conducted at the clinical base of the Moscow state medical UNIVERSITY named after N. F. Filatov. 109 children with congenital deformities of the feet were selected for treatment with the proposed methods.

During the examination, 102 children (93.6%) were diagnosed with a typical and 7 (6.4%) with an atypical form of pes equino-varus. The soft tissue form was found in 51.4% of cases (in 56 children), and in 48.6% - the bone form (53 children). In 22.0% of cases, we found a left - sided type of deformity (24 children), in 18.3% - a right-sided type (20 children), and in 59.6% of cases (65 children), a bilateral lesion.

According to the age at which the deformity was detected, the patients were distributed as follows. In 73.4% of cases, deformity was diagnosed before 3 months (80 children), in 6.4% of cases from 3 to 6 months – (7 children), in 20.2% of cases over the age of 6 months (22 children). The average start time of clinical follow-up was 1.0 (1.0; 3.5) months. The average start time of treatment was 1.0 (1.0; 4.0) months. The average duration of surgical intervention was 3.0 (2.0; 4.25) months.

Surgical correction was performed in 100% of cases (91 children) with pes equino-varus and in 50% of cases (3 children) with vertical talus. Metatarsus varus

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in 100% of cases were subjected to conservative treatment. Surgical treatment was performed in 94 children (achillotomy was performed in 91 children with pes equino-varus and 3 with vertical talus).

All children with PES equino-varus and 11 (91.7%) of 12 children with metatarsus varus used the Ponseti procedure. This is a conservative technique of plastering congenital clubfoot, which consists in gradually removing all components of the deformity to the correction position, based on the biomechanics of the ankle joint and supplemented by percutaneous achillotomy.

All children with vertical talus had the Dobbs technique applied. This is a conservative technique of plaster cast for congenital equinovalgus deformity of the feet, which consists in gradually removing all components of the deformity to the correction position, based on the biomechanics of the ankle joint, supplemented by percutaneous achillotomy and in some cases fixing the 1st leg of the foot with a Kirschner spoke.

Complex treatment of children with metatarsus varus included massage procedures. Also, 5 out of 6 children (83.3%) with vertical talus had massage. Children with pes equino-varus were not given massage treatments. Courses of physiotherapy procedures were used in 16.7% of children with metatarsus varus (2 out of 12). Complex physical therapy sessions with metatarsus varus were conducted in 70.3% of cases (64 children out of 91) and in 33.3% of cases with vertical talus (4 out of 6 children). Children with pes equino-varus did not receive comprehensive physical therapy classes.

Research results. Criteria of effectiveness of treatment was: emptiness of the heel, the degree of rigidity of the Cavus the medial folds form the lateral bending of the arch of the foot, its aquinos and degree of dorsiflexion. Changes of the foot were determined according to the classification Pirani:

1. the condition of the posterior part of the foot according to Pirani before correction had more pronounced statistical differences than after correction (according to the Wilcoxon criterion = -8.955, p<0.001);

2. the degree of Cavus rigidity according to the piranha classification before correction had more pronounced statistical differences than after correction (Wilcoxon criterion = - 9.125; p<0.001);

3. Assessment of the medial fold of the foot before correction had more pronounced statistical differences than after correction (Wilcoxon criterion = - 9.105; p<0.001);

4. the Bending of the outer edge of the foot before correction had more pronounced statistical differences than after correction (Wilcoxon criterion = - 9.364; p<0.001);

5. the Equinus of the foot before correction had more pronounced characteristics than after correction (Wilcoxon criterion = - 8.879; p<0.001);

6. The evaluation of the posterior heel fold according to the Pirani system before correction had more pronounced statistical differences than after correction (Wilcoxon criterion = - 8.791; p<0.001).

The total number of points according to the piranha criteria before the correction was 4.5 (3.0; 6.0), after the correction 0 (0;0) points. The obtained differences are statistically significant (we used Friedman's analysis of variance for related samples, p<0.001).

In our study, 61 patients received outpatient surgical treatment and 45 patients received inpatient treatment (n = 106).

The results of outpatient surgical treatment. Changes in the foot according to the piranha classification were distributed as follows:

1. heel Emptiness according to the Pirani classification before correction revealed statistically significant differences than after correction (Wilcoxon criterion = -6.705, p<0.001, 1.0 (0.5; 1.0) before correction versus 0.0 (0.0; 0.0) after correction);

2. the rigidity of the Cavus according to the Pirani classification before correction had more pronounced statistical differences than after correction (Wilcoxon criterion = - 6.628; p<0.001, 1.0 (0.5, 1.0) before correction versus 0.0 (0.0; 0.0) after correction);

3. Assessment of the medial fold of the foot before correction had more pronounced statistical differences than after correction (Wilcoxon criterion = - 6.628; p<0.001, 1.0 (0.5, 1.0) before correction vs. 0.0 (0.0; 0.0) after correction);

4. the Bending of the outer edge of the foot before correction had more pronounced statistical differences than after correction (Wilcoxon criterion = - 6.683; p<0.001, 1.0 (0.5, 1.0) before correction vs. 0.0 (0.0; 0.0) after correction);

5. Equinus of the foot before correction had more pronounced statistical differences than after correction (Wilcoxon criterion = - 6.753; p<0.001, 1.0 (0.5, 1.0) before correction vs. 0.0 (0.0; 0.0) after correction);

6. The assessment of the back heel fold according to the Pirani classification before correction had more pronounced statistical differences than after correction (Wilcoxon criterion = - 6.662; p<0.001, 1.0 (0.5, 1.0) before correction versus 0.0 (0.0; 0.0) after correction).

Thus, the total score for the piranha classification before correction was 5.0 (4.0; 6.0), after correction 0 (0;0) points. The obtained differences are statistically significant (we used Friedman's analysis of variance for related samples, p<0.001).

The results of hospital surgical treatment. Changes in the foot according to the piranha classification were distributed as follows:

1. heel Emptiness according to the Pirani classification before correction had more pronounced statistical differences than after correction (Wilcoxon criterion = -5.665, p<0.001, 1.0 (0.5;1.0) before correction versus 0.0 (0.0; 0.0) after correction);

2. the rigidity of the Cavus according to the Pirani classification before correction had more pronounced statistical differences than after correction (Wilcoxon criterion = - 5.557; p<0.001, 1.0 (0.5, 1.0) before correction versus 0.0 (0.0; 0.0) after correction);

3. Assessment of the medial fold of the foot before correction had more pronounced statistical differences than after correction (Wilcoxon criterion = - 5.516; p<0.001, 1.0 (0.5, 1.0) before correction vs. 0.0 (0.0; 0.0) after correction);

4. the Bending of the outer edge of the foot before correction had more pronounced statistical differences than after correction (Wilcoxon criterion = - 5.631; p<0.001, 1.0 (0.5, 1.0) before correction vs. 0.0 (0.0; 0.0) after correction);

5. Equinus of the foot before correction had more pronounced statistical differences than after correction (Wilcoxon criterion = - 5.674; p<0.001, 1.0 (0.5, 1.0) before correction vs. 0.0 (0.0; 0.0) after correction);

6. the evaluation of the posterior heel fold according to the Pirani classification before correction had more pronounced statistical differences than after correction (Wilcoxon criterion = - 5.631; p<0.001, 1.0 (0.5, 1.0) before treatment versus 0.0 (0.0; 0.0) after treatment).

Thus, the total score for the piranha classification before correction was 5.5 (4.0; 6.0), after correction 0 (0;0) points. The differences are statistically significant (two-factor Friedman analysis for related samples, p<0.001).

Comparison of inpatient and outpatient treatment groups. Prior to treatment, the inpatient and outpatient treatment groups were comparable in all piranha classification criteria:

1. The emptiness of the heel (the Mann-Whitney test, p=0,466);

2. Cavus Rigidity (Mann-Whitney test, p=0.611);

3. Medial fold of the foot (Mann-Whitney test, p=0.986);

4. Bending of the outer edge of the foot (Mann-Whitney test, p=0.978);

5. Equinus of the foot (Mann-Whitney test, p=0.663);

6. Back heel folds (Mann-Whitney test, p=0.671).

By total score (Mann-Whitney test, p=0.917). Thus, the groups are comparable to each other in terms of these indicators. Based on the criteria for the effectiveness of treatment according to the Pirani classification, it is possible to compare clinical comparison groups by the degree of dorsiflexia achieved.

Achieved dorsiflexia greater than 15 degrees was observed in 51 cases of surgical treatment (83.6%) in outpatient settings and in 39 cases (86.6%) of surgical treatment in inpatient settings (table 1).

Table 1 – Achieved dorsiflexia in comparison groups.

The table shows that the differences between the groups are statistically insignificant (Fisher's exact test, exact significance (2-sided) = 0.139). By total score (Mann-Whitney test, p=0.917). Thus, the groups are comparable to each other in terms of these indicators.

Adverse outcomes of inpatient and outpatient treatment. After surgical correction in a hospital setting, one child required repeated surgery due to a relapse (an additional achillotomy was performed). Based on our experience and the literature we have studied, early detection of relapses of pathology is the key to successful elimination of secondary deformity. The cause of secondary deformity is usually a violation of the rules for using rehabilitation correctors, braces, and orthopedic shoes after the main stage of surgical correction is completed. Relapse is usually detected during the period of intensive foot growth-up to 10-13 years of age. Therefore, at the beginning of adolescence, such children should be regularly monitored by an orthopedist [10].

In 9.1% of outpatient cases (5 out of 55 children), children had limited movement in the distal part of the lower leg, while the same complication in the hospital was observed in 2.6% (1 out of 39 children). There are no statistical differences in the compared groups (Fisher's criterion, exact significance (2-sided) = 0.395).

Thus, both outpatient and inpatient treatment options for children with foot pathology had an equally significant impact on the evaluation criteria for treatment effectiveness. In 100% of cases of operative correction, satisfactory results were achieved. When choosing a treatment method (outpatient or inpatient), the principal criteria should be considered not only the degree of social adaptation of the patient, but also economic factors, since the clinical effectiveness of these treatment approaches was the same.

Discussion. In modern pediatric orthopedic practice, PES equino-Varus correction using the ponseti method is the "gold standard" of treatment. To achieve complete successful correction of PES equino-Varus with the prevention of relapses or other deformities, careful compliance with the ponseti Protocol is necessary. Initially, the ponseti procedure was used only in children under two years of age, but current research on the results of PES equino-Varus correction is already focused on older age groups of children [9].

Our research results are consistent with the data obtained by other authors. The Ponseti procedure is successful and relapse-free in 94-96% of cases [7].

We believe that the most preferable age for correction of deformities is early age and adhere to the position that it is necessary to start correction of deformities early (immediately after diagnosis). Based on the literature available to us, late initiation of treatment is directly proportional to the frequency of relapses and duration of treatment [2].

Conclusion. Based on the data obtained, we recommend treating PES equino-Varus as early as possible after birth (3-5 months), to prevent relapses and ensure complete correction of the deformity. Strict compliance with the Ponset Protocol is required to prevent relapses.

When correcting vertical scree, conservative correction in combination with minimally invasive surgical techniques can prevent the development of complications that were previously observed during extensive surgical procedures.

The method of Dobbs correction used by us is simpler and more effective in young children. Our data are consistent with reports of excellent results from other authors. The Dobbs correction method is less invasive and avoids the risks associated with more extensive operations [11].

We did not find any significant differences in the choice of outpatient or inpatient treatment. Taking into account the economic factor, in conditions of statistically reliable identical clinical outcomes, outpatient treatment is most preferable.

Conflict of interest. The authors declare that there is no conflict of interest.

Achieved dorsiflexia in comparison groups

			The comparison group	
			Outpatient n=61	Stationary n=45
Achieved dorsiflexia	<15°	Number, people	4	6
		Frequency, %	6.4	3.4
	> 15°	Number, people	57	39
		Frequency, %	83.6	86.6

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CLINICAL – AUDIOLOGICAL AND CLINICAL - GENEALOGICAL ANALYSIS OF CASES OF HEARING LOSS IN THE REPUBIC OF BURYATIA

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In this paper we presented for the first time the results of the clinical-audiological and clinical-genealogical research of hearing impairments in the Republic of Buryatia. The sample included Buryats (47.9%), Russians (46.1%) and representatives of other ethnicity (6%), amounting 165 people. As the result of the clinical-audiological analysis, 70.3% (n=116) of individuals had bilateral deafness of the sensorineural type, and 29.7% (n=49) had bilateral hearing loss of varying severity. The segregation analysis was carried out in 17 Buryat and 18 Russian families made it possible to assume the hereditary nature of cases of hearing loss, segregating according to the autosomal recessive mode of inheritance only in Russian families (segregation frequency SF = 0.25, at t = 0.64). The frequency rate of segregation (SF = 35, at t = 0.38) of the pathological trait in Buryat families turned out to be higher than theoretically expected for the autosomal recessive type of inheritance (SF_0 = 0.25), which indicates the presence of other types of inheritance and other forms of hearing impairments caused by non-hereditary reasons. The results of this study and the expeditionary material will be the basis for further study of the molecular genetic etiology of deafness/hearing loss in Buryatia.

Keywords: clinical-audiological analysis, clinical-genealogical analysis, hearing impairment, hereditary burden, Republic of Buryatia

Introduction. For the majority of hereditary diseases associated with organs of hearing, a large number of genes have been identified with a significant variety of mutations contributing to their development [6, 9-11; 13, 14, 18, 22, 25-29, 31], also regional and ethnic differences in the spectrum and frequencies of identified mutations have been manifested [3, 7, 15, 19-23, 30, 36]. Hereditary hearing impairments (HI) are genetically heterogeneous and manifest with different penetrance, which requires a special approach to the development of molecular diagnostics methods for genetically different forms of deafness [12, 24]. Recently, a significant number of works have been published on the successful identification (using various strategies of WES - exome sequencing) of genetic factors leading to hearing loss (HL) and the list of genes associated with hearing loss is constantly expanding (Hereditary Hearing Loss Homepage: http://hereditaryhearingloss.org). To search for the molecular genetic causes of rare forms of deafness in humans at the first stage of research, a thorough clinical and genealogical analysis of families of deaf people with large pedigrees is required.

It is known that the accumulation of a rare genetic disease due to the founder effect can occur in small isolated human populations. Most of the genes associated with one or another rare genetic disease, including those associated with hearing impairment, were first identified in families with large branched pedigrees with numerous affected individuals from isolated populations with a high endogamy index (Ashkenazi Jews, Finns, Sami, as well as inbred families from the Middle East and South Asia) [4, 8, 11]. In such populations, there is high probability of detecting new genes for human mendelian diseases. In Russia, the study of the fundamental foundations of rare (monogenic) human diseases can be carried out using the example of endogamous populations of the peoples of the Caucasus, the Volga-Ural region, Siberia and indigenous peoples of the North.

As a result, the studies of congenital forms of deafness (as one of the most frequent mendelian human diseases) in poorly studied regions of the world, such as the territory of Siberia, are especially relevant. Earlier, according to the contribution of *GJB2* gene mutations (Cx26) among samples of patients with hearing impairments from Siberian regions, the following were described in detail: the Altai Republic [16], the Sakha Republic (Yakutia) [5, 33] and the Tyva Republic [2, 34, 37]. The study of hereditary deafness in the Republic of Buryatia is a logical continuation of research among the populations of Siberia, which makes it possible to close many “blank spots” concerning the issues of genetic epidemiology of hereditary forms of deafness.

The aim of this study is to conduct audiological and clinical-genealogical analysis of the families with hearing impairment in the Republic of Buryatia, which will serve as the basis for further study of the molecular genetic etiology of HI in peoples of Eastern Siberia.

Materials and methods.

The patients

During the expedition work of the Yakutsk Scientific Center for Complex Medical Problems (Yakutsk) in the Republic of Buryatia 165 (n=160 unrelated) deaf

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people were examined in the city of Ulan-Ude. Among them, males accounted for 41.2% (n=68), females - 58.8% (n=97). The average age 48.7±14.9 years. Ethnic composition of the sample: Buryats - 79 people (47.9%), Russians - 76 people (46.1%) and representatives of other ethnicity - 10 people (6%) (Metis (Buryats/Russian) - 2, Mongol - 3, Nanai- 1, German - 1, Uzbek -1, Chuvash -1, Evenk - 1). The characteristics of the sample of the subjects are presented in Table 1.

Audiological examination. The survey was used to find out complaints about the state of hearing, the presence of discharge from the ear, tinnitus, dizziness. For each patient, the following information was obtained: life history, medical history (including previous illnesses), information about allergic reaction, injuries and / or operations, use of ototoxic drugs, contact with industrial noise. The otologic examination was carried out by the unified algorithms on the KaWe Combilight otoscope. The complete audiological examination was performed using a tympanometer and an audiometer "AA222" ("Interacoustics", Denmark). Audibility thresholds were measured by air conduction at frequencies 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 kHz and bone conduction at frequencies 0.25, 0.5, 1.0, 4.0 kHz in 5.0 dB increments. The degree of hearing loss was assessed by hearing thresholds of better-hearing ear in the voice frequency range (VFR) in accordance with the international classification under which I degree is equal to 26-40 dB in VFR, II degree - 41-55 dB, III degree - 56-70 dB, IV degree - 71-90 dB, deafness > 90 dB.

Clinical-genealogical analysis. For each participant of the study there was an individual card that included the following information: the participant's last name, first name, and patronymic, as well as their parents; age; ethnicity up to the third generations; place of birth and residence; and profession. The individual map included information about the main ENT diagnosis, the probable cause of hearing loss, age at the time of onset of hearing loss, the presence or absence of hereditary burden and concomitant diseases. After collecting the necessary information (family history), a pedigree was compiled for each proband for clinical and genealogical analysis. The information about otorhinolaryngologic diagnosis, potential cause of HI onset of HI, presence or absence of relatives with HI, including concomitant diseases in the individual patient's card. The pedigrees compiled on the basis of all obtained data were subjected to subsequent clinical-genealogical analysis.

To confirm the hereditary nature of deafness/hearing loss and clarify the type of inheritance, a segregation analysis was performed. To take into account possible distortions related to the features of material collection (insufficient awareness of the proband about close/distant relatives and their health), the expected segregation frequency (SF) was calculated using the proband method [17, 32, 39]. The following formulae were used for calculations:

to calculate the probability of registering a trait in families:

$\pi = \sum n/\sum r$ (1)

where: π - probability of registration; n - number of all probands in all siblings; r - number of those affected in all sibstances;

to calculate the expected segregation frequency of a trait in families:

$SF = \sum r - n/\sum s - n$ (2)

where: SF - expected segregation frequency, r - number of affected individuals in all siblings, n -number of all probands in all siblings, s - total number of siblings in siblings;

calculate the standard deviation:

$\sigma = \sqrt{SF (1 - SF)/\sum s - n}$ (3)

where: σ - standard deviation, SF - segregation frequency, s - total number of siblings in siblings; n - number of all probands in all siblings;

test the hypothesis about the type of inheritance:

$t = SF0 - SF/\sigma$ (4)

where: t - student's t-test, SF_0 - theoretically expected segregation frequency, SF - segregation frequency, σ - standard deviation.

Ethical approval. All examinations provided in this study have been conducted with the informed written consent of all participants or their parents. This study was approved by the local Biomedical Ethics Committee of Federal State Budgetary Scientific Institution "Yakut Science Centre of Complex Medical Problems", Yakutsk, Russia (Protocol #7, August 27, 2019).

Results clinical-audiological analysis. Based on the results of clinical and audiological analysis, the sample was divided into two groups - deafness and hearing loss of varying severity, the criterion for distinguishing which is a different degree of HL. Of the 165 examined individuals, deafness was diagnosed in 116 (70.3%) people, the remaining 49 (29.7%) individuals - HL.The results of clinical-audiological analysis are schematically shown in Figure 1.

All 116 deaf individuals had bilateral permanent sensorineural HL. Of all patients 62 individuals believe that they have HL from birth/infancy (congenital). Prelingual (period before speech formation, up to 2-3 years) form of HL is noted by 30 people, postlingual (after speech formation) form - 24 individuals, of which 17 began to HL in preschool age (from 3 to 7 years old), the rest 7 - at school age. The main reasons for HI are subjectively considered: heredity - 26 people, past infectious diseases - 10 people, past organ/tissue inflammation - 25 people, possibly injuries received in childhood - 11 people, possibly toxic effects of antibiotics - 1 person, found it difficult to answer - 1 person, do not associate with anything - 40 people. Objectively, during otological examination, the majority of the examined among the otologic problems are noted: otitis media - 26 people, tiniitis - 32 people, dizziness - 25 people. Otologic problems are presented in detail in Figure 1.

In another group with HL (n = 49), 37 individuals have the bilateral persistent HL of the sensorineural type, 11 individuals of the mixed (conductive and sensorineural) type, and one person of the conductive type. Of these, 14 individuals have a congenital form of HI, 33 people - juvenile (15 people - prelingual, 18 people - postlingual) and 2 people noticed HL in adulthood. Subjectively, of the main causes of HI are noted: heredity - 13 people, transferred infectious diseases - 6 people, transferred inflammation of

Table1

Characteristics of the samples

Sample investigated		Total individuals n (%)	Total unrelated n (%)	Sex		Middle age:
				Male	Female	
Total		165 (100%)	160 (100%)	68 (41.2%)	97 (58.8%)	48.7±14.9
Ethnicity	Buryats	79 (47.9%)	74 (46.2%)	31 (39.2%)	48 (60.8%)	44.2±13.9
	Russians	76 (46.1%)	76 (47.6%)	32 (42.1%)	44 (57.9%)	54.7±14.0
	Other*	10 (6%)	10 (6.2%)	5 (50%)	5 (50%)	39.5±12.1

Note: * - individuals of mixed and other ethnicities

organs/tissues - 15 people. Among the otologic problems in most cases tiniitis (n=11) and dizziness (n=9) were also noted. Otologic problems and the causes of HI in this group of subjects are presented in detail in Figure 1.

Thus, in accordance with the clinical and audiological analysis of the samples (n=165), the majority of individuals (92.7%) showed a persistent bilateral impairment of sound perception according to the sensorineural type (n=116 - deafness, n=37 - hearing loss). In total, 52% (n=87) of the subjects had juvenile HL, 46% (n=76) of individuals noted congenital form, two people 1,2% (n=2) noticed HI only in adulthood (at 30 and 37 years, respectively).

Clinical-genealogical analysis. Empirical data on the pedigrees of 160 probands allowed us to assume the probability of transmission of the disease by autosomal recessive type, since 97% (n=155) of probands had hearing parents who did not complain of hearing impairment. The rest of the probands (3%, n = 5) with deaf parents (both parents are affected - in 3 probands, one parent - in 2 probands), the family had hearing siblings and/or close relatives.

To establish or refute the hypothesis of AR type of inheritance, segregation analysis was carried out. To obtain correct results, this analysis was carried out separately in 17 Buryat (Table 2) and 18 Russian families (Table 3), in which repeated cases of HL (burden) are observed. In the analysis, only siblings in each nuclear family were taken into account, excluding half-siblings and indirectly (according to relatives) registered affected. During the analysis, nuclear families with one child were excluded from the material, as well as isolated cases in three generations (unburdened), since in these families (both hearing parents) it is not possible to check the segregation of the trait.

Thus, the analysis includes burdened nuclear families with two or more children with hearing parents. As a rule, the “proband” Weinberg method is most often used to calculate the segregation frequency (SF) [1, 38]. The essence of the method consists in calculating the ratio of the total number of affected siblings (without probands) to the total number of all siblings (without probands).

When establishing the hereditary nature of the pathological trait (deafness/HL) in Buryat families, the probability of registration (π) of the trait (probability completeness) by the Fisher method was:

(1) π = 17/44 = 0.38.

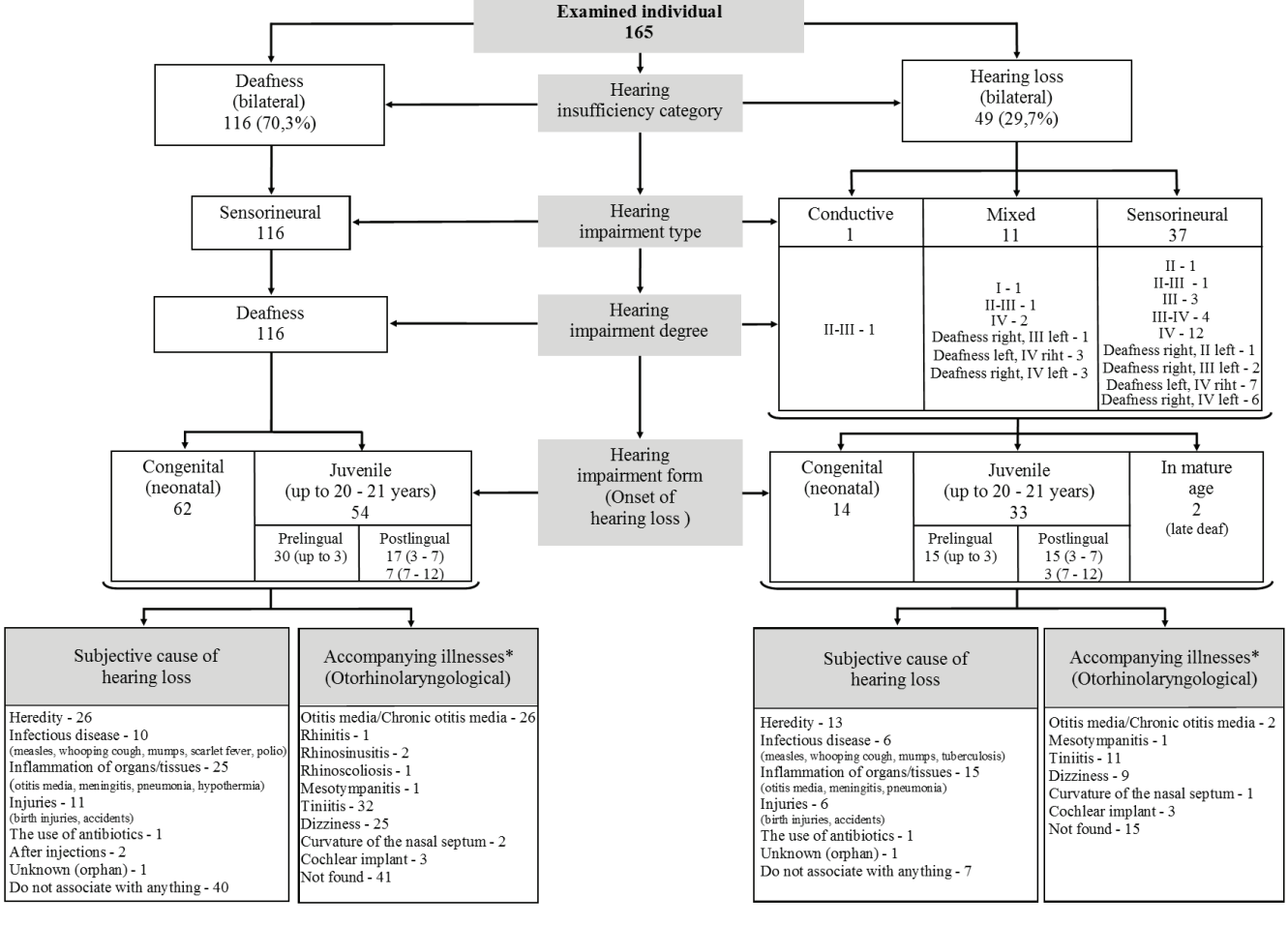
The obtained probability of registering a trait (π = 0.38) indicates its hereditary nature and corresponds to multiple incomplete registration, where $0 < \pi \leq 1$ [32, 39].

The segregation frequency (SF) or the expected proportion of the affected for all siblings was:

(2) SF = 44 - 17/94 - 17 = 27/77 = 0.35.

(3) $\sigma = \sqrt{0.35(1 - 0.35)/94 - 17} = 0.05$.

The segregation frequency (SF = 0.35) turned out to be higher than theoretically expected in autosomal recessive inheritance (SF_0 = 0.25). Further, the comparison of the obtained frequency with the theoretically expected frequency was carried out for different types of inheritance (SF_0 = 0.25 - AR, SF_0 = 0.50 - AD) using the student's t-test (4). As a result, negative values were obtained ($t = 0.25 - \sqrt{0.35/0.05}$, $t = 0.50 - \sqrt{0.35/0.05}$), which refuted these types of inheritance, where $t_{AR} > 2.58$, $t_{AD} < 2.58$.



The results of clinical and audiological analysis. * one individual may have several concomitant ENT diseases

Table2

Segregation analysis in Buryat families with signs of deafness/hearing loss

Sibship's size	Nuclear families/ probands (n)	Number of siblings with affected children				Total number children		
		2	3	4	5	Affected (r)	Healthy -	Total (s)
2	3	3				6	3	6
3	2	2				4	2	6
4	2		2			6	2	8
6	3	1	1	1		9	9	18
7	2	1	1			5	9	14
8	4	3			1	11	21	32
10	1		1			3	7	10
Total	17	10	5	1	1	44	53	94

Table3

Segregation analysis in Russian families with signs of deafness/hearing loss

Sibship's size	Nuclear families/ probands (n)	Number of siblings with affected children		Total number children		
		2	3	Affected (r)	Healthy -	Total (s)
2	10	5		10	10	20
3	2	1	1	5	2	6
4	2	2		4	2	8
5	3	2	1	5	10	15
8	1	2		4	7	8
Total	18	12	2	28	31	57

When establishing the hereditary nature of a pathological trait in Russian families, the probability of registration (π) of a trait according to Fisher's method was:

(1) π = 68/74 = 0.64.

The obtained probability of registering a trait (π = 0.64) indicates its hereditary nature and corresponds to multiple incomplete registration, where 0 <π ≤ 1.

The segregation frequency estimate, taking into account the registration probability (π = 0.64), was SF = 0.25:

(2) SF = 28 - 18/57 - 18 = 10/39 = 0.25.

The obtained values of the segregation frequency turned out to be equal for the expected AR inheritance (SF_0 = 0.25) and prove the correctness of the setting of the type of disease inheritance in the analyzed 18 Russian families.

Thus, the performed segregation analysis confirmed the hereditary character of the sign of deafness / hearing loss (according to Fisher's method 0 <π = 0.64 ≤1) segregating according to the AR type (SF = 0.25, at t = 0.64) only in Russian families. The obtained segregation frequency (SF = 0.35, at t = 0.38) of the pathological sign in 17 Buryat families turned out to be higher than the theoretically expected for the AR type of disease transmission.

Discussion. For the first time, the clinical-audiological and clinical-genealogical analysis of cases of HI in the Republic of Buryatia was carried out. As the result of this analysis of the samples (n = 165), according to the degree of HI, 70.3% (n = 116) of individuals had bilateral deafness, the remaining 29,7% (n=49) had bilateral HL, of varying severity. Among the reasons that influenced HI, 49.7% (n = 82) of the surveyed indicated exogenous factors, mainly the pathological effects of various infectious and inflammatory diseases transferred in childhood (mainly otitis media), as well as various injuries. A hereditary cause of HL was indicated by 21.8% (n = 36) of individuals. HI was not associated with anything in 28.4% (n = 47) people, in this group, in some patients, the hereditary nature of the disease may be hidden.

The segregation analysis was performed to confirm the hereditary nature of deafness/HL and to clarify the type of inheritance. Taking into account the fact that deafness/HL is extremely heterogeneous and the frequency/likelihood of the manifestation of these genes differs in ethnic populations, the segregation analysis was carried out separately for 17 Buryat and 18 Russian (n=18) families in which the inheritance of the pathological trait was noted. (repeated cases of transmission - burdened). The empirical observations of the pedigree data of families allowed us to assume the likelihood of transmission of the disease in an autosomal recessive mode of inheritance, since all probands had hearing parents, and similar signs were found in siblings, cousins, or second cousins. At the first stage of the analysis, the obtained probability of registering the trait (deafness/HL) in Buryat families was π = 0.38, in Russian families π = 0.64. These values corresponded to multiple incomplete registration (0 <π ≤ 1), which makes it possible to use this value (π) to calculate the segregation frequency. Further, the performed segregation analysis confirmed the hereditary nature of the sign of deafness/HL (according to Fisher's method 0 <π = 0.64≤1) segregating in an AR manner (SF = 0.25, at t = 0.64) in Russian families. The obtained segregation frequency (SF = 35, at t = 0.38) of the pathological trait in 17 Buryat families turned out to be higher than theoretically expected for the AR type of inheritance (SF_0 = 0.25).

Conclusion. Thus, the performed segregation analysis suggested the hereditary nature of cases of hearing impairment, segregating according to the autosomal recessive mode of inheritance only in Russian families. It should be noted that when testing one or another hypothesis of inheritance, the assessment of the segregation frequency was complicated not only by isolated cases in the family, but also by the late nature of the manifestation of the disease (in our sample, they are 52%, n=87). The difficulty lies in the fact that when compiling pedigrees in such families, siblings may not be taken into account, who do not have any symptoms or have a mild degree of HL (HI, before the progression begins). Under such circumstances, the frequency of healthy families with sick children in the sample will inevitably be overestimated. Another problem may be associated with the presence of other types of inheritance and other forms of hearing loss caused by non-hereditary reasons in some genealogies with hereditary burden.

We hope that in the future the results obtained in the course of this work will allow to develop the most optimal approach to the molecular genetic study of hereditary hearing impairments in the Republic of Buryatia, the results of which

will complement the information on the genetic etiology of deafness/hearing loss in populations of Eastern Siberia.

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HEALTHY LIFESTYLE. PREVENTION

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INFLUENCE OF SMOKING ON METABOLIC DISORDERS AND THEIR RELATIONSHIP IN INDIGENOUS POPULATION OF THE ARCTIC TERRITORY OF YAKUTIA

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The study of effect of smoking on metabolic disorders and determination of the association between them in the indigenous population of the northern territory of Yakutia was carried out. A high frequency of risk factors for the development of cardiovascular pathology, such as overweight, obesity and smoking, was revealed. Overweight is equally common in both men and women, obesity is found almost 2.5 times more often in women. An increase in body weight, systolic blood pressure is not associated with smoking, however, the simultaneous combination of all these factors can affect the risk of cardiovascular accidents. We have confirmed the data of foreign scientists on the suppression of the level of insulin secretion under the influence of nicotine. An increased index of insulin resistance is associated with the simultaneous spread of obesity among nonsmokers. The same association of glucose level with blood pressure was obtained in both smokers and non-smokers. Metabolic disorders in the indigenous population of the northern territory of Yakutia are caused by a change in the traditional way of life with a decrease in physical activity, diet, and non-observance of a healthy lifestyle.

Keywords: smoking, overweight, obesity, insulin, glucose, systolic blood pressure, indigenous population, Yakutia.

Introduction. The prevalence of overweight and obese is a global epidemic affecting both developed and developing countries [13,21,26]. Numerous researchers have proven the relationship between obesity and insulin resistance. Obesity is generally thought to lead to hyperinsulinemia based on pathophysiological and metabolic mechanisms [12]. Hyperinsulinemia, in turn, leads to the development of metabolic syndrome and type 2 diabetes mellitus. The connection between insulin resistance and the risk of developing cardiovascular pathology has been proven in many studies [7,8,10,16,25].

It is generally recognized that tobacco smoking is one of the negative factors affecting the human body. According to the WHO, more than a billion people smoke, with a steady increase every year [31]. Russia is one of the top countries with a high prevalence of tobacco smoking [22]. Scientists have received conflicting results on the effect of smoking on body mass index. For example, scientists proved that nicotine, acting on the levels of various neurotransmitters, such as catecholamines, dopamine and serotonin, suppresses appetite and, therefore, reduces food intake [4,6]. A meta-analysis of prospective cohort studies in China, Singapore, and the United States showed that smokers with type 2 diabetes mellitus had an increased relative risk of cardiovascular complications and death [19]. The relationship between body mass index and insulin levels under the direct or indirect influence of tobacco smoking is currently widely discussed in the scientific community [17]. Previously, results were published on the high prevalence of overweight and obesity among the indigenous peoples of the northern territory of Yakutia [3]. Given the change in the traditional lifestyle and nutrition, the study of the relationship between smoking, obesity and insulin levels in this population remains relevant and poorly understood.

Aim of the study: To study the influence of smoking on metabolic disorders and their relationship in the indigenous population of the Arctic territory of Yakutia.

Materials and research methods. The collection of material for the study was carried out under expeditionary conditions in the Arctic territory of Yakutia in places of compact residence of indigenous peoples (Nizhnekolymsky, Verkhnekolymsky, Tomponsky districts). 348 people were examined using the continuous method. Patient sample consisted of adult population aged from 20 up to 70 years, 225 women, and 123 men. The response made 70%. Average age of the respondents was 48,16±0,52 years, of women 49,71±0,63, of men 44,98±0,91 years.

Inclusion criteria: representatives of indigenous people of Yakutia (Evens, Chukchi, Yukaghirs, Yakuts).

Exclusion criteria: representatives of non-indigenous nationality.

Research program included the following sections: a questionnaire for objective assessment of state; informed consent of the respondent to conduct research and donate blood (according to the protocol of the Ethics committee); anthropometric examination with measurement of height and weight with calculation of body mass index, waist and hips measurements; blood sampling from the cubital vein in the morning on an empty stomach with 12-hour abstinence from food. After centrifugation, blood serum was stored in a freezer (-70°C) until analysis. In the survey, only those who smoked at least 1 cigarette per day during the last 12 months were considered smokers.

For further analysis, the traditional indicator was used - body mass index (BMI) or Quetelet index, which was calculated by the following formula: BMI (kg / m²) = body weight (kg) / height (m²). Overweight was considered to be a BMI ≥ 25 and <30 kg/m², obesity was determined at a BMI of ≥ 30 kg/m² [according to European recommendations of the III revision, 2003].

Laboratory methods included the determination of insulin and fasting glucose. The generally accepted HOMA-IR index was used to calculate insulin resistance (D. Matthews et al., 1985): fasting serum insulin (μIU / ml) x fasting plasma glucose (mmol / l) / 22.5. An index value exceeding 2.7 is considered insulin resistance [27].

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Blood pressure (BP) was measured twice with an OMRON M2 Basic automatic tonometer (Japan) in a sitting position with calculation of average blood pressure with a margin of permissible measurement error of ± 3 mm Hg. (ESH 2002) according to the instructions for the correct measurement of blood pressure, outlined in the European clinical guidelines for the diagnosis and treatment of hypertension. Hypertension is present at the 140/90 mmHg (2017 ACC/AHA Guideline).

The study was conducted according to Ethics Committee protocol YSC KMP on the respondent's informed consent to the processing of personal data and the study.

Statistical data processing was performed using standard methods of mathematical statistics using the SPSS software package (version 19.0). To define the characteristics, the arithmetic mean (M) and the characteristic's standard error of the mean (m) were calculated. Intergroup differences were evaluated using analysis of variance or non-parametric criteria. Correlation was calculated by Spearman's correlation coefficient. Differences were considered statistically significant at p <0.05.

Results and discussion. In the general population, almost half of the respondents (45.7%) were found to smoke, men had the largest number of smokers - 59.3%, women - 38.2%.

The average BMI was significantly higher in women, amounting to 28.73 ± 0.42 kg / m² compared to men (25.51 ± 0.36 kg / m²) (p <0.001). In the general population, 35.9% of the respondents were overweight, and 31.9% were obese. 36% of women were overweight, 39.6% were obese. In men, 35.8% and 17.9%, respectively. Thus, an equally high incidence of overweight BMI was found in both men and women. Obesity is most common among the female population. The high incidence of overweight BMI and obesity is due to changes in lifestyle, low physical activity of the population, especially with regard to women.

We have conducted a study of the relationship between smoking and BMI. In non-smokers, BMI was statistically significantly higher compared to smokers (28.93 ± 0.44 kg / m² and 26.01 ± 0.39 kg / m², respectively, p <0.001). In nonsmoking men, as well as in women, the mean BMI was significantly higher compared to nonsmokers (men: 27.20 ± 0.58 and 24.36 ± 0.41 kg / m2, p <0.001; women: 29, 55 ± 0.55 and 27.42 ± 0.60 kg / m², p = 0.013). A negative relationship between BMI and smoking has been confirmed in many studies [4,6,17]; nevertheless, it has been proven that smoking was an independent risk factor for the development of cardiovascular diseases, including stroke and coronary heart disease [2,11,15,20,23,28].

The table presents a comparative analysis of overweight BMI and obesity by BMI value depending on status of smoking in the general population and on gender. The frequency of overweight BMI in the general population of smokers was higher compared to nonsmokers due to female smokers; the differences are statistically insignificant. With regard to obesity, all respondents had a significantly higher incidence of obesity in non-smokers compared to smokers. In our study, smoking did not affect the development of constitutional obesity, which is confirmed by the studies of several authors on the effect of nicotine on weight loss through an increase in various neurotransmitters [6,17].

Comparing the SBP level among the respondents depending on their smoking status, the following results were obtained. In smokers, the average SBP was significantly lower than in nonsmokers (134.84 ± 1.85 and 147.83 ± 1.98, respectively, p <0.001), while the average SBP in nonsmokers was higher than normal. There was also a negative correlation between the number of smoked cigarettes and the level of SBP (r = -0.226, p <0.001), the stronger correlation was obtained in women (r = -0.220, p = 0.001) than in men (r = -0.147, p = 0.104). Accordingly, 64.2% of nonsmoking respondents had a significantly increased blood pressure compared with smokers, whose blood pressure increased in 35.8% of cases (p <0.001). Thus, we obtained results in which smoking is not associated with an increase in blood pressure, possibly due to its indirect effect on BMI reduction. Nevertheless, according to previous research, the combination of these two risk factors affects the mortality rate from cardiovascular complications [1,5,9,15].

Due to conflicting research data on the effect of smoking on the level of insulin secretion, we carried out a comparative analysis of the average values of insulin and the HOMA-IR index in respondents depending on their relationship with smoking. The average insulin concentration did not exceed the reference values, in smokers it was 7.19 ± 1.23 IU / ml, which is statistically significantly lower than in non-smokers (12.74 ± 2.35 IU / ml) (p = 0.035). By gender, only men had significant differences in mean insulin concentration; in smokers it was lower and amounted to 4.91 ± 0.75 IU / ml compared to non-smokers (8.42 ± 1.76 IU / ml, p = 0.034) There were no significant differences in women, in smokers - 8.94 ± 2.09 IU / ml, in non-smokers - 13.86 ± 2.92 IU / ml (p = 0.201). We also determined the average values of the HOMA-IR index, which was 1.48 ± 0.24 in smokers - significantly lower compared to non-smoking respondents (2.77 ± 0.46, p = 0.014). In non-smokers, these indicators were higher than the reference values. Thus, our study shows that smoking suppresses insulin secretion, which may affect the development of type 2 diabetes. Similar results were obtained in the studies of certain foreign authors [17,18,29]. The increased HOMA-IR index in nonsmoking respondents, which characterizes insulin resistance, is most likely associated with the prevalence of obesity among them.

When comparing the relationship between the number of smoked cigarettes and BMI, we obtained a statistically significant negative correlation (r = -0.318, p <0.001). This correlation was most clearly observed in men (r = -0.423, p <0.001) rather than in women (r = -0.134, p = 0.088). With insulin, a statistically significant inverse relationship was also obtained with the number of cigarettes smoked (r = -0.140, p = 0.029). Separately, in men and women, there was no clear relationship (men: r = -0.163, p = 0.153; women: r = -0.140, p = 0.029).). We did not find any particular relationship between the number of cigarettes smoked and the glucose level (r = -0.045, p = 0.489). Separately, in men and women, there was no correlation either. The obtained result does not reflect the influence of one of the risk factors on the development of atherosclerosis and type 2 diabetes mellitus, since we have not determined the duration of smoking. Thus, the number of cigarettes smoked is not associated with BMI and insulin levels.

We analyzed the association of insulin, glucose, SBP and BMI parameters under the direct influence of smoking. A study on the relationship between insulin and the level of systolic blood pressure in the general population was carried out. There was a positive correlation between insulin levels and SBP (r = 0.239, p = 0.003), which is confirmed by studies abroad [14,24]. Analysis of the effect of smoking on the relationship between insulin and SBP showed that nonsmokers had a significant correlation (r = 0.197, p = 0.034), in contrast to smokers (r = 0.116, p = 0.198).

A study of the relationship between insulin levels and BMI showed that there is a strong positive correlation between

Frequency of overweight and obesity by BMI depending on status of smoking (%)

		Overweight	p	Obesity	p
Total	smokers	37.7	0.587	20.8	**0.001**
	nonsmokers	34.4		41.3	
men	smokers	32.9	0.412	9.6	**0.042**
	nonsmokers	40.0		30.0	
women	smokers	41.9	0.122	30.2	**0.039**
	nonsmokers	32.4		45.3	

these indicators (r = 0.283, p <0.001). Depending on the adherence to smoking, it was proven that in smokers (r = 0.223, p = 0.013), as in nonsmokers, the level of insulin was equally significantly correlated with BMI (r = 0.220, p = 0.017). As for the relationship between glucose level and BMI, a strong correlation was obtained in nonsmokers (r = 0.287, p = 0.002), but not in smokers (r = 0.031, p = 0.731). Glucose was also associated with SBP in both smokers (r = 0.183, p = 0.021) and non-smokers (r = 0.420, p <0.001). Thus, an association of insulin with BMI was obtained, including smokers, which is confirmed by a number of studies abroad [17,29].

Conclusion. Summarizing the above analysis, we summarize that the indigenous population of the northern territory of Yakutia has a high frequency of risk factors for the development of cardiovascular pathology, such as overweight, obesity and smoking. Overweight is equally common in both men and women; obesity is almost 2.5 times more common in women. An increase in body weight and systolic blood pressure is not associated with smoking, however, the simultaneous combination of all these factors can affect the risk of cardiovascular accidents. We have confirmed the data of foreign scientists on the suppression of the level of insulin secretion under the influence of nicotine. An increased index of insulin resistance is associated with the simultaneous spread of obesity among nonsmokers. The same association of glucose level with blood pressure was obtained in both smokers and non-smokers. Metabolic disorders in the indigenous population of the northern territory of Yakutia are caused by a change in the traditional way of life and nutrition with a decrease in physical activity, and a lack of adherence to a healthy lifestyle. By addressing modifiable risk factors, including overweight, obesity and smoking, it is possible to prevent morbidity and premature mortality from cardiovascular disease.

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HEALTHCARE, MEDICAL SCIENCE AND EDUCATION ORGANIZATION

Davydova T.K., Kononova S.K., Sidorova O.G., Romanova A.N.

EXPERIENCE IN CREATING A SPECIALIZED MEDICAL CARE CENTRE FOR PATIENTS WITH NEURODEGENERATIVE DISEASES BASED ON THE CLINIC OF SCIENTIFIC INSTITUTION

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The article presents the experience of creating a specialized center of medical care for patients with neurodegenerative diseases on the basis of the clinic of the Federal State Budgetary Scientific Institution of the Yakutsk Scientific Center for Complex Medical Problems (YSC KMP). The aim of this work is to present a model for creating a specialized center for patients with neurodegenerative diseases, as an improved model for providing specialized care for patients with neurodegenerative diseases in the Republic of Sakha (Yakutia) and an example of the consolidation of a federal medical research institution and the regional ministry of health. The materials in the work were the register of patients with SCA 1 and MND, reporting data of regional neurologists from 2016-2018, regulatory documents of the Ministry of Health of the Russian Federation and the Republic of Sakha (Yakutia). Clinical, comparative analysis and organizational modeling were used for the study. The result of the analysis was the opening of the Center for Neurodegenerative Diseases at the YSC KMP.

Key words: neurodegenerative diseases, specialized care, type 1 spinocerebellar ataxia, medical and social care

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Relevance. Currently, one of the urgent problems of public health and social protection is the provision of high-quality medical and social assistance to the population of patients with diseases of the nervous system, including neurodegenerative diseases. It is known that neurodegenerative diseases (NDD) are age-dependent and affect people of the older age group. In most of these diseases, the etiology and pathogenesis remain unclear, despite many years of scientific research in the world [3,4,6]. Solving the issue of providing medical and social assistance to this a group of patients at the outpatient hospital stage is a difficult task for healthcare in Russia, including healthcare in the Republic of Sakha (Yakutia). The financial crisis that health care is going through affects primarily the vulnerable segments of the population suffering from various diseases, which limit their ability to receive adequate medical care.

The lack of specialized departments for patients with neurodegenerative diseases deprives this category of patients of medical care not only in the hospital, but also at the outpatient stage, because most of these patients have problems with motor, speech, and cognitive functions. Thus, patients with neurodegenerative pathology are practically deprived of medical care. In addition, this problem is interdisciplinary in nature, since neurodegenerative diseases cause disturbances not only on the part of the nervous system, but also on the part of other systems of the body, impairing vital functions. All of the above requires the organization of a set of measures in the field of practical health care and social services for citizens. In our opinion, scientific institutions of a medical direction, which have their own clinics, in which both medical care at the outpatient hospital stage and scientific research can be carried out, can make their contribution to the provision of specialized care. It is the consolidation of medical science and practical health care that could bear fruit in this direction. In this article, we want to show a joint solution to this problem by the efforts of a scientific institution of the federal state Yakutsk Scientific Center for Complex Med-

ical Problems and the Ministry of Health (MH) of the Republic of Sakha (Yakutia).

Despite the fact that to date, the epidemiological situation of neurodegenerative diseases has been studied only for individual diseases in the republic, the epidemiological indicators obtained indicate that the percentage of NDD is relatively high among all diseases of the nervous system [5]. The most studied are type 1 spinocerebellar ataxia (SCA), oculopharyngeal myodystrophy (OPMD) [7], Charcot-Marie-Tooth disease (CMT) [1], Parkinson's disease (PD) [6], amyotrophic lateral sclerosis (ALS) [2]. Yakutia is the territory of the greatest prevalence of type 1 spinocerebellar ataxia in the world, 34.4 cases per 100 thousand population [8]. The situation with Alzheimer's disease, which ranks 1st in the world among neurodegenerative diseases [9], as well as various genetic and inherited diseases of the nervous system common in Yakutia, remains unexplored. Taking into account the age-dependent nature of neurodegenerative diseases, their frequency in Russia, as well as in the world, is steadily increasing and creates a medical and social problem for the health care and social protection authorities, since the number of patients with aging of society increases rapidly. The key to the success of the development of the direction of brain research is the combination of scientific potential and health authorities. The provision of specialized medical care to patients with neurodegenerative diseases in the Russian Federation is a very urgent problem against the background of the overall optimization of hospital beds in healthcare. Therefore, the opening of a specialized department for patients with neurodegenerative diseases in a scientific medical institution is a very significant help to regional health care and is a concrete example of the consolidation of practical health care with medical science.

The aim of this work is to present a model for creating a specialized center for patients with neurodegenerative diseases, as an improved model for providing specialized care for patients with neurodegenerative diseases in the Republic of Sakha (Yakutia) and an example of the consolidation of a federal medical research institution and the regional ministry of health.

Research methodology and organization. To organize a center for neurodegenerative diseases (CNDD) at the YSC KMP Clinic, a comprehensive program was drawn up, including the following stages:

1. Analysis of the initial organizational model for the provision of neurological care to patients with neurodegenerative diseases in the RS (Y);

2. Study of the base of the Clinic of the YSC KMP to determine the potential for opening the CDCH;

3. Determine the structure of the CNDD;

4. To propose an improved model of the organization of specialized medical care for patients with neurodegenerative diseases at the Collegium of the Ministry of Health of the Republic of Sakha (Yakutia);

5. To agree on the opening of the CNDD with the Ministry of Health of the Republic of Sakha (Yakutia) and draw up an order on the procedure for routing patients with NDD at the outpatient hospital stage in the CNDD;

6. To evaluate the medical effectiveness of the implementation of the improved organizational model for the provision of neurological care to patients with NDD and the social significance of the proposed model from 2019-2021.

Materials and research methods. The materials for this study were the register of patients with SCA 1 and MND, reporting data from regional neurologists from 2016-2018, regulatory documents of the Ministry of Health of the Russian Federation and the Republic of Sakha (Yakutia).

Clinical, comparative analysis and organizational modeling were used for the study. The clinical method included studying the register of patients with SCA 1 and MND, reports of regional neurologists of the republic on other NDDs, and this method was also used to determine the list of diseases and criteria for selecting patients for hospitalization in the newly created neurological department of the CNDD. The method of comparative analysis and organizational modeling included the study of the initial organizational structure of the provision of health care to patients with NDD and the proposed improved model, as well as the study of the base of the YSC KMP Clinic, where it was planned to organize the Center NDD, as a new organizational model for providing specialized care to patients with NDD.

Results and discussion. At the first stage of the comprehensive program, the initial organizational structure of medical care for patients with NDD was studied, which revealed a number of shortcomings in both outpatient and hospital care.

For outpatient specialized care, patients can apply to municipal polyclinics and the Medical Genetic Center (MGC) Republican Hospital №1 National Center of Medicine. Primary patients go to the neurologist of the polyclinic in the direction of the therapist and narrow specialists in the order of the general queue, the repeated and those who are registered at the dispensary can contact immediately, bypassing these specialists. In the MGC, patients with NDD are referred to by a neurologist or therapist. But due to specific neurodegenerative processes leading to motor and cognitive disorders, disorders of the psycho-emotional sphere, this category of patients cannot receive sufficient assistance at the outpatient stage, because they require a long examination by a neurologist at an appointment and getting prescribed treatment on an outpatient basis for many patients is a difficult task due to the manifestations of a neurodegenerative disease. Despite the fact that by order of the Ministry of Health of the Russian Federation in 2015, the time for an outpatient visit by a neurologist to one patient was increased to 22 minutes. [10], this time is still not enough to admit a patient with a neurodegenerative disease, which affects the quality of the patient's examination by a neurologist and the establishment of a preliminary diagnosis.

Hospital stage. In the republic in the health care system there are 2 neurological hospitals for round-the-clock stay, which are based in Republican Hospital N 2 - Center for Emergency Medical Aid (RH 2-CEMA)

1. Neurological department for patients with acute cerebrovascular accident in the Regional Vascular Center (RVC) 50 beds;

2. Department of General Neurology for 30 beds for the provision of emergency care to neurological patients, of which 5 beds are allocated for patients with NDD for the entire republic. Patients with severe pain syndromes, epilepsy or a series of epileptic seizures, acute inflammatory diseases of the nervous system, exacerbations of demyelinating diseases and other conditions, except for stroke, are hospitalized in this department. Thus, the Department of Emergency Neurology, which is the only department for patients with a general neurological profile, hospitalizes patients from all over Republic the, including patients with NDD. The available 5 beds in the neurological department of the Republic of RH 2-CEMA for patients with NDD cannot cover the needs of these patients throughout the republic.

A study of the annual reports of neurologists showed not only the lack of data on the primary appealability of such NDDs as Aligheimer's disease and other

dementias, many hereditary diseases, including SCA 1, myotonic dystrophy, oculopharyngeal myodystrophy, hereditary spastic paraplegia, dystonia, motor neuron disease and many other diseases ... There is no data on the volume of care provided (number of visits per year, treatment in a day hospital or at home, data on hospitalization). Patients suffering from NDD, as a rule, have motor, speech or cognitive impairments, which is an undoubted obstacle to attending polyclinics, and the lack of hospitals for rehabilitation or rehabilitation treatment deprives them of receiving medical assistance in case of illness.

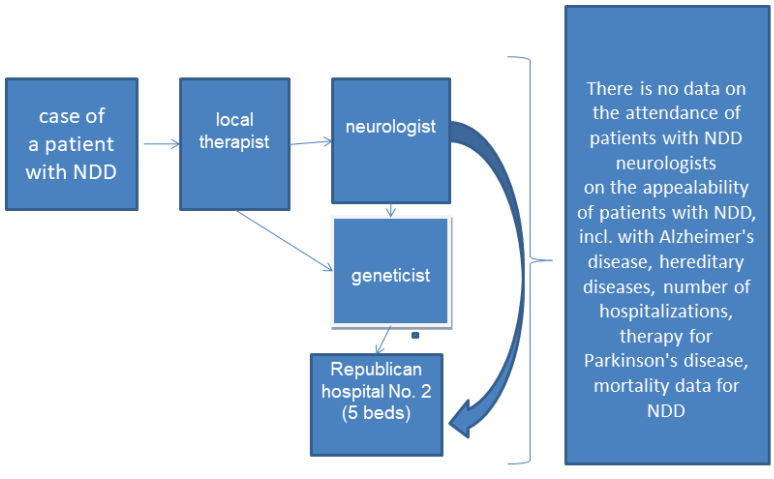

Fig. 1. Initial model of specialized care for patients with neurodegenerative diseases

Therefore, the predominant and comfortable type of medical help for such patients is treatment in a round-the-clock hospital. At the same time, it should be noted that outpatient care for patients with NDD in Yakutia became more effective after the opening in November 2017 of the Center for Extrapyramidal Disorders and Botulinum Therapy at the Clinic of the North-Eastern Federal University. The opening of such a Center in the Republic of Sakha (Yakutia) was a good alternative to providing MP to patients with NDD, despite the provision of compensated assistance. In Russia, there are examples of the opening of such specialized centers in the system of the Ministry of Science and Higher Education in medical research institutions. For example, specialized and high-tech medical care for patients with neurodegenerative diseases in Russia is provided by: Scientific Center of Neurology [11], Moscow, Institute of the Human Brain by N.P. Bekhtereva of the Russian Academy of Sciences, St. Petersburg. [12],

Thus, in the Republic of Sakha (Yakutia), if at the outpatient stage, patients with NDD have the opportunity to receive medical care, although not in full, then the issue of inpatient care is unresolved. Given the current situation, there is a need to improve the existing organizational model for the provision of neurological care to patients with NDD.

At the second stage of the comprehensive program, the base of the Clinic of the YSC KMP was investigated in order to identify the real possibilities of creating a CNDD. Not only the material and technical base was studied, but also personnel issues and issues of financing this category of patients from the Territorial Compulsory Medical Insurance Fund (TCMIF) were considered. It is known that medical organizations have single-channel funding from the TFOMI funds, and the main part of the NDH is included in the group of orphan diseases, which, due to the severity of the course, expensive treatment and examination, are among the highly paid clinical statistical groups (CSG) in the TCMIF system.

The clinic of YSC KMP occupies the 1st and 2nd floors of a 4-storey building of a standard hostel. The 1st floor is reserved for a polyclinic, and the 2nd floor is occupied by a round-the-clock hospital for 110 beds, including (at the time of the study) a therapeutic department for 40 beds, of which 10 are neurological, a gynecological department for 25 beds, a cardiology department for 35 beds. The Clinic has a physiotherapy department, a clinical diagnostic laboratory, which serve the clinic and the hospital. In addition, the structure of the YSC KMP includes the department of medical genetics, which includes a laboratory of hereditary pathology. The lack of MRI and the Department of Radiation Diagnostics at the Clinic is compensated by the conclusion of bilateral agreements with medical institutions that have this equipment. In general, given the availability of space for the proposed center for patients with NDD, this task could be successfully solved.

When analyzing the volume of financing of the Clinic from the funds of the TCMIF, the administration of the YSC KMP found effective ways to solve the release of funds and direct them to the solution of strategic tasks for the further development of the YSC KMP Clinic. First, a decision was made to reduce the catering unit and turn to outsourcing services for organizing meals for patients. Secondly, an unprofitable bed capacity of the gynecological and cardiological departments was identified. This was due to the fact that in the republic in 2011. Within the framework of the National Project "Health" on the basis of RH 2-CEMA the Regional Vascular Center (RVC) was opened, equipped with the most modern equipment, designed to provide specialized high-tech round-the-clock medical care for patients with acute cerebrovascular accidents and acute coronary syndrome (ACS). Thus, the cardiology department of the YSC KMP Clinic, excluding patients with ACS, began to admit patients with chronic ischemic heart disease, hypertension and other diseases of the heart and blood vessels, which are referred to as “therapy” during hospitalization. In addition, in Yakutsk in March 2018 year the Republican Perinatal Center (RPC) was opened, with a hospital for 130 beds (department of pregnancy pathology, obstetric physiological department, maternity department, department of pathology of newborns and premature babies), a consultative and diagnostic department for 150 visits per shift, an intensive care and intensive care department for women and newborns, as well as a follow-up department for young children, etc. The opening of the RPC also affected the unprofitability of the beds of the gynecological department of the YSC KMP Clinic.

The above objective reasons led to the decision to reduce the gynecological department and 25 beds in the cardiological department and place the Center for Neurodegenerative Diseases on their base.

The third stage of the comprehensive program was to define the structure of the CNDD, as a module that would include both outpatient and inpatient care, and provide assistance in social issues. Therefore, it was decided to allocate a separate block on one floor for the central oil refinery. In the structure of the CNDD, an important role is played by the location of the office for cognitive disorders, the office for bioethics and medical and social care and the inpatient neurological

department on the same floor, which is important for patients with limited mobility. As a result of this location, the CNDD is an integral section, isolated from other premises of the Clinic. Figure: 2 The structure of the CNDD

The CNDD is the main link in our proposed improved organizational model of specialized care (IOMSC) for patients with NDD and is a single unit for the provision of specialized care, where all stages of the provision of medical aid are interconnected. Figure: 3 Perfect the model.

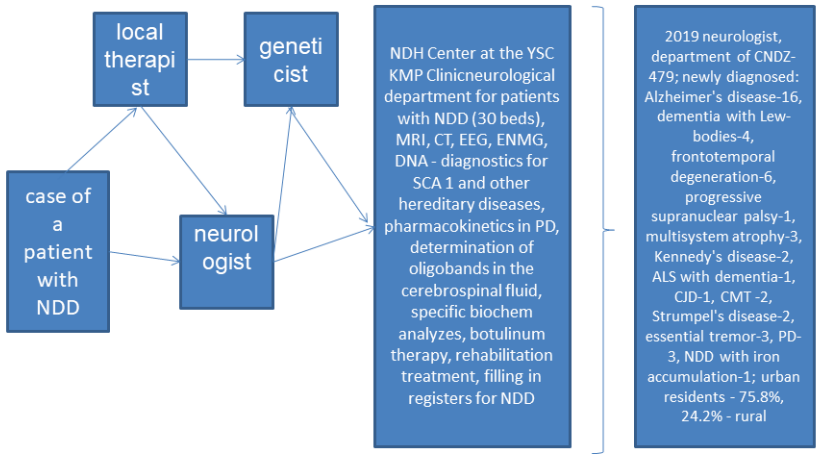

Fig. 2 An improved model of medical care for patients with neurodegenerative diseases in the RS (Y)

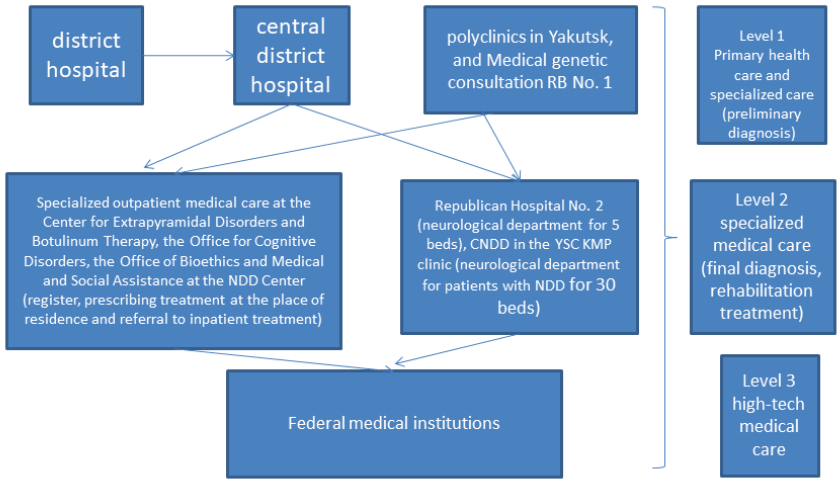

Fig 3. Patient routing scheme in an improved model of MP delivery to patients with neurodegenerative diseases

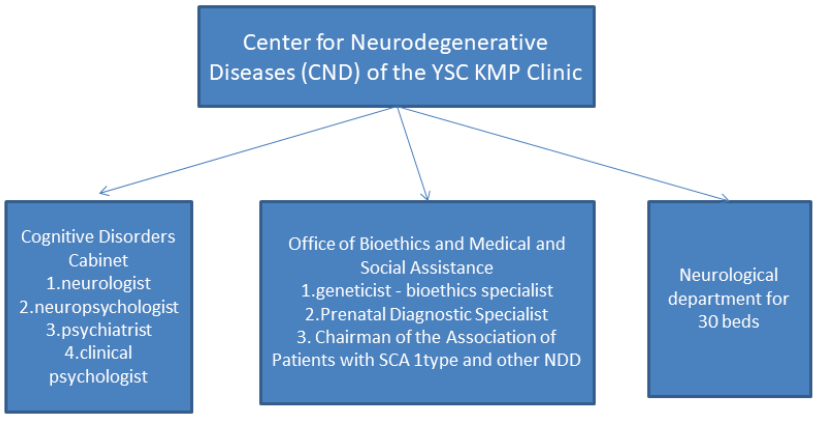

Fig 4. Structure of the Center for Neurodegenerative Diseases of the Clinic of the YSC KMP

At the fourth and fifth stages of the comprehensive program, joint work was carried out with the Ministry of Health of the Republic of Sakha (Yakutia) and the TCMIF, for the Republic of Sakha (Yakutia) to clarify the amount of funding for neurological beds. The functional structure of the CNDD was presented by us at the Collegium of the Ministry of Health of the Republic of Sakha (Yakutia) in December 2018. Considering that the neurological department will serve patients from all over the republic and for its full functioning, at the College of the Ministry of Health of the Republic of Sakha (Yakutia), it was recommended to draw up a draft order on the procedure for routing patients suffering from neurodegenerative diseases. Thus, based on the Decree of the Head of the Republic of Sakha (Yakutia) dated December 27, 2016. "On the approval of the regulations of the Ministry of Health and its collegium" (Appendix 1, paragraphs 3.11, 3.19, 3.20) and in pursuance of the order of the Ministry of Health of the Russian Federation dated November 15, 2011 No. 926n "On the approval of the Procedure for providing medical care to the adult population with diseases of the nervous system, the order of the Ministry of Health of the Republic of Sakha (Yakutia) No. 01-07 / 184 dated 02.14.2019 was drawn up and approved. "On the procedure for routing neurological patients suffering from neurodegenerative diseases at the outpatient and hospital stages."

The above-issued order of the Ministry of Health of the Republic of Sakha (Yakutia) allows you to gradually concentrate patients in one medical institution, which will make it possible to create a unified database of neurodegenerative diseases, track new cases, consult patients and maintain direct communication with neurologists using telemedicine. The data of the created registers will also make it possible to provide restorative and rehabilitative treatment to patients in need, monitor their condition in dynamics, identify the peculiarities of the clinical picture, and track families with genetic diseases. On the basis of this knowledge, an assessment will be made of the current state of the epidemiological situation of NDD in the regions of Yakutia and the prospects for the development of early (preclinical) diagnostics, approaches to personalized treatment of neurodegenerative diseases, primarily Parkinson's disease, Alzheimer's disease and type 1 spinocerebellar ataxia, have been developed.

The moral and ethical side of this problem is also an important factor, since the introduction into healthcare practice

of this order on the routing procedure and the creation of the CNDD, in fact shows that there is a search in solving the problems of providing health care to this category of patients, who until that moment were practically deprived of it, will make them feel like full-fledged members of society, which means that will improve their quality of life.

The preliminary results presented above show that the implemented improved model of specialized care for patients with neurodegenerative diseases may justify itself in the future.

Conclusion. Thus, the opening of the specialized center for patients with neurodegenerative diseases is the example of the consolidated interaction of a federal scientific medical institution and regional healthcare in solving a medical and social problem.

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HYGIENE, SANITATION, EPIDEMIOLOGY AND MEDICAL ECOLOGY

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SYMPTOMS OF DYSPEPSIA AMONG FIRST-YEAR STUDENTS IN THE REPUBLIC OF SAKHA (YAKUTIA)

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To study the symptoms of dyspepsia among first-year students of the Medical Institute there was a survey among 51 students, with an average age of 18 years, 30 of them are female, and 21 are male. The study included dyspepsia issues, its duration, nature, periodicity, frequency, seasonality, relationship with food intake and concomitant diseases of the digestive system. Symptoms of dyspepsia were determined in 33.6% of the respondents. The most common symptom of dyspepsia was a feeling of heaviness in the epigastric region, nausea, pain in the epigastric region, sometimes heartburn, nausea and belching. According to our data, functional dyspepsia is more common than organic dyspepsia, which amounted to 9.8%, possibly due to previously diagnosed diseases of the gastrointestinal tract. In the last two years, the overwhelming majority of respondents have developed symptoms of dyspepsia, which coincides with the high level of stress and psycho-emotional stress during the school period and with the adaptation of the freshman. For the prevention of symptoms of dyspepsia, the recommendations for first-year students are: regularly undergo preventive medical examinations, observe the regime and principles of rational nutrition. We have developed practical recommendations for the prevention of symptoms of dyspepsia among first-year students.

Key words: questioning, adaptation. dyspepsia, students, diseases of the gastrointestinal tract, prevention.

Introduction. The urgency of the symptom of dyspepsia is due to the high prevalence among the population and can lead to various disorders of the gastrointestinal tract and largely determine the overall health potential of students, as well as adversely affect the quality of life.

In recent years, the study of functional diseases of the gastrointestinal tract (GIT), which occurs quite often, remains relevant [2]. Interest in these diseases is associated with the high prevalence of dyspeptic complaints among the population. The modern rhythm of life, saturated with constant stress, poor ecology, improper and irrational nutrition leads to the fact that by the age of 30, every fourth person has one of the gastrointestinal diseases in his anamnesis. Dyspepsia occurs mainly at a young age, and affects mostly women [6].

The term "dyspepsia" translated from Greek as "impaired digestion". In different periods of history, this term meant different conditions. According to the modern concept, dyspepsia is called unpleasant sensations (pain, burning, bloating, a feeling of fullness after eating, a feeling of quick satiety), localized in the epigastric region. Dyspepsia is considered chronic, if symptoms bother the patient for at least 3 months. [5].

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According to the latest data, the prevalence of dyspepsia syndrome is 8.5-35.5% in the general population [7] and from 2 to 5% of the total number of visits to doctors. Also, symptoms of dyspepsia are one of the most common causes of temporary disability [1].

If the causes of organic dyspepsia occur due to organic lesions of the gastrointestinal tract, the causes of functional dyspepsia have not been sufficiently studied. However, it is important to note that one of the leading etiological factors in both cases of dyspepsia is the psychoemotional factor.

Students are one of the social groups that have a high rate of gastrointestinal morbidity. This is explained by physiological characteristics, social, everyday, psycho-emotional factors and irrational nutrition of students.

By origin, dyspepsia can be secondary (organic) and functional (idiopathic). Based on the nature of the complaints, age, anamnesis data, the results of physical and general clinical research methods, the doctor must exclude, first of all, the organic nature of the symptoms of dyspepsia. Secondary dyspepsia is diagnosed in patients with organic, systemic, or metabolic diseases.

The causes of organic (secondary) dyspepsia are most often stomach and duodenal ulcers; diseases of the biliary tract; chronic pancreatitis; malignant tumors of the stomach, pancreas, colon; taking medications (non-steroidal anti-inflammatory drugs, antibiotics, theophylline, digitalis, iron, etc.); alcohol; diabetes; hyper- or hypothyroidism; hyperparathyroidism; electrolyte disturbances; diseases of the connective tissue, liver and other organic pathology. In patients with new-onset dyspepsia over the age of 45, its functional nature is unlikely; therefore, in this clinical situation, taking into account the patient's age, the physician should have a reasonable oncological alertness [3].

The prevalence of FD in different populations varies greatly, which is associated with different interpretation and severity of symptoms, different diagnostic criteria, environmental factors, local prevalence of organic diseases such as peptic ulcer, stomach cancer. In patients with PD, the quality of life is significantly reduced, which is associated with emotional distress due to persistent symptoms, treatment costs, and reduced ability to work [7].

Thus, all of the above determines the relevance of studying the selected topic and introducing some practical recommendations that can have a positive effect on the gastroenterological situation in general, and sufficiently describe and disclose this problem among students.

The purpose of study is to investigate dyspeptic symptoms and risk factors among first-year students.

Materials and research methods. The data of the questionnaire survey of 51 students, the average age of 18 years, 30 - female and 21- male, freshmen of the Medical Institute of NEFU named after M.K. Ammosov, Yakutsk, Republic of Sakha (Yakutia).

In the survey, we selected questions such as symptoms of dyspepsia, their duration, nature, frequency, frequency, seasonality, connection with food intake, concomitant diseases of the digestive tract. We identified the most significant for the study of the symptom of dyspepsia: a feeling of heaviness after eating, epigastric pain, heartburn, belching, nausea, vomiting and girdle pain. All students

who participated in the survey signed an informed consent form of participation in the study.

Statistical processing of the material was carried out on a personal computer using the Statistica version 12 software package. During the statistical processing of the research results, the shares were calculated according to the frequency tables. Proportion comparisons were made using the Difference tests. The critical value of the significance level (p) was taken equal to 5%.

Results and discussion. Pain symptoms are more often localized mainly in the epigastric region (43.1%), are not seasonal (92.1%), in most cases are characterized by a moderate frequency and duration of several hours (54.9%) (table 1). By the nature of the pain, it is mainly dull (52.9%) and appears periodically (68.6%). These symptoms are diagnostic criteria for dyspepsia, which allows us to assume the presence of dyspepsia syndrome in these students. In addition to pain, the most common symptoms are nausea, sometimes heartburn, belching, and a feeling of heaviness after eating. Earlier, the students were diagnosed with gastrointestinal diseases (table 2).

90.2% of respondents have at least one of the symptoms of dyspepsia. And only 9.8% of the respondents noted the absence of any dyspeptic manifestations. Such a high frequency of dyspepsia can be explained with the characteristics of the social, emotional and psychological status and irrational nutrition of the respondents (Fig. 1), which is consistent with the literature [8].

In addition, we analyzed various symptoms and the frequency of their manifestations in the respondents (Fig. 2, Fig. 3). 31.4% of students have epigastric pain, 17.6% - girdle pain, 39.2% - belching, 45.1% - heartburn, 43.1% - nausea, 27.5% - vomiting and in 31.4% - a feeling of heaviness after eating. At the same time, pain bothered our freshmen less often than Omsk sophomores - 31.4% versus 54 (p = 0.0077), and heartburn appeared more often - 45.1% versus 23 (p = 0.0046) [4].

As seen from the pic. 2, most common symptoms are heartburn, nausea, and belching. On average, 33.6% of respondents have symptoms of dyspepsia. The number corresponds to the prevalence of dyspeptic syndrome in the population as a whole [1, 7], and according to the results of the work of other authors, among students of other universities [8].

Students most often noted the following symptoms of dyspepsia: feeling of heaviness after eating in 17.6% of cases, epigastric pain in 11.8%, girdle pain in 2%, nausea in 13.7%, and belching in 7.8% of cases , heartburn and vomiting. (pic. 3). The frequency of PD was similar to the frequency among the 2nd year students of the Omsk Medical University - 36% and in the Ural population - 23.4% (p = 0.77 and 0.097, respectively) [9]. When comparing the frequency of FD symptoms with the results of studies by Belgian scientists, it was found that our respondents were much less likely to have symptoms such as feeling of heaviness and epigastric pain - 31.4% each versus 88 and 68, respectively (p <0.0001) [4].

Dyspepsia symptoms are often detected in 9.8% of students. This may indicate the presence of any organic lesion, and,

Table1

Characteristics of pain symptom

Symptom	Frequency	%	χ^2 Пирсона	p
Stomach pain:	not - 5 is in the epigastrium- 22 is in other areas -6	9.8 43.14 11.76	21.01	<0.001
Pain appears:	fasting - 12 after meal - 18 at night - 2 pass after eating - 4	23.53 35.29 3.92 7.84	22.13	<0.001
Girdle pain:	sometimes - 9 often - 1	17.65 1.96	*	0.0011*
There was an attack of severe girdle pain:	once - 5 repeatedly - 2	9.8 3.92	*	0.29*
There was an attack of severe pain in the right hypochondrium:	once - 8 repeatedly - 2	15.69 3.92	*	0.023*
During the period of pain. they disturb:	periodically - 35 constantly - 0	68.63 0	*	<0.0001*
How long have you had stomach pains. heartburn. nausea	0-2 years - 23 2-5 years - 3 5-10 years - 1 Over 10 years - 2	45.1 5.88 1.96 3.92	53.5	<0.001
Pain predominantly	sharp- 12 stupid - 27	23.53 52.94	11.54	<0.001
Duration of pain	few hours - 28 day - 5 a week - 1 month - 1	54.9 9.8 1.96 1.96	69.63	<0.001
They give off pain	in the shoulder - 3 in the back- 2	5.88 3.92	*	1.0*
The pains are seasonal	yes - 4 no - 47	7.84 92.16	*	<0.0001*

Table2

Symptoms of dyspepsia and previously diagnosed diseases

Symptoms and previously diagnosed illnesses	Частота (n=51), абс.		%	χ2 Пирсона	p
Belching	-sometimes -often	20 4		*	<0.0001*
Nausea:	-sometimes -often	22 7		13.52**	<0.001**
Vomiting	-sometimes -often	14 4		*	0.0022*
Heartburn	-sometimes -often	23 4		*	<0.0001*
Heartburn while lying down	-sometimes -often -constantly	10 4		*	0.055*
Feeling of heaviness after eating	- ulcer disease chronic gastritis -cholecystitis -pancreatitis	16 9 –3		11.1	0.004

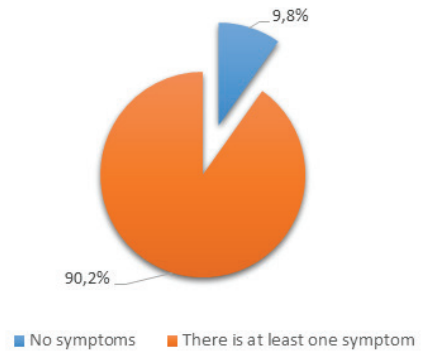

Fig. 1. Frequency of dyspepsia symptoms among respondents

as a result, organic dyspepsia. Students with frequent manifestations of dyspeptic symptoms require further research and additional diagnostic methods. Also, the presence of gastrointestinal tract pathology is shown by the survey data on previously diagnosed diseases. Out of 33.6% with symptoms of dyspepsia, 9.8% are associated with organic dyspepsia and corresponds to the literature data. Organic dyspepsia accounts for about 40% of all cases of dyspepsia diagnosed [7]. According to the questionnaire, students had a history of peptic ulcer disease in 1.9%, chronic gastritis in 3.9%, cholecystitis in 1.9%, and pancreatitis in 5.8%. Thus, functional dyspepsia is more common in students. According to the duration of the onset of symptoms of dyspepsia over the past 2 years, they were identified in 45%, from 2 to 6 years in 6%, from 5 to 10 years - 2% and in 4% - more than 10 years (pic. 4). Also statistically significantly less frequent were: nausea - 43.1% versus 63 (p = 0.02), belching - 39.1% versus 58 (p = 0.03). Heartburn and vomiting appeared with a similar frequency - 45.1% versus 56 and 27.5% versus 30 (p = 0.2 and 0.75, respectively) [4].

The overwhelming majority of the surveyed students (45%) had symptoms of dyspepsia over the past 2 years. Perhaps this is due to the high level of stress and significant changes during the period of schooling, with a significant increase in psychoemotional loads and with the adaptation of freshmen to the new rhythm of life at the institute.

In this research, we studied the frequency of manifestations of dyspepsia symptoms among freshmen of a medical school, outlined the main forms and symptoms, and developed practical recommendations aimed at informing students about the symptom of dyspepsia and prevention of the disease.

Conclusion. As a result of the study, it was found that among first-year students, the symptoms of dyspepsia are 33.6%. Functional dyspepsia predominates, while organic dyspepsia accounted for 9.8% of the respondents with a history of gastrointestinal diseases. The most common symptoms of dyspepsia are nausea, heartburn, feeling of heaviness, belching, and pain. In terms of the duration of the symptoms of dyspepsia, most have worried about the last 2 years. We have developed practical recommendations informing about the symptom of dyspepsia and methods of its prevention. Students are encouraged to pay attention to the symptoms of dyspepsia, regularly undergo preventive medical examinations, follow a balanced diet and recommendations for the prevention of dyspepsia symptoms.

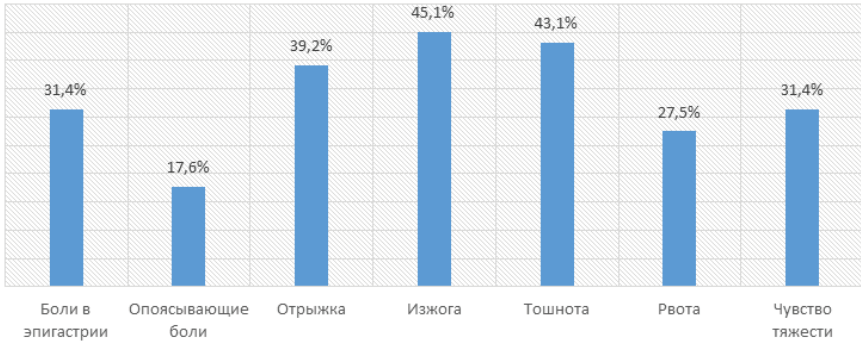

Fig. 2. Symptoms of functional dyspepsia

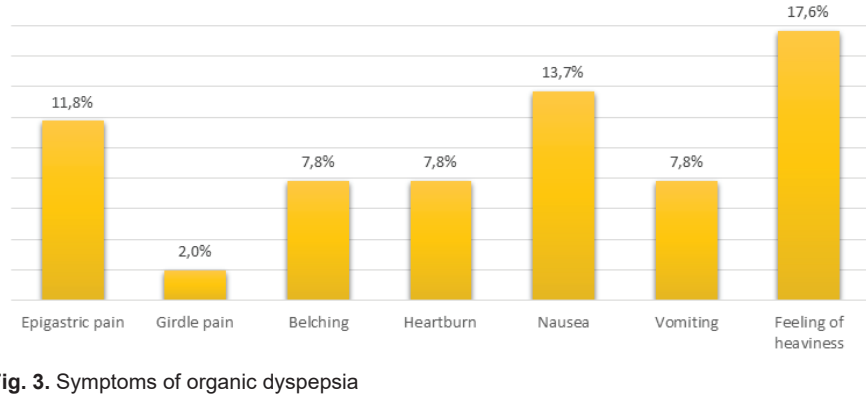

Fig. 3. Symptoms of organic dyspepsia

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COMPARATIVE CHARACTERISTICS OF MORPHOFUNCTIONAL INDICATORS OF PHYSICAL DEVELOPMENT OF YOUNG MEN

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Abstract. This article presents the comparative characteristics of the dynamics of indicators of physical development of young men. The study aims to carry out a comparative analysis of the dynamics of morphological and functional indicators of physical development and the functional state of male bodies at the youthful stage of ontogenesis. We have analysed the results of the one-time study (1999 and 2019) of 1st-year students; in total, 760 young male students were examined in 1999, and 273 students in 2019 (aged 17-18). To reveal research results of physical development indicators, we have carried out a comprehensive study of somatoscopic, somatometric, and functional indicators of young men. Analysis of the morphological and functional indicators of men in Yakutia at the youthful stage of ontogenesis over time (20 years of difference). We take into account the individual typological characteristics of the organism, manifested at the structural and functional level (somatotype), and reflected in the main anatomical and anthropological indicators of the organism. This makes it possible to state the impact of external factors (environmental, social influences; a complex of genetic and cultural characteristics) on the indigenous people of the North.

Keywords: morphological and functional indicators, sociotype, physical development, individual-typological characteristics.

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Relevance. The centuries-old long-term residing of indigenous peoples on the territory of the Sakha Republic has contributed to the formation of a morphological and functional ecotype that is maximally adapted to local extreme climatic and geographical conditions. However, recently there have been significant socio-economic changes in the areas of residence of the peoples of Yakutia, which had a significant impact on the health of the population. [9]

One has to agree that any hasty breakdown of an existing lifestyle can lead to changes in the morpho-functional state of the human body. [6] Recently, a decrease in the level of physical activity has been recorded; along with this, malnutrition and an improper lifestyle have been noted, which in turn leads to a decrease in the health indicators of learning youth. [1, 2, 5, 8].

In this regard, it became relevant to study the individual typological characteristics of the physical development of the indigenous population of Yakutia in specific periods of ontogenesis (adolescence). The revealed features are necessary for preventive medicine and the development of targeted programs of physical education for the younger generation in the North-East of Russia.

Research Objective is a comparative assessment of the morphological and functional indicators of physical development and the functional state of male bodies at the youthful stage of ontogenesis.

Research Materials and Methods. In this work, we have analysed the results of a one-step study (1999 and 2019). The age range of the subjects is 17-18 years old. We included first-year students in random sampling; in total, 760 young male students (Group 1) were surveyed in 1999 and 273 students in 2019 (Group 2).

This study uses data from the work by Permyakova S.P. titled "Patterns of Morphological and Functional Development of Sakha Republic's Indigenous Population's Young Men" (2002) [9].

The research was carried out according to a comprehensive program; at the initial stage we carried out a questionnaire survey and analysis of the student's medical data. The second stage involved the study of the physical development of students, which was carried out using standard anthropometric techniques – somatometric indicators were determined: total body dimensions (body weight, height, chest circumference); partial body dimensions characterizing the dimensions of individual parts of the body, longitudinal, latitudinal and girth dimensions of the body, and caliperometry data. In addition, we assessed the physiometric parameters of young men (hand strength, back strength, lung capacity, heart rate, and blood pressure). Statistical processing of the results obtained was carried out using the SOMAX database analytics system. [4]. For data with a normal distribution, we calculated the mean (M) and standard deviation (σ). When comparing sample means for data with a normal distribution, we used the *t*-test. For analysis, p values < 0.05 were considered statistically significant.

Results and Discussion. The analysis of the morphological parameters of young men's organisms was carried out taking into account the ethnic character and revealed differences in the physical development of young men in the Sakha Republic during different periods of the study. As a result of the study, it was found that in the analysed groups there were significant differences in the length of the body depending on the year of the study. Our data show that the greatest indicators of body length are determined in the second group of boys, and lower numbers – in the young men of the first group (Table 1).

The body length of the men of the second group exceeds (on average) the given indicator of the men of the first group by 3.64 cm, which characterizes the accelerated growth rate and energy expenditures of the body and is of hygienic significance. The body mass index in the young men of the second group is 4.36 kg higher than in the men of the first group, which also has significant differences (P < 0.05). Higher values of body weight in young men of the second group is explained by their longer body length since these indicators have a high correlation. At the same time, the BMI value (the ratio of body weight in kg to the square of the body length) also has differences in the studied groups, but these indicators can be seen as the "norm" when assessing the physical development of young men in the territory of the Sakha Republic.

Table1

Main Morphological Indicators of Young Men's Bodies (M±σ)

	Group 1 (1999) N = 760 (M±σ)	Group 2 (2019) N = 273 (M±σ)	P <
Body Length, cm	170.05±6.16	173.69±5.59	0.05
Body Weight, kg	59.87±8.0	64.23±10.42	0.05
Chest Circumferemce, cm	85.70±6.05	87.59±6.71	0.05
Quetelet Index	351.62±41.02	369.57±56.07	0.05
Body Mass Index	20.68±2.27	21.27±3.15	0.05
Rohrer Index	1.22±0.14	1.22±0.19	-
Body Area*	1.70+0.12	1.78±0.14	0.05
Specific Weight	1.04±0.01	1.03±0.05	0.05

Since bodyweight is an indicator that is sensitive to changes in the intensity of metabolic processes in the body, we carried out a deeper analysis of this indicator. The component composition of body weight was studied and analysed, the data on which are presented in Table 2. As can be seen from the Table, the absolute and relative content of bone, adipose, and muscle tissue also have significant differences between the 2 groups.

Analysis of the component composition of body weight indicates an increase in the bone and fat components and a decrease in the content of muscle tissue after 20 years (P < 0.05). Perhaps this is due to the peculiarities of nutrition and metabolism of modern youth.

Analysis of fat fold thickness indices and assessment of absolute and relative values of body fat in the study groups also revealed statistically significant differences (P > 0.05) (Table 3).

The Rohrer index, which reflects the density of the body, largely depends on the linear dimensions and body weight. The results of our study did not reveal statistically significant differences in young men in both groups. They tend to have high rates of the Rohrer index (1.22 ± 0.01) and (1.22 ± 0.19), which in turn is the main feature determining the medical anthropology characteristics of the northern ecotype. This is explained by the high ecological valency of the organism of young men of the Sakha Republic's indigenous population, and it characterizes the high adaptability of the organism [4]. When analysing body length, it is of interest to clarify the question of how this indicator is predominantly formed. The main segments of body length are leg length and body length. From Table 4, it can be seen that significant differences were revealed in the indicators of the length-wise body size of young men. In the young of the second group, the indicators of the length of the body, arms, legs, hands,

Table2

Young Men's Body Composition (M±σ)

	1-я группа (1999г) N=760	2-я группа (2019г) N=273	P<
Absolute Bone Mineral Density. kg	11.09±1.21	14.21±18.07	0.05
Relative Bone Mineral Density. %	18.67	21.75	0.05
Absolute Body Fat. kg	7.30±2.97	11.09±6.13	0.05
Relative Body Fat. %	12.03	16.66	0.05
Absolute Muscle Density. kg	28.86±4.32	29.61±5.07	0.05
Relative Muscle Density. %	48.21	46.39	0.05

Table3

Indicators of Young Men's Bodies' Fat Folds Thickness (M±σ)

	1-я группа (1999 г.) N=760 (M±σ)	2-я группа (2019 г.) N=273 (M±σ)	P<
Chest Fat Folds	5.51±2.16	9.06±5.86	0.05
Shoulder Fat Folds (Front)	4.25±1.75	6.24±3.5	0.05
Shoulder Fat Folds (Back)	8.21±3.48	9.27±4.94	0.05
Forearm Fat Folds	5.14±1.56	5.59±2.6	0.05
Back Fat Folds	7.49±2.35	10.74±7.08	0.05
Abdomen Fat Folds	9.17±4.71	14.13±9.04	0.05
Thigh Fat Folds	6.87±3.22	10.61±5.66	0.05
Knee Fat Folds	5.73±2.52	9.17±5.01	0.05

Table4

Young Men's Length-Wise Body Sizes (M±σ)

	1-я группа (1999 г.) N=760	2-я группа (2019 г.) N=273	P<
Body Length, cm	170.05±6.16	173.69±5.59	0.05
Body Length, cm	89.87±3.68	91.39±11.54	0.05
Arm Length, cm	75.28±3.25	76.78±3.86	0.05
Brush Length, cm	17.30±0.93	17.10±3.35	-
Leg Length, cm	88.0±4.95	91.93±6.45	0.05
Foot Length, cm	25.08±1.16	25.08±2.77	-
Skelic Index, %	89.26±17.92	92.2±18.26	0.05

It is known that the transverse dimensions of the body have a significant effect on the body's overall proportionality (Table 5). Of the transverse body dimensions, the data on the shoulder width indicators (acromial diameter meter) are of greatest interest. According to our data, the shoulder width in the first group of young men was 39.84 ± 2.56 cm, and significantly exceeds this indicator when compared to the second group (38.24 ± 0.16 cm; (P < 0.05).

Almost all latitudinal dimensions of the bodies of young men, except for the width of the pelvis and the diameter of the shoulders, are significantly larger in young men of the second group (P < 0.05).

The magnitude of body massiveness and its segments is characterized by girth dimensions, which make it possible to more objectively assess both the body type and the expressiveness of individual components of body weight (table 6).

As can be seen from Table 6, the girth dimensions of the forearm, wrist, abdomen, thigh, lower leg, and above the ankles in young men of the first group are lower than in the second group (P < 0.05). This corresponds to the main dimensions of their body (length, body weight, thoracic organs).

According to the absolute indicators of the size of the shoulder girth, there were no significant differences between the young men of both groups (P> 0.05). But at the same time, reliable differences were obtained in the indicators of the shoulder girth when tensed; in the second group, this indicator is significantly higher than in the young men of the first group.

The main indicators characterizing the functional state of the body of the studied groups of young men are presented in Table 7.

As can be seen from Table 7, higher values of the absolute muscle strength (right and left hand) were observed in young men of the second group (P < 0.05). The absolute value of the back strength in the second group is significantly lower and amounted to 97.03 ± 48.74 kg, while in the first group this indicator was 121.56 ± 26.94 kg.

The study of lung capacity allowed us to reveal the following features: higher values of the absolute indicator were in the young men of the second group (4024.56 ± 1290.48 ml). The representatives of the first group showed indicators of 3573.30 ± 611.04 ml; we also revealed significant differences (P> 0.05) in relative terms (LC per kg of body weight).

Analysis of hemodynamic parameters of the body (heart rate, blood pressure) in the studied groups showed that heart rate is statistically significantly higher in modern young men (P > 0.05), which is presented in Table 7.

Thus, a comparative analysis of the functional indicators of the organism of young men, which are to a greater extent a phenotypic trait, has revealed significant differences in the studied groups. This may be due to a change in lifestyle

Table5

Young Men's Width-Wise Body Sizes (M±σ)

	1-я группа (1999 г.) N=760	2-я группа (2019 г.) N=273	P<
Acromial Diameter	38.24±2.28	39.84±2.56	0.05
Cross-Section Diameter	26.75±1.62	27.27±2.39	0.05
Front-to-Back Diameter	17.78±1.59	18.2±1.99	0.05
Pelvis Width	27.52±1.49	27.36±2.35	-
Shoulder Diameter	6.91±0.41	7.04±1.17	-
Forearm Diameter	5.69+0.32	5.58±0.42	0.05
Thigh Diameter	9.67+0.51	10.82±6.26	0.05
Shin Diameter	7.16±0.43	7.8±4.48	0.05
Shoulder Width Index	22.49±1.23	22.94±1.38	0.05
Pelvis Width Index	16.08±0.8	15.76±1.27	0.05

and feet were significantly higher than in the young men of the first group.

Manouvrier's Skelic Index in young men of the second group is 92.2%, which indicates macroskelia (90% and higher indicates macroskelia).

Among the representatives of the first group, it corresponds to 89.26% and speaks of mesoskelia (85-89.9% – mesoskelia). In other words, modern youths of indigenous nationality are characterized by the high length of the lower limbs, even though in many literary sources of the 20th century (Shreiber S.E., 1931; Levin M.G., 1945; Klevtsova N.I., 1976), attention is drawn to locals' "short legs". [9].

Table6

Girth Measurements of Young Men's Bodies (M±σ)

Обхват	1-я группа (1999 г.) N=760	2-я группа (2019 г.) N=273	P<
Chest	85.70±6.05	87.59±6.71	0.05
Shoulder	26.12±2.53	26.25±2.55	-
Shoulders (Tensed)	28.69±2.7	29.52±3.71	0.05
Forearms	25.09±1.74	24.76±1.77	0.05
Wrists	16.42±0.86	16.12±1.54	0.05
Abdomen	72.26±5.91	75.39±11.68	0.05
Hips	50.77±4.07	52.31±6.53	0.05
Shins	33.59±2.21	35.29±4.57	0.05

Table7

Young Men's Functional Indicators (M±σ)

	1-я группа (1999 г.) N=760	2-я группа (2019 г.) N=273	P<
Right Hand Strength, kg	38.67±7.81	40.5±7.94	0.05
Left Hand Strength, kg	35.91±7.72	39.35±7.23	0.05
Stan Force, kg	121.56±26.94	97.03±48.74	0.05
Lung Capacity, ml	3573.30±611.04	4024.56±1290.48	0.05
Life Index (LC / BW), ml/kg	60.04±11.54	63.68±20.93	0.05
Diaphragmatic Excursion, cm	6.59±1.91	6.25±1.91	0.05
Heart Rate, beats per minute	73.19±7.78	81.7±14.96	0.05
Systolic Blood Pressure, mm Hg	112.87+12.5	112.39±12.3	-
Diastolic Blood Pressure, mm Hg	71.67±8.86	72.43±11.58	-
Pulse Blood Pressure, mm Hg	41.20±9.84	39.95±12.37	-

(dietary habits, physical activity, and other factors). The revealed significantly low values of the absolute backbone muscle strength in the second group indicate a decrease in the level of development of skeletal muscles.

In clinical practice abroad, the method of somatotyping according to the Rice-Eysenck index (1945) is widely used. [10] In accordance with this scheme of constitution diagnostics, the surveyed contingent of young men was divided into the following groups: dolichomorphic, mesomorphic, and brachymorphic somatotypes. Among young men of the first group, the dolicomorphic type was found in 51.32% of cases, mesomorphic – 43.95%, and brachymorphic – 4.73%. Among the young men of the second group, there were more dolichomorphic and brachymorphic somatotypes – 58.33% and 9.09%, respectively. The number of mesomorphic types decreased and amounted to 32.58% of cases. As can be seen from the presented material, the manifestation of dolicomorphic and brachymorphic types was the most frequent among today's young men, which is combined with the highest indicators of longitudinal and latitudinal body dimensions revealed in the latter, as well as higher values of body fat folds.

Conclusions. Thus, we have analysed of morphological and functional indicators of young men in the Sakha Republic over time (difference of 20 years). We took into account the individual typological characteristics of the organism, manifested at the structural and functional level (somatotype) and reflected in the main anatomical and anthropological indicators of the body (body length, weight body, chest circumference, body surface area). This allowed us to state the impact of external factors (environmental, social influences, a complex of genetic, cultural characteristics of the way of life) on the indigenous people of the North. Constitutional diagnostics showed that over 20 years there has been an increase in the number of dolichomorphic and brachymorphic body types, along with a decrease in the number of mesomorphic body types. The revealed individual typological features of the physical development of the male population of Yakutia at the adolescent stage of ontogenesis. This makes it possible to assess their physical development as disharmonious. The data obtained can be used in practical work (preventive and military medicine, medical control of physical education courses).

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PHYSICAL RISK FACTORS IN OCCUPATIONAL SENSORINEURAL HEARING DISORDERS

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We have studied the impact of harmful physical factors of production on the development of occupational sensorineural hearing loss (OSHL). A retrospective analysis of 537 cases of civil aviation flight personnel and car drivers of technological transport in the mining industry of Yakutia with the first diagnosis of OSHL was carried out. Among these two groups of workers, a comparative analysis of the severity of hearing loss and its dependence on age, length of service, and the level of excessive industrial noise was carried out. It was found that among flight personnel, OSHL is the only diagnosis of an occupational disease, develops after 24.69±7.05 years of work in this position. In car drivers, OSHL is combined with other diagnoses caused by exposure to local and/or general vibration, develops with a work experience of 26.02±6.69 years with a predominance of IInd degree of hearing loss. As a result, it was concluded that the typical pattern and prevalence of the Ist degree of OSHL is possible in the absence of the impact of other harmful physical factors of production, such as local and general vibration. When exposed, along with excessive noise of local and general vibration, the patterns of OSHL development are blurred, the clinical course is more severe, with a predominance of IInd and IIIrd degrees.

Keywords: occupational sensorineural hearing loss; occupational noise, occupational factor, degree of hearing loss.

Introduction. Hearing loss from exposure to occupational noise has recently become a common occupational disease. Occupational sensorineural hearing loss (OSHL; synonyms – perceptual hearing loss, sensorineural hearing loss) is a chronic disease of the hearing organ, characterized by bilateral impairment of the auditory function of a sound-perceiving nature, which develops under prolonged exposure to occupational noise exceeding the maximum permissible levels [1]. In the Russian Federation, the maximum permissible noise level at workplaces is established by the sanitary standards SN2.2.4 / 2.1.8.562-96 «Noise at workplaces, in residential, public buildings and on the territory of residential development» and is 80 dB. Currently, the incidence of OSHL is growing and affects mainly working-age workers [2]. Occupational risks play an important role in the complex of factors affecting health at working age: from 20 to 40% of all labor losses are caused by diseases directly or indirectly associated with unsatisfactory working conditions, among which noise occupies one of the leading places. Currently, as a rule, there is a combined effect of noise on the body of workers in combination with vibration (general and local), air pollution in the working area with harmful substances and aerosols, unfavorable microclimate, high severity and intensity of work and other harmful factors [2]. In Yakutia, those working in harmful and/or dangerous working conditions are mainly engaged in three economic activity's types – mineral extraction, transport and communications, production and distribution of electricity, gas and water. Recently, a lot of efforts have been made to minimize harmful production factors in the workplace. However, some technological processes have irreparable factors, such as unrecoverable engine noise in transport. All workers employed at work with harmful and/or hazardous working conditions annually undergo periodic medical examinations, with mandatory audiological examination. If an occupational disease is suspected, including sensorineural hearing loss, the employee is sent to the center of occupational pathology for examination of the connection of the disease with the profession, according to the results of which the employee is diagnosed with a chronic occupational disease.

Currently, the clinical picture of OSHL is well studied [3], therefore, many researchers are interested in the issues of epidemiology, the effectiveness of treatment and rehabilitation methods in OSHL [2]. Main methods in the diagnosis of OSHL are a thorough study of the patient's professional route, working conditions and audiological research methods. The purpose of this work is to analyze OSHL in civil aviation flight personnel and drivers of technological transport in the mining industry of Yakutia, depending on age, length of service in this position, the level of excessive noise and other physical factors.

Material and methods. As a result of the examination of the connection between the disease and the profession for the period from 2010 to 2019, at the Yakutsk Republican Center for Occupational Pathology 1181 patients were diagnosed for the first time with various diagnoses of a chronic occupational disease. For the analysis, we selected 537 civil aviation flight personnel and technological transport's drivers in the mining industry of Yakutia, who were first diagnosed with OSHL. From this number were formed two observation groups.

The first group consisted of 305 males working at the examination's time in the positions of «pilot», «co-pilot», «aircraft commander», «pilot-instructor», «navigator», «senior navigator», «navigator-in-

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structor», «onboard radio operator», «onboard mechanic», «onboard engineer», «aviation technician», «aviation mechanic», «senior onboard engineer», «onboard operator».

The second group consisted of 232 males working at the examination's time in the positions of «driver», «car driver», «driver for all brands of cars».

The age of the patients was identified using passport data. Work experience is calculated according to the entries in the work book. The median of the noise affecting the employee during the working life is taken as the level of excessive noise during his work in this position according to the sanitary and hygienic characteristics of the working conditions of each employee, approved by the Federal service for supervision of consumer rights protection and human welfare and additionally for Ist group – according to the protocol for calculating the equivalent noise level in flight approved by the labor protection department employer. Indicators of other physical factors are also taken from the sanitary and hygienic characteristics of working conditions.

In the retrospective analysis of the outpatient's card, all 537 participants underwent a standard examination by an audiologist-otorhinolaryngologist (using the «KaWe Combilight» otoscope, «KaWe», Germany). The auditory status was confirmed by an audiological study, including tuning fork tests (tuning fork C_{128}), impedance measurement (tympanometer and audiometer «AA222», «Interacoustics», Denmark), threshold tonal audiometry (tympanometer and audiometer «AA222», «Interacoustics», Denmark; audiometer «AC40», «Interacoustics», Denmark) on air conduction at frequencies 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0 kHz and bone conduction at frequencies 0.25, 0.5, 1.0, 4.0, 6.0 kHz with a step of 5.0 dB [4]. The type of hearing loss was considered conductive – with an increase in air conduction thresholds on audiograms, sensorineural – with an increase in bone and air conduction thresholds on audiograms, mixed – with an increase in bone and air conduction thresholds with an interval exceeding 20.0 dB in total in $PTA_{0.5, 1.0, 2.0, 4.0}$ kHz. Hearing loss was considered symmetric when the difference in the hearing thresholds in the $PTA_{0.5, 1.0, 2.0, 4.0}$ kHz did not exceed 15.0 dB. Considering that we are studying the damaging effect of occupational noise, the degree of hearing loss was assessed by the thresholds of hearing worse than the hearing ear in the $PTA_{0.5, 1.0, 2.0, 4.0}$ kHz. When assessing the severity of the disease, were used two classifications: 1) the classification proposed by Ostapkovich V.E. for ICO 12.4.062-78 «Noise. Methods for determining human hearing loss», according to which 0 degree (signs of noise exposure to the hearing organ) – up to 10 dB (arithmetic mean 0.5, 1.0, 2.0 kHz) and 50±20 dB (4.0 kHz), Ist degree (sensorineural hearing loss with mild the degree of hearing loss) – 11-20 dB and 60±20 dB, IInd degree (sensorineural hearing loss with a moderate degree of hearing loss) – 21-30 dB and 65±20 dB, IIIrd degree (sensorineural hearing loss with a significant degree of hearing loss) – 31-45 dB and 70±20 dB [5]; 2) international classification, according to which the Ist degree corresponds to 26-40 dB in $PTA_{0.5, 1.0, 2.0, 4.0}$ kHz, IInd degree – 41-55 dB, IIIrd degree – 56-70 dB, IVth degree – 71-90 dB, deafness – > 90 dB.

sions of the expert commission showed that in Ist group OSHL is the only established diagnosis of an occupational disease, and in IInd group – in all cases (100%) OSHL was found in combination with other diseases, such as «vibration disease», «radiculopathy», «polyneuropathy» of varying severity and stage of the course.

Comparison of the degrees of hearing loss in two groups showed that during the initial diagnosis of OSHL, more severe hearing loss is observed in the second group (p<0.05): significantly less Ist degree (29.3% versus 63.9%), significantly more IInd degree (48.7% versus 25.9%) and IIIrd degree (22.0% versus 9.8%) (Fig. 1). Profound hearing loss (IVth degree) was observed in only one case, and is most likely of a casuistic nature.

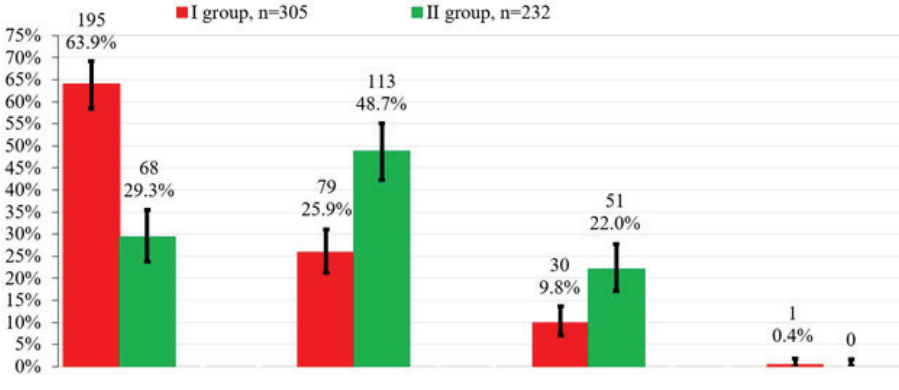

Figure 1. Comparison of degrees of hearing loss.

Statistical processing was performed using the software Biostatd (McGraw-Hill, Inc. Version 3.03), Sampling (kindly provided by V. Macaulau and M. Metspalu), and STATISTICA version 8.0 (StatSoft Inc, USA). Differences were considered statistically significant at p<0.05. The audiograms with a break were normalized by introducing the maximum values (120.0 dB) in places where the patient did not respond.

Each case contains a written informed voluntary consent of the patient to medical intervention, which provides for the anonymous use of the examination's results and treatment for scientific purposes.

Results. The analysis of the conclu-

Considering that noise is the only adequate stimulus to the auditory analyzer, we attempted to establish the cause of the observed difference in the severity of hearing loss in the second group. Comparison of age and length of service in this position showed that older (p<0.05) and more trained (p<0.05) patients work in the second group, while exceeding the maximum permissible level is more significant (p<0.05) in the first group (Table 1).

At the same time, correlation analysis shows that the relationship between age, work experience and the level of excess noise can be traced only in Ist group (Fig. 2, 3, 4).

Thus, in civil aviation flight personnel

Table1

Comparison of the two observation groups in terms of age, length of service, and excessive noise

Criterion	I group	II group	P
age	50.64±6.04	53.18±5.69	**0.000001**
experience	24.69±7.05	26.02±6.69	**0.028189**
excess noise	10.80±4.72	1.20±0.40	**0.000000**

Table2

Analysis of the presence of other physical factors

Physical factor	Working conditions class *	
	I group	II group
aerosols of predominantly fibrogenic action	1	2
noise	**3.1 – 3.3**	**3.1-3.2**
infrasound	1 – 2	1
ultrasound air	1	1
vibration general and local	1	**2 – 3.2**
non-ionizing radiation	1	1
ionizing radiation	1	1
microclimate	2	2
light environment	2	2
working posture (fixed or forced)	**3.1**	**2 – 3.2**

*1 – optimal, 2 – permissible, 3 (4 subclasses) – harmful, 4 – dangerous. Indicators exceeding the maximum permissible levels are highlighted in bold.

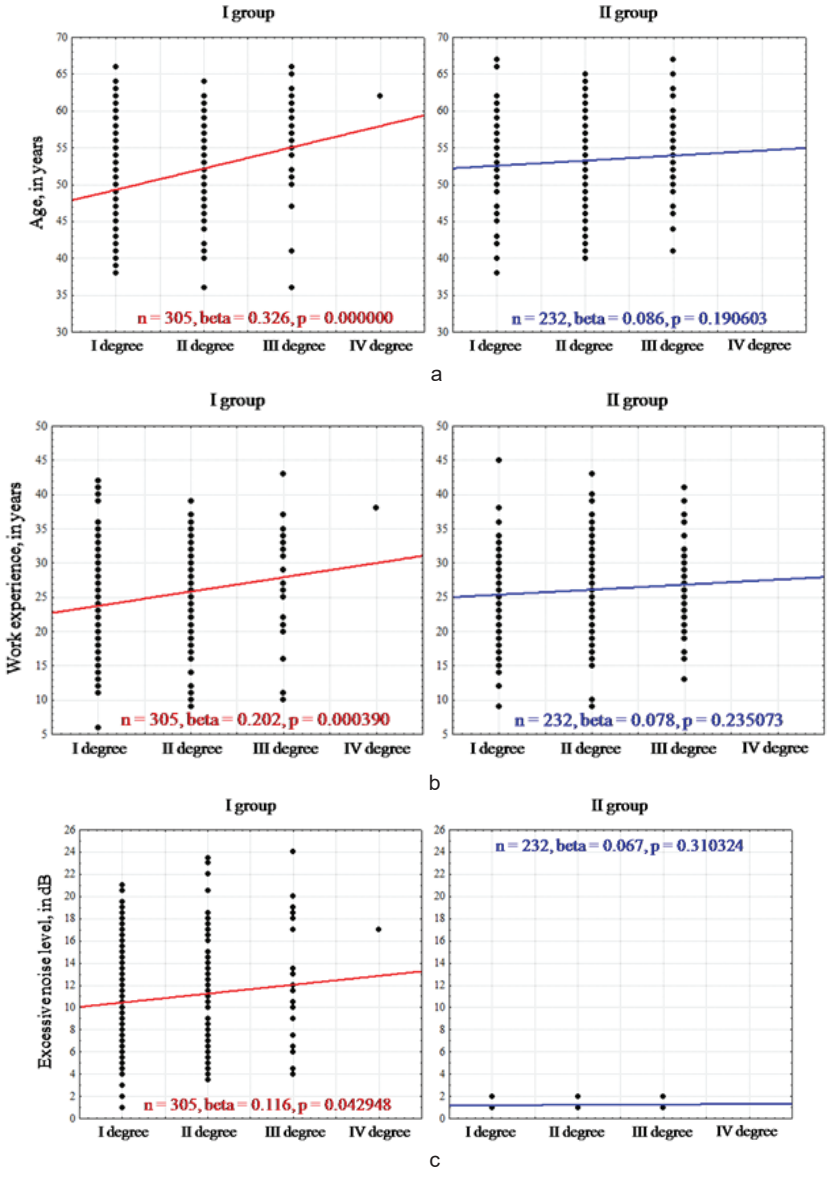

Figure 2. a) Correlation analysis of the dependence of the degree of hearing loss on age, b) Correlation analysis of the dependence of the degree of hearing loss on the length of service in this position, c) Correlation analysis of the dependence of the degree of hearing loss on the level of excess occupational noise.

(group I) the hearing loss is lighter and depends on age, experience and the level of excessive noise, and in car drivers (group II) the hearing loss is more severe, and does not depend on age, experience and level of excess noise. Since the results obtained do not fully explain the difference in the severity of hearing loss in observed groups, we additionally studied their working conditions and revealed the presence of other physical factors exceeding the maximum permissible levels in group II (Table 2).

Discussion. OSHL in all highly developed countries, including Russia, occupies one of the first places in the structure of all occupational diseases. Thus, in the all-Russian structure of occupational diseases, OSHL is about 16-17%. However, in some industries these figures reach 35-40% (weaving, forging and pressing production; aviation, automobile, mining, etc.). OSHL usually develops after a more or less long period (10-14 years) of work under conditions of industrial noise exposure. However, almost all researchers are unanimous in the opinion that increased sensitivity of the hearing organ to the adverse effects of noise, the additional effect of other factors of production (vibration, working posture, microclimate, neuro-emotional overstrain, etc.), and others play an important role in the development of OSHL risk factors (obesity, cardiovascular diseases, household noise, tobacco smoking, etc.) [6, 7, 8, 9].

The results of our study largely coincide with recent studies [10] and once again confirm that the severity of hearing loss with OSHL, obviously, depends on the potentiating effect of local vibration, general vibration, forced and/or fixed working posture. Hearing loss with OSHL in civil aviation flight personnel (group I) is lighter and depends on age, work experience and the level of excess noise, while the levels of local and general vibration are at the optimal level. For car drivers (group II), probably due to exceeding the maximum permissible level of local and general vibration at their workplaces, such patterns are blurred, and hearing loss becomes more severe. It should be noted that OSHL in car drivers (group II) is not the only diagnosis of an occupational disease, it was in all cases combined with other diseases, such as vibration disease or radiculopathy, caused by exposure to vibration and working posture. Perhaps the severity of hearing loss does not directly depend on the impact of these factors, but is a consequence of the disease «vibration disease» or «radiculopathy». These issues, as well as the genetic aspects of OSHL,

are the subject of more detailed study.

Conclusions. Typical flow and prevalence of Ist degree OSHL is possible in the absence of exposure to other harmful physical factors of production, such as local and general vibration. When exposed, along with excessive noise of local and general vibration, the patterns of OSHL development are blurred, the clinical course is more severe, with a predominance of II and III degrees.

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TOPICAL ISSUE

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ANALYSIS OF CLINICAL OUTCOMES IN PATIENTS WITH GASTRODUODENAL DISEASES DEPENDING ON *HELYCOBACTER PYLORY vaCA* GENE VARIANTS IN YAKUTIA

Vacuolating cytotoxin (VacA) is an important virulence factor coding for vacuolizing toxin and present in all *Helicobacter pylori* strains. There is a significant difference in vacuolating activity among strains due to the heterogeneity of the sequence in the m- and s- regions of the *vacA* gene of *Helicobacter pylori* strains. The m region has allelic types *m1* and *m2*, and the s region located at the 5'-end of the gene has alleles *s1* or *s2*. Strains carrying the mosaic combination of the *vacAs1m1* gene demonstrate higher levels of cytotoxic activity than *s1m2* strains, while *s2m2* strains do not secrete vacuolating cytotoxin. The effect of the *vacA* gene alleles on gastroduodenal diseases in patients in Yakutia has not been studied. The aim of this study is analysis of clinical outcomes in patients with gastroduodenal diseases depending on the variants of the *Helicobacter pylori vacA* gene in Yakutia.

According to the results of the histological report, 154 patients (out of 322 examined) were included in the study, in whom the presence of *Helicobacter pylori* in the antrum of the stomach was revealed. The patients were divided into two groups: chronic gastritis (n=65) and chronic gastritis with erosive and ulcerative lesions (n=89). The average age was 26.6 years (range 5 to 70). The diagnosis of chronic gastritis was established in 65 cases (42.2%), and chronic gastritis with erosive and ulcerative lesions was diagnosed in 89 cases (57.7%). The *vacAs1m1* allele was found in the sample with erosive gastritis in 30 patients among 39 (76.9%) and 9 out of 39 patients in the sample with chronic gastritis (23.1%) ($p<0.01$). In relation to other allelic variants of *vacA*, no statistically significant differences were found. The high rate of the *vacAs1m1* allele in Yakut patients confirms the hypothesis that the *vacAs1m1* alleles of *Helicobacter pylori* are the most cytotoxic and are more common in the sample of patients with more severe gastritis in peptic ulcer disease.

Keywords: *Helicobacter pylori*, gastroduodenal diseases, *vacA* gene, Yakutia.

Introduction. *Helicobacter pylori* (*H. pylori*) is a spiral-shaped gram-negative bacterium that infects various areas of the stomach and duodenum. Many cases of gastric and duodenal ulcers, gastritis, duodenitis, gastric cancer, and possibly some cases of gastric lymphomas are etiologically associated with *H. pylori* infection. However, most infected carriers of *H. pylori* show no symptoms of disease [2]. The genome of *H. pylori* strain "26695" is represented by a circular double-stranded DNA molecule with a size of 1667867 base pairs, and contains 1630 genes, of which 1576 encode proteins [19]. The results of two sequences of *H. pylori* genomes were published, in which a large family of 32 bound outer membrane proteins (HOP) was discovered, including the most famous *H. pylori* adhesins [19].

It is found that *H. pylori* has a number of the most characteristic adhesins that have focused on the action of human gastric epithelial cells. *H. pylori* produces specific proteins that can presumably be attributed to virulence and pathogenicity factors. Vacuolating cytotoxin (VacA) is an important virulence factor encoding vacuolizing toxin and is present in all strains of *H. pylori* [7]. There is a significant difference in vacuolizing activity among *H. pylori* strains [1, 3] due to the heterogeneity of the sequence in the m and s regions of the *vacA* gene. Region m has allelic types *m1* and *m2*, and s region located at the 5'-end of the gene has alleles *s1* or *s2*. Strains carrying a mosaic combination of the *vacAs1m1* gene demonstrate higher levels of cytotoxic activity than *s1m2* strains, while *s2m2* strains do not secrete vacuolizing cytotoxin [15, 20]. Infection with vacuolizing cytotoxin-positive strains is associated with certain gastroduodenal diseases [19, 21].

Most *H. pylori* strains deliver the 95kDa vacuolating cytotoxin VacA secreted by the exotoxin. The toxin is injected into the epithelial cell membranes and forms hexameric anion-selective, voltage-gated channels through which bicarbonate and organic anions can be released, with the ability to provide nutrients. VacA toxin causes the release of cytochrome C and induces apoptosis [19].

In addition to vacuolization of gastric epithelial cells, VacA inhibits acid secretion in the stomach, increases the secretion of pepsinogen, inhibits cell proliferation, damages mitochondria, disorganizes the cytoskeleton of gastric epithelium cells, and provides nutrients to the bacteria. *H. pylori* can "feed" on the exudate of the host tissue, while certain importance has the inflammatory process in the gastric mucosa, which is caused by the bacterium. Damage to the gastric mucosa is facilitated by the formation of proinflammatory cytokines: IL-1β, IL6, IL-8, and tumor necrosis factor (TNF-α) [18, 22].

The production of *H. pylori vacA* exotoxins causes vacuolization, damage and death of cells of the gastric mucosa. The clinical outcomes of gastroduodenal diseases, depending on the variants of the *H. pylori vacA* gene, common in Yakutia, have not been previously studied. The aim of this study is analysis of clinical outcomes in patients with gastroduodenal diseases depending on the variants of the *Helicobacter pylori vacA* gene in Yakutia.

Materials and methods. Gastric biopsy specimens were obtained from 322 patients that admitted to the endoscopic department for fibrogastroduodenoscopy (FGDS) in endoscopic department of State autonomous institution of Republic Sakha (Yakutia) «Republican Hospital No. 1 – National Center of Medicine» (RH No.-1 NCM). To confirm the presence of *H. pylori* infection, gastric biopsy specimens were sent for histological examination to the pathoanatomical department of RH No.-1 NCM.

According to the results of histological analysis, 154 patients (out of 322) were included in the study, who had the presence of *H. pylori* in the antrum. The average age was 26,6 years (from 5 to 70). In accordance with macroscopic analysis of the mucosa and histological results, patients were divided into two groups: chronic gastritis and chronic gastritis with erosive and ulcerative lesions.

Genomic DNA of *H. pylori* was isolated from frozen gastrobiopsies of the examined patients by using phenol-chloroform extraction [10].

Fibrogastroduodenoscopy was performed in the morning on an empty stomach. Biopsy specimens were taken from the antrum in amount of 2-3 pieces during endoscopic examination using a GIF-P3 fiberscope (Olympus, Japan).

Obtained biopsies of the gastric mucosa were fixed in 10% formalin solution. Dewaxing of sections and staining with hematoxylin and eosin were carried out according to the standard technique. For targeted bacterioscopy, sections are stained according to the Romanovsky-Giemsa method. The study was performed under magnification x100, X400 and X1000 on the "Axioskop" microscope "Opton" company. The morphological criteria of chronic gastritis were assessed in accordance with the visual analogue scale according to the modified Sydney system (Houston, USA, 1996).

The genomic DNA of *H. pylori* was isolated from frozen gastrobiopsy specimens from patients with a confirmed histological diagnosis of chronic gastritis and chronic gastritis with erosions and ulcers using phenol-chloroform extraction.

Amplification of the required *H. pylori* DNA fragments was performed using the Bio-Rad PCR amplifier. The detection of alleles of the *vacA* gene was carried out using the original sequences of oligonucleotide primers previously proposed [Hou et al., 2000], that flank the DNA region of the *H. pylori* containing *vacA* gene. Separation of amplification products was carried out in horizontal electrophoresis chambers in 3% agarose gel. Visualization of PCR products was carried out using a Bio-Rad gel-video documentation device using Image Lab ™ Software.

Differences between groups were evaluated by x-square test using Biostatd software (McGraw-Hill, Inc. Version 3.03). Differences were considered statistically significant at $p < 0.05$.

The surveys, provided by the framework of research work, were carried out strictly after the informed consent of participants, parents or legal representatives of minor patients without violations of ethical standards. This study was approved by the local committee on biomedical ethics of the Yakutsk Scientific Center for Complex Medical Problems. Protocol No. 41 of November 12, 2015. Decision No. 5.

Results and Discussion. *Analysis of the frequency of H. pylori vacA gene variants in patients with gastroduodenal diseases*

Endoscopic and histological examinations carried out at the first stage of the work showed that only 154 (out of 322 patients) were confirmed to have the *H. pylori*. The diagnosis of chronic gastritis was established in 65 cases (42.2%), and chronic gastritis with erosive and ulcerative lesions was diagnosed in 89 cas-

Table1

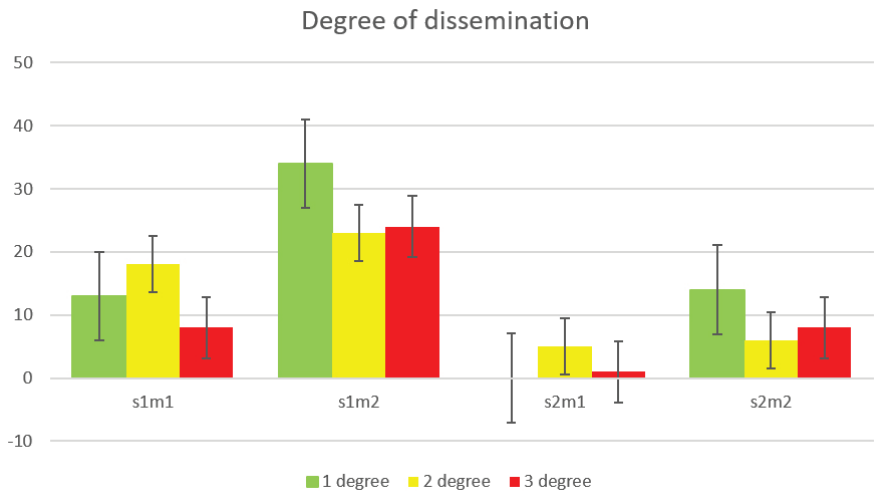
Design of oligonucleotide primers for the detection of alleles of the *vacA* gene

Gene	Primer	Sequence from 5' → 3' end	Fragment size	Reference
vacA	*vacAs1*	F5'-ATGGAAATACAACAAACACAC-3'	259 п.н.	[13]
		R5'-CTGCTTGAATGCGCCAAAC-3'		
	vacAs2	F5'-ATGGAAATACAACAAACACAC-3'	286 п.н.	
		R5'-CTGCTTGAATGCGCCAAAC-3'		
	vacAm1	F5'-GGTCAAAATGCGGTCATGG-3'	290 п.н.	
		R5'-CCATTGGTACCTGTAGAAAC-3'		
	vacAm2	F5'-GGAGCCCCAGGAAACATTG-3'	352 п.н.	
		R5'-CATAACTAGCGCCTTGCAC-3'		

Table2

Prevalence of *H. pylor*i genotypes in detected strains

Gene	*H. pylori vacA* strains	Prevalence
vacA (154)	*s1m1*	39 (25.2)
	s1m2	81 (52.4)
	s2m1	6 (4.0)
	s2m2	28 (18.3)



Comparative analysis of vacA gene with dissemination degree in patients with gastroduodenal diseases.

es (57.7%). Next, we analyzed the frequencies of *H. pylori vacA* gene variants among 154 samples with histologically confirmed *H. pylori* infection. Regions of the s- and m- genotype *vacA* were determined in all studied strains of H. pylori, among which four genotypes were found: *s1m1* (25.2%), *s1m2* (52.4%), *s2m1* (4.0%) and *s2m2* (18.3%) (Table 2).

Comparison of the occurrence of allelic variants of the H. pylori vacA gene in patients depending on the course of gastroduodenal diseases (erosive or chronic gastritis)

It was found that the *vacAs1m1* gene variant was significantly more frequent in the sample of patients diagnosed with chronic gastritis with erosive and ulcerative lesions of the stomach and duode-

Table3

Comparison of the frequency of *vacA* genes depending on the presence of erosions and ulcers, age and sex and demographic factors

Factors	n (154)	CG with erosions and GU/DU	CG	χ^2	*p*
Dependence on the presence of erosions and ulcers					
vacAs1m1	39	30 (76.9)	9 (23.1)	**8.215**	**<0.01**
vacAs1m2	81	40 (49.3)	41 (50.6)		
vacAs2m1	6	4 (66.6)	2 (33.3)	0.334	>0.05
vacAs2m2	28	15 (53.5)	13 (46.4)		
Dependence of age					
Factors	n (154)	Children	Adults	χ^2	*p*
vacAs1m1	39	20 (51.3)	19 (48.6)	0.755	>0.05
vacAs1m2	81	46 (59.7)	35 (40.2)		
vacAs2m1	6	4 (60.0)	2 (40.0)	0.170	>0.05
vacAs2m2	28	14 (51.8)	14 (48.1)		
Comparison by gender					
Factors	n (154)	♂	♀	χ^2	*p*
vacAs1m1	39	20 (51.2)	19 (48.7)	0.201	>0.05
vacAs1m2	81	38 (46.9)	43 (53.1)		
vacAs2m1	6	0 (0)	6 (100)	5.100	<0.05
vacAs2m2	28	14 (50.0)	14 (50.0)		
Comparison on place of residence					
Factors	n (154)	Urban population	Rural population	χ^2	*p*
vacAs1m1	39	6 (15.3)	33 (84.6)	0.179	>0.05
vacAs1m2	81	15 (18.5)	66 (81.4)		
vacAs2m1	6	0 (0)	6 (100)	1.561	>0.05
vacAs2m2	28	6 (21.4)	22 (78.5)		

num (76.9%) than in patients diagnosed with chronic gastritis, in whom the *vacAs1m2* gene variant was more common (50,6%) (p<0.01) (Table 3).

Comparison of the occurrence of allelic variants of the H. pylori vacA gene in patients depending on the degree of contamination

No statistically significant differences were found when comparing groups of patients with different degrees of contamination and the presence of variants of the gene *vacAs1m1*, *vacAs1m2*, *vacAs2m1* and *vacAs2m2 H. pylori*. However, there was a slight predominance of grade 2 contamination in patients with the *vacAs1m1* gene variant (рис. 1).

Comparison of allelic variants of the H. pylori vacA gene in group of patients depending on gender, age, place of birth and residence

There were no significant differences depending on age, place of birth and residence in patients with *vacA* gene variants of the *H. pylori* (p>0.05) however, statistically significant differences were found when comparing groups of men and women (p<0.05) (Table 3). Thus, the frequency of occurrence of the *vacAs2m1* gene variant in the group of female patients was 100% (p <0.05), while in male patients this allelic variant was not recorded (0%) (Table 3).

In this study the incidence of gene variants *vacA H. pylori* has been studied and analyzed for the first time in patients with chronic gastritis in Yakutia. It was found that when analyzing a sample of patients with different variants of the *H. pylori vacA* gene, statistically significant differences were found in the group of patients, depending on the presence of erosive and ulcerative manifestations (Table 3). Thus, in patients with erosive and ulcerative lesions, the *H. pylori vacAs1m1* variant was significantly more often identified than in patients with chronic gastritis (76.9%) (p<0.01). Obtained results are consistent with the results of similar studies in other populations, where the frequency of the *vacAs1m1* gene variant was higher than the frequency of *vacAs1m2* in patients with chronic gastritis complicated by erosions and ulcers of the stomach and duodenum. (In Russia: Rostov-on-Don – 48.0%, Vladivostok – 57.2%, St. Petersburg – 55.5%; in Thailand – 58.0%, in Southern Mexico – 61.4%) [4-6, 14, 17].

This result may indicate a more pronounced pathogenic potential of the *H. pylori vacAs1m1* gene variant. The results of our study are in accordance with the previously obtained data that the presence of the *vacAs1m1* gene variant in the *H. pylori* genome is associated with a higher incidence of duodenal ulcer disease, complicated course of *H. pylori* infection, as well as with gastric adenocarcinoma. [8, 12, 14, 16].

When comparing *vacA* gene variants with inflammation activity, no statistically significant differences were found. However, a slight difference was found when comparing the *vacAs1m1* gene variant with the 2nd degree of contamination (Fig. 1). The insignificant difference is most likely explained by the small number of compared subgroups in the studied sample.

When comparing *H. pylori vacA* gene variants in patients depending on age, place of birth and residence, no statistically significant differences were obtained. (p>0,05). However, when analyzed according to gender in women, the allelic variant *vacAs2m1* of *H. pylori* was 100% and most likely due to stochastic reasons (p<0,05) (Table 3). The *vacAs2m1* gene variant is very rare and also belongs to non-virulent variants. Previous studies indicate a very low cytotoxic activity and a very low frequency of this allelic variant [9, 11].

With help of the obtained results for the alleles of the vacA gene and it will be possible to obtain data that will make it possible to determine how virulent and pathogenic the identified strain of H. pylori is, thereby making it possible to choose a more appropriate tactics for treating patients with chronic gastritis in Yakutia.

Conclusion. We have shown a relationship between a high rate of the *vacAs1m1* gene variant with a more severe clinical course of gastroduodenal diseases in Yakut patients. The obtained result confirms the hypothesis that the *vacAs1m1* alleles are the most cytotoxic and are more common in patients with more severe gastritis in peptic ulcer disease.

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DIFFERENTIAL EXPRESSION OF MICRO-RNA IN TUMOR AND NORMAL COLON TISSUES

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Non-coding RNAs (miRNAs and long non-coding RNAs (lncRNA)) play an important role in many biological processes, and dysregulation can lead to various diseases, including colorectal cancer (CRC). The aim of the study was to analyze the differential expression of miRNAs in the tumor and normal tissues of patients with CRC, as well as the identification of potential lncRNA targets and target genes using methods of machine learning. Analysis of miRNA expression was performed by multiple parallel sequencing on a MiSeq instrument. For bioinformation analysis, DESeq2, TarPmiR, ORA (Over-Representation Analysis) and FMD (Functional module detection) algorithms were used. Sequencing revealed 6 differentially expressed microRNAs (hsa-miR-143-3p, hsa-miR-26a-5p, hsa-miR-25-3p, hsa-miR-92a-3p, hsa-miR-21-5p, hsa -let-7i-5p) in the tumor tissue of the colon is relatively normal. For these microRNAs, 97 target genes and 23 potentially interacting long non-coding RNAs were identified. Together, they form a network of competitively expressed RNA characteristic of CRC, which is involved in the implementation of signaling cascades such as regulation of cell adhesion, activation of the immune response, regulation of the cellular response to hormones and stress, Wnt signaling pathway and cell migration regulation, regulation of proliferation and cell cycle, regulation interaction with viral agents, regulation of apoptosis and response to hypoxia. The data obtained expand the understanding of the mechanisms of gene expression regulation in CRC and can become the basis for a panel of tumor markers.

Keywords: colorectal cancer, microRNA, non-coding RNA, expression, metastases, multiple parallel sequencing.

Introduction. Currently, there is a stable increase in the incidence of colorectal cancer (CRC): worldwide, the number of annually registered cases is approaching 1.4 million [6]. CRC is a heterogeneous multifactorial disease, which in about 35% of cases is caused by genetic factors, including changes in the expression of noncoding RNAs (ncRNAs) [25].

The transformation of normal colon mucosa into adenocarcinoma occurs in several stages that take decades [25]. Although numerous therapies such as surgery, chemotherapy, radiation therapy, targeted therapy, and immunotherapy have been shown to reduce relapse rates and improve patient survival, 5-year survival in CRC patients is still low [24]. This emphasizes the need to search for new biomarkers that can be used for early diagnosis and prognosis of the course of this disease. A large number of studies have revealed that ncRNAs (miRNAs and long noncoding RNAs (lncRNAs)) play an important role in many biological processes, and their dysregulation can lead to various diseases, including colon cancer [29].

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MicroRNAs are short non-coding RNAs that regulate gene expression by catalyzing the destruction of mRNA or by inhibiting the translation of mRNA into protein [4]. MicroRNAs make a significant contribution to the initiation and development of various molecular events, including the initiation of oncogenesis, progression and metastasis of tumors, which makes microRNAs potential biomarkers for assessing the progression and prognosis of CRC. Although miRNAs regulate the expression of genes encoding proteins, mainly through degradation or silencing of mRNA, there is growing evidence that miRNAs can interact with lncRNA, which, in turn, also regulates the expression of target genes [1].

The study of the regulatory network of microRNA-long non-coding RNA-mRNA is of great importance both for elucidating the molecular mechanisms underlying carcinogenesis and for creating a panel of new biomarkers. Therefore, the aim of the study was to analyze the differential expression of microRNA in tumor and normal tissues of CRC patients, as well as to identify potential lncRNA targets and target genes using machine learning methods.

Materials and methods.

Patients. For multiple parallel microRNA sequencing, 80 patients (adenocarcinoma of the colon G2) aged 45-69 (median age 65) were selected, from which paired tissue biopsies (tumor and normal, 160 samples in total) were obtained.

Multiple parallel microRNA sequencing. The mirVana miRNA Isolation Kit (Ambion, Life Science Technologies, USA) was used to isolate the miRNA fraction. The creation and subsequent purification of the miRNA transcriptome library was performed using the TruSeq Small RNASample Preparation Kit (Illumina, United States). The 3'- and 5'-adapters were sequentially sewn to the miRNA fraction, reverse transcription and amplification of the created miRNA construct were carried out according to the attached protocol. The cDNA copies were purified by electrophoresis in 6% PAGE. cDNA from the gel was extracted with water and precipitated with 95% ethanol (glycogen was used as a co-precipitant). The amount of cDNA was determined on a Qubit 2.0® fluorometer using the Qubit dsDNA HS Assay Kit (Invitrogen, USA). Multiple parallel sequencing of the nucleotide sequences of the cDNA libraries was performed on a MiSeq instrument (Illumina, United States). The method of parallel multiple sequencing identified the nucleotide sequence in a random sample of generated cDNA copies. The expression of miRNA was determined by comparing the nucleotide sequence of the sequenced molecules in each sample with the known nucleotide sequences of miRNA presented in the miRBase and mirGeneDB databases. The approach is based on the algorithm implemented in miRanalyzer [14].

Statistical / bioinformatic analysis.

DESeq2 algorithm. When analyzing differential expression of miRNAs, the DESeq2 algorithm was used, implemented in the R. DESeq2 environment. DESeq2 provides a method for checking differential expression using negative binomial generalized linear models; variance estimates and changes in logarithmic fold. The analysis included microRNAs represented in the samples by

at least 10 copies. To take into account the effect of integral expression of miRNAs on the expression of compared miRNAs, the compared libraries were normalized using the RLE (Relative Log Expression) method. The Wald test was used to assess the statistical significance of the differences. To take into account the multiplicity of comparisons, we used the method of assessing the probability of false-positive results (false discovery rate (FDR, Benjamini-Hochberg)) [14].

Search for target genes. The search for target genes was carried out using the TarPmiR algorithm using the TargetScan, mirDB, and miRTarBase databases. TarPmiR uses a random forest approach to predict the target microRNA site. Random forest (from English - "random forest") is a machine learning algorithm that uses an ensemble of decision trees and combines the Breiman bagging method and the random subspace method. The result of the random forest model is the predicted probability that the candidate target site is the true target site. TarPmiR integrates 13 traditional functions for predicting microRNA target sites, including the availability of binding sites and thermodynamic properties such as free energy [5].

Over-Representation Analysis (ORA). This method measures the percentage of genes (or microRNAs) in the signaling pathway that are differentially expressed. The goal of ORA is to obtain a list of the most important signaling pathways, ordered according to p-value [2].

Clustering of target genes by function (Algorithm FMD (Functional module detection)). The algorithm is based on the k-nearest neighbors (KNN) algorithm and Louvain's community search algorithm to cluster closely related genes into separate functional modules. Representative processes and pathways enriched in each cluster are presented along with the cluster and their resulting Q value. The Q value of each member of the functional module is calculated using Fisher's one-sided exact test and Benjamin-Hochberg correction to correct multiple comparisons [13].

Analysis of microRNA-lncRNA interaction. HITS-CLIP, PAR-CLIP and CLASH data were obtained from the Gene Expression Omnibus database and pre-processed using FASTX-Toolkit v0.0.13 and analyzed using PARalyzer v1.1. All binding site coordinates were converted to assemblies hg19, mm9 / mm10 and ce6 / ce10, respectively, using the UCSC LiftOver Tool. The genomic coordinates of the conserved miRNA target sites predicted by TargetScan, miRanda / mirSVR, PITA, Pictar, and RNA22 were also assembled and transformed into hg19, mm9 / mm10, and ce6 / ce10 assemblies using LiftOver. The obtained coordinates were compared with the previously described CLIP clusters using BEDTools [21].

Results and discussion. As a result of multiple parallel sequencing of 160 samples, the following categories of RNA were found (average percentage is shown): microRNA (0.5%), mRNA (0.03%), antisense mRNA (0.01%), tRNA (86.7%), transcripts of unclear significance (0.03%)), other RNAs (12.7%).

The DEseq2 algorithm was used to analyze the differential expression of the detected miRNAs in tumor and normal tissue of the colon of 80 patients (Fig. 1). Six differentially expressed miRNAs were found: the expression of two, hsa-miR-143-3p and hsa-miR-26a-5p, was reduced, and four, hsa-miR-25-3p, hsa-miR-92a-3p, hsa-miR -21-5p and hsa-let-7i-5p are elevated in tumor tissue compared to normal.

The highest expression level in normal tissue was found for hsa-miR-143-3p, the lowest for hsa-miR-92a-3p; in tumor tissue, these were, respectively, hsa-miR-10b-5p and hsa-miR-25-3p (Table . one). The largest difference in miRNA expression in tumor tissue relative to normal was recorded for hsa-miR-92a-3p (7.2 times, p = 0.02), the smallest one for hsa-miR-26a-5p (3.6 times, p = 0.01) (Table 1). 1, fig. 1).

target genes presented in Table 2 made it possible to identify 3 functional clusters, including 65 genes (Fig. 2, Table 3)

From the data presented in Table 3 and Figure 2, it can be seen that the main signaling pathways in which target genes are involved are (Q <0.05): regulation of intracellular signal transmission, regulation of cell adhesion, protein dephosphorylation, activation of the immune response, regulation of the organization of the plasma membrane, regulation of cell growth, regulation of cellular response to hormones, cellular response to stress, and regulation of the Wnt signaling pathway.

An alternative FMD method for assessing the significance of differentially expressed microRNAs is overrepresentation in signaling pathways (ORA). The data obtained during the implementation of the ORA algorithm are presented in Table 4.

The main signaling cascades involving microRNAs hsa-miR-143-3p, hsa-miR-26a-5p, hsa-miR-25-3p, hsa-miR-92a-3p, hsa-miR-21-5p and hsa- let-7i-5p include (p <0.05): regulation of proliferation and the cell cycle, immune response and response to inflammation, interaction with viral agents, regulation of apoptosis, response to hypoxia, and regulation of cell migration.

Analysis of the possibility of interaction of 6 differentially expressed miRNAs with lncRNA revealed the following miRNA-lncRNA pairs (Table 5).

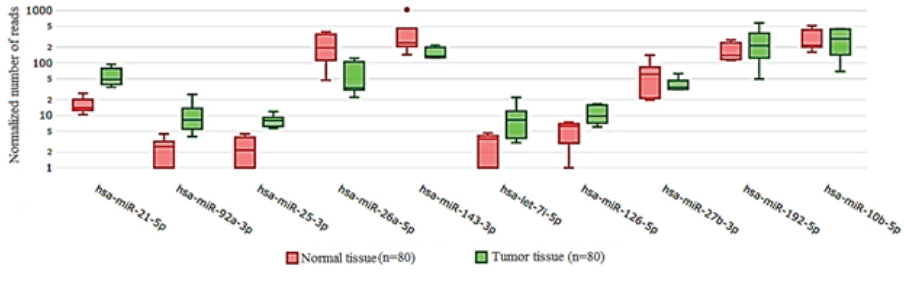

Figure 1. Expression of 10 miRNAs with the lowest FDR (6 significant miRNAs).

For microRNAs differentially expressed in tumors and in normal tissue, a search for target genes was carried out using the TarPmiR algorithm.

For 6 miRNAs that statistically significantly alter the expression in colon tumors, 5,360 target genes were predicted, of which were validated (confirmed) in the TargetScan, mirDB and miRTarBase databases of miRNA-mRNA interactions for 116 target genes, including 97 targets with minimal free energy of microRNA-mRNA interaction (Table 2).

Application of the FMD (Functional module detection) algorithm to the list of

As can be seen from the data presented in Table 5, five lncRNAs have binding sites common to several microRNAs. For example, lncRNA MALAT1 can interact with 5 out of 6 microRNAs (hsa-miR-143-3p, hsa-miR-26a-5p, hsa-miR-25-3p, hsa-miR-92a-3p, hsa-miR- 21-5p).

Significant progress in molecular biology in the last 10 years has revealed a link between microRNA and CRC. Stable dysregulation of miRNA expression in CRC leads to the stimulation of oncogenes or tumor suppressors [6].

Different studies of microRNA sequencing in CRC have identified dif-

Table1

Expression of microRNA in normal and tumor tissues *

microRNA	Normal tissue	Tumor tissue	FC	$\log_2$FC	*p*-value	P_{adj}
hsa-let-7i-5p*	1.83	8.20	4.481	2.164	0.02	0.04
hsa-miR-10a-5p	36.91	37.00	1.002	0.004	0.99	0.99
hsa-miR-10b-5p	301.14	283.17	0.940	– 0.089	0.87	0.96
hsa-miR-126-5p	4.02	10.09	2.510	1.328	0.03	0.05
hsa-miR-143-3p*	382.87	159.01	0.415	– 1.268	0.02	0.04
hsa-miR-192-5p	174.70	258.4	1.479	0.565	0.34	0.42
hsa-miR-21-5p*	15.41	57.40	3.725	1.897	0.0	0.0
hsa-miR-25-3p*	1.44	7.05	4.896	2.292	0.01	0.02
hsa-miR-26a-5p*	220.06	61.45	0.279	– 1.840	0.01	0.02
hsa-miR-27b-3p	60.77	39.16	0.644	– 0.634	0.3	0.41
hsa-miR-92a-3p*	1.35	9.65	7.148	2.838	0.0	0.02

*Note. Expression Minimum Threshold = 1; FC (fold change) - change rate; Padj is the p-value adjusted for multiple comparisons using the Benjamini-Hochberg method.

ferent specific expression profiles that may be associated with clinical or prognostic features. So Hamfjord et al. [9] identified 19 hypo-expressed and 18 hyper-expressed miRNAs in CRC. Later Schee et al. [23] identified the 5 most expressed miRNAs (hsa-miR-10a-5p, hsa-miR-21-5p, hsa-miR-22-3p, hsa-miR-143-3p, and hsa-miR-192-5p) in 88 CRC samples that the number of identified microRNAs is close to our study.

In our study, the expression of 2 microRNAs (miR-143-3p and miR-26a-5p) was decreased, and 4 (miR-25-3p, miR-92a-3p, miR-21-5p, and let-7i-5p) in tumor tissue compared to normal. The results obtained are confirmed in the works of other authors. Thus, miR-143-3p is part of a cluster associated with oncogenes, DNA repair genes, and genes that regulate the WNT and MAPK signaling pathways. Tokarz P and Blasiak J. [26] showed that hsa-miR-143-3p in CRC cells reduces the expression of DNA methyltransferases 3A (DNMT3A) and changes the phenotype of malignant transformation, and hsa-miR-143-3p is negative a prognostic factor for survival in patients with CRC. [20].

Hernández R. et al. showed that the overexpression of miR-21 leads to tumor formation, increasing invasion and metastasis [10]. Previously, Liu et al. [16] after analyzing the concentrations of miR-21 and miR-92a in the serum of CRC patients, they found higher levels of these miRNAs compared to healthy subjects. In CRC, the expression of miR-92a is significantly increased in tissues, which leads to a decrease in E-cadherin and increases the level of β-catenin and vimentin, which are involved in the regulation of the epithelial-mesenchymal transition [31]. In addition, Nishida et al. reported an association between high levels of miR-92a and lymph node and liver metastases [19]. The above results indicate that high expression of miR-92a in CRC patients is associated with poor survival, and miR-92a may be a potential biomarker for CRC.

In colon tumors, let-7 suppresses KRAS and its expression is associated with better patient survival [22].

Table2

Target genes of 6 differentially expressed microRNAs

Overexpressed microRNA						Hypoexpressed microRNA	
hsa-92a-3p		hsa-let-7i-5p		hsa-miR-25-3p	hsa-miR-21-5p	hsa-miR-26a-5p	hsa-miR-143-3p
Гены-мишени							
SPOCK2	*KLF2*	*ZNF644*	*PDP2*	*GALNT7*	*ST6GAL1*	*CHAC1*	*ZBTB44*
MEF2D	*OTUD3*	*TGFBR3*	*NR6A1*	*PER2*	*KLHL42*	*CDK8*	*MAPK7*
SNN	*CPEB4*	*SMC1A*	*PLAGL2*	*SRPRA*	*PAN3*	*TET2*	*SECISBP2L*
ATXN1	*GNAQ*	*POTEM*	*ARID3B*	*SLX4*	*GID4*	*PDE4B*	
FAR1	*PHTF2*	*AGO1*	*SMCR8*	*TMEM184B*		*ZNF608*	
GLYR1	*TSC1*	*ONECUT2*	*MBD2*	*LHFPL2*		*AGPAT5*	
PTPRJ	*G3BP2*	*BACH1*		*CPEB4*		*ESR1*	
DAB2IP	*YIPF4*	*CCNT2*		*EIF4G2*		*PIM1*	
RRBP1	*ELOVL6*	*CBX5*		*PKDCC*			
EIF4G2	*GOLGA8J*	*GRPEL2*		*C5orf24*			
PLEKHA1	*UBE2Z*	*ADIPOR2*		*FNIP1*			
SPRYD4	*PPP1R37*	*RBFOX2*		*PHLPP2*			
EVI5	*USP28*	*PDE12*		*SERTAD3*			
PER2	*KCNC4*	*KREMEN1*					
NOL4L	*FAM20C*	*CRY2*					
DNAJB12	*SOCS5*	*SURF4*					
TRAM2	*SLX4*	*IGDCC4*					
ACTC1	*MAN2A1*	*CEP135*					
CREB3L2	*ATXN7*	*SYT1*					
SOX4	*GRAMD1B*	*USP47*					
TECPR2	*PCMTD1*	*SEMA4C*					

Table3

Functional clusters and signaling pathways target-genes of microRNA differentiall

Cluster	Function / signal pathway	Q value*	Number of genes	Гены
M1	downregulation of the protein kinase B signaling pathway	0.00056	3	*PLEKHA1,PTPRJ,OTUD3*
	protein dephosphorylation	0.00096	4	*TSC1,PDP2,PTPRJ,CRY2*
	negative regulation of intracellular signaling	0.00110	5	*PTPRJ,PLEKHA1,TSC1, ESR1, OTUD3*
	positive regulation of focal adhesion	0.00253	2	*TSC1,PTPRJ*
	positive regulation of the assembly of cellular compounds	0.00325	2	*TSC1,PTPRJ*
	positive regulation of biogenesis of cellular com-ponents	0.00422	4	*TSC1,ESR1,PTPR-J,CEP135*
	regulation of phosphoprotein phosphatase activity	0.00658	2	*CRY2,TSC1*
	cellular response to dsRNA	0.00914	2	*PDE12,ESR1*
	cell surface receptor signaling pathway that acti-vates the immune response	0.01281	2	*PLEKHA1,PTPRJ*
	regulation of the organization of the plasma mem-brane	0.02375	2	*SYT1,CEP135*
	protein stabilization	0.02442	2	*TSC1,OTUD3*
	transmission of a signal that activates the immune response	0.02984	2	*PLEKHA1,PTPRJ*
	regulation of cell growth	0.03313	2	*SYT1,PTPRJ*
	activation of the immune response	0.03807	2	*PLEKHA1,PTPRJ*
	cellular response to hormones	0.05441	2	*TSC1,ESR1*
M2	negative regulation of the cell cycle	0.02375	2	*SOX4,SMC1A*
	organization of the endomembrane system	0.03258	2	*TRAM2,SURF4*
	growth regulation	0.03429	2	*SERTAD3,PIM1*
	cellular response to organic cyclic compound	0.04654	2	*PIM1,RBFOX2*
	drug metabolism	0.04814	2	*ST6GAL1,KLF2*
M3	positive regulation of cell growth	0.00914	2	*EIF4G2,USP47*
	regulation of the canonical Wnt signaling pathway	0.02083	2	*GNAQ,USP47*
	positive regulation of GTPase activity	0.02233	2	*GNAQ,EVI5*
	lipid biosynthesis process	0.03125	2	*FAR1,ELOVL6*

*Q value - P-value adjusted using Benjamini-Hochberg correction.

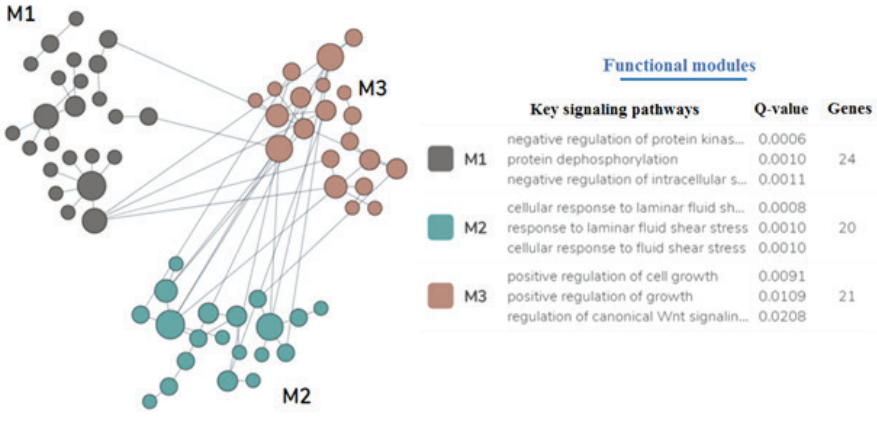

Figure 2. Functional clusters of target genes of 5 differentially expressed microRNAs in KPP

Thus, the investigated microRNAs can be divided into oncogenic and oncosuppressive ones. Those. oncogenic microRNAs mainly target and inhibit the expression of suppressor genes. The activation of these miRNAs has a significant effect on the progression of CRC (for example, hsa-miR-21 and hsa-miR-92a). However, a number of miRNAs (oncosuppressive miRNAs) play an important role in slowing tumor progression by suppressing oncogenes associated with proliferation, apoptosis, invasion, and migration (for example, hsa-miR-143-3p and hsa-let-7i-5p).

Let us consider in more detail one of the functional clusters (M3) identified by us and the effect of 5 microRNAs on it (Figure 3).

Figure 3 shows that the overexpression of miR-92a-3p can lead to suppression of the expression of the FAR1, EVI5, MAN2A1, PCMTD1, GNAQ, PHTF2, and ELOVL6 genes. The ELOVL6 gene (Fatty Acid Elongase 6) encodes an enzyme that catalyzes the first and rate-limiting reaction of the fatty acid elongation cycle and is involved in the synthesis of fatty acids that are essential in numerous biological processes as precursors of membrane lipids and lipid mediators. The FAR1 gene encodes a protein necessary for the reduction of fatty acids to

Table4

Results of analysis of overrepresentation in signaling pathways (ORA)

Signaling pathways	p-value	microRNAs
positive regulation of the activity of the tran-scription factor nf kappab	0.017	miR-143; miR-26a; miR-25; miR-92a; miR-21; let-7i
reaction to ethanol	0.017	miR-143; miR-26a; miR-25; miR-92a; miR-21; let-7i
inflammatory response	0.019	miR-143; miR-26a; miR-25; miR-92a; miR-21; let-7i
cell migration	0.020	miR-143; miR-26a; miR-25; miR-92a; miR-21; let-7i
regulation of cell proliferation	0.021	miR-143; miR-26a; miR-25; miR-92a; miR-21; let-7i
phosphatidylinositol-mediated signaling	0.023	miR-143; miR-26a; miR-25; miR-92a; miR-21; let-7i
cellular response to hypoxia	0.026	miR-143; miR-26a; miR-25; miR-92a; miR-21; let-7i
cytokine-mediated signaling pathway	0.027	miR-143; miR-26a; miR-25; miR-92a; miR-21; let-7i
ubiquitination of proteins	0.028	miR-143; miR-26a; miR-25; miR-92a; miR-21; let-7i
epidermal growth factor receptor signaling pathway	0.030	miR-143; miR-26a; miR-25; miR-92a; miR-21; let-7i
regulation of the apoptotic process	0.030	miR-143; miR-26a; miR-25; miR-92a; miR-21; let-7i
immune response	0.030	miR-143; miR-26a; miR-25; miR-92a; miR-21; let-7i
cell cycle control	0.031	miR-143; miR-26a; miR-25; miR-92a; miR-21; let-7i
interaction with the virus	0.040	miR-143; miR-26a; miR-25; miR-92a; miR-21; let-7i

Table5

MicroRNA-lncRNA interactions

microRNA	lncRNA	Genomic coordinates	Alignment
1	2	3	4
hsa-let-7i-5p	XIST	chrX:73046461-73046481	LncRNA: 5' agaACCCUAAAC-CCUACCUCu 3' | | :|||| |||||| miRNA : 3' uugUCGUGUUUGAUGAUGGAGu 5'
	NEAT1	chr11:65205185-65205207	LncRNA: 5' cugguCUUGGUCUGUCUACCUCg 3' | ::: ||: |||||| miRNA : 3' uugucGUGUUUGAU-GAUGGAGu 5'
	HELLPAR	chr12:102625721-102625741	LncRNA: 5' uuCAGAUCUCAUU-CUACCUCc 3' ||| | |:| |||||| miRNA : 3' uuGUCGUGUUUGAUGAUGGAGu 5'
	AP000766.1	chr11:107185038-107185058	LncRNA: 5' uuCACUCUUAAAU-CUACCUCa 3' || : : || | |||||| miRNA : 3' uuGUCGUGUUUGAUGAUGGAGu 5'
	LINC00265	chr7:39831428-39831448	LncRNA: 5' uuaGGCCCAUAC-CCUACCUCa 3' :|| || || |||||| miRNA : 3' uugUCGUGUUUGAUGAUGGAGu 5'
	AC124045.1	chr3:44710371-44710393	LncRNA: 5' auUAGCCUGUAAGAU-CUACCUCa 3' :||| ::||: | |||||| miRNA : 3' uuGUCG--UGUUUGAUGAUGGAGu 5'
	MIRLET7BHG	chr22:46509673-46509694	LncRNA: 5' gggAGGGCCGCCCCCUACCUCa 3' || :| : | |||||| miRNA : 3' uugUCGUGUUUGAUGAUGGAGu 5'
	OLMALINC	chr10:102133372-102133384	LncRNA: 5' ---------cuCUCCUACCUCa 3' || |||||| miRNA : 3' uugucguguuuGAUGAUGGAGu 5'
hsa-miR-143-3p	HELLPAR	chr12:102714596-102714616	LncRNA: 5' agggcaaaccuaUUCAUCUCa 3' ||||||| miRNA : 3' cucgaugucacgAAGUAGAGu 5'
	MALAT1	chr11:65269208-65269229	LncRNA: 5' ugGUUCAUAUUCAGUCAUCUCa 3' |:| |:| | |||||| miRNA : 3' cuCGA-UGUCACGAAGUAGAGu 5'

Continuation of table. 5

1	2	3	4
	AC245884.8	chr19:54950318-54950338	LncRNA: 5' ucauguCUGUAAGUCAUCUCc 3' | || |||||| miRNA : 3' cucgauGUCACGAAGUAGAGu 5'
	MEOX2-AS1	chr7:15734898-15734919	LncRNA: 5' gcGUUGUGAAUCAGUCAUCUCa 3' |:|:: | | |||||| miRNA : 3' cuCGAUG-UCACGAAGUAGAGu 5'
	MEG3	chr14:101315825-101315845	LncRNA: 5' augggAUUGUCAUUCAUCUCa 3' |: || ||||||| miRNA : 3' cucgaUGUCACGAAGUAGAGu 5'
hsa-miR-26a-5p	TUG1	chr22:31371700-31371719	LncRNA: 5' augCUCUACUGG--UACUUGAa 3' || | |||| |||||| miRNA : 3' ucgGAUAGGACCUAAUGAACUu 5
	NORAD	chr20:34637129-34637150	LncRNA: 5' acaaauuaacuccUUACUUGAa 3' |||||||| miRNA : 3' ucggauaggaccuAAUGAACUu 5'
	HCG11	chr6:26523678-26523694	LncRNA: 5' -----uuaacuccUUACUUGAa 3' |||||||| miRNA : 3' ucggauaggaccuAAUGAACUu 5'
	MALAT1	chr11:65268969-65268990	LncRNA: 5' aaCUUGUUAUUUUUUACUUGAa 3' |:|:|: | ||||||| miRNA : 3' ucGGAUAGGACCUAAUGAACUu 5
	WASIR2	chr16:75102-75123	LncRNA: 5' uuauaccauaaaAUUACUUGAa 3' |||||||| miRNA : 3' ucggauaggaccUAAUGAACUu 5
	AC005332.6	chr17:66132006-66132023	LncRNA: 5' acaUUAUCU----UUACUUGAg 3' :||||: ||||||| miRNA : 3' ucgGAUAGGACCUAAUGAACUu 5'
	AC005332.7	chr17:66125163-66125184	LncRNA: 5' uucuggauaaaacAUUACUUGAg 3' |||||||| miRNA : 3' ucggauaggaccUAAUGAACUu 5'
	EBLN3P	chr9:37087347-37087368	LncRNA: 5' uccagggaaagagcUACUUGAc 3' |||||| miRNA : 3' ucggauaggaccuaAUGAACUu 5'
hsa-miR-25-3p	NORAD	chr20:34636526-34636547	LncRNA: 5' ccauuguuAUGUGUGUGCAAUu 3' | ::: |||||| miRNA : 3' agucuggcUCUGUUCACGUUAc 5'
	XIST	chrX:73063643-73063665	LncRNA: 5' uuGGACUGUUAAUAUGUGCAAUu 3' :|||:| |:| |||||| miRNA : 3' agUCUGGC-UCUGUUCACGUUAc 5'
	MALAT1	chr11:65268090-65268110	LncRNA: 5' caGGAAGGAG-CGAGUGCAAUu 3' :|| ||| |:||||||| miRNA : 3' agUCUGGCUCUGUUCACGUUAc 5'
	PURPL	chr5:27494380-27494405	LncRNA: 5' gaAGACCUGAUUUAUCAUGUGCAAUa 3' ||||| || | || |||||| miRNA : 3' agUCUGG-CU--CU-GUUCACGUUAc 5'
hsa-miR-92a-3p	OIP5-AS1	chr15:41592688-41592709	LncRNA: 5' uaaaaCCGGGAUAUGUGCAAUa 3' |||||:| |||||| miRNA : 3' uguccGGCCCUGUUCACGUUAu 5'
	NORAD	chr20:34636526-34636547	LncRNA: 5' ccauuguuauguguGUGCAAUu 3' |||||| miRNA : 3' uguccggcccuguuCACGUUAu 5'
	XIST	chrX:73063643-73063664	LncRNA: 5' uggacuguuaAUAUGUGCAAUu 3' |:| |||||| miRNA : 3' uguccggcccUGUUCACGUUAu 5'
	MALAT1	chr11:65268089-65268110	LncRNA: 5' ccAGGAAGGAGCGAGUGCAAUu 3' ||| || :|:||||||| miRNA : 3' ugUCCGGCCCUGUUCACGUUAu 5'

The end of the table. 5

1	2	3	4
	SNHG14	chr15:25364949-25364970	LncRNA: 5' agcaccaaauuucuGUGCAAUg 3' |||||| miRNA : 3' uguccggcccuguuCACGUUAu 5'
hsa-miR-21-5p	TUG1	chr22:31372028-31372048	LncRNA: 5' gauAAAUGAG-CUAAUAAGCUu 3' | || | | |||||| miRNA : 3' aguUGUAGUCAGACUAUUCGAu 5'
	XIST	chrX:73043927-73043947	LncRNA: 5' cugcCACCCAUAU-AUAAGCUa 3' || | | |||||| miRNA : 3' aguuGUAGUCAGACUAUUCGAu 5'
	MALAT1	chr11:65266342-65266365	LncRNA: 5' uuuGCAUUCAAGUUCCAUAAGCUg 3' :|||: |||: |||||| miRNA : 3' aguUGUAG--UCAGACUAUUCGAu 5'
	FAM66E	chr8:7841811-7841833	LncRNA: 5' acuugAUUGUGGUGGAUAAGCUu 3' ||:: | : ||||||| miRNA : 3' aguugUAGU-CAGACUAUUCGAu 5'

fatty alcohols, which is necessary for lipid synthesis. Suppression of expression of the FAR1 and ELOVL6 genes can lead to changes in the lipid composition of cell membranes and disruption of the normal presentation of cellular antigens, which is important for masking tumor cells from the immune system [8]. The PHTF2 (Putative Homeodomain Transcription Factor 2) and EVI5 (Ecotropic Viral Integration Site 5) genes function as regulators of the cell cycle and transcription of a wide range of genes associated with tumor development (according to https://www.genecards.org). GNAQ (G Protein Subunit Alpha Q) is involved in the modulation of various transmembrane signaling cascades, regulates the chemotaxis of dendritic cells, and is also involved in the regulation (agonist) of the canonical Wnt signaling pathway.

At the same time, miR-26a-5p hypoexpression can lead to an increase in the expression of the CDK8, TET2, and AGPAT5 genes (Figure 3). TET2 encodes a protein that catalyzes the conversion of the modified DNA base methylcytosine to 5-hydroxymethylcytosine, i.e. plays a key role in active demethylation of DNA, which leads to changes in the epigenetic regulation of gene expression during cell differentiation [11]. AGPAT5 encodes an integral membrane protein that converts lysophosphatidic acid to phosphatidic acid, which is a step 2 of de novo phospholipid biosynthesis. This can also contribute to a change in the representation of tumor antigens on the cell surface. The protein encoded by the CDK8 gene is a member of the cyclin-dependent protein kinase family. CDK8 can have an activating or inhibiting effect on the function of transcription factors [18]. CDK8 can act as an oncogene in colorectal cancer (transcription of the CDK8 gene in tumors of this type is significantly increased), caused by over-activation of the Wnt / β-catenin signaling pathway. [7].

Thus, changes in the expression of these microRNAs in CRC can promote epithelial-mesenchymal transition and masking of tumor cells from the immune system, which is also confirmed in other studies [10].

However, it is obvious that data on microRNA-mRNA interactions are insufficient for a complete understanding of the mechanisms of carcinogenesis. In recent years, data have been obtained on complex regulatory interactions (by competitive binding) between lncRNA, microRNA, and the target genome in CRC patients [29, 3, 16, 25].

In our study, 6 lncRNAs interact with more than one differentially expressed microRNA. One of these lncRNAs, MALAT1, has binding sites for miR-143-3p, miR-26a-5p, miR-25-3p, miR-92a-3p, and miR-21-5p. MALAT1 (metastasis associated lung adenocarcinoma transcript 1) is a long spliced non-coding RNA that is involved in the epigenetic modulation of gene expression (in particular, those associated with cell migration) and is closely associated with the development of cancer. [thirty]. Long noncoding RNA XIST (X-inactive specific transcript) has binding sites for 4 microRNAs (let-7i-5p, miR-25-3p, miR-92a-3p, and miR-21-5p). Xist is a key effector in the inactivation of the X chromosome, thereby providing dose equality (ie, the number of active variants of one gene) [12]. Long non-coding RNA NORAD (Non-Coding RNA Activated By DNA Damage) has binding sites for 3 microRNAs (miR-26a-5p, miR-25-3p, and miR-92a-3p) and is associated with cancers such as pancreatic cancer and bladder. Its overexpression reduces the invasive ability of tumor cells, while the rate of apoptosis increases significantly [15]. Long noncoding RNA HELLP, potentially interacting with let-7i-5p and miR-143-3p, activates a large set of genes that are involved in the cell cycle and regulate the invasive ability of cells [27]. Long noncoding RNA TUG1 (taurine upregulated gene 1), potentially interacting with miR-26a-5p and miR-21-5p, plays an import-

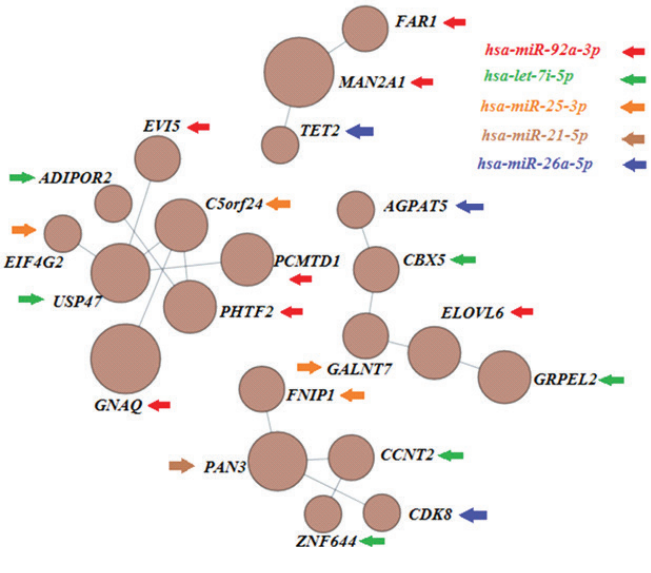

Figure 3. Functional cluster M3 consisting of 21 target genes and 5 microRNAs differentially expressed in CRC

ant role in the epigenetic regulation of transcription, promotes proliferation, and is activated in tumor cells [25].

Currently, the role of the eRNA network (a network of competitively expressed RNAs) consisting of microRNAs (miR-143-3p, miR-26a-5p, miR-25-3p, miR-92a-3p, miR-21-5p and let- 7i-5p), long non-coding RNAs (listed in Table 5), and messenger RNAs encoded by 97 target genes (listed in Table 2), the development of CRC remains completely unclear. And the data obtained in the course of bioinformatic analysis can become the basis for further studies of the complex regulatory network of mRNA-lncRNA-miRNA for a better understanding of the mechanisms of carcinogenesis and identification of new CRC biomarkers.

Conclusion. Thus, as a result of multiple parallel sequencing, 6 differentially expressed microRNAs (hsa-miR-143-3p and hsa-miR-26a-5p, hsa-miR-25-3p, hsa-miR-92a-3p, hsa- miR-21-5p, hsa-let-7i-5p) in the tumor tissue of the colon is relatively normal. For these micro-RNAs, 97 target genes were identified, belonging to 3 functional clusters, and 23 long non-coding RNAs potentially interacting with them. Together they constitute a network of competitively expressed RNAs (keRNA network) characteristic of CRC, which is involved in the implementation of signaling cascades such as the regulation of cell adhesion, activation of the immune response, regulation of the cellular response to hormones and stress, regulation of the Wnt signaling pathway and cell migration, regulation of proliferation and cell cycle, regulation of interaction with viral agents, regulation of apoptosis and response to hypoxia. The data obtained broaden the understanding of the mechanisms of gene regulation in the conditions of this oncopathology and can become the basis for a panel of highly specific tumor markers.

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HUMAN PAPILLOMAVIRUS AS A FACTOR IN THE DEVELOPMENT OF CERVICAL CANCER

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The analysis of cytological samples of 100 women aged 23 to 60 years was carried out by liquid-based cytology. The predominance of NILM in the samples in comparison with intraepithelial lesions of the cervix was noted. Among intraepithelial lesions, low-grade LSIL was significantly more common. Positive HPV tests were found in less than half of the cases in tested women. Among the positive tests of high oncogenic risk the types 16 and 51 were considered as high-risk HPV types. There is approximately equal positivity of the high-risk HPV test in women with NILM and intraepithelial lesions (LSIL, HSIL, ASCUS). The positivity of the high-risk HPV test depends on age: in women under 45 years this indicator is higher than in women 46 years and older.

According to our survey, with cytological study it is necessary to apply integrated test approach when screening high-risk HPV infection. The combined use of liquid-based cytology and HPV testing can improve the effectiveness of diagnostics by reducing the amount of uninformative material and allows to detect pathological changes at an earlier stage. It will significantly increase the value of diagnostic measures and will improve cytologist's experience in diagnosing and giving recommendations in clinical practice of cervical pathologies.

Keywords: human papillomavirus, cervical cancer, diagnostics, liquid-based cytology.

Cervical cancer is in the 2nd place among malignant neoplasms of female reproductive organs and 4th in the incidence structure of cancer among the female population, accounting for about 12% of malignant tumors in women and 60-80% of all forms of genital cancer [7]. Every year, 440 thousand new cases of cervical cancer are detected in the world, which is 5.8% of the total incidence of cancer [4]. Epidemiological and molecular-biological data indicate an important role of HPV in the occurrence of cervical intraepithelial neoplasia (CIN) and cervical cancer (CC) [5]. In the 70s herpes simplex virus serotype 2 (HSV-2) was suspected to be a carcinogenic agent. However, 20 years of experience in studying the role of this virus did not come to a positive conclusion [1]. Possibly, the virus associated with cytomegalovirus, bacteria, and protozoa can act as a co-factor of carcinogenesis, causing the development of dysplasia, and keeping it in a state of stabilization. Numerous papers published in 1980-1990 found that human papillomavirus (HPV) had links with dysplasia and squamous cell carcinoma of the cervix. Hybridization methods showed that 80 to 100% of cervical cancers contained DNA of HPV. A rough correlation was found between the frequency of CC and the detection of HPV in the population. For example, in countries with a high frequency of CC, the detection rate of HPV infection was about 10-20%, while in countries with a low frequency - 5-10% [10].

Characteristics of the aetiopathogenesis of squamous cell carcinoma in distal segments of female genitals are determined by external causes, where the viral infection is the most important factor. The causes of infection occurrence: decreasing the body defenses after infectious diseases, smoking, alcohol, young age, endometriosis, a large number of sexual partners, the presence of partners who had contact with a woman suffering from cervical cancer, anogenital condyloma, repeated abortions, inflammatory diseases, genital infections (chlamydia, trichomoniasis, genital herpes, etc.). The virus is 2 times more common in women who use oral contraceptives than in those who use barrier contraceptives or are postmenopausal [25].

It's been proven that HPV replication at the initial stage leads to an inflammatory reaction in the cervix, and later to the appearance of atypical cells in the thickness of the epithelial layer with varying disorder severity of differentiation, to changes in the layerage of epithelial cells. HPV-associated cervical dysplasia CIN develops [2]. According to C.M. Wheeler (2006) 3 years after HPV infection, one in four women has the progressing CIN II-III [32]. Human papillomavirus is a DNA-containing virus consisting of two structural genes (L1 and L2) encoding capsid proteins, and seven functional genes (E17) involved in HPV genome replication and transcription, cell cycle, signal transmission and control of apoptosis, immune modulation, and structural modification of the infected cell. More than 200 types of HPV are known, and more than 40 of them can affect the mucous membranes of the genitals [17]. HPV is divided into viruses of "high oncogenic risk" (16, 18, 31, 33, 35, 39, 45, 50, 51, 52, 56, 58, 59, 64, 68, 70) and "low oncogenic risk" (3, 6, 11,13, 32, 42, 43, 44, 72, 73). The "high-risk" viruses are more often detected in severe dysplasia, preinvasive and invasive carcinoma. In squamous cell carcinoma, type 16 HPV is most common (in more than 50% of cases), while type 18 HPV is more often associated with adenocarcinoma and low-grade cancer. The "low risk" viruses are identified with pointed and flat condyloma, mild dysplasia, and rarely with invasive cancer [9, 10].

The most dangerous for the development of a precancerous state – cervical epithelial dysplasia – is a long-term infection caused by type 16 HPV. Long-term HPV18 infection leads to the development of cancer in the endocervix. Infection with type 16 papillomavirus is common to find among all types of oncogenic HPV. It is found in more than 20% of women infected with papillomaviruses. As a comparison, HPV18 was found in 7% of the examined women with viral infection [34]. The epidemiological situation is showing the result of the tendency that has been emerging over the past twenty years. It is based on a steady increase in the frequency of infection caused by

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HPV16 with a constant percentage of infection associated with non-oncogenic types of HPV (HPV 6, HPV 11). This is all the more concerning since this infection affects young women aged 20-30-year-old. The incidence of HPV infection is the highest in the age group of 15-19-year-old: about 40% of positive tests indicate the presence of HPV DNA in material taken from the cervix. The percentage of positive results decreases with the increase of the surveyed women age: more than 30% among the 20-24 age group, and less than 30% among the 25-29 age group. The group of women under the age of 30 shows a further decrease in the frequency of HPV infections to 15-17% of the population. Papillomavirus infection is very rare in post-menopausal women but has an important prognostic value due to the risk of developing cervical pathology. Papillomavirus infection is very rare in post-menopausal women but has an important prognostic value due to the risk of developing cervical pathology. As a rule, human papillomavirus infection in postmenopausal women has the character of long-term persistent infection. This is very dangerous in terms of the occurrence of molecular changes leading to the initiation of the carcinogenesis process [35].

The peculiarity of HPV infection is the fact that epitheliotropy of the virus is not detected in the blood, and the production of antibodies by the immune system is not observed in all infected patients [31]. At the same time, the antibodies level is very low and is not able to provide long-term and reliable immunity. It can be explained by the ability of HPV to "escape" from the immune system of the macroorganism, which allows it to persist for a long time due to evolutionary acquired features (the replication cycle is limited by the epithelium, there is no viremia, cytolysis, peritransplant immunosuppression due to viral proteins) [19,40]. The persistence of highly oncogenic HPV types for more than two years is the most dangerous factor in the progression of precancerous cervical cancer. When HPV type 16 persists, the risk of developing cervical intraepithelial neoplasia (CIN) is 40-50%; when HPV type 26 is 30-40%; HPV type 31, 58, 82 is 20-30%; HPV type 18, 33, 35, 51, 52 is 10-20% [32].

The target for papillomavirus exposure is the transformation zone, the area of contact between the multilayer flat and prismatic epithelium of the cervix. It is the place of development of precancerous conditions. It was found that up to 94% of papillomas are located in the transformation zone or distal [14]. Cervical damage caused by HPV, in the form of cervical intraepithelial neoplasia (CIN), can be mild, moderate, or severe. Women with low-grade CIN are at risk for severe CIN and cervical cancer. However, CIN 1 does not always progress to CIN 2-3. The age of women with cervical intraepithelial neoplasia is usually 5-10 years lower than with grade of CIN1, which indicates a gradual progression of changes in the cervical epithelium [41]. Probably the oncogenic types of HPV infection, which affects the cervix, are determining the risk of severe cervical intraepithelial neoplasia in women [29]. The frequency of detection of HPV DNA increases as the degree of dysplasia worsens and the transition of dysplastic epithelium changes into the tumor. HPV DNA in CIN 2 is detected 24-25%, in CIN 3 - 61-80%, and in microinvasive carcinoma - 83-88% [28, 30]. In invasive carcinoma and carcinoma in situ, HPV DNA was detected in more than 90% of cases. Papillomaviruses are known to belong to the family Papovaviridae. The genome is a double-stranded circular DNA, and reproduction occurs in the nucleus of host cells. The human papillomavirus, like many DNA viruses (herpes simplex, Epstein-Barr virus, etc.), is a biological agent that can modify the growth, differentiation, and morphology of cells. When a virus enters cell, it causes changes in its structural, biochemical, and genetic organization, and introduces foreign genetic information into it. The formation of binuclear and polynuclear cells is a characteristic feature of the cytodestructive action of the virus. The formation of binuclear cells is usually based on the fusion mechanism of plasma membranes of two cells; a number of viruses contain enzymes that can lyse cell membranes [29]. The formation of intranuclear and cytoplasmic inclusions, vacuoles is the main characteristic of many DNA viruses. It is possible that the mechanism of vacuolated cell formation is based on the water and energy metabolism process of cells. The formation dynamics of inclusions, their shape, size, and content of nucleic acids and proteins in them are of great diagnostic importance since they differ in various groups of viruses. The cytoplasm of cells accumulates oxyphilic masses containing viral protein and RNA, numerous viral particles that form vacuoles. Chromosome pulverization, karyolysis, karyorrhexis, pyknosis develop in the nuclei of cells; nuclei move to the periphery. Under the cytopathogenic action of viruses, cell destruction is directly or indirectly related to the penetration of the virus genome and its functioning. The resulting changes in cell morphology may be the result of one or more factors: inhibition of the synthesis of cellular DNA, RNA, and proteins [43]. Infection with the virus is a necessary but insufficient condition for the development of malignant neoplasms. Along with this, there are additional factors, including genetic ones, involved in the transformation of normal cells [31,37,39]. Two genes present in the HPV genome cause malignant cell transformation [18]. These viral oncogenes are named E6 and E7. The E6 and E7 genes are multifunctional. The ability to activate cellular telomerase is the one of the mechanisms of their action. Telomerase is an important enzyme for maintaining the stability of chromosome telomeres during multiple cycles of cellular proliferation. Increased telomerase levels are observed in severe intraepithelial injuries [13, 27].

Another point of influence of the E6 and E7 genes is the suppressor proteins p53 and Rb (retinoblastoma gene), which induce apoptosis. The effect of the virus is manifested in the inactivation of suppressor proteins and inhibition of apoptosis. Increased expression of the p53 gene in CIN I, compared with more marked injuries, indicates its protective role in the process of carcinogenesis and may be one of the diagnostic criteria for damage assessment and disease progression [38,44] However, the expression of E6, E7 genes is not enough to cause not only proliferation but also the transformation of cells. The "High-risk" HPV invades the host cell to induce chromosomal aberrations and gene mutations. The accumulation of cell mutations with a latently persistent virus subsequently serves as an endogenous factor in the progression of tumor cells [23].

Cervical carcinogenesis initiated by human papillomaviruses can be divided into several stages [4]: 1) primary infection with the virus; 2) persistence of the human papillomavirus genome in the episomal form and the capability of viral particles production with subsequent secondary infection; 3) integration of viral DNA into the cell genome without visible specificity of the integration site; at II and III stages, the functions of E6 and E7 begin to manifest, violating the control of cell division; 4) induction of mutations in cell DNA, causing instability of the cell genome; 5) selection of a clone of cells with mutant DNA containing integrated DNA of human papillomaviruses; 6) active reproduction of this clone of cells and tumor growth. This mechanism explains the fact that from the moment of viral infection to the appearance of a tumor takes a long time – 5-10 years.

Diagnostics of HPV-associated diseases of the cervix should be complex and based on several studies: detection of HPV DNA using a real-time polymerase chain reaction with typing and determination of the number of genomic equivalents of the virus; enzyme immunoassay (ELISA) - an immunological method for the qualitative or quantitative determination of E7 HPV 16 and 18 type cancer proteins based on a specific antigen-antibody reaction; cytological examination (traditional or liquid-based); expression assessment of P16/Ki67 oncoproteins by immunocytochemical (ICC) and immunohistochemical (IHC) methods; extended colposcopy; histological examination of a cervical biopsy [3].

The main methods for diagnosing HPV include polymerase chain reaction (PCR), which allows genotyping and quantification of 21 type of HPV. The method is based on an amplification reaction, during which primer molecules bind to fragments of DNA virus in a sample and in with the addition of catalysts, and under the influence of temperature, daughter complementary double-stranded DNA molecules are formed [12]. According to several researchers, high-grade dysplasia and CC determination needs to conduct a cytological study in combination with HPV testing, the sensitivity reaches up to 96% [33Confirmation of the etiological role of human papillomavirus (HPV) in the development of CC has now led to HPV testing being considered as a possible component of screening for this disease. It is important to separate HPV infection and HPV-associated disease (precancer) and to determine the risk of its progression. Практически любые методы выявления ДНК ВПЧ обладают 95–100 %-ной диагностической чувствительностью по отношению к тяжелым дисплазиям и раку шейки матки [6]. Almost all methods for detecting HPV DNA have a 95-100% diagnostic sensitivity to severe dysplasia and cervical cancer [6]. But the qualitative determination of HPV DNA has controversial clinical significance, since it does not allow predicting the course of infection. It is considered that a negative HPV test means the patient has a low risk of developing cervical cancer in the next 3 years. However, if the HPV test is positive, this does not mean that the patient has a high risk of developing cervical cancer. Primary cytological examination of the cervix and cervical canal is a classic screening method for detecting changes in the cervical epithelium [8, 11, 22]. Despite the exceptional value of cytological examination of cervical smears for the preinvasive form diagnosis of the tumor process, the frequency of false-negative results of the test can reach 50% in the invasive form diagnosis of CC [10, 24, 26, 36, 45].

In recent years, the method of liquid-based cytology (LBC) has been actively introduced in addition to traditional cytology, which contributes to ensuring the quality of sampling, storage, and transportation of material [12]. An important technological feature of the LBC method that improves the quality of the study is that the test material is placed in a special stabilizing solution during standardized sampling, which ensures its safety without changing its morphological and immunocytochemical properties. According to generalized data, the sensitivity of the traditional method was less than 60%, and the LBC method was 95 %. The specificity of the methods was 40 % and 66%, respectively. The correlation with the results of the histological examination does not exceed 60 % by the traditional cytological method; it is close to 100 % (99 %) by the LBC method [20]. The authors of the research concluded that the LBC method is a more reliable laboratory test: reduces the number of false negative results, reduces the number of unsatisfactory drugs for analysis, and reduces the time required for a cytologist to evaluate cell material. In addition, the same sample taken for LBC can be used for detection of HPV of high oncogenic risk and determination of ICC using CIN cancer markers [4].

In recent years, one of the most promising methods for the diagnosis of HPV-associated cervical lesions is immunocytochemical analysis. The determination of proliferation markers P16 and Ki-67 expression can determine not only the presence of viral proteins in the cell but also the degree of violations of cellular regulation in response to the persistence of the virus in the cell. The atypical squamous cells cervix expressing both P16 and Ki-67 indicates induced cell cycle dysregulation and provides a more accurate diagnosis of high-severity lesions. The first studies on the determination of P16 and Ki-67 showed high specificity of the method [12]. The combined use of LBC and immunocytochemical analysis of the tumor marker p16ipk4a in the screening of CC will allow detecting tumors at the early stages of development, and at the stages preceding it when the curability of this disease is close to 100% [16].

An additional examination method of patients with cervical pathology is extended colposcopy. According to S. I. Rogovskaya, the sensitivity of colposcopy for determining subclinical human papillomavirus infection, precancerous cancer, and breast cancer is 80-90%, and the specificity is 30-60% [15]. The advantages of this method include the possibility of targeted biopsy from the most suspicious areas of the cervix for further histological examination. However, it is known that even if the diagnosis of dysplasia is morphologically verified, the probability of dysplasia turning into cancer is less than 50 % [42].

Therefore, confirmation of the causation of HPV in cervical cancer development has led to the fact that the diagnosis of papillomavirus infection, along with cytological studies, has become considered an essential element of screening and prevention of this disease. At the present stage of the occurrence of cervical cancer, it is safe to assume that long-term infection with HPV 16 and 18 is the most important risk factor for the development of this tumor. The success of new diagnostic methods will lead to early detection of the atypical transformation of cervical epithelial cells, and risk assessment factors for tumor transformation. Modern methods of the diagnosis and treatment of papillomavirus infection would open up new opportunities for understanding and controlling malignant pathology of the reproductive system in women caused by papillomavirus infection.

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MUTATIONS IN BRCA1/2 GENES IN PATIENTS OF SOUTHERN RUSSIA WITH MALIGNANT OVARIAN TUMORS

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The article presents the results of a study of the spectrum of mutations in the BRCA1 / 2 genes associated with the development of hereditary breast and ovarian cancer in patients of South Russia with malignant ovarian neoplasms.

Mutations in the BRCA1 gene were determined by real-time PCR: 185delAG, 300T> C, 2080delA, 4153delA, 5382insC, 3819delGTAAA, 3875delGTCT; in the BRCA2 gene - 6174delT in 178 patients with a histologically verified diagnosis of ovarian cancer.

The study included epithelial tumors (malignant) - 98.1%, and granulosa cell tumors - 1.9%. Of the epithelial tumors, the most common was high-grade serous carcinoma (78%). Based on the results of genotyping, the prevalence of germline mutations in the BRCA1 / 2 genes was revealed at 20.8%. The higher rate of genetic changes is obviously associated with hereditary history (40% of patients). Of the seven identified mutations, 5382insC (67.6%) was revealed more frequently. All patients confirmed the same mutation in the tumor. There were no cases of somatic changes in BRCA1 / 2. The prevalence of BRCA1 mutations was noted in the group of patients with low-grade serous carcinoma, in which all cases of mutations in the BRCA2 gene were identified.

Thus, in patients with OC living in the south of Russia, the mutation frequency in the BRCA1 / 2 genes was 20.8%. The distribution of mutation types with predominance of 5382insC BRCA1 (67.6%) corresponds to the ratio of their occurrence in populations of European countries. BRCA1 / 2 mutations were recorded more frequently in the group of patients with high-grade serous carcinoma.

Keywords: ovarian cancer, mutations, BRCA1 / 2, PCR.

Introduction. Ovarian cancer (OC) is a malignant tumor and the first leading cause of death among gynecologic cancers due to a large number of patients with advanced disease characterized by metastases into the abdominal cavity. About 10-15% of ovarian tumors are associated with hereditary diseases, and about 65-85% of patients with hereditary ovarian carcinomas have a mutation in the BRCA1 and BRCA2 genes involved in DNA repairing and genomic stability maintenance [16]. The lifetime risk of OC developing in women with mutations in the BRCA1 or BRCA2 gene is 35-70% and 10-30%, respectively [16]. The total lifetime risk of developing breast cancer and OC increases to 85% and 60%, respectively, in carriers of BRCA1 and BRCA2 mutations [8]. Hereditary OC was reported to be associated with the presence of gene aberrations related to other hereditary syndromes (TP53 mutations in Li-Fraumeni syndrome, mismatch repair genes (MMR) in Lynch syndrome, double-strand break repair genes BARD1, CHEK2, RAD51, and PALB2) [17].

Aim of the study. The aim of the study was to analyze the spectrum of mutations in the BRCA1/2 genes associated with the development of hereditary breast cancer and ovarian cancer in patients with ovarian cancer in the South of Russia.

Material and methods. The study included 178 women aged 27-71 years old diagnosed with ovarian cancer T1-4N0-1M0-1, gr. 2 (stage I-IV), who received treatment in National Medical Research Centre for Oncology in 2015-2019. The survey revealed one or more risk factors in 71 cases: the age of onset up to 45 years, multiple tumors, bilateral lesions, and hereditary burden. All patients gave their informed consent to the processing of their personal and medical data, as well as to the use of biological material. EDTA venous blood and paraffin-embedded ovarian tissue blocks were studied. Genomic DNA was isolated from whole blood leukocytes and tumor samples using the DNA-sorb-B kit (AmpliSens, Russia). For DNA isolation from paraffin blocks (tissue samples fixed in 10% buffered formalin), 5-8 slices 3 μm thick were obtained using a microtome, dewaxed with o-xylene and 95% ethyl alcohol, lysed overnight in 200 μl of lysis solution with the addition of 20 μl of

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proteinase K (10 mg/ml) at 58°C, then heated at 90°C for 1 hour to eliminate DNA-protein cross-links, and processed using the DNA-sorb-B kit according to the instructions. Mutations were detected by real-time PCR using the OncoGenetics BRCA kit (DNA-Technology, Russia). Mutations were detected in all patients in the BRCA1 gene: 185delAG, 300T>C, 2080delA, 4153delA, 5382insC, 3819delGTAAA, 3875delGTCT; in BRCA2 – 6174delT. The registration and interpretation of the reaction results was performed automatically using the software supplied with the detecting amplifier DTprime 5M1 (OOO NPO DNA-Technology, Russia).

Statistical analysis of the results was performed using applied statistical programs Microsoft Excel 2013 (Microsoft Corporation, USA) and STATISTICA 8.0 (Stat Soft Inc., USA). Differences were evaluated with the nonparametric Mann-Whitney U-test; the frequencies of characteristics were compared using the χ^2 test.

Results and discussion. The data on genotyping of DNA extracted from the blood and tumor tissue were obtained for all 178 patients. Differences between the mutation statuses in the blood and tumor material were not registered. Table 1 presents the distribution of BRCA1/2 mutations among patients of various age groups.

Analysis of OC patients' age demonstrated that the mean age of the disease onset in wild-type BRCA1/2 was 52.4 years, for the mutant type – 54 years. As a result, no differences were found in the age of onset between two groups depending on the status of the BRCA1/2 gene (U=-0.133 at p=0.894). However, distribution of patients with different mutation statuses by age groups revealed statistically significant differences (χ^2=18.47 at p<0.01). Mutations were most frequent in patients aged 45-64 years old, while in elder women (>65 years) wild-type BRCA1/2 prevailed (Table 1).

Mutations in the BRCA1/2 genes were found in 37 (20.8%) OC patients. Germline mutations in the BRCA1 gene were detected in 35 women, in BRCA2 – in 2 women. Six out of eight studied mutation types were found in OC patients (Table 2). BRCA1 5382insC mutation was the most frequent (67.6%).

OC is a heterogenic disease with morphological as well as molecular genetic features. Most OC types are classified as epithelial (90%), with histological subtypes such as serous (68-71%), endometrioid (9-11%), clear cell (12-13%), mucinous (3%), malignant Brenner tumors (1 %) and mixed carcinomas (6%) [12]. Depending on the genetic profile, serous carcinomas are divided into clearly distinguishable serous carcinomas with low malignant potential including lesions associated with borderline ovarian tumors and G1 adenocarcinomas (genetic type I), and barely distinguishable serous carcinomas with high malignant potential including G2 and G3 tumors (genetic type II) [1, 2].

In this study, epithelial (malignant) tumors were histologically verified in 98.1% cases, and adult granulosa cell tumors – in 1.9%. Barely distinguishable serous carcinoma was the most frequent epithelial tumor (78%).

The main proportion of mutations in the BRCA1 gene was observed in the largest group of high-grade serous carcinomas (67.6%), and mutations in the BRCA2 gene were identified only in this group (Table 2). However, no statistically significant differences in mutational status were found between different histological subtypes (Table 3).

The BRCA1/2 genes play an important role in maintaining DNA integrity by participating in the repair of double-strand breaks by homologous recombination, which, when disrupted, is responsible for the accumulation of genomic changes and eventual genomic instability [4]. The identification of pathogenic mutations allowed determining the mechanisms of the pathology development, as well as predicting the risks for carriers of germline mutations. While the general population risk of ovarian cancer averages 1-2%, it increases in families with mutations identified in these genes to 39-63% for BRCA1 and 16-27% for BRCA2 [8].

In this study, mutations in the BRCA1/2 genes were detected in 20.8% of OC patients. An increased rate of genetic changes was apparently associated with a large number of patients with hereditary burden (40%). All people with pathogenic BRCA1/2 mutations were referred for a genetic counseling to form the groups with high risks of breast cancer, OC and other tumors, including pancreatic [14,

Table1

Status of the *BRCA1/2* genes in various age groups of OC patients

Age group*	Wild type (%)	Mutant type (%)
<34	11.5	0
35-44	19.2	11.1
45-54	15.4	44.4
55-64	23.1	29.6
>65	30.8	14.8
χ^2 (p)	18.47 (<0.01)	

Note. *Here and in Tables 2 and 3: the age at initial diagnosis is given.

Table2

Rates of genotypes with a mutant allele of the *BRCA1/2* genes in patients with different histological types of OC.

Studied mutations		Barely distinguishable serous carcinoma (%)	Clearly distinguishable serous carcinoma (%)	Mixed epithelial tumors (%)
BRCA1	185delAG	2.7 (1/37)	0	0
	4153delA	13.5 (5/37)	0	0
	5382insC	41.1 (15/37)	13.5 (5/37)	13.5 (5/37)
	3819delGTAAA	0	0	0
	3875delGTCT	0	0	0
	300T>C	2.7 (1/37)	0	0
	2080delA	8.1 (3/37)	0	0
BRCA2	6174delT	5.4 (2/37)	0	0

Table3

Status of the BRCA1/2 genes in different histological types of OC

Histological subtype of OC	Wild type (%)	Mutant type (%)
Barely distinguishable serous carcinoma	50.6	15.2
Clearly distinguishable serous carcinoma	15.2	2.8
Mucinous tumors	4.5	0
Endometrioid tumors	6.2	0
Mixed epithelial tumors	1.7	2.8
Adult granulosa cell tumor	1.1	0
Total	79.2	20.8
χ^2 (p)	9.377 (>0.05)	

6] and prostate [11] cancer. 2 patients (5.4%) developed other tumors during clinical observation.

Results of BRCA genetic tests are important for OC patients, for example, as a prognostic biomarker of response to specific antitumor therapy [5]. Studies have shown that patients with a positive test for pathogenic BRCA1/2 variants demonstrate higher sensitivity to combination therapy with platinum derivatives, and higher sensitivity to treatment with PARP inhibitors compared to patients without such variants [5]. Inability to repair chemotherapy-induced DNA damage results in significantly better prognosis in BRCA-positive patients with progressing disease compared to wild-type patients. BRCA-positive OC patients are at risk of developing secondary cancers. BRCA-positive test in patients with OC is also important for assessing the risk of cancer development and its prevention among relatives [5].

Recent studies showed that the prevalence of hereditary pathogenic variants and assessment of gene-specific risk may vary based on family history and type/molecular subtype of tumors, as well as on the race, ethnicity and geographic place of residence [3, 7, 13]. In this study, rates of the BRCA1 gene mutations (Table 2) generally corresponded to that for European countries, and the most common mutation 5382insC occurred with the same frequency as in the previously published data (67.6% vs 68.3%) [10].

Conclusion. Thus, the study demonstrated that the frequency of mutations in the BRCA1/2 genes in OC patients living in the South of Russia was 20.8%. The distribution of mutation types with a predominance of 5382insC BRCA1 (67.6%) corresponded to their rates in populations of European countries. Carriers of mutations in the BRCA1/2 genes were more often observed in the group of patients aged 45-64 years old with barely distinguishable serous carcinoma.

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ARCTIC MEDICINE

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CHANGES IN IMMUNE PARAMETERS AND LYMPHOCYTE ATP LEVEL OF PERIPHERAL BLOOD IN RESIDENTS OF THE NORTH DURING SHORT-TERM COLD EPXPOSURE TO THE BODY

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The immune system constantly responds to various environmental stimuli. The aim of the study was to investigate immune effects of short-term cold exposure taking into account ATP level in peripheral blood lymphocytes in healthy residents of the North. So, the total number of lymphocytes and their phenotypes, as well as the content of cytokines and the concentration of ATP in the lymphocytes were determined in 38 volunteers twice (before and after their short-term stay for 5 minutes in a cold chamber at t = -25°C). Cluster analysis revealed two statistically different groups. The first group with an initially higher ATP level in lymphocytes responded to hypothermia by lower ATP concentration with the unchanging total number of lymphocytes as well as by a decrease in predominantly $CD16^+$ killer-cells. The other group reacted by an increase in ATP concentration with a decrease in the number of lymphocytes and by a pronounced decrease in $CD4^+$ helper-cells and in $CD71^+$ cells with a transferrin receptor. Also, the proinflammatory cytokines TNFα and IL-6 increased in the first group, while the second group showed a decrease in the level of lymphocyte-activating cytokine IL-1β. It can be assumed that the response to hypothermia in the first group is provided through the CIRP-NFkB-TNFα axis and leads to an increase in the risk of non-infectious inflammation. For the second group, a protective mechanism is triggered to restrain lymphocyte activity and the development of T-cell-mediated inflammation through regulation by means of T-effector and T-regulatory cells AMPK balance, autophagy, mitophagy and mitochondrial biogenesis. The study of the immune response to hypothermia is important for understanding the cellular mechanisms of adaptation as well as for the search of targets to correct the immune response.

Keywords: hypothermia, adaptation, T-cells, signaling mechanisms, ATP.

Introduction. A key mechanism of adaptive process is a rapid initiation of transient reactions responsible for homeostasis regulation, that is required to the body to adapt for continuously changing environment [10]. The immune system significantly impacts on the success of the body's adaptation to environmental factors and immune system responses to non-immunological stimuli continue to be comprehensively studied. Lymphocyte metabolism has sufficient plasticity to ensure demands of T cells for energy and biosynthesis, which considerably vary depending on their state and function. Intracellular metabolic regulators, in particular AMP-activated protein kinase (AMPK) being an energy sensor of the cell, play a key role in adaptation processes. AMPK is responsible for cell energy balance maintenance, by activating metabolic pathways of ATP production through oxidative phosphorylation (OXPHOX) and by inhibiting energy-consuming biosynthetic processes [7]. AMPK stimulates autophagia by means of positive regulation of ULK1 (unc-51-like autophagy-activating kinase 1) and formation of autophagosome complexes [2]. AMPK also facilitates mitophagy by triggering mitochondrial sorting through activation of MFF (mitochondrial fission factor) [11]. TRP-family receptors (transient receptor potential), being highly sensitive to environmental changes, play an important role in metabolic rearrangement of T cells [3]. TRP receptors (in particular TRPV3,4 and TRPM8) in addition to their main ion-channel function facilitate activation of cold-inducible proteins (CIPs), that protect cells during cold, hypoxia and other types of stress [8]. The study of immune responses to cold exposure is of prime importance for better understanding of adaptation mechanisms to the North environment and for developing ways to increase the body's resistance to low temperatures.

The aim of the study was to investigate short-term cold exposure effect on immune system taking into consideration ATP level in peripheral blood lymphocytes in healthy residents of the North.

Materials and methods 38 volunteers, residents of the Arkhangelsk city of both sexes, were examined. The average age was 35 (8.7) years. All volunteers gave their consent to participate and were informed about the results of the study in accordance with the requirements of the Declaration of Helsinki (the World Medical Association) on the ethical principles of medical research (2000).

Venous blood was collected twice, before and after the volunteers underwent short-term cold exposure in a cold chamber (time duration – 5 min; temperature – minus 25 ˚C). In both samples, total number of lymphocytes and their individual phenotypes were evaluated. The content of cytokines and adenosine triphosphate (ATP) in lymphocytes were also measured.

The complex of immunological studies included isolation of lymphocytic fraction from the peripheral blood followed by identification of lymphocyte phenotypes ($CD3^+$, $CD4^+$, $CD8^+$, $CD10^+$, $CD16^+$, $CD71^+$, $CD23^+$, $CD25^+$, $CD95^+$, HLA DR) by indirect immuno-peroxidase reaction (reagents of Sorbent LLC, Russia). The concentration of cytokines (IL-1β, IL-6, TNF-α, IL-10) in blood serum was measured by ELISA assay on an automatic analyzer "Evolis" "Bio-RAD" (Germany). ATP concentration in lymphocytes was

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measured on luminometer by bioluminescence using luciferin-luciferase reaction (reagents Lumtek LLC (Russia)).

Statistical analysis was performed using «Statistica 10.0» software («StatSoft», USA). In the multivariate exploratory analysis module, the data was clustered using the "K means" method. In the descriptive statistics module, mean values (M) and standard deviation (SD) were calculated; the Kolmogorov-Smirnov and Lilliefors test for normality was used to check the data for normality of distribution. When the distribution was close to normal, the Student's t-test was used to compare the results of the samples, the differences were considered significant at p<0,05.

Results. A statistical analysis of the obtained data using descriptive statistics and clustering by the "K-means" method helped us to reveal two groups. These groups were statistically different in lymphocyte ATP level and in the most of the determined parameters, which changed ambiguously after cold exposure (table 1).

The data in the table shows that after a cold exposure in the first cluster group, the ATP level in lymphocytes significantly decreased, while their total number in peripheral blood did not change. Concentration of helper-cells ($CD4^+$), killer-cells ($CD16^+$) and cells with IL-2 receptor ($CD25^+$) significantly reduced, however, the content of the other determined phenotypes remained at the same level. In the second cluster group concentration of ATP after a cold exposure, on the contrary, increased against significant decrease in both the total number of lymphocytes and all determined phenotypes. Changes in the total number of lymphocytes and the level of ATP in lymphocytes during cold exposure is shown in the diagram (Fig. 1)

In the plasma cytokine profile of the first group, the background concentration of the pro-inflammatory cytokines IL-6 and TNF-α significantly increased relative to their values before cold exposure. In the second group, the concentration of the lymphocyte-activating factor IL-1β decreased, and the level of the other cytokines did not change.

The calculation of relative content of lymphocyte phenotypes within the groups showed that the proportion of the majority of phenotypes in the groups was equal. However, in the first cluster group, compared to the second cluster group, the content of $CD16^+$ and $CD25^+$ cells were higher on 4.6 and 2.4% respectively and the content of $CD71^+$ cells with a transferrin receptor was lower on 2.5%. After cold exposure some changes were observed in the content of lymphocytes, which had their own characteristics for each group. In the first group, the content of $CD16^+$ killer-cells, $CD4^+$ helper-cells, and activated $CD25^+$ cells with IL-2 receptor predominantly decreased by 5.8, 3.9, and 3%, respectively (Fig. 2). In the second group, the proportion of $CD4^+$ cells decreased significantly (by 11.2%), the content of $CD8^+$ cytotoxic cells and $CD71^+$ cells with a transferrin receptor decreased by 4.4 and 4.7%, respectively (Fig. 2). For the other types of cells, the differences were smaller both within each group and between groups.

Discussion of results. An adaptive reaction to cold exposure leads to the induction of the so-called cold shock proteins. As soon as the body is exposed to low temperatures, these specific proteins immediately react to allow cells to quickly adapt to environmental conditions. One of them is the cold-inducible RNA binding protein (CIRP) which activity is enhanced by hypothermia and, in addition, it promotes translation of some specific mRNAs [17]. It is estimated that at low temperatures, regardless of the global suppression of protein expression, transcription of CIRP mRNA is significantly increased due to the CIRP-mediated transcriptional activation of alternative promoters [1]. Enhanced expression of CIRP leads to its accumulation in the cytoplasm, where CIRP exerts its cytoprotective effect: it increases the activation of anti-apoptotic proteins Bcl-2 and Bcl-x1 through activation of the MAPK / ERK1/2 pathway and nuclear factor NF-kB [5] and inhibits caspase cascade through suppression of Bax and Bad pro-apoptotic factors [9]. In addition, CIRP promotes

Changes of measured parameters in groups before and after cold exposure

Parameters	before cold exposure			after cold exposure			
	cluster 1 (N=11)	cluster 2 (N=27)	Signif. level P	cluster 1 (N=11)	Signif. level P*	cluster 2 (N=27)	Signif. level P**
ATP μmol /106 cells	3.81 (1.389)	0.77 (0.563)	0.00002	2.59 (1.415)	0.0272	1.58 (1.544)	0.0071
Lymph×106 cells/ml	1.06 (0.344)	1.81 (0.780)	0.0002	0.94 (0.260)	0.187	1.34 (0.503)	0.0087
CD4 ×106 cells/ml	0.21 (0.085)	0.34 (0.176)	0.0052	0.15 (0.080)	0.0436	0.10 (0.087)	0.0002
CD8 ×106 cells/ml	0.20 (0.102)	0.31 (0.149)	0.0095	0.15 (0.077)	0.119	0.17 (0.073)	0.0001
CD10 ×106 cells/ml	0.20 (0.067)	0.32 (0.123)	0.0008	0.15 (0.089)	0.094	0.20 (0.093)	0.0004
CD95 ×106 cells/ml	0.15 (0.058)	0.27 (0.103)	0.0007	0.12 (0.051)	0.142	0.17 (0.083)	0.0011
CD16 ×106 cells/ml	0.23 (0.105)	0.31 (0.144)	0.058	0.15 (0.065)	0.0257	0.19 (0.093)	0.0008
CD23 ×106 cells/ml	0.18 (0.058)	0.32 (0.131)	0.0004	0.17 (0.113)	0.372	0.22 (0.107)	0.0069
CD25 ×106 cells/ ml	0.20 (0.061)	0.30 (0.142)	0.0072	0.15 (0.061)	0.0415	0.21 (0.104)	0.0143
CD71 ×106 cells/ml	0.19 (0.058)	0.37 (0.163)	0.00002	0.15 (0.072)	0.083	0.21 (0.091)	0.00003
HLAD×106 cells/ml	0.21 (0.081)	0.32 (0.153)	0.0073	0.18 (0.093)	0.267	0.23 (0.114)	0.0205
IL-1β pcg/ml	5.34 (0.497)	5.88 (0.876)	0.0250	5.36 (0.303)	0.454	5.40 (0.713)	0.0163
IL-6 pcg/ml	0.57 (0.416)	1.18 (0.877)	0.0081	1.41 (0.563)	0.0005	1.28 (1.141)	0.365
IL-10 pcg/ml	3.04 (1.549)	4.20 (3.159)	0.139	2.90 (2.128)	0.434	3.57 (2.852)	0.225
TNF-α pcg/ml	1.20 (0.867)	2.11 (1.544)	0.0398	2.18 (1.209)	0.0287	2.49 (2.535)	0.279

- significance level relative to values before cold exposure of *- cluster 1,**- cluster 2.

the activation of antioxidant defense, reducing the negative effects of reactive oxygen forms, production of which increases under conditions of cold stress [16]. At the same time, getting into the circulation by lysosomal secretion, CIRP manifests itself as a Danger-associated molecular pattern (DAMP) in a non-cellular environment. By interacting with TLR4 receptors and leading, through stimulation of NF-kB, to the production of pro-inflammatory cytokines CIRP is capable of initiating a non-infectious inflammatory response [4,16]. Obtained results showed an increase in the content of pro-inflammatory cytokines (TNF-α and IL-6) in the blood of the first group of volunteers.

Lymphocytes need a certain balance of production and energy consumption associated with their current state, differentiation and functioning. The fluctuations of the relative content of lymphocyte phenotypes in groups with different background levels of ATP and its change in response to cold are noteworthy. In the first cluster group, where initial ATP level of lymphocytes was high, the significant decrease was in content of $CD16^+$ killer-cells with a decrease in the concentration of ATP in lymphocytes. While in the second cluster group (with an initially low ATP level) there was a sharp decrease in the content of $CD4^+$ helper-cells with an increase in lymphocytes ATP concentration. AMPK plays an important role in T-cell metabolism, affects T-cell development and fate, and has both positive and negative effects in relation to growth, differentiation and functions of T-cells [15]. Suppressing the activity of mTOR complex 1 and acetyl-coenzyme A carboxylase, AMPK inhibits energy-consuming syntheses of proteins and fatty acids, reducing the growth and functioning of T-effector cells [14]. On the other hand, by stimulating β-oxidation of fatty acids and OXPHOS in $CD4^+$ cells, AMPK directs cell differentiation from Th17 to Treg cells [6], influences T-cell-mediated inflammation through a change in the balance of T-effector and T-regulatory cells [13]. AMRK also stimulates autophagy (through activation of ULK1) [2], mitophagy (through the induction of mitochondrial factor MFF) [11], mitochondrial biogenesis (via direct and NAD^+-mediated SIRT1 regulation of the mitochondrial modulator PGC-1α) [4], and thus, contributes to the increase in ATP production in cells. An increase in ATP level in lymphocytes was observed in the second group.

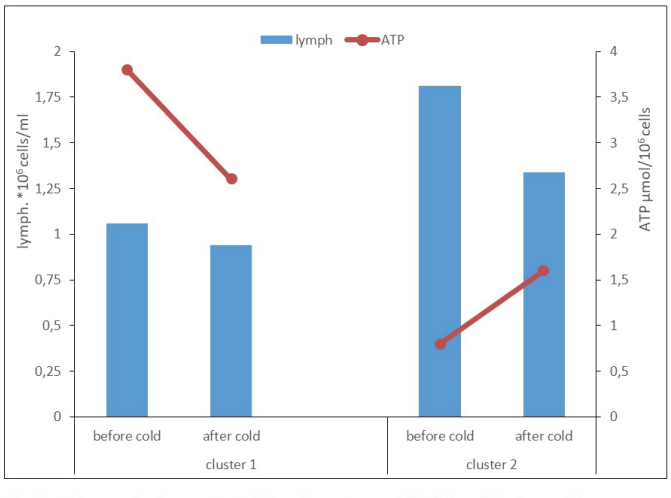

Fig. 1. Changes in the content of lymphocytes and ATP level during cold exposure.

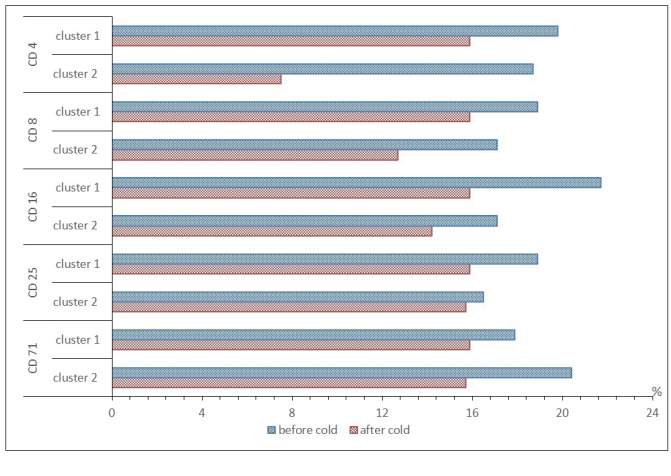

Fig. 2. Changes in content of individual lymphocyte phenotypes in cluster groups.

Conclusion. The immune response on influence of low temperature is expressed in quantitative changes of T cells, their phenotypes and cytokines and is associated with intracellular ATP level. The results show that after cold exposure in the group with an initially higher level of ATP, the levels of pro-inflammatory cytokines (IL-6 and TNF-α) increase. Also, the content of mainly $CD16^+$ killer-cells decreases and the concentration of ATP in lymphocytes decreases while the total number of lymphocytes in the peripheral blood does not change. In the group with lower ATP values, the level of lymphocyte-activating IL-1β decreases, the content of $CD4^+$ helper-cells and activated $CD71^+$ cells with a transferrin receptor decreases significantly. At the same time, the concentration of ATP in lymphocytes increases with a decrease in the total number of lymphocytes in the blood. These differences may be due to the response associated with the energy status of cells, metabolic pathway activity, and transduction signals. It can be assumed that for the first group, the action of cold leads to the production of pro-inflammatory cytokines through the axis of TRP - CIRP - NF-kB - TNF-α and a decrease in the lymphocytes ATP level. For the second group, it is possible that a mechanism of restraining lymphocytes activity and T-cell-mediated inflammation is triggered through AMPK regulation presenting an increase in ATP concentration accompanied by the decrease in the total number of lymphocytes and their phenotypes.

A more detailed study of the T-cell response to low temperature exposure can provide a better understanding of adaptation mechanisms and detection of targets for specific correction of possible immune disorders in people living in the North.

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AGE-RELATED FEATURES OF THE ACTIVITY OF LYMPHOCYTE ENZYMES AND THEIR INTERCONNECTION IN CHILDREN OF THE FAR NORTH

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The research was conducted to study activity indicators of lymphocyte enzymes in 99 healthy children aged 3 to 15 years living in town Tynda in the summer season. The increase of enzymes activity in children's lymphocytes was noted as they mature. Differences in indicators of enzyme activity were manifested in a lower level of dehydrogenases in the group of 3-year-old children, higher acid phosphatase, and lower glycerol-3-phosphate dehydrogenase in all age groups. Some features of age-related dynamics of the correlation relationships of the studied indicators are noted and periods of greatest adaptive tension in children are determined.

Keywords: North, children, lymphocytes, enzymes, correlation

Introduction. The regions of the Far North and equivalent areas cover more than 64% of the territory of Russia [11]. Issues of human full life activity and protection of his health in extreme climatic conditions of the North are far from being resolved. The health of the population, especially children's is under the constant influence of changeable parameters of the climate system, which often leads to the formation of various pathologies.

The city Tynda, in which the studies were conducted, is located in the most northern city of the Amur Region and, according to a number of climatic and geographical features, is assigned to areas equated to the North.

For assessing a degree of impact of the extreme environment per person, the characteristics of homeostatic systems reflecting the adaptive capabilities of the organism should be included. Blood cells are considered as components of the immune system involved in adaptive reactions [4].

Lymphocytes are the main morphological substrate of the immune system. And the regulation of the immune response is determined by functional capabilities of these cells, which are based on intracellular metabolic reactions. The latter ones, to a large extent, are provided by a certain level of intracellular enzymes activity [2,16].

The level of functioning of these cells is supported by the mechanisms of neuroendocrine regulation, in the complex interaction of intracellular relationships, wherein the variability of the level of these connections is the most important reserve of target results effects on the organism [3].

The urgency of the problem lies in the fact that children's organism is labile to the effects of the environment and its climatic features. Children of preschool and school age are characterized, on the one hand, by intensive growth and development rates, and, on the other hand, by insufficiently high resistance to adverse factors during this period of ontogenesis.

We have chosen the metabolic parameters of blood lymphocytes and the correlation between them as integral indicators of the degree of environmental impact on the organism. A change in the correlations between the physiological parameters of the organism under the influence of various systems of adverse environmental factors has been repeatedly proven in adult populations [3, 5, 10].

Studies dedicated to the study on the characteristics of the activity of enzymes of lymphocytes, taking into account their relationships in children in the North, are few and poor in content.

Aim of the study: to reveal the features of age-related dynamics of the activity of lymphocyte enzymes and their relationships in children of the alien population of the North.

Material and methods of research. 99 children aged from 3 to 15 were examined years living in the city of Tynda, Amur Region. All children were divided into groups: 3, 5, 7, 10 and 15 years. The

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studies were conducted in the summer season (June). It is the most stable on fluctuations in weather conditions a period of time.

The criterion for inclusion in the study was that the children were of the 1st and 2nd health groups and did not get sick in the previous two months, and also were not vaccinated during this period. All children attended kindergartens or schools. The examination of children was carried out in agreement with the administration of kindergartens and schools, when parents signed informed consent to conduct this study. The research protocol of healthy individuals was in line with ethical standards. developed in accordance with the Helsinki Declaration of the World Medical Association and was approved by the Biomedical Ethics Committee of the NIIMPS SB RAS.

The criterions for exclusion from the study were deviations in the children's health status and disagreement of children and parents at each stage of the study.

Cytochemical method was determined in lymphocytes peripheral blood activity enzymes markers metabolic pathways:: Krebs cycle – succinate dehydrogenase, (SDG); glycerophosphate shunt connecting glycolysis with the Krebs cycle – mitochondrial glycerol-3-phosphate dehydrogenase (G3PDG); glycolysis – lactate dehydrogenase (LDG) and its aerobic isoenzyme – (H-LDG); pentose phosphate pathway – $NADPH_2$-diaphorase ($NADPH_2$-D). catabolism – non-specific acidphosphatase, (ACP), according to [9]; catabolism of monoamines – monoamine oxidase (MAO) according to [7]. The activity of dehydrogenases and MAO was expressed by the number of formazan granules in 1 cell (gr./ cell), and ACP in Kaplow units.

The connectivity of the studied indicators was assessed by correlation analysis. The intensity of adaptive reactions in children studied by method correlation adaptometry using the G-criterion. The weight of the correlation graph − G, was calculated by the formula − $G = \Sigma|rij|, |rij| | 0,5$, где rij | are the pairwise correlation coefficients between the i-th and j-th parameters, and Σ – is the sum of the moduli of these coefficients [5,10]. The work analyzed only statistically significant correlation coefficients (r), $p < 0.05$ and higher, where p – is the achieved level of statistical significance. Statistical processing was performed using the software package “Statistica v. 6.1 ". The data are presented in the form X ± x, where X– is the arithmetic mean, x – is the error of the mean. To assess the difference in means in pairwise unrelated samples, the Mann – Whitney U-test was used, the difference in values was considered significant at $p < 0.05$.

Results and discussion When assessing the age-related features of the enzymatic metabolism of lymphocytes, it was found that the lowest activity of enzymes in the group of children 3 years old, while in older age groups, the level of enzymes increased (table 1). The low activity of energy and plastic metabolism enzymes in preschool children indicates a decrease in the functional capabilities of cells and their low immunoreactivity, which gives reason to regard this period as critical.

A feature of the metabolic system of lymphocytes in children of 5 years is a sharp jump in the level of activity of SDG and LDG by 1,5 times ($p<0,01$) relative to the level of enzymes in children of 3 years, whereas in the middle-latitude zone a shift of the ontogenetic curve is observed at an older age [6,9].

At the studied stage of ontogenesis the activity of enzymes constantly increased, and in children 5, 7, 10, and 15 years old it was significantly different from the group of children 3 years old.

However, with increasing age the higher level of enzyme activity of the Krebs cycle and glycolysis in lymphocytes of children of Tynda was combined with inhibition of the glycerophosphate shunt. The activity of mitochondrial G3FDG was significantly lower than the enzyme indices of children living in middle latitudes [1,6,9].

The weakening of the catalytic function of the shuttle system G3FDG in mitochondria of lymphocytes in all age groups also violated the physiological ratio of G3PDG/SDG, which is normal – 0.59-0.65 [6,9], and in children of Tynda it was less than 1.5-2 times, which allows us to judge the decrease in the energy potential of mitochondria. The low G3PDG activity of the lymphocytes of the children of the North is probably due; on the one hand, to the substrate outflow of glycerophosphate to the regenerative synthesis of membrane lipids, On the other hand, it is possible competition between SDG and MAO for the cofactor, given their localization in mitochondria.

In the age dynamics of the children of Tynda, an increase in LDG activity in lymphocytes was probably due to the growth of anaerobic forms of the enzyme. The proportion of the aerobic component of H-LDG decreased with age from 56% in children 3 years old to 39% in children 7-15 years old. Such a decrease in the content of the H-LDG isoenzyme indicates an increase in the intensity of anaerobic glycolysis with a decrease in the level of aerobic, which is the leading form of energy supply for lymphocytes [2]. Exactly with the activation of glycolysis with age is associated with a decrease in the aerobic fraction of LDG, because an increased concentration of pyruvate inhibits this isoenzyme [12].

In all the examined children in Tynda have a high activity of NADPH2-D, which reaches maximum values in adolescents, exceeding the value of the indicator of the lymphocyte children 3-year in 2,5 times ($p<0,01$). Such activation of the enzyme is apparently determined by an increased substrate flow through the pentose phosphate shunt and the corresponding synthesis of NADPH. Moreover, it is known that in the lymphocytes in he North the content of total lipids is increased, the synthesis of which depends on NADPH [1].

Table1

Indicators of enzyme activity in blood lymphocytes of children of different age groups of the alien population of the North (Tynda city, (X ± x)

Indicator	Age (years)				
	3 (1 group)	5 (2 group)	7 (3 group)	10 (4 group)	15 (5 group)
	n=15	n=25	n=15	n=15	n=29
SDG, gr./cl.	7.93±	11.75±	13.29±	17.20±	16.25±
G3FDG, gr./cl	3.40±	3.38±	4.61±	6.35±	6.07±
LDG, gr./cl.	5.98±	8.73±	10.15±	10.53±	12.21±
H-LDG, gr./cl.	3.37±	4.59±	5.19±	4.14±	5.20±
NADPH2-D gr./cl.	4.19±	5.07±	5.81±	5.30±	10.25±
MAO, gr./cl.	1.82±	2.15±	1.66±	3.69±	–
				p1.2.3<0.05	
ACP, units Kaplow	162.0±	179.26.51	201.1±	167.69±	–

The age-related dynamics of the MAO activity level of lymphocytes in children of the city of Tynda was oscillatory in nature with a maximum enzyme activity in children of 10 years old, which reflects the peculiarity of the regulation of lymphocyte metabolism by biogenic amines in this region [15].

The activity of ACP in the lymphocytes of children of the city of Tynda is in 1,5-2 times higher than the indicators of age norms for children living in temperate latitudes [1,6,9]. Such high enzyme activity in lymphocytes indicates their accelerated maturation and entry into the bloodstream of young immunocompetent cells, and is also usually observed with inflammation and allergies [13,14].

The enzymatic systems of lymphocytes in children of Tynda are functioning with agreed coordination of the individual components, which is expressed in the presence of numerous relationships between them. Correlation analysis data showed that in all age groups of children of Tynda there are no indicators that are constantly interconnected with each other (table 2). So, in 3-year-old children, statistically significant correlations are not determined in lymphocytes. Such a decline correlation is most likely associated with a violation of intracellular regulation [8].

In the age group of children of 5 years, negative MAO-ACP relationship is revealed in lymphocytes, which indicates inhibition of catabolic reactions with an increase in the level of monoamines, as well as an increase in the interaction between energy and plastic metabolism - LDG-NADPH2-D, an increase in the intensity of aerobic glycolysis − a positive relationship LDG-H-LDG.

In the blood lymphocytes of children of 7 years old, close negative correlations of MAO and H-LDG with NADPH2-D are revealed, which indicate inhibition of biological synthesis reactions with an increase in the level of monoamines and with the intensification of aerobic glycolysis, closely associated with the work of shuttle systems (G3PDG- H-LDG).

In lymphocytes of children of 10 years of age there are more correlations than in other groups. The closest of them are noted between enzymes of energy metabolism. More weak ties in this age period are negative correlations − MAO-NADPH2-D and MAO-H-LDG, which reflect the inhibition of monoamine reactions of biological synthesis and glycolysis, and the connection H-LDG-NADPH2-D testifies i the conjugacy of these processes.

In adolescence, the number of correlation relationships between enzymes decreases by 6 times, relative to children of 10 years,, which reflects an increase in the number of possible options for the operation of intracellular systems and an increase in the metabolism capacity of immunocompetent cells. In lymphocytes adolescent is determined, only one positive correlation of SDG-G3PDH between enzymes of energy metabolism. Usually, such a connection arises when the level of cellular energetics changes. Probably, toward teenage age are formed new neuro-humoral regulatory impacts on lymphocyte metabolism when happens intensification of most types of metabolism [1].

When assessing the intensity of adaptive reactions in children using the G−criterion, it was found that in the group of children 3 years old G = 0, and in children 5,7,10 years old there is an increase in the weight of the correlation graph (table 2).. The maximum G (and, therefore, adaptive tension) is determined in the group of children of 10 years old − G = 3.59.

In the group of adolescents, the weight of the correlation graph is 6,9 times lower relative to children of 10 years, which testifies a decrease in the level of adaptive tension.

Thus, the weight of the correlation graph serves as a rather sensitive criterion for adaptive tension, revealing the features of the process of adaptation of children to the conditions of the North at each stage of ontogenesis.

Conclusion. The research has shown the features of the formation of age-related dynamics of enzyme activity in the lymphocytes of children living in the Far North. In children of 3 years old the lowest enzyme activity is revealed, which indicates reduced functional activity of these cells.

With the age of children, the increase in the enzyme activity of energy, plastic metabolism and the decrease in the proportion of aerobic glycolysis (H-LDG) were identified at higher level of acid phosphatase, which characterizes the peculiarities of the functioning of metabolic pathways in children in the North. The enzyme activity and their correlations determine the variants of the metabolic response of the cells of the immune system at each stage of ontogenesis. By the weight of the correlation graph, the periods of the greatest adaptation tension were identified the maximum of which was noted in children of 10 years old.

The parameters of lymphocyte metabolism in children from Tynda presented in this work as well as the nature of their interconnection, reflect the regional features of the immune status of children living in the Far North.

Table2

Correlation dependence of enzyme activity indicators in blood lymphocytes in children of different age groups of the alien population of the North (Tynda)

Age, years	Enzymes	r	G
3		–	–
5	MAO - ACP LDG - NADPH2-D LDG - H-LDG	-0.55* 0.52* 0.54*	1.61
7	MAO - NADPH2-D H-LDG - NADPH2-D G3FDG - H-LDG	-0.63* -0.61* 0.60*	1.84
10	SDG - G3FDG SDG - LDG MAO - H-LDG MAO- NADPH2-D H-LDG - NADPH2-D LDG - H-LDG	0.63* 0.69** -0.53* -0.51* 0.55* 0.68**	3.59
15	SDG - G3FDG	0.52*	0.52

Note: r − the correlation coefficient; statistical significance of correlations:* − p <0.05; ** − p <0.01; G − correlation graph weight

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FEATURES OF THE DAILY PROFILE OF ARTERIAL BLOOD PRESSURE AT MIGRANTS OF FAR NORTH DEPENDING ON ACCOMMODATION TERMS IN NEW KLIMATOGEOGRAPHIC CONDITIONS

The aim of the study was to study features of the daily blood pressure profile of migrants in the Far North, depending on the period of residence in the new climatic and geographical conditions. Materials and methods: 267 patients of both sexes with hypertension, who arrived from the regions of the Far North for permanent residence in Central Siberia, the average age of 64,0 years, were examined. Indicators of the daily blood pressure profile were studied by the method of daily blood pressure monitoring using the BPLab MnSDP-2 device for 24 hours. Results: high frequency of the daily non-dipper profile was noted in both migrants and permanent residents of Krasnoyarsk. The non-dipper profile was more common among migrants who lived for six or more years after moving, which may indicate a more severe course of arterial hypertension during this period. Discussion: the prevalence of persons with non-dipper and night-piker profiles among migrants in the Far North indicates a more pronounced lesion of target organs and a more severe course of hypertension. Conclusions: Among migrants with longer periods of residence after moving (more than 10 years), there was an increase of indicators of the daily blood pressure profile, but the largest number of persons with the changed daily profile was found in migrants in the first 5 years after moving from the Far North. This may be due to increased adaptation processes (readaptation) to new living conditions, due to a high level of neuroticism and stress during this period.

Keywords: migrants, Far North, arterial hypertension, daily blood pressure profile.

Introduction. Arterial hypertension (AH) remains one of the most pressing modern problems, due to the high population frequency of AH, its negative impact on the state of health, working capacity and life expectancy of the population [5, 8]. The study of the characteristics of AH among the population living in harsh conditions of the North and Siberia is [3, 4, 6, 9, 10]. In harsh climatic conditions, the cardiovascular system (as highly reactive) is one ofof great importance the first to be included in adaptive reactions [3, 11]. This cause certainly affects productivity and ability to work [2, 11]. In regions with extreme climatic conditions, this can cause a negative migration flow and destabilization of the population, in particular in the regions of the Far North [10]. Climatic conditions can also play a role in the re-adaptation of the human body in the new living conditions [10].

Currently, 24-h ambulatory BP monitoring (ABPM) is a valuable diagnostic

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method that allows identifying individual features of daily BP profile [1, 7]. The studies made it possible to distinguish the "northern" variant of AH [5], characterized by disruption of circadian of the daily rhythm, consistency of the daily profile of BP, increased weather stability, tougher current and earlier damage to target organs: left ventricular myocardial hypertrophy, trophic changes of the vascular wall [5, 11]. In this regard, it is interesting to study the features of the daily BP profile in migrants of the Far North with AH during their stay in new climatic conditions, for a personalized assessment of cardiovascular risk and to decide on the need for prescribing and correcting antihypertensive therapy.

Aim. The aim of the study was to study features of the daily blood pressure profile of migrants in the Far North, depending on the period of residence in the new climatic and geographical conditions.

Materials and methods. The research included 267 patients of arrived population (Caucasians) of both sexes with stage AH II-III (according to the recommendations of ESH/ESC, 2013) [8], arrived from regions of Far North for the permanent residence to the Central Siberia (Krasnoyarsk, Minusinsk), average age of 64,0 [59,0;73,0] years. According to research goals and objectives the migrants were divided into groups depending on accommodation terms in new the climatic and geographic conditions after moving from the region of Far North: the 1st group – with staying for 5 years, the 2nd group – staying from 6 to 10 years and the 3rd group – about 10 years . The group of comparison was comprised of 267 patients with arterial hypertension of similar age range (65,0 [59,0;74,0] years) constantly living in Krasnoyarsk. All patients gave the written informed consent. The research was conducted according to ethical principles of the Helsinki declaration and approved by local ethical committee.

Indicators of the daily BP profile were revealed by a method of ABPM with use of the BPLab MnSDP-2 ("Pyotr Telegin", Russia) within 24 hours. The average values of systolic and diastolic arterial blood pressure (SBP and DBP), the time index of high BP, the variability of BP for three periods of monitoring (day, day, night) were estimated as well as indicators of the BP daily index and the morning BP. Depending on the size of nocturnal BP fall, patients were categorized into four groups: dippers, non-dippers, over-dippers and night-peaker.

Statistical processing of the research results was carried out by means of the Statistica 6,1 software package. The obtained data were presented in the form of the median (Me) and the interquartile interval [Q_1; Q_3]. Two independent groups were compared my means of the U-criterion of Mann-Whitney. The analysis of different frequencies in two independent groups was carried out by means of criterion χ^2 with Yates's amendment. There were significant distinctions $p < 0,05$.

Results and discussion. The analysis of BP indicators during the ABPM showed that among migrants and residents of Krasnoyarsk, the average daily indicators of SBP and the values of SBP in the daytime corresponded to the breakpoints (≥130 mm Hg per day and ≥135 mm Hg per day, respectively). For migrants, the values of SBP during night hours corresponded to increased values (≥125 mmHg), in contrast to the breakpoints of similar indicators (≥120 mmHg) for residents of Krasnoyarsk (table 1).

Considering the variability of both systolic, and diastolic BP at daily monitoring, it is established that these indicators in both groups corresponded to normal values both in the afternoon, and at night at least no any of their four critical values were revealed. At the same time, values of variability at night and per day in general were higher among residents of Krasnoyarsk (table 1) but at the same time had no statistical significance.

To assess the dynamics of BP in the morning, MSBP and the rate of morning BR rise were calculated. Importance of assessment of these indicators was caused by high risk of emergence of the events leading to sudden death (strokes, myocardial infarctions, heart rhythm disorder). In both groups, the values of rise BP indicators corresponded to normal values, since they did not exceed 56 mmHg for SBP and 36 mmHg for DBP, respectively. But the speed of rise BP indicators among migrants exceeded the standard values and were higher in comparison with residents of Krasnoyarsk, both for SBP (> 10 mmHg) and for DBP (> 6 mmHg) (table 1).

For the purpose of quantitative assessment of episodes of increase BP indicators "loadings by pressure" analyzed that more precisely, than average AH values, characterize load of target organs (table. 1). It was shown that values of the average daily index of time both for the SBP and for DBP in both groups exceeded referents values (> 30%). Indicators of index of time was higher in-group of migrants in the afternoon and corresponded to the increased values, however these distinctions had statistically no importance. Indicators of the time index in both groups did not exceed at night the referents of values and were higher among migrants, but had no significant distinctions.

The frequency of various options of the daily BP profile among the examined persons (figure. 1) was analyzed. It was established that there was prevalence of insufficient night decrease BP (non-dipper) and significant amount of persons in each group with the daily night-piker profile – 20,7% and 23,4%. among both migrants (49,4%), and residents of Krasnoyarsk (42,6%). Change of the daily BP profile was revealed in 74,7% of the examined migrants and 71,3% – (figure 1) constantly living in Krasnoyarsk ($p=0,731$). According to data of the literature, insufficient decrease of night BP at patients with AH can cause a risk of organ defeats and mortality [1, 2, 11]. Owing to the reduced extent of night, the decrease of BP increased afterload of left ventricle is noted, it affected by actual increase in myocardial mass of left ventricle, in comparison with patients with normal decrease BP [11] at night.

The results about the frequency of the adverse daily AD profiles at migrants will be coordinated with data of the researches conducted earlier [1, 2, 3]. So, according to Zapesochnaya I. L. et al. (2015) patients from AG living in Khanty-Mansi Autonomous Okrug (KhMAO – Ugra), had the following frequency of the AD profiles: dipper – 19,1%, non-dipper – 57,3%, over-dipper – 7,4%, night-peaker – 16,2% [2]. According results to Polyakov's V. Ya. et al. (2011) [3], among the sick AH living in North conditions the broken daily non-dipper profile – 48% prevailed. The daily dipper profile was noted at 43% examined and the over-dipper profile – at 9%. According to Gapon L. I. et al. (2014) [1] only 1/3 of all patients (25,6% native and 28% alien) were registered with a normal degree of reduction in night BP. The predominance of individuals with non-dipper and night-piker profiles, both among indigenous and newcomers, indicates a more pronounced lesion of target organs and a more severe course of hypertension [11].

The carried-out analysis of indicators BP at migrants depending on terms of accommodation at new the climatic and geographic conditions (table 2) showed that the SBP among migrants the faces of the 3rd group had the greatest average daily, day and night indicators, and corresponded to boundary values.

The patients of 1st and 2nd of groups had similar indicators normal. The values of night SBP exceeded the standard indicators in groups 1st and 3rd, and in the second group corresponded

Table1

Comparative characteristics of the daily blood pressure profile in the surveyed migrants and residents of Krasnoyarsk with hypertension

	Indicators	Migrants (n=87)	Krasnoyarsk (n=94)	*p*
Day	SBP, mmHg	131 [122;142.6]	132.0 [121.8;139.3]	p=0.743
	DBP, mmHg	82 [73.8;87]	79.3 [73.2;84.3]	p=0.060
	VSBP, mmHg	12.8 [10.9;15.5]	14.6 [11.6;17]	p=0.012
	VDBP, mmHg	10.5 [8.7;12.8]	11.7 [9.6;13.7]	p=0.004
	TI SBP, %	38 [14.9;66]	39.9 [20.7;67.7]	p=0.647
	TI DBP, %	38 [17;61.4]	38.9 [16.9;52.5]	p=0.570
Daytime	SBP, mmHg	134 [124;143]	133.0 [123.3;142]	p=0.848
	DBP, mmHg	84 [77;89.6]	81.7 [75.1;87]	p=0.087
	VSBP, mmHg	13 [11;16]	12.9 [11;17.2]	p=0.607
	VDBP, mmHg	10.1 [9;13.1]	11.1 [8.9;14.2]	p=0.327
	TI SBP, %	31.8 [7.7;65]	24.8 [5.9;57.5]	p=0.658
	TI DBP, %	24.5 [6;51]	19.0 [5.91;37.2]	p=0.207
Night	SBP, mmHg	125 [115;136]	124.4 [117;135]	p=0.767
	DBP, mmHg	77 [70;83]	72.7 [68.6;80]	p=0.077
	VSBP, mmHg	11 [9;14]	12.6 [10.2;14.9]	p=0.008
	VDBP, mmHg	9 [7;11]	9.9 [8.2;12]	p=0.029
	TI SBP, %	52 [17;90]	59.6 [34.8;90.1]	p=0.269
	TI DBP, %	63.5 [22.1;90]	59.6 [39.2;79.4]	p=0.924
DND SBP, %		5.9 [0;13.3]	5.6 [0.1;11.7]	p=0.939
DND DBP, %		8.8 [3.6;15.9]	9.5 [4.3;16.9]	p=0.838
VMR SBP, mmHg		39.5 [31;55]	40.5 [21;53]	p=0.602
VMR DBP, mmHg		31 [22.2;42]	32.5 [15;40]	p=0.302
SMR SBP, mmHg/hour		11.9 [7;18.6]	8.4 [3.7;11.9]	p=0.004
SMR DBP, mmHg/hour		8 [4;16]	6.7 [2.7;9.6]	p=0.034

to breakpoints. The day indicators of DBP in all groups corresponded to normal amounts while DBP in the first and second were at night boundary, and in the third group corresponded to the increased values. In spite of the fact that the variabilities SBP and variabilities DBP values in all examined groups corresponded to normal values both in the afternoon, and at night (table 2), the highest values of the specified variability indicators BP were noted among the persons who lived after moving> 10 years.

The analysis of indicators of BP morning dynamics showed that the values of rise BP values both for SBP and DBP in the compared groups had no distinctions (table 2). However, the largest values of rise SBP were noted in the third group and values of rise DBP in the second group. In the analysis of indicators the speed of rise BP it is established that among migrants in the 2nd and 3rd groups both for SBP, and DBP the studied values exceeded standard values, and the highest parameters were in the 3rd group for the SBP and in the 2nd group for DBP respectively.

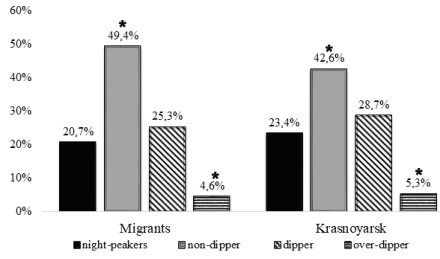

Figure. 1. Indicators of the daily blood pressure rhythm in the surveyed migrants and residents of Krasnoyarsk with hypertension. Note: * - differences within groups p<0,05

It is established that the average daily indices of time for SBP at migrants of the first and second groups did not exceed the referents of values, whereas in the third group they were raised (table 2). The average daily indices of time for DBP were increased among migrants of the first and third groups. The indicators of time index of SBP in day and night time corresponded to standard values in all three groups, except the time values of SBP in day at migrants of the 3rd group with the exceeded values. As for the indicators of time at night, in all allocated groups both for SBP and DBP, their values exceeded twice the standard both in the first and third groups. The differences between groups on all indicators of time had no statistical significance.

The frequency of various options of the daily BP profile depending on accommodation terms after moving (figure 2) was analyzed.

In all 3 groups the changes of BP daily profile due to the insufficient decrease BP at night – non-dipper as well as the daily profile from 13,6 to 36,4% patients with increase BP at night – night-piker were observed. There was change of BP daily profile in the 1st group in 81,8% of people, among surveyed the 2nd group of change of the BP profile were noted at 63,6% and in the 3rd group at 78,8% examined according to (p_{1-2}=0,284; p_{1-3}=0,678; p_{2-3}=0,332;) (figure 2).

Conclusion. According to the results of the study, it was found that migrants had higher values of nocturnal SBP, higher values of the time index in daytime, higher and exceeded standard for both SBP and DBP indicators of EMS, unlike residents of Krasnoyarsk, and along with residents of Krasnoyarsk, had a high frequency of the daily profile "non-dipper." When analyzing the ABPM indicators among the surveyed migrants, it was found that the average daily, day and

Table2

Comparative characteristics of the daily blood pressure profile in the examined migrants with hypertension depending on the period of residence in the new climate conditions

Index		Time of residence after moving			*p*
		≤5 лет (1-я группа)	6-10 лет (2-я группа)	>10 лет (3-я группа)	
Day	SBP, mmHg	122.4 [117.0;138.0]	125.0 [119.5;135.0]	131.0 [125.0;142.6]	p_{1-2}=0.819; p_{1-3}=0.098; p_{2-3}=0.097
	DBP, mmHg	79.0 [73.8;87.0]	76.3 [70.0;87.0]	82.6 [77.0;86.0]	p_{1-2}=0.606; p_{1-3}=0.440; p_{2-3}=0.232
	VSBP, mmHg	11.1 [11.0;12.8]	12.0 [10.0;15.4]	13.9 [11.0;15.6]	p_{1-2}=0.954; p_{1-3}=0.238; p_{2-3}=0.260
	VDBP, mmHg	10.3 [8.5;10.9]	10.0 [9.0;12.7]	10.5 [8.9;12.8]	p_{1-2}=0.834; p_{1-3}=0.507; p_{2-3}=0.810
	TI SBP, %	22.0 [7.0;65.0]	25.4 [11.0;59.0]	38.0 [22.0;64.2]	p_{1-2}=0.924; p_{1-3}=0.416; p_{2-3}=0.239
	TI DBP, %	38.0 [9.6;50.0]	26.0 [7.0;53.0]	40.0 [23.0;57.5]	p_{1-2}=0.804; p_{1-3}=0.401; p_{2-3}=0.229
Daytime	SBP, mmHg	129.0 [119.0;137.0]	128.0 [121.2;137.0]	134.0 [127.0;143.0]	p_{1-2}=0.760; p_{1-3}=0.136; p_{2-3}=0.092
	DBP, mmHg	82.0 [75.0;87.0]	80.5 [71.0;87.0]	84.4 [80.0;89.5]	p_{1-2}=0.731; p_{1-3}=0.343; p_{2-3}=0.183
	VSBP, mmHg	12.0 [10.3;13.5]	11.5 [10.0;15.1]	14.0 [12.0;16.3]	p_{1-2}=0.879; p_{1-3}=0.071; p_{2-3}=0.094
	VDBP, mmHg	10.1 [8.1;12.4]	9.8 [8.0;13.0]	11.0 [9.0;14.0]	p_{1-2}=0.954; p_{1-3}=0.336 p_{2-3}=0.226
	TI SBP, %	12.8 [0.0;48.0]	19.7 [5.0;65.0]	32.0 [12.0;57.0]	p_{1-2}=0.492; p_{1-3}=0.184; p_{2-3}=0.419
	TI DBP, %	16.0 [1.7;35.0]	18.3 [4.0;53.0]	25.2 [7.0;48.0]	p_{1-2}=0.516; p_{1-3}=0.208; p_{2-3}=0.542
Night	SBP, mmHg	125.0 [112.4;133.0]	121.5 [111.0;129.0]	128.0 [117.0;135.0]	p_{1-2}=0.606; p_{1-3}=0.489; p_{2-3}=0.144
	DBP, mmHg	74.8 [66.1;87.0]	72.5 [65.0;79.0]	77.0 [71.0;82.0]	p_{1-2}=0.445; p_{1-3}=0.776; p_{2-3}=0.128
	VSBP, mmHg	10.9 [9.7;11.5]	9.0 [8.0;12.5]	12.0 [9.0;14.0]	p_{1-2}=0.244; p_{1-3}=0.725; p_{2-3}=0.319
	VDBP, mmHg	8.2 [7.0;11.7]	8.6 [7.0;11.8]	9.3 [7.0;11.0]	p_{1-2}=0.954; p_{1-3}=0.755; p_{2-3}=0.938
	TI SBP, %	58.0 [15.0;87.0]	36.0 [14.0;53.0]	61.0 [23.0;91.0]	p_{1-2}=0.423; p_{1-3}=0.588; p_{2-3}=0.077
	TI DBP, %	58.2 [24.3;89.0]	33.0 [9.0;80.0]	65.2 [28.0;89.0]	p_{1-2}=0.222; p_{1-3}=0.850; p_{2-3}=0.084
DND SBP, %		5.2 [-2.0;9.4]	6.1 [1.7;14.1]	6.0 [1.5;10.0]	p_{1-2}=0.302; p_{1-3}=0.473; p_{2-3}=0.797
DND DBP, %		12.5 [1.4;17.8]	12.4 [6.2;15.4]	8.3 [3.3;18.3]	p_{1-2}=0.620; p_{1-3}=0.860; p_{2-3}=0.542
VMR SBP, mmHg		32.0 [22.0;41.0]	35.0 [26.0;53.0]	40.5 [34.0;56.0]	p_{1-2}=0.311; p_{1-3}=0.069; p_{2-3}=0.272
VMR DBP, mmHg		28.0 [22.2;32.0]	36.0 [24.0;42.5]	31.0 [22.0;43.0]	p_{1-2}=0.203; p_{1-3}=0.279; p_{2-3}=0.797
SMR SBP, mmHg/hour		8.0 [3.5;15.0]	11.5 [3.7;15.9]	12.0 [10.0;22.0]	p_{1-2}=0.588; p_{1-3}=0.157; p_{2-3}=0.276
SMR DBP, mmHg/ hour		5.1 [1.0;9.0]	9.7 [2.0;16.5]	8.0 [5.0;14.0]	p_{1-2}=0.187; p_{1-3}=0.205; p_{2-3}=0.913

night indicators of SBP, DBP, the values of rise index SBP, speed of rise SBP and DBP, as well as the index of time SBP were the highest in people who lived after moving >10 years, and corresponded to the increased values. This may indicate a more severe course of arterial hypertension in this group. In all three groups, changes in the daily BP profile were observed due to insufficient reduction of BP during the night hours – non-dipper. Among migrants with longer periods of residence after moving (more than 10 years), there was an increase in the number of indicators of the daily blood pressure profile, but the largest number of persons with the changed daily profile observed in migrants in the first 5 years

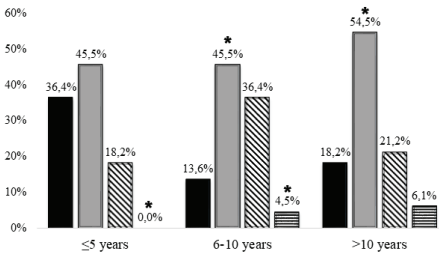

Figure. 2. Indicators of the daily blood pressure rhythm in the examined migrants with hypertension, depending on the period of residence in the new climate conditions. Note: * - differences within groups p<0,05

after moving from the Far North. This may be due to increased adaptation processes (readaptation) to new living conditions, due to a high level of neuroticism and stress during this period.

Conflict of interest. The authors declare no conflict of interest.

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NUTRITION IN THE NORTH

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ANALYSIS OF THE MACRO- AND MICRONUTRIENT DIET PROFILE OF YOUNG MALE INDIGENES IN THE NORTHEAST OF RUSSIA

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Each ecological region has individual characteristics of metabolism particularly because of the indefinite landscape zone that can be inhabited by people of different ethnic and racial origin. The environmental factor of nutrition has a great influence on the metabolism characteristics. What macro- and micronutrients are consumed with food becomes a prognostic sign of the development of alimentary-dependent deviations that directly affect functional parameters of the body systems. The diet of 17-21 year old male indigenous residents of Magadan region (n = 38) and Chukotka Autonomous District (n = 52) has been analyzed. All the examinees were full-time students having similar living conditions. The ASPON-Nutrition program (Russia) was used to evaluate the young subjects' nutritional profiles by analyzing their daily diets. The studied parameters were proteins, fats, carbohydrates, fiber, vitamins, and macro- and microelements. In addition, the energy value of the daily diet was assessed in kcal / day. Larger deviations from the current normative values of the body physiological needs in macro- and micronutrients have been found. The nutritional imbalance characterized by insufficient intake of proteins, fats, essential trace elements, and vitamins against the background of reduced energy value of daily calorie content has been shown. The revealed deficiency is much more typical at young men living in the city of Magadan. In general, the multilateral imbalance established in the diet of young indigenes indicates a violation of the basic principles of healthy eating (satisfying energy needs, balancing proteins, fats, carbohydrates) and can be associated with avoiding the traditional type of the diet by the indigenous population and turning to the classical Caucasian one which does not provide all the needs of the body of this ethnicity.

Keywords: young men, indigenes, diet, macro-, micronutrient diet profile.

Introduction. In contrast to all abiotic factors that constantly affect a person, food is transformed in the body into an internal factor as a source of energy and plastic material for building cells [1]. Being environmental factor nutrition has a great influence on the metabolism characteristics [8]. What macro- and micronutrients enter the body with food becomes a prognostic characteristics of the development of alimentary-dependent deviations that directly affect functional parameters of the body systems [15].

The youth are known to keep to a special pattern of eating behavior which is characterized by irrational, monotonous nutrition with the dominance of carbohydrates [16]. That can lead to nutritional deficiency and imbalance [18]. According to WHO avoiding a balanced diet is one of the main risk factors for the development of chronic diseases [25]. Such diet unbalanced by the consumption of micro- and macronutrients is a very important component in the etiology of cardiovascular, oncological diseases as well as obesity [19].

It is obvious that even within one ecological region there are individual characteristics of metabolism particularly because of the considered landscape zone that can be inhabited by people of different ethnic and racial origin [14]. In this regard, the aim of our research was to identify regional characteristics of the macro- and micronutrient composition of the diet of young indigenous men born in the northeast of Russia.

Methodology. The diet assessment was carried out among young men from the indigenous population of the city of Magadan (n = 38) as well as representatives of the Chukotka Autonomous District (ChAD) aged 17-21 (n = 52). All the examinees were full-time students having similar living conditions. The ASPON-Nutrition program (Russia) was used to evaluate the young subjects' nutritional profiles by analyzing their daily diets. The studied parameters were proteins, fats, carbohydrates, fiber, vitamins, and macro- and microelements. In addition, the energy value of the daily diet was assessed in kcal / day. Besides, the frequency of deficiency or excess in macro- and micronutrients intake were recorded as compared to the normative ranges.

The study was carried out in accordance with the principles of the Helsinki Declaration (2008). The study protocol was approved by the Ethics Committee of Biomedical Research at the NESC, FEB RAS (No. 004/013 dated 12/10/2013). All the volunteers gave their written informed consent of being included in the study. The results underwent statistical processing using the software package of Statistica 7.0. The distribution of the measured variables was checked for normality based on the Shapiro-Wilk test. The processing parametric methods are presented as the average value (M) and the arithmetic mean error (± m). The statistical significance of differences was determined using the Student's t-test for independent samples. The critical level of significance (p) was taken equal to 0.05.

Results and discussion. Tables 1 and 2 show the diet macronutrition facts of young male natives from both the city of Magadan and Chukotka Autonomous District. Proteins are known to play a leading role in biological systems since they determine the plastic function of cellular and extracellular structures [24]. It ought to be noted that food should contain at least 140 g of protein per day for normal body functioning in the North [9]. We have found the protein content per day to be no more than 112.1 ± 9.9 g. The young men of Magadan were significantly lower in protein than the young men of the ChAD. The same picture has been observed for lipids as the indigenous residents of the city of Magadan demonstrated the smallest animal and vegetable fats in their diets. Of note that diets of residents of Magadan region have proved to be low in polyunsaturated fatty acids which are important nutrients and the lack of them can lead to significant consequences, in particular, a shift in the blood lipid spectrum - an increase

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in total cholesterol, triglycerides, and atherogenic fractions of lipoproteins [11]. No statistically significant differences in carbohydrates which are the main energy supplier to the body have been found.

The protein-lipid type of nutrition of the indigenous population, which evolved as man started developing the Arctic, was a natural basis for a fairly quick adaptation of the ethnic group to a cold climate [10, 12]. According to the researchers, the type of aboriginal diet focused on high intake of protein and animal fats is the only way to maintain the energy balance of the body in the North even with low carbohydrate content in food [22].

The previously conducted research on nutrition features typical for the native population in the European and Far North showed nearly complete absence of traditional dishes in the diet of younger people of these regions [4, 5]. Our data also indicate transition from the traditional protein-lipid to the urbanized diet of younger representatives of indigenes of northeast Russia, which is more frequent among young male residents of Magadan region. In the future that can lead to significant metabolic disorders significantly worsening health and reducing quality of life of the indigenous population of Russia's northeast [13].

There should be also noted the extreme lack of fiber consumption (both among residents of Magadan and among young men from ChAD), which is considered a risk factor for the development of a number of non-infectious diseases [7]. That indicates a low variety of food consumed and may be associated with the high cost of them especially fresh fruit and vegetables.

The analysis of macro- and microelement composition of the diet of young men is presented in Table 3, and Table 4 shows the frequency of deficit, excess and normal values of the studied indicators. It can be seen that only the copper and nickel values demonstrated no statistically significant differences. The values of manganese and fluorine were higher among young men from the city of Magadan. The indicators of the rest 12 macro- and microelements were significantly higher among the inhabitants of ChAD, and in some cases this difference had multiple values. Such an essential element in maintaining the optimal level of bone mineralization [6] as calcium was found in the diet of Magadan subjects almost two times less than among the inhabitants of Chukotka. Analysis of deviations from normative indicators revealed that calcium deficiency was found in 100% of Magadan subjects and 90% of Chukchi. The examinees from Magadan were also lower in potassium and sodium which are the most important intra- and intercellular cations playing a key role in maintaining a stable volume of cells [21]. It must be emphasized that, 61% of them from Magadan Region were very deficient in sodium while the great majority (86%) of those from ChAD had an excess of it in their diet being two times higher than the daily norm recommended by WHO for adults [26]. Chlorine which regulates (along with calcium and sodium) the water-electrolyte balance in the body and is of importance in the development of neuromuscular inhibition had an even more pronounced excess in the subjects of that group, its intake with food exceeded the normal values in almost all examinees and was 4.5 times higher than that of Magadan subjects. The latter were totally deficit in the intake of iodine while in Chukotka such a shortage was found only in half the cases. Apparently such a low intake of this important trace element is associated with shortage in seafood in the diet especially fish. Of note is the pronounced deficiency of selenium which is a cofactor for iodine and therefore influences the functioning of the thyroid gland [17]. In our studies, 94% of Magadan subjects have proved to be low in this microelement as well as 100% of the inhabitants of Chukotka.

Tables 5 and 6 present data on the vitamin profile in the diet of the examined young men. Vitamins are one of the most important micronutrients that play a key role in many biochemical reactions of the functional state of cells [23]. We could observe significant differences in almost all indicators. It should be noted that most of the highest values were demonstrated by residents of the ChAD. Only vitamin C tended to increase among residents of Magadan, but that deviation did not have a statistically significant difference. At the same time, the lowest values for all water-soluble and three fat-soluble vitamins A, D, and K were recorded for male indigenes of Magadan. It is known that almost all vitamins that are water-soluble, unlike fat-soluble vitamins, cannot accumulate in the body, therefore their insufficient daily intake with food leads to disorganization of redox processes and disruption of the body functional systems [3]. Vitamin deficiency is one of the causes of metabolic disorders, reduced physical and mental activity, and rapid fatigue [2]. Moreover, even a perfectly designed adult diet, calculated at 2500 kcal per day, is deficient in most vitamins by at least 20% [20]. According to our studies, the energy value of the daily diet of Magadan residents did not reach that value (and was significantly lower than that of residents of the city of Anadyr), which again indicates the pronounced depletion of the vitamin profile characteristic of people living in the city of Magadan (Table 1).

Conclusion. The conducted studies allowed us to establish that the diet of young male residents of northeast Russia is characterized by the large deviations from the current normative values of the body physiological needs for macro- and micronutrients. The current nutrition of young residents from the indigenous population of Magadan Region and Chukotka Autonomous District is characterized by the low proportion of proteins and fats, the shortage of polyunsaturated fatty acids, and the complete lack of fiber

Table1

Macronutrient composition of the diet in young male indigenes of Magadan region and ChAD

Indicators	Cohort		Difference Significance between Cohorts
	Indigenes, Magadan	Indigenes, Chukotka	
Proteins, g	74.2±1.9	112.1±9.9	p<0.001
Fats, g	35.2±1.6	58.7±4.6	p<0.001
Arachidonic Acid, g	0.3±0.0	0.5±0.0	p<0.001
Docosahexaenoic Acid, g	0.04±0.01	2.36±1.75	p<0.001
Linoleic Acid, g	8.9±0.5	8.5±1.3	p=0.79
Linolenic Acid, g	0.22±0.01	0.31±0.04	p<0.05
Carbohydrates, g	327.2±8.2	351.0±14.6	p=0.15
Fiber, g	3.9±0.2	6.1±0.8	p<0.01
Energy Value, kcal	2310.5±44.3	2807.2±89.1	p<0.001

Note. Table 1-3 the significance of differences between groups is indicated at $p < 0.05$, $p < 0.01$, $p < 0.001$.

Table2

Microelement profile of the diet in young male indigenes of Magadan Region and ChAD

Indicators	Cohort		Difference Significance between Cohorts
	Indigenes, Magadan	Indigenes, Chukotka	
Iron, mg	14.9±0.5	24.1±4.5	p<0.05
Potassium, mg	2013±82.2	3346.4±142.4	p<0.001
Calcium, mg	355.5±19.0	670.7±58.0	p<0.001
Magnesium, mg	278.9±13.4	340.8±27.0	p<0.05
Manganese, mg	4.1±0.2	3.3±0.2	p<0.05
Sodium, mg	1433.4±89.8	4274.7±240.8	p<0.001
Phosphorus, mg	1043.8±29.9	1542.7±114.5	p<0.001
Fluorine, mg	22.3±4.9	10.8±1.9	p<0.05
Chlorine, mg	1342.3±84.1	6213.7±396.8	p<0.001
Zinc, mg	9.5±0.4	12.5±0.6	p<0.001
Iodine, mcg	45.4±2.9	116.2±9.6	p<0.001
Cobalt, mcg	22.8±1.1	51.2±4.1	p<0.001
Copper, mcg	1900±70	5145±2640	p=0.22
Molybdenum, mcg	74.1±3	87.7±4.0	p<0.05
Nickel, mcg	47.1±3.5	49.1±6.3	p=0.77
Selenium, mcg	21.1±1.9	28.7±2.9	p<0.05
Chromium, mcg	46.6±2.9	146.0±13	p<0.001

intake against the background of the relatively high proportion of carbohydrates. It should be noted the reduced energy value of the daily calorie intake necessary for young men at this age who tend to follow active lifestyle. The macro- and microelement nutritional profile is also characterized by the obvious deficit in the most essential elements and lacks in vitamin composition of consumed food products. The indicated deviations have high prevalence among male indigenes residing in Magadan Region. The imbalances of their nutrition profile in macro- and micronutrient composition have proved to reach the highest values among all the examined men, in some cases demonstrating a 100 percent deficit.

In general, the multilateral imbalance established in the diet of young indigenes indicates the violation of the basic principles of healthy eating (satisfying energy needs, balancing proteins, fats, carbohydrates) and can be associated with avoiding by the Indigenous population the traditional type of the diet and turning to the classical Caucasian one which does not provide all the needs of the body of this ethnicity.

Table3

Vitamin profile of the diet in young male indigenes of Magadan Region and ChAD

Indicators	Cohort		Difference Significance between Cohorts
	Indigenes, Magadan	Indigenes, Chukotka	
Biotin, mcg	29.3±2.1	42.5±6.5	p<0.05
Vitamin B1, mg	0.82±0.03	8.59±4.27	p<0.05
Vitamin B12, mcg	2.9±0.1	17.1±4.0	p<0.01
Vitamin B6, mg	1.5±0.0	2.5±0.1	p<0.01
Vitamin C, mg	61.9±9.5	55.8±14.5	p=0.72
Vitamin D, mcg	5.5±0.8	21.5±2.8	p<0.001
Vitamin E, mg	15.7±0.7	17.2±1.4	p=0.35
Vitamin PP, mg	12.4±0.4	17.9±1.0	p<0.001
Vitamin A, mcg	359.8±28.0	870.25±80.2	p<0.01
Vitamin B2, mg	0.76±0.04	1.38±0.13	p<0.001
Vitamin K, mcg	13.3±2.2	49.1±5.7	p<0.001
Pantothenic Acid, mg	3.1±0.1	5.5±0.5	p<0.001
Folacin, mcg	102.2±3.4	174.8±17.9	p<0.001

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SCIENTIFIC REVIEWS AND LECTURES

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BIOMARKERS OF EPILEPSY: MICRO-RNA (PART II)

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This article considers the use of microRNAs as a possible biomarker of epilepsy.

The presented studies have shown that microRNAs can be involved in the process of epileptogenesis by regulating the inflammatory response, apoptosis of neurons, and transcription factors involved in cell differentiation. Biological fluids (blood and CSF) of patients with epilepsy showed differences in the number of circulating microRNAs, which may allow further use of microRNAs as a diagnostic biomarker. Recent discoveries providethe sufficient source of new microRNA targets, but there are still significant problems of studying their role in pathogenesis and the possibility of their application in clinical practice.

Keywords: epilepsy, epileptic seizures, biomarker, microRNA, epileptogenesis.

Introduction. Epilepsy is a chronic neurological disease characterized by recurrent seizures resulted from pathological synchronous excitation of brain neurons [26]. The diagnosis of epilepsy is usually based on clinical manifistations of the disease, electroencephalogram (EEG) data, and magnetic resonance imaging (MRI) of the brain. In some cases, clarifying a seizure type is not an easy task and it makes the choice of the therapy even more challenging.

There is not enough evidence proving that antiepileptic drugs affect the process of epileptogenesis. No biomarkers of epileptogenesis to prevent the development of the disease and assess the effectiveness of therapy are revealed, it allowing to predict the response to antiepileptic drugs. There is a great need to search for new biomarkers of epileptic seizure, status epilepticus, as well as of epileptogenesis [25]. Moreover, molecular biomarkers are especially attractive, since their determination is possible using minimally invasive methods with the study of biofluids, e.g. blood [9].

The usage of gene expression profiling, which dates back to early 2000s, has made the understanding of the scale of changes in the brain of patients with epilepsy [1, 49]. Nowadays, a number of molecules have been studied, including proteins and ribonucleic acids (RNA), but most of them not characterized with specificity and sensitivity [47]. The research results published about 10 years ago showed changes in the levels of microRNA in blood after an epileptic seizure in rats [13].

MicroRNA: definition and mechanisms of functioning. The existence of microRNAs was first reported in 1993 by Victor Ambros and Gary Ruvkun [34, 58]. Currently, miRNAs are recognized as the main regulators of the translation stability of messenger RNA (mRNA) [20].

MicroRNAs are molecules from a class of short noncoding RNAs that regulate the level of gene expression by affecting the mRNA stability. Specific microRNAs bind to complementary sequences of the non-coding part of mRNA (3'-untranslated region, 3'-UTR) . It was shown that the synthesis of about 60% of all human proteins is regulated by microRNA molecules [13].

MicroRNA sinthesis occurs in the nucleus by transcription of the encoding gene. When entering in the cytoplasm of the cell and reaching its functional state, the miRNA molecule is able to bind to the matrix molecule (mRNA), inhibiting the subsequent translation of the protein of this mRNA, which is a matrix for protein's synthesis in the ribosome with the transport RNAs' involvement [4, 5, 19, 56].

MicroRNA's role in the pathogenesis of epilepsy. Over the past 5 years, several targeted and genome-wide studies of the microRNA level of expression in epilepsy have been carried out. According to the obtained data, changes were found in more than 100 different miRNAs in animal modelx and in patients with epilepsy, which proves the correlation between miRNA expression and epilepsy [24, 39, 55]. The very first study of miRNAs in human epilepsy was published in 2010, when an increase in hippocampal miRNA-146a levels associated with the control of inflammatory responses was reported [29].

Recently, a potential clinical use of miRNAs has been proposed using agomyres or inhibitory miRNA sequences as potential therapeutic molecules in epilepsy [2, 14, 21]. Altered microRNA profiles in biological fluids can be useful biomarkers of epileptogenesis [40].

The control of microRNA expression in epilepsy. The mechanism of changes in microRNA expression in patients with epilepsy remains uncertaion. Both direct and indirect disregulation theouries have been discussed [18].

Epigenetic mechanisms may be important regulators of microRNA expression in epilepsy. Epigenetic processes comprise DNA methylation and histone protein modification. Increased DNA methylation generally promotes chromatin compaction and decreases gene transcription in these regions. For example, after an epileptic seizure induced by kainic acid in rats, the acetylation of histones of the microRNA-124 gene locus decreases. This epigenetic mechanism of expression's suppression may explain the decrease in the level of miR-124 in the hippocampus [17]. Genomic analysis

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of DNA methylation in hippocampal tissue in patients with temporal lobe epilepsy revealed differences in the methylation state of several miRNA genes. Inverse correlations were found between methylation status and miRNA expression in hippocampal samples [37].

Mutations in microRNA genes can also affect the risk of epilepsy development. A number of studies have reported the association of candidate genes carriage and the disease's manifestation [7, 42]. Single-nucleotide variants of genes encoding microRNAs can influence the function of microRNAs in one of the following ways: altering the primary transcription of miRNAs, processing primary miRNAs (pri-microRNA) and precursor miRNAs (pre-miRNA), and by indirect modulation of microRNA – mRNA interactions [8, 10]. Changes in the global profiles of microRNA expression in various animal models of epilepsy have been shown [27]. Specific miRNAs in brain tissue have been associated with seizure-induced neuronal death or neuroprotection [28]. But still, no convincing evidence has been found that genetic forms of epilepsy are caused by mutations or single nucleotide variants' carriage of genes encoding microRNAs (Fig. 1).

Figure 1 - This schematic representation illustrates the potential epigenetic mechanisms, including DNA methylation, histone alterations, RNA-based transcriptional control, and bro domain reading activity which can alter the cellular gene expression profile and thus promote inhibition of epileptogenesis progression.). Figure has been adaptated from the article of Younus I. et al. 2017 [35].

The EpimiRNA Consortium studies the role of changes in genes encoding microRNAs in epilepsy development. In the project, a group of patients with epilepsy is compared with healthy individuals from the control group. The sequencing strategy focuses on those genomic regions in which changes are most likely to cause epilepsy. The changes affecting miRNAs are analyzed together with the entire subset of their predicted mRNA targets [59]. These microRNAs are expressed in the hippocampus of the human brain [33] and are likely to take part in the development of epilepsy. Thus, the search and selection of possible microRNA targets is carried out, the role of which has been confirmed in functional studies [60]. It is expected that the EpimiRNA sequencing strategy will highlight the regions of the genome encoding microRNAs, variations in which contribute to the development of epilepsy [15].

On the other hand, experimental and

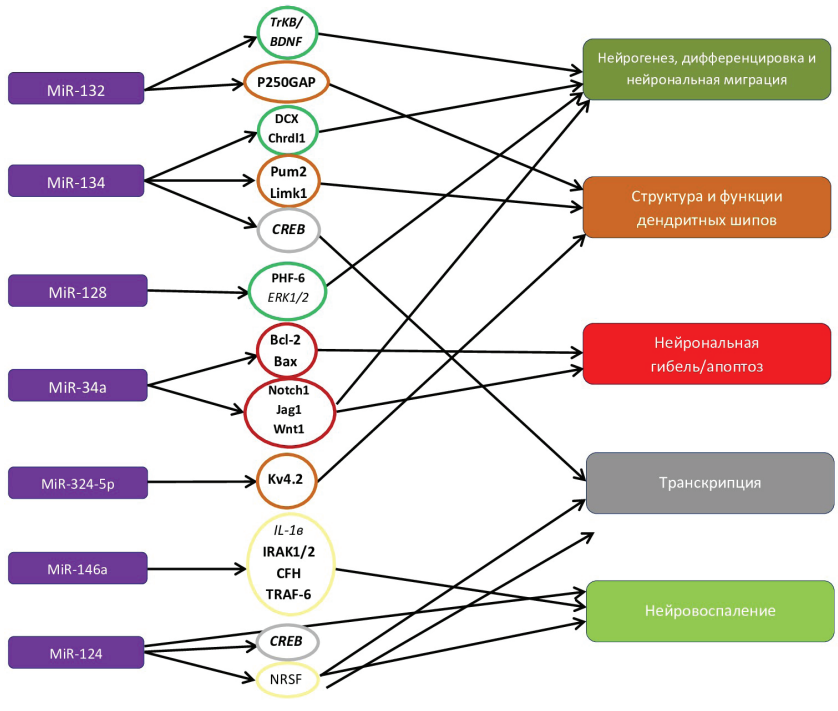

Fig. 1. Targeted approaches to the discovery of epigenetic inhibitors in epilepsy

human epilepsy are associated with network-wide changes in the levels of transcripts encoding proteins and aberrant protein production, which leads to significant suppression of transcription. Thus, seizures are likely to increase the levels of microRNA, which will subsequently lead to a decrease in the amount of mRNA of the genes encoding the protein. It was shown that epileptic seizures increase the levels of 28 miRNAs in the CA3 subfield of the mouse hippocampus [45]. For some epilepsy-related miRNAs, such as miRNA-134, transcriptional mechanisms are well-known. The Mef2 protein is activated by neuronal activity and stimulates the expression of miRNA-134 in neurons [31].

However, the question is not as simole as it seems, it has been proven that one microRNA molecule can have dozens of targets that regulate several genes of the same pathogenetic pathway or separate genes belonging to different pathogenetic pathways, which allows it to act at different levels of the pathological process [60].

The targets of microRNA in epileptogenesis. The studies have shown that microRNAs can be involved in the process of epileptogenesis by regulating the inflammatory response, apoptosis of neurons, and transcription factors involved in cell differentiation [38, 46]. The role of various miRNAs in epileptogenesis is shown on Figures 2 and 3.

Figure 2 - Simplified model depicting targets of candidate microRNAs and the associated epileptogenic pathways. Targets that have not been shown to be directly regulated by the respective microRNA are printed in italics). Figure has been adaptated from the article of Tiwari D. et al. 2018 [34].

Early functional studies linked the influence of microRNAs on the development of epileptic seizures with neuroinflammation and changes in the microstructure of neurons. For example, dysregulation of miRNA-134 changed the number and volume of dendritic spines of excitatory neurons, presumably through the target kinase of the LIM-domain [16, 43, 48], whereas miRNA-146a, miRNA-221, and miRNA-222 can control immune responses through interleukin-1β and cell adhesion molecules [6, 23, 52].

Moreover, the altered microRNA levels are likely to affect a wide range of molecular and cellular pathways in epilepsy, including cell differentiation, migration, and proliferation. For example, the identification of the axon guidance occurs under the microRNA contro, which underlines that if miRNAs associated with epilepsy were identidied, the mechanisms underlying epilepsy would be discovered [9, 43]. Although the effect of expression's deregulation of axon guidance signals on epileptogenesis is not well understood, these molecular signals contribute to various neurological disorders due to their ability to control axon growth, neuronal migration, and synapse development and functioning [36, 41]. Axon-guiding molecules may be important for the inte-

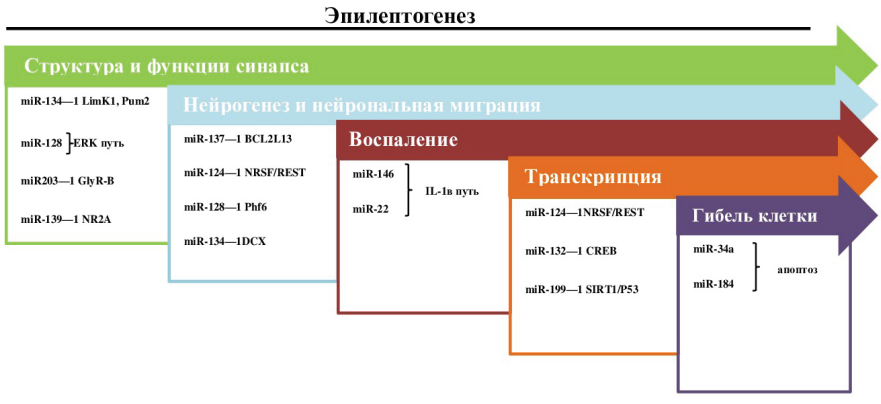

Fig. 2. The simplified model depicting candidate micro-RNA targets and associated epileptogenic pathways

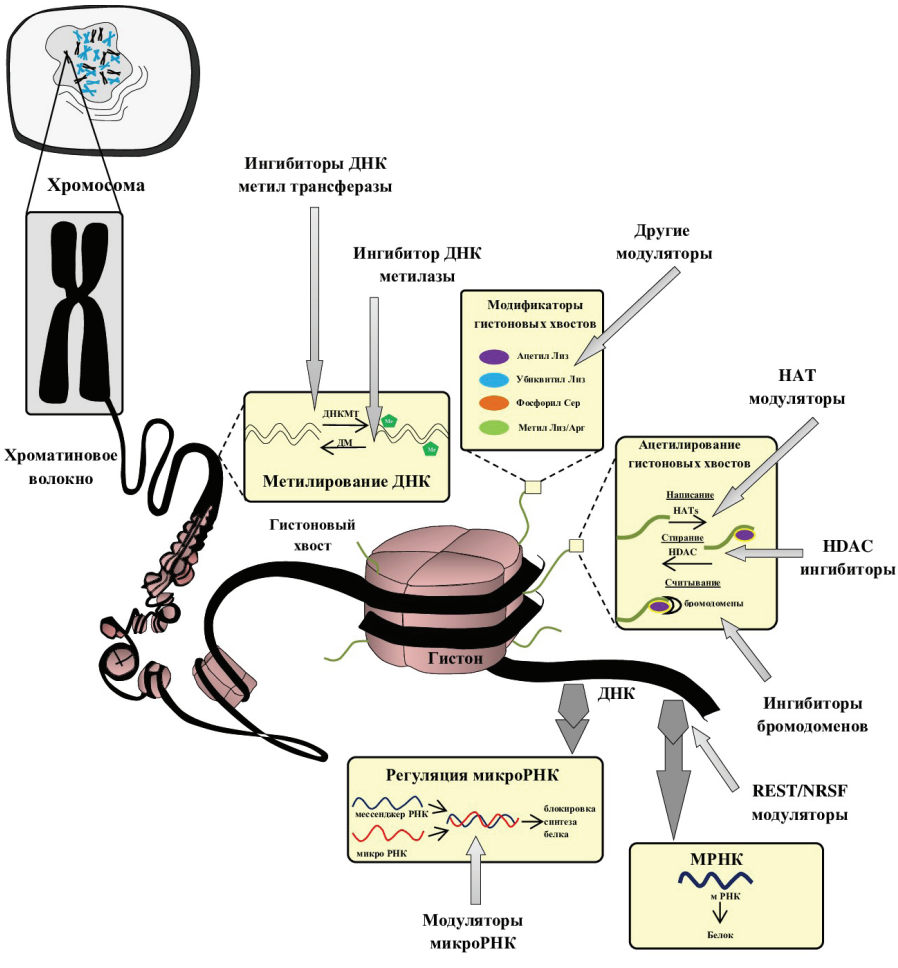

Fig. 2. Micro-RNA and their targets in epileptogenesis

gration of newly differentiated neurons, for example, in the dentate gyrus of the hippocampus. These changes are traced in models of epilepsy and in patients with mediobasal temporal lobe epilepsy [3, 4, 54] (Fig. 3).

Figure 3 – microRNAs and their targets in epileptogenesis. This figure summarise examples of microRNAs and their mRNA targets and pathways associated with key processes in epileptogenesis). Figure has been adaptated from the article of Brennan G.P. et al. 2018 [14].

In an animal sample of epilepsy and in patients, it was shown that microRNAs are differentially expressed in the brain, and functional studies have shown their role in modulating the susceptibility and severity of epileptic seizures [14, 30, 53]. The main microRNA-regulated pathogenetic pathways of epilepsy are: control of the cell cycle; ion channel modification; tissue remodeling and neural plasticity; regulation of transcription and gene expression; neuroinflammation; apoptosis; the emergence of stem cells (stemness). The immune system, cell cycle, apoptosis as well as neurotrophin signaling pathway were identified as main and most inriched pathways [15].

Neuroinflammation. The importance of the adaptive and innate immune system is increasingly recognized in the pathogenesis and constant maintenance of the epileptic state. MicroRNAs have become potent regulators of inflammation, targeting components of both innate and adaptive immune responses. Thus, it was shown that miRNA-132 has an anti-inflammatory effect by acting on acetylcholinesterase and multiple miRNAs involved in the regulation of the transcription factor NF-kB and other pathways [33].

The first inflammation-related miRNA that was identified in epilepsy was miRNA-146a, which regulates the expression of toll-like receptors (TLRs) and cytokine signaling pathways [33]. Toll-like receptors (TLRs) have been identified as direct targets for microRNAs in the brain. It has been shown that TLR4 is activated after experimentally induced seizures in mice. TLR4 levels are partially controlled by microRNAs, including let-7i. Apparently, the ligand for TLR4 is the HMGB1 protein, which is thought to be released from damaged neurons upon stimulation of seizures. Thus, RNA-sensitive TLRs are expressed in the brain both in glia and in neurons. MicroRNA let-7b expressed in the brain can activate TLR-7 to promote neuronal death. Evidence that this is a relevant mechanism *in vivo* has been presented in an intrathecal model of neurodegeneration [15].

It was also shown that interleukin 1β regulates the expression of microRNA - 146a in cultures of human astrocytes. There is an assumption that the immune system is not only regulated by microRNA, but can regulate microRNA itself [23]. It was revealed that in the normal brain microRNA-146a is expressed by neurons, not glia. After the status epilepticus, hippocampal levels of microRNA-146a were increased both in rats of different ages and in the resected hippocampus in adults and children with drug-resistant temporal lobe epilepsy. An increased level of miRNA-146a was present in neurons and astrocytes, but not in microglia, which indicates the specificity of the cell types producing this microRNA. However, these studies involved patients with hippocampal sclerosis, so it is un-

clear whether microRNA-146a increases in epilepsy without this pathology. The mechanism of an increase in the level of microRNA can be mediated through IL-1, whereas TNFα does not stimulate the expression of microRNA-146a at all [56

On the other hand, microRNA-15b-5p has been shown to be specifically activated in the cerebrospinal fluid of patients with Alzheimer's disease [11]. In epilepsy, it was found that this miRNA is suppressed, which suggests a possible loss of control over the ASM enzyme and leads to the conversion of sphingomyelin into a proinflammatory and proapoptotic ceramide [57]. Studies have also shown that microRNA-106b is involved in AD-associated inflammation in epilepsy [22], and miRNA-451 is involved in maintaining the inflammatory status in several brain pathologies. Additionally, some works describe the activation of microRNA-451 in temporal lobe epilepsy [23] and its connection with the inflammation in the brain [44].

Cell cycle. Another target for microRNA in epileptogenesis is neurogenesis, control of the cell cycle and cell proliferation, called "cell cycle". This pathway includes all those genes that are involved in the proliferation and differentiation of neuronal cells, which are controlled by microRNA-15a-5p, microRNA-34a, microRNA-106b-5p, and microRNA-146. Thus, in epilepsy, the observed suppression of microRNA-15a-5p can lead to a decrease in its control activity on targets, including the ubiquitin ligase FBXW7, which destabilizes Cyclin E and leads to the blockage of the cell cycle in the S-phase. This may partially reflect the inhibition of neurogenesis, which, with a parallel increase in neuronal apoptosis, leads to the loss of neurons observed in patients with epilepsy [57].

It has been demonstrated recently that the microRNA-34/449 family is a key regulator of mitotic spindle orientation during cerebral cortex development. In addition to this, members of the microRNA-34 family are the most activated microRNAs in differentiated neurons. They play a role in controlling the cell cycle and blocking apoptosis, suggesting that the observed suppression of miR-34a in epilepsy may lead to the termination of the cell cycle, activation of apoptosis, and consequently to the neurons' loss. What is more, in a rat sample of epilepsy induced by electrical stimulation, it was shown that microRNA -106b-5p is activated at an early stage of epilepsy development, which indicates a potential role of this microRNA in the induction of neuronal cell cycle termination and neuronal apoptosis [44].

Apoptosis. Apoptosis includes apoptosis-associated genes and pathways involved in pro- or anti-apoptotic signaling, which are confirmed targets for microRNA-15a-5p (downregulated), microRNA-106b-5p, microRNA-146 and microRNA-451 [31].

MiRNA-15a-5p has been as a modulator of ischemia in the human brain: the level of the miR-15a / 16 cluster usually increases after cerebral ischemia. It has been observed that antagomir treatment or genetic loss of this microRNA cluster is capable of inducing the activation of anti-apoptotic proteins (such as Bcl2 and Bcl-w) and suppressing pro-inflammatory molecules. It is possible that the observed suppression of miRNA-15a-5p in epilepsy mainly works due to the effect of miRNA-15a-5p decrease on the modulation of neuroinflammatory cytokines [59].

MicroRNA-106b-5p regulates caspase 6 (CASP6) and MAPK-binding protein 1 (MAPKBP1) (inflammation and apoptosis of neurons). Status epilepticus induces the expression and activation of CASP6 in the rat hippocampus, which leads to neuronal apoptosis in various models of epilepsy [50].

Upregulation of microRNA-146 has been found in several models of epilepsy and probably also plays a role in the regulation of neuronal apoptosis [57] as it reduces proliferation and promotes apoptosis in other pathologies. For instance, activation of microRNA-451a in the cerebrospinal fluid was associated with several pathologies of the central nervous system. Regarding the role of microRNA-451, it is known that this microRNA is able to control the AMPK-mTOR pathway. The increase in microRNA-451 observed in patients with epilepsy may modulate autophagy and neuronal loss that is observed in the brain after cerebral ischemia [58].

Synaptic structures & functions. MicroRNA-134 is constitutively expressed in the adult brain in neurons and is found in the body of the neuron as well as in their dendrites [15]. It was found that overexpression of miR-134 in neurons *in vitro* reduces the volume of the neuronal dendritic process, whereas the inhibition of miR-134 leads to a slight increase in its volume [45]. The mechanism of these changes was determined by local directed translation of miRNA-134 of the Lim domain containing kinase 1 (Limk1) inside the dendrites. Limk1 phosphorylates and inhibits actin depolymerization factor (ADF / cofilin), thereby promoting the formation of F-actin, which is critical for enlargement of the dendrite (and induction of long-term depression). By inhibiting Limk1, microRNA-134 promotes process collapse by increasing G-actin in neuronal processes. It is noteworthy that the dendritic phenotype of miRNA-134 overexpression is similar to that in Limk1 mice. The relationship between the volume of the dendritic process and excitatory synaptic strength may implicate hyperexcitability pathology such as epilepsy. Overexpression of microRNA-134 *in vivo* using viral vectors led to a small but still significant decrease in the complexity of basal dendrites in pyramidal neurons of MicroRNA-134 is constitutively expressed in the adult brain in neurons and is found in the body of the neuron, as well as in dendrites. It was found that overexpression of miR-134 in neurons in vitro reduces the volume of the neuronal dendritic process, while inhibition of miR-134 leads to a slight increase in its volume. The mechanism of these changes was determined by local directed translation of miRNA-134 of the Lim domain containing kinase 1 (Limk1) inside the dendrites. Limk1 phosphorylates and inhibits actin depolymerization factor (ADF / cofilin), thereby promoting the formation of F-actin, which is critical for enlargement of the appendix (and induction of long-term depression). By inhibiting Limk1, microRNA-134 promotes process collapse by increasing G-actin in neuronal processes. It is noteworthy that the dendritic phenotype of miRNA-134 overexpression is similar to that in Limk1 mice. Given the relationship between the volume of the dendritic process and excitatory synaptic strength, this has obvious implications for hyperexcitability pathology such as epilepsy. Overexpression of microRNA-134 in vivo using viral vectors led to a small but significant decrease in the complexity of basal dendrites in pyramidal neurons of layer V of the cerebral cortex. Since then, other miRNA-134 targets have been identified, including the Pum2 RNA-binding protein, CREB, and DCX. Thus, miR-134 is a potentially important regulator of brain development and synaptic plasticity V layer of the cerebral cortex. Since then, other miRNA-134 targets have been identified, including the Pum2 RNA-binding protein, CREB, and DCX. Thus, miR-134 is a potentially important regulator of brain development and synaptic plasticity [15].

The initial studies have shown that neuronal depolarization causes a significant increase in neuronal miR-134 levels, but it is not known whether increased neuronal activity *in vivo* affects miR-134 expression. Expression profiling studies have identified miR-134 among activated miRNAs in mice and rat models of status

epilepticus. Detailed studies showed that miR-134 induction occurred in areas of the hippocampus that were damaged by seizures, as well as in less damaged populations of neurons. The increase in miR-134 was accompanied by a decrease in the protein level in both Limk1 and CREB, which suggests that they may be targets *in vivo*. Seizures also increased miR-134 levels, implying functional absorption. MiR-134 levels were also increased in the hippocampus of epileptic mice and in the surgically obtained temporal neocortex from patients with drug-resistant epilepsy. Thus, miR-134 activation appears to be a general response to pathological brain activity. However, inhibition of miR-134 can affect other targets when it is used *in vivo*. Although the functional significance of dendrite alteration is unknown, temporary contractions of dendritic spines are belived to unleash NMDA-dependent signaling and create protection against excitotoxic damage. Consistent with this hypothesis, the mice in which miR-134 was silent were highly seizure-resistant in the kainic acid model of status epilepticus, experiencing less than 50% of normal seizures and significantly reducing hippocampal damage. Antagomirs of miR-134 also prevented the toxicity of kainic acid *in vitro*. These results showed for the first time that targeting one microRNA (provided that antagomir only affects this microRNA) can reduce pathological brain activity *in vivo* [15].

Conclusion. These studies have significantly expanded the number of microRNAs with a potential role in epileptogenesis and improved our understanding of their practical use. The crucial test for miRs clinical translation will be an evidence of whether treatment can affect or reverse epilepsy in epileptogenic tissue. The results of the presented studies are an abundant source of new targets for microRNAs, but significant problems are about to solve before their role in the pathogenesis, diagnosis, and treatment of epilepsy can be used in clinical practice. There is currently insufficient evidence that miRNAs have multiple targets in patients with epilepsy. Moreover, antiepileptic drugs may affect microRNA levels in the brain, which requires more research.

Although the data are promising, detailed microRNA validation will be essential for the clinical use of these biomarkers.

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GENETIC ASPECTS OF THE REGULATION OF CIRCADIAN RHYTHMS AFFECTING STRESS RESISTANCE

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The article presents the results of the analysis of the literature data on the molecular genetic mechanisms of control and regulation of circadian rhythms that determine the chronotype of a person.

It has been established that disturbance of circadian rhythms arising as a result of psychoemotional and physical stress expressed in degree and / or duration, can lead to a decrease in the body's resistance to stress factors with the subsequent development of maladjustment processes, which in turn can be a trigger mechanism for the onset of the development of such pathological conditions as diseases of the cardiovascular system, gastrointestinal tract, autoimmune, mental, neurodegenerative, oncological and other diseases.

According to the results of numerous studies, the molecular genetic control of circadian rhythms consists of interacting positive and negative feedbacks of regulatory loops of clock genes, such as *genes Bmal1, Clock, Per1, Per2, Per3, TIM, Npas2, Cry1 and Cry2, Csnk1d, Csnk1e, Rev-erba, Rora, Bhlhe40, Bhlhe41*, as well as genes *FBXL3, FTO, MADD, CYP2A6, ARNTL, Mel1a, Mel1b and GPR50*. The transcription factors encoded by these genes, enzymes, transporters, prohormones, signaling and other proteins are involved in regulation of daily frequency.

Despite the fact that the main components of the molecular genetic mechanisms of circadian rhythms are already known, the issue of regulating their work remains relevant today. The results of studies carried out on different populations with respect to individual polymorphic variants of genes are not always unambiguous and require a more detailed study, since it is the result of the interaction of genes that can determine their phenotypic effect.

Thus, understanding the molecular mechanisms and identifying genetic markers that cause disturbances in circadian rhythms is an important step in the development of methods aimed at preventing and correcting pathological conditions caused by maladjustment processes.

Keywords: adaptation, stress factors, circadian rhythm, clock genes.

Reforms of the socio-economic system, actively taking place in the world in recent decades, lead to the renewal of conditions and principles of organizing human life. Life in conditions of a huge flow of information and fierce competition has become the norm, a modern person lives and works in a "round-the-clock readiness" mode, which requires intense and well-functioning work from the internal systems of the body, the failure of which can cause a violation of adaptation processes and, as a result, the development of stress. In this regard, the problem of resistance to stress is currently considered one of the most serious and increasingly important for public health.

According to G. Selye's definition "stress or general adaptation syndrome is a set of nonspecific adaptive neurohumoral reactions that arise in response to exposure to adverse factors (stressors) that are significant in strength and duration, and body systems that counteract extreme stimuli causing stress are aimed at maintaining constancy the internal environment of the body - homeostasis" [16]. Today it is already known that the biological meaning of these processes at the early stages of the development of a stress reaction is aimed at maintaining the functions of vital organs and systems by increasing the availability of energy resources, regulating regional blood flow, activating enzymes of cellular metabolism and other factors of biological adaptation. In other words, the cascade of neurohumoral and metabolic events arising in response to the action of stressors is designed to provide an urgent and then long-term adaptation to new environmental requirements. At the same time, the same adaptive reactions, reaching a certain intensity, can acquire a damaging character and be included in almost any pathological process. According to the results of numerous studies, it has been shown that stress caused by excessive exposure to irritating factors can be a triggering mechanism in the development of such pathological conditions as diseases of the cardiovascular system, gastrointestinal tract, autoimmune, mental, neurodegenerative, oncological and other diseases [9, 11, 18]. That is why, modern biomedical science pays more and more attention to the pathogenesis mechanisms of the development of diseases, namely, the study of the possibilities of adaptive potential. Studies have shown that nonspecific characteristics of influences are of decisive importance in the manifestation of the body's response to a particular stimulus: the relative strength or intensity of the acting stimulus; relative amplitude-time dynamic characteristics of the stimulus (duration of exposure, rate of rise, reaching a "plateau", rate of decline, total dose); preferential localization of the application of the impact (central nervous system, respiratory tract, gastrointestinal tract, skin and others). Depending on these characteristics of the impacts, a systemic response of the organism is formed, which is imprinted by its individual characteristics caused by genetic factors [3]. According to the concept of systemic (neuroendocrine) regulation of genetic processes and the implementation of genetic information, it is the nervous system that triggers and connects the components of the stress response at all levels, up to the genome of nerve cells and cells of peripheral organs and tissues "according to the feedback principle in accordance with the requirements of the environment, current the needs of the body and its individual experience" [2]. The genetic component of the organismic stress response includes both regulatory changes within the normal range of the target cell genotype response and structural changes outside of it. The study of the stress response in humans and animals has shown that in addition to the changes that occur in the body during acute stress exposure, there are delayed effects of stress caused by long-term changes in the functioning of the neuro-endocrine-immune system, suggesting long-term modification of gene activity. The continuous extreme action of the stressor can lead to depletion of the reserves of responding cells and induce the restructuring of the genetic material, up to its partial or complete disintegration in case of impossibility of adaptation to external conditions, it is this mechanism that underlies the formation of stress-de-

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pendent pathology [15]. Thus, taking into account the rhythm of work of a modern person in conditions of high psychoemotional and physical stress, due to the frequent change of time zones when flying on airplanes, the leveling of time frames associated with the need and ability to work via the Internet at any time of the day, the problem of violation of circadian rhythms as the risk factor for the development of pathological conditions is most relevant today.

According to the definition of the British neurobiologist, specialist in circadian rhythms Russell Foster, "circadian rhythms" are internal biological rhythms of the body with a period of about 24 hours. They adjust all physiological processes of the body in accordance with the daily rotation of the Earth and are present in almost all living organisms on the planet, including bacteria. In humans, the main circadian rhythm is the cycle of sleep and wakefulness, but at the same time, the influence of circadian rhythms on the body is not limited only to sleep and waking up, they affect the work of hormones, heart, digestion, immunity, and even body temperature and much more. The regulation of circadian rhythms is carried out by means of a "biological clock" consisting of a large number (about 20 thousand) of nerve cells located in the hypothalamic part of the brain, called the suprachiasmatic nucleus. From the cells of the retina, the suprachiasmatic nucleus receives information about the light, adjusts the neurons in it, which send signals that coordinate the work of all other processes in the body. In other words, the work of the circadian rhythms must coincide with the work of the biological clock, and they, in turn, with the signals of the environment. However, this system does not work well for all people, many are faced with such a phenomenon as "jetlag" which occurs as a result of a person's rhythm mismatch with the daytime rhythm, due to night work or a quick change of time zones. To a large extent, the individual variability of adaptive capabilities is associated with genetic characteristics that form the chorotypes of a person [5, 14, 19].

The first assumption about the existence of biological clock genes was made by the english biorhythmologist Colin Pittendrigh. Guided by the results of studies carried out in the 1960s, he formulated the main provisions of biorhythmology, according to which circadian rhythms are independent oscillations of endogenous origin and have autonomy [12]. Several years later, in 1971, Ron Konopka and Seymour Benzer's experiments with the fruit fly Drozophila malanogaster provided convincing evidence of the genetic nature of circadian rhythms. In their experiments, they found 3 different mutations in the X chromosome region associated with deviations in the periodicity of circadian rhythms and designated the gene responsible for this as *Per* (from the engl. *period*) encoding the Per protein [8]. *Clork* - circadian rhythm gene was discovered later in 1990. A little later, Jeffrey Hall and Michael Rosbash suggested that the protein of the same name encoded by the PER gene blocks the work of its own gene, and such a feedback loop allows the protein to prevent its own synthesis and cyclically, continuously regulate its level in cells, but for this the protein needs to get into the cell nucleus. where the genetic material is stored (Fig. 1). In their experiments, they showed that the PER protein accumulates in the nucleus overnight, but how it gets there remained a mystery until in 1994 Michael Young discovered another "clock gene" of the circadian rhythm, *Timeless*, encoding the TIM protein necessary for normal circadian rhythm. Michael Young showed that when the TIM protein is bound to the *PER* protein, both proteins can enter the cell nucleus, where they block the activity of the Per gene, thus closing an inhibitory feedback loop (Figure 2). After some time, he identified another gene and named it *Doubletime*, his eponymous protein DBT, was able to delay the accumulation of the PER protein. Subsequently, these results were confirmed by other scientists, it was found that the molecular clockwork consists of not one, but at least nine interacting positive and negative feedbacks of the regulating loops of circadian clock genes. These include the *Bmal1* and *Clock* genes, which form heterodimers and trigger transcription; period transcription factor genes (*Per1, Per2, Per3); Timeless* gene (*TIM*); the *Npas2* gene; genes of proteins of cryptochromes 1 and 2 (*Cry1 and Cry2*) involved in the process of light capture; casein kinase genes (*Csnk1d, Csnk1e*), as well as *Rev-erbα, Rora, Bhlhe40 and Bhlhe41* genes. Transcription factors encoded by these genes, enzymes, transporters, prohormones, signaling and other proteins are involved in the regulation of the diurnal periodicity [7,10]. The key role of the regulator of the circadian mechanism today is assigned to genes associated with melatonin metabolism. Two of them encode the enzymes arylalkylamine-N-acetyltransferase (AANAT) and hydroxyindole-o-methyltransferase (ASMT), which are responsible for the formation of melatonin from serotonin. The genes *Mel 1a, Mel 1b* and *GPR50* provide the synthesis of melatonin receptor proteins located on the surface of the cell membranes of the suprachiasmatic nucleus of the hypothalamus, hippocampus, cerebral cortex and cerebellum. The interaction of melatonin with these receptors activates the signaling systems of the cell and the synthesis of secondary messengers of cyclic adenosine monophosphate (cAMP), a change in the concentration of calcium ions [19]. The expression of genes *Mel 1* is found in the coronary arteries, *Mel 2* - in the aorta, left ventricle, coronary arteries of healthy people and patients with coronary artery disease [6]. It is assumed that these receptors provide the vasodilating effect of melatonin and the circadian rhythm of hemodynamics, making the connection between the melatoninergic system and the suprachiasmatic nucleus of the hypothalamus. Melatonin is also able to bind to receptor proteins on the surface of the nucleus and act at the chromatin level, directly affecting protein synthesis. The genes *Ror α, Ror β, Ror γ*, found in various organs and tissues, including the suprachiasmatic nucleus of the hypothalamus, the retina and the pineal gland, encode proteins of nuclear receptors (the so-called orphan nuclear retinoid receptors Ror / Rzr), in relation to which melatonin acts in the role of the ligand [13]. In addition, the effect of melatonin on the expression of some mitochondrial genes, as well as genes that control the cell cycle, adhesion and transport, cell proliferation and apoptosis, was noted, and a direct link between melatonin and genes related to oncogenesis was revealed [1]. Given the cyclical nature of gene expression due to the synchronization of the central regulator of the circadian rhythm of the suparchiasmatic nucleus with light information, the negative effect of artificial lighting cannot be underestimated as lamps, TV screens, computer monitors and telephones, which are essentially light “pollutants”, significantly increasing the proportion of people experiencing chronic lack of sleep. In experiments on rats, it was found that constant illumination increases the threshold of sensitivity of the hypothalamus to the inhibitory effect of estrogens, and this is a key mechanism in the aging of the reproductive system in female rats. Similar results have been obtained for women, it has been proven that the influence of light at night leads to the development of dysmenorrhea [4, 17].

In this regard, today no one doubts that the regulation of circadian rhythms is carried out at a well-systematized and

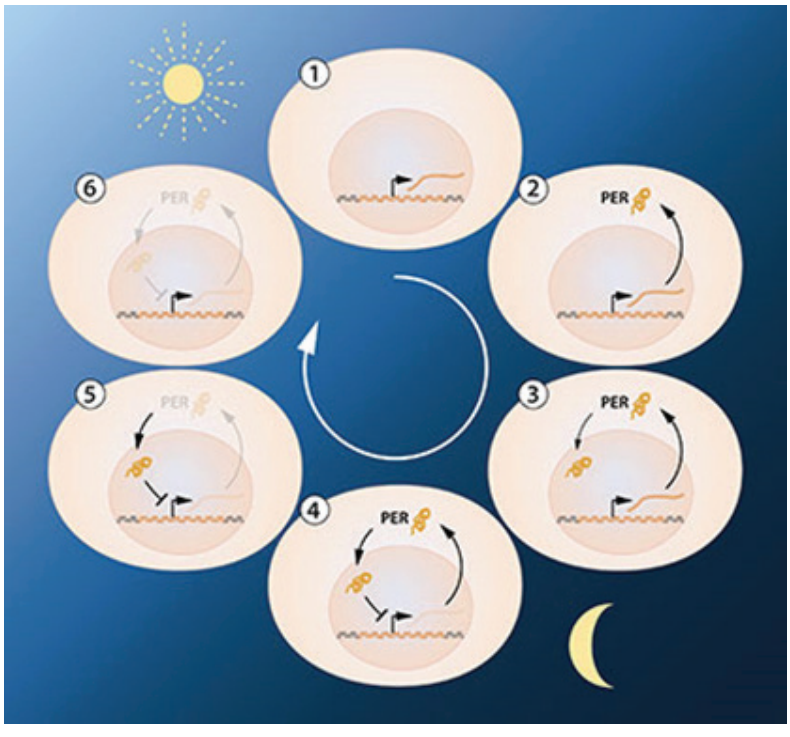

Fig. 1. Scheme of the PER gene according to the principle of "feedback" showing the sequence of events in 24 hours
Note. 1. The PER gene is active, its mRNA is produced; 2, 3. mRNA of the PER gene leaves the cell nucleus into the cytoplasm, becoming a matrix for the production of the PER protein; 4. PER protein accumulates in the cell nucleus; 5. The activity of the PER gene decreases; 6. The work of the PER gene is blocked, a feedback loop is closed.

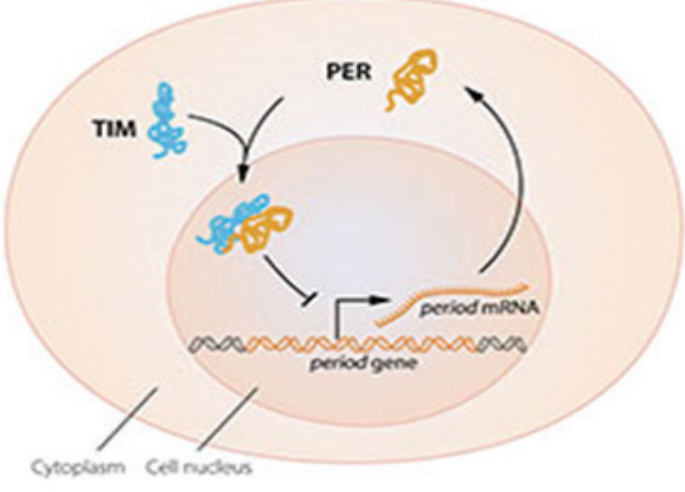

Fig. 2. Molecular mechanism of circadian rhythms by means of communication between PER and TIM proteins.

ordered molecular genetic level, and its violation entails negative consequences. Insufficient level of wakefulness, drowsiness and accompanying attention deficit, rapid fatigue and chronic fatigue are important risk factors for the development of disorders of the body's adaptation, which in turn can become a trigger mechanism of the disease.

Thus, understanding the molecular mechanisms and determining the genetic markers associated with the chronotype of individuals is an important step for the development of methods aimed at preventing and correcting pathological conditions caused by maladjustment processes.

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POINT OF VIEW

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ANALYSIS OF LIFE EXPECTANCY OF POPULATION OF THE IRKUTSK REGION

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The purpose of the research is to analyze life expectancy (LE) of the population of the Irkutsk region and to identify factors affecting the indicator at the municipal level.

Materials and methods of research. The data were used for LE of the Irkutsk region and the Russian Federation for 1990-2019, municipalities of the region for 2018; methods of descriptive statistics, correlation and regression analysis, criterion *W* of the Shapiro – Wilk test to check the normal distribution of 42 indicators.

Results and discussion. In the Irkutsk region, LE of the population increased from 66.5 years in 1990 to 69.60 years in 2019. The difference in LE in the region was lower than the national average by 3.8 years, for men and women - by 4.2 and 2.9 years. In 2018, the following characteristics of LE were recorded for the municipalities of the region: 1) maximum - 75.8 years; minimum - 58.3; 2) 18 municipalities with LE of men below 60 years old; 3) the amplitude of LE of men was 20.3 years, women - 14.1 years; 4) the maximum gender difference is 18.1 years, the minimum is 6.6 years. Due to the uneven development of the territories of the Irkutsk region, the indicators had high variability. This made it difficult to select them to study the influence of factors on LE at the municipal level. The resulting multiple regression models include: general mortality rate (LE of both sexes, men, women); mortality from diseases of the circulatory system (LE of both sexes and men), total fertility rate (LE of women); provision of hospital beds (LE of both sexes, men), provision of paramedical personnel (LE of women).

Conclusion: Irkutsk region in terms of LE is one of the last places among the subjects of the Russian Federation. 70% of the main measures to increase LE are included in state programs for the development of the region, but unsolved problems of socio-economic development impede their effective implementation. According to the results of the correlation and regression analysis, the links between LE at the municipal level and the general indicators of fertility and mortality, mortality from diseases of the circulatory system, provision of hospital beds and paramedical personnel were established. To achieve LE of 80 years in 2030, a differentiated socio-demographic policy is required for individual municipalities of the region.

Keywords: life expectancy, region, municipal level, socio-economic indicators, correlation and regression analysis

Introduction. Life expectancy (LE) of the population is an integral indicator of the state of health, quality of life of the population, an assessment of the level of socio-economic well-being of the state, and is used to calculate the human development index [3, 10, 13]. Despite the gradual growth, LE in the Russian Federation remains quite low, as well as lagging behind developed countries, including by gender gap, interregional variation, difference for urban and rural population [3, 6, 8-10, 13, 15, 23]. Efforts of all levels of government are required to increase LE with significant geographical differences [17, 19, 20]. Due to the special significance of the indicator, it is important to monitor trends and find out the reasons for the decline and stagnation [21, 25].

The purpose of the research is to analyze LE of the population of the Irkutsk region and to identify factors affecting the indicator at the municipal level.

Materials and methods of research. The Rosstat data on life expectancy of the population of the Irkutsk region and the Russian Federation for 1990-2019 have been applied; morbidity, mortality, socio-economic indicators for 42 municipalities for 2018. Life expectancy indicators for medical organizations for 2018 were calculated on the basis of the age and sex composition of the resident population of the Irkutsk region as of 01.01.2019 and the number of deaths from tables C51 «Distribution of deaths by sex, age groups and causes of death». The ranking of the subjects of the Russian Federation to determine the rating was carried out in descending order of the indicator.

Statistical analysis of the data was carried out using Microsoft Excel 10 and the Shapiro-Wilk Test calculator (https://www.statskingdom.com/320ShapiroWilk.html). Checking the correspondence of the distribution of variables to the law of normal distribution was carried out using the Shapiro-Wilk test (W). Descriptive statistics were calculated, an analysis of the variability of variables by coefficients of variation (Cv), Pearson correlation analysis, multiple regression analysis using the method of successive exclusion of variables was carried out. Before building regression models, the applicability of the multiple regression method was tested. The statistical significance of the constructed model was assessed using Fisher's F-test. The quality of fitting a linear function was assessed by the multiple determination coefficient (R2). The

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critical value of the level of significance when testing statistical hypotheses was taken equal to 5%.

Results and discussion. In the Irkutsk Region, the LE of the population decreased from 66.5 years in 1990 to 60.0 years in 1994, then the indicator increased and reached its maximum value in 2019 - 69.6 years. During 1990-2019 life expectancy indicators of the population of the region were below the national indicators by an average of 3.8 years. According to the three forecast variants of Rosstat, in 2030 the LE of the population of the Irkutsk region will be: 77.6 years (high option), 74.8 years (middling) or 71.3 years (low option) (Fig. 1).

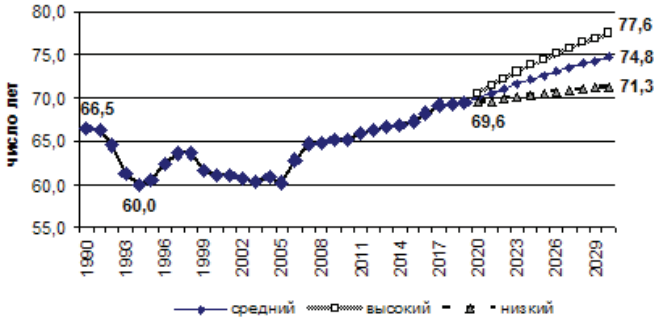

Fig.1. The dynamics of life expectancy of the population of the Irkutsk region for 1990-2019 and forecast for 2020-2030 in three options (number of years)

For the study period, the minimum values of LE were recorded in men of the Irkutsk region in 2005 (53.3 years), women in 1994 (68.1 years). In 2018, the LE of the population of the Irkutsk region was 69.3 years, for men and women - 63.3 and 75.2 years, respectively. According to the medium version of the forecast, the LE of women in the Irkutsk region will reach 79.6 years by 2030; men - 69.8 years (Table 1).

In 2018, according to the LE of the population, the Irkutsk region occupied the 80th place among 85 constituent entities of the Russian Federation (2019 the 80th); 83rd - for the LE of men, 79th - for LE of women. The Irkutsk region was assigned to the "catching up" group of regions with LE below the average for Russia and a relatively low standard of living [14].

The difference in LE of the population of the Irkutsk region with all-Russian values during 1990-2018. fluctuated: for men from 2.8 to 5.6 years (on average for 4.2 years), for women from 2.1 to 4.2 years (on average for 2.9 years); in 2018, the difference was 3.6 years, for men and women 4.4 and 2.6 years. In 2030, the difference in LE of the population of the Irkutsk region and the Russian Federation, according to all forecast variants, will be 2.2-3.5 years, for men and women - 2.9-4.2 and 1.4-2.6 years, respectively (with a low version - 3.5; 4.2 and 2.6 years).

Zaitseva N.V. et al. [5] note that «for the Russian Federation, the regional aspect of the demographic issue is particularly relevant, reflecting significant differences both in socio-economic indicators and in the indicator of LE».

In 2018, the following LE characteristics were recorded for the municipalities of the Irkutsk region: 1) the maximum age in the Irkutsk municipal district (75.8 years); the minimum age in Katanga municipal district (58.3 years); 2) 18 municipalities with LE of men below 60 years; 3) the amplitude of the LE of men was 20.3 years, for women - 14.1 years (Table 2); 4) the maximum gender difference in the Bodaibo municipal district (18.1 years), the minimum - in Bayandaevsky municipal district (6.6).

The contemporary studies have found that life expectancy is influenced by a variety of factors: heredity; natural ecological; level of development and health system services; macroeconomic, socio-economic; Lifestyle; socio-psychological, racial / ethnic, behavioral, metabolic and other risk factors [3, 4, 6-8, 10, 13, 14, 17-20].

Difficulties in selecting indicators for the correlation and regression analysis are associated with the lack of information at the municipal level and the high variability of available data. According to the results of descriptive statistics, half of the socio-economic indicators of the municipalities of the Irkutsk region were excluded for further analysis due to a high coefficient of variation: investment in fixed assets per capita – 477 %; the introduction of living space for 1 person. - 213%; emissions, kg / person - 175%; migration increase / decrease - 150%; unemployment rate - 60%, indicators of home improvement - from 54 to 60%; education expenses - 49%, etc. Despite the significance of such a factor as health care costs [8, 10, 19, 24], the latter was not included in the analysis due to the high coefficient of variation (41%), right asymmetry, and the presence of three pop-up values. 17 analyzed parameters were rejected that did not have a normal distribution according to the criteria of the W Shapiro – Wilk test. All of the above allows us to conclude about the extremely uneven development of the municipalities of the region.

According to the results of the correlation analysis, the aggregate linear dependence of the average strength between **the LE of the population** and the two studied factors with the correlation coefficient of 0.67 was established. As a result of applying the selection of factors, a linear model of

Table 1

Life expectancy of the population of the Irkutsk region for 1990-2018 and forecast for 2030 (number of years)

Indicators and forecast options	both sexes	men	women
for 1990-2018			
minimum	60.0	53.3	68.6
maximum	69.3	63.3	75.2
M (SD)	64.1 (2.9)	57.7 (3.2)	71.0 (2.1)
forecast for 2030			
low	71.3	66.1	76.6
average	74.8	69.8	79.6
high	77.6	73.2	81.7

Table 2

Life expectancy of the population of municipalities of the Irkutsk region in 2018 (number of years)

Indicators	both sexes	men	women
minimum	58.3	51.9	66.1
maximum	75.8	72.2	80.2
M (SD)	66.6 (3.6)	60.4 (3.9)	72.9 (3.2)

multiple regression was constructed:

$y_1 = 83{,}833 – 1{,}537\ x_2 – 0{,}007\ x_{21}$,

where y_1 - life expectancy (both sexes), the number of years; x_2 - total mortality rate per 1000 people; x_{21} - mortality rate from diseases of the circulatory system per 100 thousand. All factors are statistically significant with a probability of 0.95. The multiple correlation coefficient confirms the feasibility of including the above factors in this model (R = 0.82). Determination coefficient R2 = 0.67. The average approximation error is 2.56%. The F-stat value of 39.74 indicates the statistical significance of the model as a whole. A similar linear model of multiple regression was obtained for **the LE of men**:

$y_2 = 78{,}822 – 1{,}665\ x_2 – 0{,}009\ x_{21}$.

The model of multiple regression about the influence of factors on **the LE of women** includes two factors:

$y_3 = 93{,}098 – 0{,}383\ x_1 – 1{,}036\ x_2$,

where y_3 is the expected duration of women, the number of years; x_1 - total fertility rate per 1000 people; x_2 - total mortality rate per 1000 people. The multiple correlation coefficient is 0.81; coefficient of determination - 0.66; F- statistics - 38.53; the average approximation error is 2.05%, factors are significant with a probability of 95%. Thus, the expected result of the association of LE and the overall mortality rate [7], as well as LE of men and the whole population and mortality from diseases of the circulatory system, was obtained. Often, the causes of a decrease in LE include external causes of death: homicide, suicide, drug overdose, etc. [5, 16, 21, 25].

It was found that among the factors describing the availability of medical care, the **LE of the population** is affected only by the availability of beds (R = 0.65); the coefficient of determination of R2 is 0.42. The multiple regression model is obtained:

$y_1 = 74{,}752 – 0{,}119\ x_{12}$,

where life expectancy (both sexes), a number of age; x_{12} - hospital bed provision per 10 thousand population. The indicator «hospital bed provision» x_{12}, included in the regression model, and the model itself are statistically significant. Therefore, there is a connection between LE of the whole population of the Irkutsk region and the hospital bed provision for the population. A similar linear model of multiple regression was obtained for **the LE of men**:

$y_2 = 69{,}446 – 0{,}133\ x_{12}$.

The linear model of multiple regression **of LE of women** is represented by the following equation:

$y_3 = 79{,}141 – 0{,}132\ x_{11} – 0{,}053\ x_{12}$,

where y_3 is the LE of women, the number of years; x_{11} - availability of paramedical personnel for 10 thousand; x_{12} - hospital bed provision per 10 thousand. All factors are statistically significant with a probability of 0.95. The multiple correlation coefficient confirms the feasibility of including the above factors in this model (R = 0.48). The determination coefficient R2 = 0.23. The average approximation error is 3.31%. The F-stat value of 4.97 indicates the statistical significance of the model as a whole.

Conclusion: At present, the LE of the population of the Irkutsk region is one of the lowest in the Russian Federation - 69.6 years (80th place). Despite the fact that about 70% of the main directions for implementing measures to increase LE were included in state programs for the development of the region, the presence of unsolved problems of socio-economic development impedes the success of the measures taken [14]. Irkutsk region in 2030 will not reach the target of 2024 (78 years), even according to the high version of the forecast. According to the results of the correlation and regression analysis, the links of LE of the population of the municipalities of the Irkutsk region with the general indicators of fertility and mortality, mortality from diseases of the circulatory system, hospital bed provision and availability of paramedical personnel were established.

To achieve the indicator of LE of 80 years by 2030 [8-10, 12], a scientifically sound state and regional socio-demographic policy, investments, time, and differentiated decisions for individual territories are required [1, 3, 13]; a significant increase in government spending on the maintenance and development of the health system, including the municipal level, the effective use of financial resources to ensure the availability of medical care to the population. Therefore, the socio-economic well-being of the population is considered as an important factor affecting health and longevity, and as the main measure of impact in the system of managerial decisions [17, 24].

In recent years, much attention has been paid to such important socio-economic measures to increase LE, such as reducing poverty, crime, improving housing conditions and improving housing, organizing safe working conditions, recreation, achieving sanitary and epidemiological well-being, public health education, and the development of specialized medical care et al. [14]. But reserves for increasing LE remain, therefore, the use and activation of all reserves is an important condition for the further growth of LE of the population [9].

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CLINICAL CASE

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DIFFICULTIES IN DIAGNOSING MULTISYSTEM ATROPHY AT EARLY STAGES (CLINICAL OBSERVATIONS)

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Multisystem atrophy (MCA) is a rare neurodegenerative disease characterized by a predominant lesion of the basal ganglia, nuclei of the brain stem, cerebellar systems, autonomic neurons of the trunk and spinal cord. Clinically, the disease is manifested by a combination of parkinsonism with autonomic failure, cerebellar and pyramidal disorders.

The late age of onset of the disease and the early development of autonomic insufficiency should alert physicians and neurologists with respect to MSA, since this rare disease has a rapidly progressive course and leads to death. In turn, early diagnosis of the disease contributes to the timely correction of autonomic and motor disorders and, ultimately, to an increase in the quality and life expectancy of patients. The article presents its own observations of patients with various forms of MSA, examines its clinical features and provides modern diagnostic criteria for this disease.

Key words: multisystem atrophy, autonomic insufficiency, parkinsonism, ataxia, movement disorders, pyramidal insufficiency.

Introduction. Multisystem atrophy (MSA) is a rare neurodegenerative disease characterized by progressive autonomic failure (AF), parkinsonism, cerebellar and pyramidal syndromes in various combinations with a fatal outcome. The incidence is registered on average 0.6-0.7 cases per 100 thousand population a year, however, it increases with age and reaches till 3 cases per 100 thousands people a year among the population over 50 years old [10, 11]. MSA with the same indicator affects both sexes, most often there is increase in mortality within 5-7 years [9, 12, 14].

In MSA, in 20–75% of cases, motor manifestations of the disease are preceded by a prodromal stage, which may be characterized by the development of orthostatic hypotension, neurogenic urination disorders, inspiratory stridor, erectile dysfunction, as well as behavioral disturbances in the sleep phase with rapid eye movements (REM sleep disturbance) [10]. The disease is characterized by rapid and steady progression and 5 years after the first manifestations of the disease, 60% of patients find themselves in a wheelchair, and after 6–8 years most of them become bedridden [10]. The factors of poor prognosis include late age at the time of debut and early development of severe autonomic failure [8, 9, 14, 15, 16]. The most common causes of death in patients with MSA are sudden death during sleep of unclear etiology, aspiration pneumonia due to swallowing disorders, orthostatic hypotension, and other causes [13].

Patients with MSA have a wide range of disorders of the autonomic nervous system, but the most characteristic is the lesion of the genitourinary and cardiovascular systems [11]. More often, patients with the development of these symptoms do not always seek medical help, reducing their complaints to age-related changes in the body. However, it is the disorders of the autonomic system that are most significant for patients with the prodromal stage of MSA.

Description of clinical cases of MCA. *Clinical case No. 1.* Patient I., 71 years old, from the age of 61 began to notice pronounced fluctuations in blood pressure from 60/40 mm Hg up to 230/120 mm Hg. He did not take antihypertensive drugs regularly, he was observed with the diagnosis of hypertension by a local therapist. At the age of 65, urinary incontinence appeared, but did not pay special attention to urinary disorders. A noticeable deterioration in the 7th year of the disease at the age of 68: due to urinary incontinence, he began to wear diapers, slowed down in movements, unsteadiness when walking with periodic falls. The patient was also worried about severe general weakness and non-systemic dizziness, which regressed in the supine position.

By the specialized appointment of a neurologist-parkinsonologist of the Center for Extrapyramidal Disorders and Botulinum Therapy of the Clinic of the M.K. Ammosov North-Eastern Federal University he underwent a medical examination for the 8th year of his illness. The examination revealed symmetric akinetic-rigid syndrome, signs of cerebellar ataxia. The test for orthostatic hypotension revealed a decrease in blood pressure from 130/80 mm Hg. Art. (in prone position) up to 90/60 mm Hg (after 3 minutes in upright position). Cognitive function tests: within normal limits except for a slight decrease in phonetic speech activity. HADS - 5/5 (normal).

The patient for further examination was hospitalized in the Center for Neurodegenerative Diseases of the Yakutsk Scientific Center for Complex Medical Problems (YSC KMP).

The daily monitoring of blood pressure revealed episodes of blood pressure decrease in the morning and afternoon hours with a minimum value of up to 75/53 mm Hg and nocturnal hypertension up to 210/103 mm Hg. MRI of the brain revealed diffuse atrophy of the cerebral cortex, cerebellum.

Taking into account the patient's autonomic insufficiency, akinetic-rigid syndrome, cerebellar signs and characteristic changes on the MRI of the brain, 'Multisystem atrophy, Shai-Dreiger syndrome' was diagnosed.

At discharge, it is recommended to take α-adrenergic agonist (midodrine) under the supervision of a neurologist in the home area, as well

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as symptomatic therapy was appointed.

Clinical case No. 2. Patient P., 61 years old, was admitted to the Center for Neurodegenerative Diseases of the YSC KMP with complaints of slowness and stiffness in movements, severe anxiety, inability to sleep lying down due to severe anxiety, frequent urination and constipation.

From the anamnesis of the disease it is known that from the age of 58 he began to notice unsteadiness when walking, frequent urination, constipation, non-systemic dizziness and rapid fatigue.

Over the next two years, he was observed by a therapist with chronic constipation and by a neurologist diagnosed with dyscirculatory encephalopathy. The symptoms were slowly increasing. At the age of 61, slowness and stiffness in movements joined, so 'Vascular Parkinsonism' was diagnosed. Pramipexole was prescribed in a daily dose of 0.75 mg / day without effect. Neuropsychological tests: MoCA - 24 points (signs of moderate cognitive impairment), HADS - Anxiety - 6 points, depression - 18 points. Pronounced clinical evidence of depression.

The neurological status revealed a bilateral symmetric akinetic-rigid syndrome, pyramidal insufficiency (in the form of hyperreflexia and pathological extensor signs), mild cerebellar symptoms (intention when performing the fingernasal and knee-heel tests) and pelvic disorders in the form of frequent urination with episodes of incontinence. The orthostatic test did not record a clinically significant decrease in blood pressure.

The MRI of the brain revealed changes characteristic of MCA (Fig. 1).

The patient was prescribed levodopa / carbidopa with titration up to 750 mg + 75 mg / day, against which the manifestations of akinetic-rigid syndrome significantly decreased. In addition, buspirone was prescribed at a dose of 10 mg / day to relieve anxiety.

Thus, taking into account of the prevalence of symmetric akinetic-rigid syndrome, pyramidal insufficiency, early development of symptoms of autonomic failure, a positive response to levodopa therapy, 'Multisystem atrophy, nigrostriatal variant' was diagnosed to the patient.

Clinical case No. 3. Patient N., 59 years old, was admitted to the Center for Neurodegenerative Diseases of the YSC KMP with complaints of speech changes, unsteadiness and instability when walking, choking when eating liquid food, frequent urination with episodes of urinary incontinence, interrupted sleep due to frequent urination.

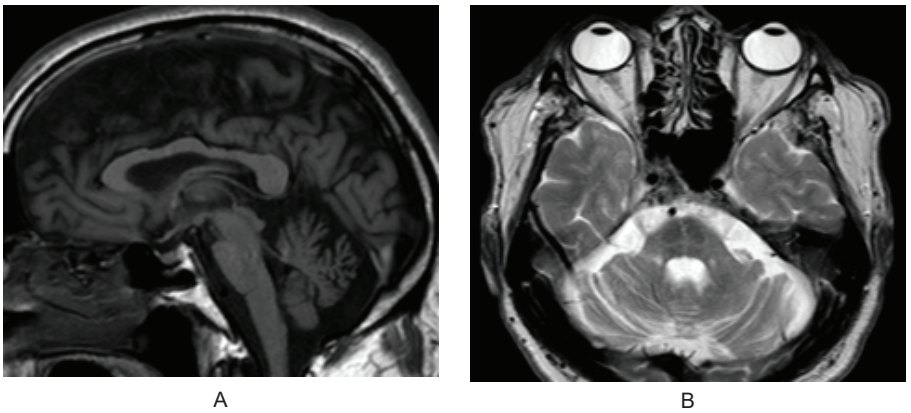
The onset of the disease at the age of 57 with changes in speech, voice, choking when eating and gait instability. At the age of 58, unsteadiness when walking with falls, frequent urination, episodes of urinary incontinence joined. Due to the presence of cerebellar ataxia, the patient was excluded from the diagnosis of type 1 spinocerebellar ataxia.

The neurological status revealed: signs of stato-locomotor ataxia, dysphonia, dysphagia, dysarthria, pelvic incontinence disorders. The orthostatic test did not record a clinically significant decrease in blood pressure.

Neuropsychological tests were conducted: MoCA - 22 points (signs of moderate cognitive impairment), HADS - 6/4 (normal).

MRI of the brain revealed signs of atrophy of the cerebellum and pons with the formation of a "cross" symptom.

Taking into account the prevalence of cerebellar symptoms, the presence of autonomic disorders, characteristic MRI signs, the patient was diagnosed as 'Multisystem atrophy, olivopontocerebellar type'.



A B

Figure: 1. MRI of the patient's head P: atrophy of vermis cerebellum and pons is determined on sagittal sections (A), atrophy of the cerebellar hemispheres with widening of furrows, cavity of the fourth ventricle and atrophy of pons with the formation of a 'cross' symptom (B).

Discussion. Orthostatic hypotension is the most frequent and at the same time severe cardiovascular autonomic disorder, that develops as a result of impaired activation of sympathetic vasoconstrictor neurons [10]. Symptoms can be latent and are expressed by general weakness, tremors in the body, headache, nausea, as well as discomfort in the neck and shoulders when changing from horizontal to vertical. With a more pronounced decrease in blood pressure, syncope and falls are possible. In addition to orthostatic hypotension, cardiovascular disorders in the framework of autonomic insufficiency can be manifested by arterial hypertension in the supine position (especially at night), a fixed pulse, postprandial hypotension (hypotension after a meal) [1].

In MSA, other autonomic disorders can also develop: weakening of gastrointestinal tract motility, impaired pupillary reactions, thermoregulatory disorders, anhidrosis or hyperhidrosis, acrohypothermia [10].

Vegetative disorders are also characteristic of Parkinson's disease, which requires differential diagnosis, especially in the absence of cerebellar and pyramidal signs. At the same time, in MSA, autonomic disorders develop at an early stage, may precede the development of symptoms of parkinsonism, and make a greater contribution to a decrease in the quality of life of patients than motor deficits [2].

The clinical cases described by us demonstrate the necessity of careful observation and examination of patients with autonomic disorders. In all three cases, cardiovascular and pelvic disorders preceded the diagnosis of MCA. At the same time, it should be pointed out that the diagnosis of MSA requires the presence of not only autonomic failure, but also cerebellar ataxia and / or parkinsonism (Table) [10].

Respiratory disorders also play a significant role among the non-motor manifestations of MSA. So, in almost half of cases at the advanced stage of MSA, there is an inspiratory stridor resulting from dystonia of the vocal cords or denervation of the laryngeal muscles, a little less often (in 40% of patients) there are sleep apnea syndromes of both obstructive and central genesis [1].

Cognitive and affective impairments are characteristic of MSA. Despite the fact that dementia is considered an exclusionary symptom in MSA, it should be

Diagnostic criteria for multisystem atrophy (by Fanciulli A., Wenning G., 2015)

Category	Features
Credible ISA	Posthumous detection: 1) numerous a-synuclein; 2) neurodegenerative changes in the strionigral and olivopontocerebral areas;
Probable ISA	A sporadic progressive disease (onset after age 30) characterized by autonomic failure, including urinary incontinence (with erectile dysfunction in men) or orthostatic blood pressure drop of at least 30 mm Hg. systolic or 15 mm Hg. diastolic after 3 minutes of standing plus one of the following: 1) parkinsonism with a poor response to levodopa (subtype MSA-P), 2) cerebellar syndrome (subtype MSA-C);
Supporting criteria	Dystonia of the muscles of the head and neck region; disproportionate antecollis; bent spine (forward, sideways, or both); contractures of the arms or legs; breathing sighs; severe dysphonia; severe dysarthria; new or increased snoring; cold hands and feet; incontinence with emotional reactions (laughing or crying); postural or kinetic tremor;
Not characteristic signs	Rest tremor, pill rolling; clinically significant neuropathy; hallucinations not caused by drugs; onset after 75 years; positive family history; dementia (according to DSM-IV criteria); changes in the white matter of the brain, characteristic of multiple sclerosis.

remembered that in one third of patients there is a moderate decrease in cognitive functions, usually of the frontal type. In later stages, 4.5% of patients may develop dementia [9]. Depression, anxiety and panic attacks are found among emotional disorders in MSA [1].

Treatment for MSA remains symptomatic. Orthostatic hypotension therapy often improves the quality of life of patients with MSA. Non-drug methods include applying compression bandages to the lower limbs, increasing the salt in the diet, raising the head end of the bed. Drug therapy for orthostatic hypotension includes the administration of α-adrenergic agonists (midodrine), mineralocorticoids (fludrocortisone), and acetylcholinesterase inhibitors (pyridostigmine) [4].

Anticholinergics of peripheral action are effective in urinary incontinence, but often induce urinary retention; Desmopressin taken at night provides regression of nocturia. In case of incomplete emptying of the bladder, intermittent catheterization is necessary [6].

Currently, there is no effective treatment for cerebellar disorders of MCA. To reduce the symptoms of parkinsonism, levodopa preparations are used at a dose of up to 1000 mg / day, provided that it is well tolerated [6].

Conclusion. Thus, rapidly progressive and difficult to treat symptoms of autonomic failure may be the initial manifestations of MSA. Unfortunately, the treatment of this disease remains symptomatic, however, the timely initiation of therapy can somewhat improve the quality of life of patients.

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CLINICAL CASE OF EOSINOPHILIC GRANULOMATOSIS WITH POLYANGITIS: DIAGNOSTIC DIFFICULTIES AND CLINICAL MANIFESTATIONS

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The article presents a clinical case of eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome), which was diagnosed for the first time in a 27-year-old man with bronchial asthma, eosinophilia, and gastrointestinal tract involvement. A retrospective analysis of the disease demonstrates the difficulties in diagnosing this disease. In this clinical case, the diagnosis was made 1.5-2 years after the onset of the first manifestations of the disease, in the second period of the development of the disease, based on the symptoms of the disease that were identified in the patient: bronchial asthma, rhinosinusopathy, pulmonary infiltrates, hypereosinophilia, despite the absence of biological markers of vasculitis. This syndrome is infrequently in clinical practice; however, doctors of various specialties should be aware of identifying this syndrome in their patients.

Keywords: Churg-Strauss syndrome, eosinophilic granulomatosis with polyangiitis, EGPA, Anti-neutrophil cytoplasm antibody (ANCA)-associated vasculitis, bronchial asthma.

Introduction. Eosinophilic granulomatosis with polyangiitis (EGPA) (formerly Chard-Strauss syndrome) was first described by J. Churg and L. Strauss in 1951. They presented a triad of histopathological features: necrotizing vasculitis, eosinophilic inflammation and extravascular granulomas at autopsy in 13 patients who had similar clinical manifestations: severe bronchial asthma, fever, eosinophilia, heart and renal failure, and peripheral neuropathy [1,2,6].

Currently, the disease is a form of vasculitis associated with antibodies against neutrophilic cytoplasm (ANCA), which is characterized by eosinophilic granulomatous inflammation and small and medium-sized vasculitis associated with asthma and eosinophilia [3-5].

Depending on the geographic regions and the criteria used, the annual incidence and prevalence of EGPA is 0.9-2.4 and 10.7-17.8% per million population, respectively. Men and women get sick equally often. The average age of the onset of the disease is 38–49 years [1,2,4,6].

The pathogenesis of EGPA is still not fully understood, and there is no standard therapy, which is based on the results of clinical studies [3-7]. Patients with EGPA usually see general practitioners and physicians with clinical symptoms of one or more internal organ involvement. Damage to many organs and systems for a long time can proceed under the guise of other nosologies, which presents significant difficulties in diagnosing a true disease [1,2,6].

The rare occurrence of this pathology, the variety of symptoms with the involvement of many organs and systems emphasizes the relevance of studies that are not only scientific, but, above all, of practical interest.

Material and research methods. A retrospective analysis was made of the medical history of a patient (male, 27 years old) who was treated at the Yakutsk Republican Clinical Hospital in 2019, where a full examination was carried out according to all standards and clinical guidelines.

Case description and discussion. The patient was admitted to the gastroenterology department at the end of February 2019. Complaints at admission: constant, aching pain in the epigastrium and along the colon; diarrhea up to 10-15 times a day, without pathological impurities; periodic nausea and vomiting; episodes of dizziness; frequent headaches; pain in the left maxillary sinus and discharge from the nose of a purulent character with a fetid odor, mainly from the left nasal passage; aching pains in the lumbar parts and knee joints; general weakness; decrease in body weight by 8 kg over the past six months.

Medical history: In the fall of 2017, the patient received ambulatory treatment for an unproductive cough at the place of residence. His condition was regarded as an exacerbation of chronic bronchitis. Despite the treatment, the disease progressed, episodes of shortness of breath, attacks of suffocation, cough with difficult to separate viscous sputum began to disturb for the first time. In the future, treatment was prescribed Symbicort 80 mcg / 4.5 mcg / dose, 1 breath 2 times a day. There was some improvement in the condition, which was manifested by a decrease in the frequency of asthma attacks and a decrease in the cough syndrome. Pain appeared in the epigastrium, which arose regardless of food intake, bloating, since the spring of 2018. In this regard, with an increase in the intensity of abdominal pain, the appearance of loose stools up to 3-4 times a day, an increase in general weakness and a decrease in body weight, the patient was referred in July 2018 for examination at the Republican Hospital № 2. The patient was diagnosed based on laboratory and instrumental studies: Ulcerative colitis with lesions of the rectosigmoid part, bronchial asthma, allergic genesis, partially controlled and recommended treatment at the place of residence: Mesakol 400 mg / day, Symbicort 80 mcg / 4.5 mcg / dose, 2 breaths 2 times a day. The patient was discharged with improvement. Despite constant medication, the patient's condition worsened since the beginning of 2019. Among the background of constant abdominal pain, the frequency of diarrhea increased up to 8-10 times a day, pain in the left maxillary sinus appeared for the first time, as well as purulent discharge from the left nasal passage, episodes of dizziness, headaches, knee pain and daily bouts of unproductive cough.

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Life history*:* The patient grew and developed according to his age. He did not work. He smoked, 1 pack of cigarettes a day, smoking experience more than 5 years, he drank alcohol in moderation. Heredity is not burdened.

The patient's condition is grave upon admission. His consciousness is complete. BMI 18.4 kg / m² (underweight). The skin is pale, clean. Respiration rate 17 / min. The lungs have normal boundaries. In the lungs: percussion sound is dulled below 10 ribs from the posterior axillary to the paravertebral line on both sides; in the same place, weakened vesicular respiration, vesicular respiration over the rest of the lung surface, no wheezing. Heart sounds are sonorous, the rhythm is correct, heart rate is 70 per minute. BP 70/50 mm Hg. Art. The tongue is moist, coated with a grayish-white bloom. The abdomen is soft, moderately distended, painful in the iliac parts, along the colon. The liver and spleen are of normal size. Beating symptom is positive on the left. Diarrhea was up to 10-15 times a day, without pathological impurities. Urination is free, painless.

The following deviations were found in the analyzes dated 02/28/19: eosinophilia 28.6%, accelerated erythrocyte sedimentation rate 62.0 mm / h, low hemoglobin 112 g / l, high fibrinogen 10.626 g / l. The fecal occult blood test was negative; coprogram: digested plant fiber was moderate; there was little neutral fat; the consistency was mushy; the color was light brown.

Superficial erosive proctitis is on rectoscopy from 02/28/19.

Therapy with sulfasalazine, ciprofloxacin and metronidazole, parenteral administration of electrolytes and glucose was started.

Considering the patient's complaints, anamnesis data (bronchial asthma, lesions of the large intestine), hypereosinophilia, accelerated erythrocyte sedimentation rate and changes in the coagulogram, the diagnostic search was continued in the hospital.

In the biochemical blood test from 03.03.19, there is an increase in C-reactive protein (9.54 mg / l) and immunoglobulins (IgM - 4.1 mg / ml; IgE - 741.7 IU / ml).

Esophagogastroduodenoscopy from 03/04/19 - superficial gastritis. Duodeno-gastric reflux.

Echocardiography from 03/04/19 - the cavities of the heart are not dilated, the ejection fraction is 48%. The global contractility of the left ventricle is slightly reduced. Diffuse hypokinesia of the left ventricle. The effect of spontaneous contrasting left ventricle. Regurgitation on the mitral valve is stage 0-1, on the pulmonary trunk there is stage 1, on the tricuspid valve there is stage 1.

X-ray of the paranasal sinus from 03/05/19 - symptoms of bilateral maxillary sinusitis.

Computed tomography (CT) of the chest organs with contrast from 03/06/19: pulmonary embolism (PE) in both lungs. There are multiple focuses on both sides. Lymph nodes are enlarged in the mediastinum and roots of the lungs. Bilateral pleural effusion.

CT scan of the abdominal cavity with contrast from 03/06/19: there are signs of a defect in the filling of the contrast agent in the preparation of the lumen of both renal veins - thromboembolism. There is a hypodenseous zone in the left kidney, differentiating between infarction and nephritis.

Ultrasound examination of leg veins dated 03/06/19 - a developing non-occlusive thrombosis of the superficial vein of the thigh is on the right.

Considering the clinical manifestations of the disease (bronchial asthma, sinusitis, pulmonary infiltrates, hypereosinophilia, gastroenteritis, high CRP and IgE levels, accelerated ESR, changes in the coagulogram), the concilium was convened on 03/06/19 to clarify the diagnosis and decide on the patient's treatment tactics. Diagnosis: Eosinophilic granulomatosis with polyangiitis (Chard-Strauss syndrome). Complications: thromboembolism of the pulmonary arteries in both lungs, non-occlusive thrombosis of the superficial vein of the thigh on the right, reactive pleurisy, secondary enteropathy, erosive proctitis. The transfer was recommended to the rheumatology department on 03/07/19. In the department, basic therapy was prescribed with prednisolone at the rate of 1 mg / kg / day, symptomatic therapy was continued, which is aimed at correcting the manifestations of the activity of the systemic vascular immune inflammatory process.

The patient's examination continued to verify the diagnosis and assess the effectiveness of the basic therapy.

In the analyzes dated 03/11/19, there is a violation of hemostasis (D-dimer 8618.9 ng / ml; fibrinogen 7.804 g / l; thrombin time 20.7 sec). In immunoblotting, antinuclear, antimitochondrial, antineutrophilic cytoplasmic antibodies and antibodies to cyclic citrullinated peptide were not detected; class G antibodies to double-stranded DNA, IgG antibodies to myeloperoxidase, and antibodies to nucleosomes are normal.

CT scan of the paranasal sinuses dated 03/14/19: signs of pathological contents are present in the right maxillary sinus, the mucous membrane is thickened in the frontal sinus, the sinuses of the main bone and ethmoid cells. These disorders were confirmed by MRI of the brain. Also, for the first time, pathological foci were found in the bones of the skull on the right. Signs of right-sided sinusitis and multiple round-shaped defects with clear uneven contours, ranging in size from 9.3 * 15 mm to 10 * 13 mm in the bones of the skull on the right were detected on X-ray CT of the skull bones 03/23/19

Trepanobiopsy from the iliac crest was performed: signs of plasma cell myeloma were not detected in the punctate. The histological picture and immunophenotype of the hypocellular bone marrow with diffuse infiltration of plasma cells without signs of monoclonality were regarded by the Hematologist as reactive changes.

In laboratory tests, changes were revealed that were carried out after 1 month against the background of basic therapy, in the general blood test: the level of leukocytes was normalized to 9.03x109 / l, hemoglobin was 130 / l, eosinophil was 0.6%, while maintaining an accelerated ESR of 23 mm / hour; in the biochemical blood test there is a tendency to an increase in the level of total protein and albumin, the level of CRP normalized to 3.7 mg / l, the rheumatoid factor values were high 16.3 IU / ml, total cholesterol was high 10.7 mmol / l, ALT was 90, 8 units / l. The coagulogram showed the normalization of indicators, with the exception of the level of fibrinogen (5.180 g / l) and D-dimer (4555.6 ng / ml).

The patient was discharged from the rheumatology department in April 2019 with positive dynamics, the level of eosinophils returned to normal, the inflammatory process regressed, the CT picture of the chest organs improved (infiltration in the lungs was partial regression), clinical symptoms and general well-being improved significantly. The patient was recommended to continue treatment at the place of residence. In the future, he is scheduled for a consultation at the Clinic of Rheumatology, Nephrology and Occupational Pathology named after N. Tareeva.

The difficulty of diagnosis and the characteristic staging of the Chardzha-Strauss syndrome are demonstrated in this clinical case.

According to the literature, three stages are distinguished in the development of the disease: at the initial stage, the disease debuts with bronchial asthma, allergic rhinitis. The severity of the

course of bronchial asthma increases gradually, and resistance to therapy often appears [1,5]. Hypereosinophilic syndrome with various clinical manifestations (Leffler's syndrome, eosinophilic pneumonia, eosinophilic gastroenteritis, etc.) is noted in the second stage and at this stage the maximum number of diagnostic errors is allowed. Clinical signs of systemic vasculitis (fever, systemic inflammatory reaction syndrome, nephritis, cutaneous manifestations, etc.) manifest themselves at the third stage. The severity of bronchial asthma may regress [1,4,6].

In clinical practice, the diagnosis of systemic vasculitis is always difficult and long-term to establish. For several months (sometimes years), the patient can be observed by many specialists, as the presented clinical case demonstrates.

In this case, the diagnosis was made at the second stage of the development of the disease, 1.5-2 years after the appearance of the first clinical manifestations, based on the classification criteria of the disease identified in the patient: bronchial asthma, rhinosinusopathy, pulmonary infiltrates and hypereosinophilia, despite the absence of biological vasculitis markers.

In the diagnosis of EGPA, like other vasculitis, an increase in the level of antineutrophilic cytoplasmic antibodies, the action of which is directed against various cytoplasmic antigens - myeloperoxidase, elastase, protease, is of great importance [1,3-7]. ANCA-positive patients (70 to 75%) have antibodies to myeloperoxidase with perinuclear staining (pANCA) and more often kidneys (especially necrotizing glomerulonephritis), the central nervous system, peripheral polyneuropathy, purpura and other skin manifestations are affected [3,7].

In ANCA-negative variant, the process begins with the lungs (pulmonary infiltrates, pleurisy) and the heart (myocarditis, pericarditis, cardiomyopathy, heart rhythm disturbances, etc.). According to the literature, the absence of ANCA does not exclude the diagnosis, since autoantibodies are found in 50-70% of cases with a single study [3,6,7].

This patient has ANCA-negative EGPA type, the disease began with the lungs (bronchial asthma, sinusitis, pulmonary infiltrates, pleurisy) and the gastrointestinal tract (gastroenterocolitis), hypereosinophilia, with a predominance of the clinic of eosinophilic infiltration into organs and tissues, as well as the absence of ANCA in blood.

The variety of clinical and immunological forms of the disease necessitates a differential diagnostic search to exclude a wide range of diseases and pathological conditions.

Broncho-obstructive syndrome, first of all, requires a differential diagnosis between bronchial asthma and chronic obstructive pulmonary disease, as well as allergic bronchopulmonary aspergillosis and chronic eosinophilic pneumonia. In contrast to these conditions, bronchial asthma with EGPA from the moment of onset becomes difficult for therapy, is characterized by the development of pulmonary infiltrates with the involvement of other organs and systems.

The revealed erosive-ulcerative ulceration in the mucous membrane of the rectosigmoid colon was initially regarded as manifestations of ulcerative colitis. At the same time, a timely biopsy of the intestinal mucosa is important in establishing the true cause of destructive changes in the intestinal wall, revealing granulomatous vascular inflammation and eosinophilic tissue infiltration in the biopsy specimen.

Treatment tactics depend on the clinical course of the disease. In this case, the patient was prescribed prednisolone at a dose of 1 mg / kg / day as a basic therapy. This tactic is indicated for patients with a good prognosis, without systemic manifestations of the disease, within 1 month or until the signs of process activity are reduced (up to 1 year) [1,4].

At the same time, high levels of D-dimer and fibrinogen in the blood plasma revealed in our patient, accompanied by systemic thrombosis (PE, thrombosis of the renal veins, forming non-occlusive thrombosis of the superficial vein of the thigh), local impaired contractility of the left ventricle, a decrease in ejection fraction up to 48%, are poor prognostic factors of EGPA, when the most effective pulse therapy with methylprednisolone (1g intravenously for 3 days), followed by 40-60 mg of prednisolone, for a long time [1,3,4].

Conclusion. In the diagnosis of EGPA (Chardzha-Strauss syndrome), a detailed examination of the patient with a targeted search for pathognomonic symptoms of damage to organs and systems is of decisive importance. From the moment of the onset of the disease, the patient was observed by various specialists (pulmonologists, gastroenterologists, therapists), for a long time he was treated for bronchial asthma and ulcerative colitis without positive dynamics. Given the presence of several competing diagnoses, a comprehensive laboratory and instrumental examination was carried out, which made it possible to make a diagnosis.

The presence of this rare disease should be suspected in patients with bronchial asthma with the appearance of infiltrates in the lungs, against the background of high eosinophilia and accelerated ESR in the peripheral blood. Timely diagnosis and adequate therapy can prevent irreversible organ damage and significantly improve the prognosis of the course of the disease.

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FEATURES OF SPINOCEREBELLAR ATAXIA TYPE 17 IN YAKUTIA (CLINICAL OBSERVATION)

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A literature review of the disease type 17 spinocerebellar ataxia (SCA 17) was carried out. The authors present their own clinical observation of SCA 17 with the absence of characteristic signs of SCA - an inverse correlation between the degree of expansion and the age of manifestation of symptoms of the disease, as well as a direct relationship between the degree of expansion of repeats and the severity of clinical manifestations, confirming the existence of differences in the clinical picture of different types of SCA.

Keywords: autosomal dominant spinocerebellar degenerations, expansion of trinucleotide repeats, spinocerebellar ataxia type 17.

Introduction. To date, 47 subtypes of spinocerebellar ataxias (SCA) have been described. SCA is considered a rare group of cerebellar ataxias, with an average prevalence 2.7 cases per 100,000 population. The most common forms are polyglutamine dilatation diseases (ATXN1 / SCA1, ATXN2 / SCA2, ATXN3 / SCA3, CACNA1A / SCA6, ATXN7 / SCA7, TBP / SCA17 , and ATN1 / DRPLA) [2]. Yakutia is a hotbed of SCA1 accumulation in Russia, the prevalence is 46 cases per 100,000 [12]. Spinocerebellar ataxia type 17 (SCA17, MIM 607136) is a severe neurodegenerative disease, a rare variant of ataxia caused by the expansion of trinucleotide CAG repeats in the TBP gene (TATA-binding protein) on chromosome 6q27. The protein product of the gene belongs to transcription factors and specifically binds to the regulatory TATA site of the transcribed segments [6].

The first case of the disease was described in 1999 by R. Koide et al. in a 14-year-old Japanese patient, who, from the age of 6, had impaired coordination of movements and intellect, and then ataxia of the trunk, spasticity, muscle weakness, dysphagia and dysarthria, and atypical absences were added. The patient was found to have an expansion of tandem trinucleotide CAG repeats in the *TBP* gene (63 copies of repeats at a rate of 25–42 repeats) [4]. Later, a similar mutation was found in other families of Japanese and European origin [7, 9, 10, 14, 19]. The age of onset of symptoms is usually 19–48 years, but an extremely severe phenotype of type 17 spinocerebellar ataxia (SCA17) with the onset of the disease at 3 years of age and the maximum number of CAG repeats known to date is 66 copies [4]. Late variants of type 17 spinocerebellar ataxia (SCA17) with manifestation of symptoms at the 7th decade of life are also known. In the examined families with SCA17, there was a clear anticipation, as well as an inverse correlation between the age of disease onset and the number of trinucleotide repeats[25] .

In 2016, S.A. Klyushnikov et al. identified the first case of SCA 17 in the Russian population [2]. The gene responsible for the development of the disease encodes the TATA-box-binding protein; therefore, it was named *TBP* (TATA-binding protein) [7]. The site with microsatellite CAG / CAA repeats, the expansion of which causes the development of the disease, is located in exon 3. The range of normal values for the size of the polyglutamine site in the protein is between 25 and 44 amino acid residues. Alleles causing the development of the disease contain 43–63 CAG / CAA repeats. In this case, the level of expansion of 43–49 repeats is considered a zone of incomplete gene penetrance. In a patient - a man of 27 years old, suffering from a real disease for 7 years, a heterozygous carriage of a mutant allele in the *TBP* gene with a number of CAG / CAA repeats of 45 was revealed. disease caused by a mutation with incomplete penetrance (the number of CAG / CAA repeats is less than 49). Such a disease did not occur in the patient's family, however, during DNA diagnostics of the parents, an identical mutant allele was found in the patient's healthy mother, which clearly demonstrates the phenomenon of incomplete penetrance of the mutation in this molecular form of AD-CCA. Subsequently, the presence of SCA 17 was found in 2 more families [2].

Clinical features. Spinocerebellar ataxia type 17 is a neurodegenerative disease with an autosomal dominant mode of inheritance, with extensive phenotypic variability and age at the beginning of several decades. The main clinical manifestations, as for other types of ataxia, are impaired coordination of movements due to damage to the structures of the cerebellum, as well as its afferent and efferent connections, and dementia [25].

In this literature review, patients with SCA17 (Tab.) ranged from 3 to 60 years of age at onset, and about half of the patients developed ataxia as an initial symptom. The age of onset of the disease weakly correlated with the age of the number of repeats (Tab.) [25]. During the course of the disease, the majority of patients (> 90%) developed ataxia, which was manifested by gait instability and speech impairment. Cognitive dysfunctions and memory impairment were also observed as an initial symptom [20]. Dementia is the second most common symptom (73%) during the course of the disease. In childhood - dementia. Psychiatric symptoms such as aggression [16], paranoia [8], euphoria [24] and depression [17, 22] are common. Behavioral disorders or personality changes can be diagnosed as mental disorders. Involuntary movement is one of the characteristic features of SCA17 [13, 18]. Since chorea is a well-known symptom of SCA17, the clinical phenotype sometimes overlaps with Huntington's disease (HD) and is characterized by the triad of movement

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disorder, mental manifestations, and cognitive impairment [27]. In many cases of clinically suspected HD, patients lack the CAG re-expansion that causes HD. It is believed that such people suffer from HD or HD-like phenocopy syndromes. disorders [25, 26]. Therefore, SCA17 is also called Huntington's disease.

The relationship between the number of repetitions and clinical symptoms of SCA17. Previously, Japanese scientists investigated the relationship between the number of repetitions and clinical symptoms and found that more than 75% of patients with a CAG / CAA repeat size of 43-50 had intellectual impairment; in some people, intellectual problems and involuntary movements were the only signs. Psychiatric problems or dementia, parkinsonism and chorea, a clinical combination similar to Huntington's disease, are more common in people with CAG / CAA repeats in this range than in people with more repeats. All people with a CAG / CAA repeat size of 50-60 have ataxia and 75% have decreased intellectual function. Pyramidal signs (eg, increased deep tendon reflexes) and dystonia are more common in these people than in people with fewer repetitions. These features are confirmed in this literature review. (Tab.) [23]. Two children were reported with more than 60 repetitions. One, family case, with a 66 repetition extension of CAG / CAA, a gait disorder developed at age 3, followed by spasticity, dementia, and psychiatric symptoms [16]. Another, with a de novo CAG / CAA expansion of 63 repetitions, developed ataxia, an intellectual impairment at age 6, followed by spasticity [4].

Less common symptoms are epilepsy [3, 4, 8, 9, 14, 19, 22] (20%), vegetative symptoms [9, 10, 11, 24] (9%), apraxia [9] (7%) and symptoms of peripheral nerve damage [3, 17, 22] (3%). Lin et al. [22] reported a patient who developed ophthalmoplegia with parkinsonism and Rolfs et al. [8] reported a patient with symptomatic hypogonadism.

CT and MRI in patients with type 17 spinocerebellar ataxia (SCA17) of the hemispheres show gross cerebellar atrophy and less pronounced cerebral atrophy [4]. The section reveals a general decrease in the volume and mass of the brain, microscopically - the death of neurons and gliosis in the region of the caudate nucleus, shell, thalamus, inferior olives, frontal and temporal cortex. In the cerebellum, there is degeneration and death of Purkinje cells, growth of Bergmann's clays. Immunohistochemical analysis reveals intranuclear neuronal inclusions typical of polyglutamine diseases, containing ubiquitin and polyglutamine epitopes [7, 9, 19].

Thus, there are several characteristic differences between SCA17 and other polyglutamine diseases.

First, SCA17 exhibits a complex and variable clinical phenotype, in some cases overlapping the phenotype of Huntington's disease.

Second, compared to other subtypes of SCA caused by increased trinucleotide repeats, expectation in the relationship of SCA17 is rare due to the characteristic structure of the TBP gene.

Third, SCA17 patients often have diagnostic problems that can arise from nonpenetrance. Since the gap between normal and abnormal repeat numbers is very small, it is difficult to determine the threshold for the number of abnormal CAG repeats in SCA17.

Clinical case. A woman of Yakut nationality, 49 years old, retired in service in the Ministry of Internal Affairs, was hospitalized in the neurological department at the end of 2019 at the Hospital of the Yakut Scientific Center of the CMP with complaints of impaired coordination, ataxic gait, speech impairment, general weakness, rapid fatigue, decreased vision, constant dizziness of a non-systemic nature, lowering blood pressure to 90/60 mm Hg, pain in the right knee joint, difficulty in bending the legs in both knee joints, apathy, depression, irritability, sleep disturbance, cannot fall asleep, falls asleep in the morning.

From the anamnesis of the disease: the first manifestations of the disease appeared in 2014 at the age of 44. When turning the head, dizziness appeared, thought from cervical osteochondrosis, gait slowed down, could not run. During the year, speech and gait disorders gradually appeared, colleagues at work noticed. She was examined and treated in the neurological department of the medical unit of the Ministry of Internal Affairs, then in the neurological department of the Republican Hospital №2- - EMP with a diagnosis of Cerebellar syndrome. In 2015, she underwent DNA diagnostics for the carriage of a mutation in the *ATXN1* gene (26/27) at the Medical Genetic Center Republican Hospital №1-NCM, SCA type 1 was excluded. In 2016, in the same place, the patient's DNA was checked for the carriage of mutations in the genes *ATXN2* (20/27 repeats), *ATXN3* (22/29 repeats) and in the gene *TATA-binding protein (TBP*), as a result of determining the size of alleles on an automatic DNA analyzer ABI Prism 3500 (Applied Biosystems) identified 35/65 repeats of the 6q27 locus in the *TATA-binding protein (TBP)* gene, normally 29-42 repeats. Gradually the disease progresses. I started walking with a cane 2 years ago. Decreased mood since last year, could lie for days, not eat. Sleep disturbances appeared in late spring 2019, took glycine, before that tenoten, became less irritable, was touchy, tearful. For the last year he has not moved independently on the street, only with an accompanying person on one side. Disability 2 g since 2018, b / s. She underwent treatment in neurological department of Republican Hospital №2- - EMP in September 2019, without positive dynamics, she began to stagger more. He does not know the pedigree from his father's side, his mother told me that she met in Tiksi. The mother died of three ischemic strokes in 2010 at the age of 65. She was born the first child of two in Pokrovsk. Has a brother, is clinically healthy. She grew and developed with age. Has two secondary specialized educations, a paramedic and a lawyer. She worked in the police as an inspector for administrative law. Divorced, 1 son, adult, DNA diagnostics did not pass. She lives alone in Pokrovsk, in a partially furnished house with central heating. He cannot cope with the household, a social worker comes. The son wants to take him to Yakutsk, the patient does not want to be a burden. Past illnesses: he does not know childhood infections, hepatitis during pregnancy, Tuberculosis, diabetes mellitus, stroke, he denies head trauma. In 2015, under general anesthesia, uterine polyps were removed in 2015 under general anesthesia. Gynecological history: P-3, m / abortions - 2, childbirth - 1, natural. She is allergic to household dust, animal hair in the form of a runny nose, sore throat. I used to smoke.

Neurological status: clear consciousness. It is oriented correctly in time, space and self. The behavior is adequate. The mood background is slightly lowered. FMN: distinguishes smells, vision is reduced due to presbyopia. The eye slits are equal. Pupils are rounded, equal, FTR is reduced. The movement of the eyeballs is slightly limited in the extreme abduction. Nystagmus is horizontal and more vertical. There is no diplopia. The exit points of the trigeminal nerve are painless. The sensitivity on the face is not disturbed. The nasolabial folds are symmetrical. Hearing saved. The pharyngeal reflex is triggered. There is no dysphagia. Tongue in the midline, agitated with fasciculations at the edges. Dysarthria is mild, without chanting. ROA Marinescu-Radovici ++. Flaccid tetraparesis. Hand dynamometry on the right is 18 kg, on the left is 13 kg. The tone is diffusely

Brief clinical signs of patients with SCA 17.

1	2	3	4	5	6	7	8	9	10	11
Sex	Age of onset	Initial symptom	Ataxis	Dementia	Involuntary movement	Pyramidal signs	Extra py-ramidal signs	Psychiatric symptoms	CAG \CAA	References
F	60	chorea	-	nc	Chorea	-	nc	depression	41	Park et al. [5]
F	35	Gait instability	н/д	nc	Chorea, trunk titubation	+	+	depression	41	Herrema et al. [20]
M	59	Slurred speech	+	nc	mioclonus	-	-	-	41	Dohertyet al [11]
M	42	Reduced operating speed	+	++	-	+	+	nc	43	Nielsen et al. [21]
F	29	Gait instability, behavior change	++	+	Chorea	+	-	-	44	Stearinet al. [13]
M	48	Gait instability	+	+++	Dyskinesias	nc	nc	nc	44	Mariottiet al. [22]
F	40	Gait instability	+	nc	nc	nc	nc	nc	44	
F	38	Gait instability	+	nc	nc	nc	nc	nc	44	
M	41	behavior change	+	+	Dyskinesias	+	nc	nc	45	
F	38	depression	nc	+++	nc	+++	nc	depression	45	
F	40	Gait instability	+	+	Dyskinesias	nc	nc	nc	45	
м	34	Gait instability	+	-	Dyskinesias, tremor	+	nc	nc	45	
м	55	Chorea	+	Нет данных	Chorea, Dyskinesias	nc	nc	nc	45	
F	52	Gait instability	+	+	Dyskinesias	nc	nc	nc	45	
м	55	Gait instability	+	+	nc	nc	nc	nc	45	
м	nc	Ataxia, urination disorder	+++	+	-	+	++	Euphoria	45	Lin ett al. [24]
F	30	Speech impairment, depression	+	nc	nc	nc	++	depression	45	Rolfs et al. [9]
M	43	Ataxia	+	nc	nc	nc	nc	depression	45	
F	30	depression	+	+	Dystonia, chorea	+	nc	Depress.,aggression, paranoia	45	
M	23	Speech impairment Ataxia	+	nc	nc	+	nc	-	45	
M	19	Behavioral disorders	+	+	Dyskinesias	nc	nc	nc	46	Mariotti et al [22]
M	37	Paranoia psychosis	+	+	choreoathetosis	+	-	Paranoia psychosis	46	Fujigasakiet al. [8]
F	55	Ataxia, dementia	+	+	-	+	+	nc	46	
nc	40	nc	+	+	Chorea	+	+	nc	47	Nakamura et al. [19]
nc	28	nc	-	+	-	+	+	nc	47	
nc	39	nc	+	+	Dystonia,	+	+	nc	48	
nc	48	nc	+	+	Chorea	+	+	nc	48	
F	38	depression	-	nc	nc	nc	nc	Hallucinations, suicidal attempts	49	Rolfs et al. [9]
F	16	Cognitive impairment, ataxia	+	+++	nc	nc	nc	nc	49	
F	48	Chorea	+	н/д	Chorea	nc	+	paranoia	50	Mariotti et al [22]
м	55	Behavioral disorders	+	+	Dystonia, dyskinesia	nc	nc	nc	50	
м	13	mental retardation	+	+	-	+	+	nc	51	Zuhlke et al. [10]
F	35	depression	+	+	dyskinesia	nc	nc	depression	52	Mariotti et al []
F	25	Paranoia	н/д	+++	nc	+++	nc	Paranoia	53	
F	35	ataxia	+	+++	Dystonia,	nc	nc	++	53	Maltecca et al [14]
м	34	ataxia	+	++	Dystonia,	nc	nc	++	53	
м	23	ataxia	+	++	Dystonia,	+	+	++	53	
F	34	Personality disorders	+	+	nc	+	+	Euphoria	53	Fujigasakiet al. [8]
M	35	Ataxia, psychiatric symptoms	+	+++	Dystonia,, Chorea	+	nc	Aggression. Paranoia	54	Rolfs et al. [9]

End of the table

1	2	3	4	5	6	7	8	9	10	11
M	18	Ataxia, dementia	+++	++	nc	+	nc	nc	54	Rolfs et al. [9]
F	23	Hallucinations	++	+	Dystonia,, Chorea	+	nc	Mania	54	
F	18	Speech impairment	+	nc	nc	nc	nc	-	54	
M	18	Ataxia	+	nc	Dystonia	nc	nc	-	54	
F	20	dystonia	+	-	Dystonia corticollis	-	-	nc	55	Zuhlke et al [10]
nc	19	nc	+	+	dystonia	+	+	nc	55	Nakamura et al. [19]
nc	25	nc	+	+	dystonia	+	+	nc	55	
F	6	Mental retardation, ataxia	+	++	-	+	-	nc	63	Koide et al.. [4]
F	3	Ataxia	+	+++	dystonia	+	+	++	65	Maltecca et al. [14]

reduced. SPR from arms and legs alive, equal. In the Romberg position, stands with legs together, but with pronounced ataxia. Falls in tandem. The finger test is performed with light intention on both sides. A knee-calcaneal test with moderate ataxia on both sides. There are no meningeal symptoms. There are no sensitive violations. Gait is ataxic, with a cane; on the right, the legs are raised in a semicircle. There are no pelvic disorders.

The patient underwent studies on the following scales:

Morse fall assessment sheet from –60 points (high),

Ataxia scales: SARA -15 points from 12/09/19 and 12.5 points from 12/19/19, positive dynamics, decrease by 2.5 points (maximum 40 points).

ICARS - 25 points (maximum 100 points).

Hospital Anxiety and Depression Scale (HADS): Anxiety - 3 points, Depression - 7 points, where 0-7 points are normal, 8-10 points - subclinically expressed anxiety / depression.

The Montreal Cognitive Assessment Scale - 23 points (norm 30 points). On the attention test, the patient did not cope with serial subtraction and with one of the lists of numbers. There was also a decrease in memory in the stitched work.

A.M. Wayne assessment of vegetative changes – 39 meaning a pathological increase in points (the norm is up to 15 points). The sympatatonic variant of the vegetative tone prevailed.

According to instrumental studies: *ECG* from 10.12.19: Rhythm - sinus with a heart rate of 81 beats per minute. Metabolic changes in the myocardium of the lower-lateral regions.

Spirography from 10.12.19 conclusion: lung capacity within normal limits. Indicators of airway patency at the lower limit of the norm.

Electroneuromyography, conclusion: SPI on the motor fibers of the median, ulnar, tibial nerves on the right and left without pathology, peroneal nerves on the right and left reveals moderate axonal disorders at the distal level in the form of a decrease in the amplitude of the M-response by 55.5-62% (more pronounced on the left).

SPI on the sensory fibers of the median, ulnar and peroneal nerves on the right and left - without pathology.

The F-wave along the motor fibers of the ulnar nerve on the right reveals signs of reinnervation in the form of repeated waves up to 15.4%.

F-wave along the motor fibers of the ulnar nerve on the left, median, tibial nerves on the right and on the left - without clinically significant disorders. In the clinical aspect, there are signs of symmetric motor axonal neuropathy on the distal segments of the peroneal nerves of a moderate degree.

EEG from 09/23/2020. Conclusion: general characteristics of EEG at rest: a low-amplitude EEG is recorded. Significantly disorganized alpha activity is observed in the form of separate waves of medium amplitude, low index, irregular, most pronounced in the frontal region. No amplitude modulation. Beta-activity dominates in the form of a rhythm of a high index, medium amplitude, low frequency, with an area of expression in the anteroposterior region (Fp1 Fp2).

MRI of the brain from September 24, 2019 revealed a small congenital retrocerebellar cyst and an expansion of the cerebellar grooves, probably as a manifestation of atrophic changes.

In the hospital, the following consultations were held: ophthalmologist from 10.12.19 D: Vertical nystagmus. OI hypertensive angiopathy.

Psychiatrist from 12/11/19D-z: Anxiety-subdepressive syndrome.

Physiotherapist on 11/12/19 prescribed massage of the lower extremities.

Clinical psychologist from 12/13/19 Endorsement: Reduced emotional background, experiences in connection with the disease, aggravated by a feeling of loneliness. General bypass with bypass with deputy.

Geneticist 12/18/19 Cerebellar ataxia, late form, unspecified type of inheritance. Type 17 - SCA 17 is not excluded. Progressive course, flaccid tetraparesis, cerebellar syndrome. The offspring risk for late cerebellar ataxia is high.

Treatment performed: Aminoplasmal 250.0 intravenous drip slowly, after 2 days No. 5 - a course of amino acids for basic diagnosis, Mexidol 5.0 intravenous cap No. 10 for antioxidant therapy, Atoris 20 mg in the evening, Phenibut 250 mg 1 ton at night , Grandaxin 50 mg 1t * 2rvd (y, d), Vit B12 i / m, exercise therapy. massage of the lower extremities.

Against the background of treatment, he was discharged with some improvement, in the form of a decrease in intention when performing coordination tests, the speech became slightly clearer.

Conclusion. Thus, SCA17 is considered to be a complex and variable clinical phenotype, in some cases overlapping the phenotype of Huntington's disease. Compared to other subtypes of SCA, caused by increased trinucleotide repeats, expectation in the relationship of SCA17 is rare due to the characteristic structure of the TBP gene. SCA17 patients often have diagnostic problems that can arise from nonpenetrance. Since the gap between normal and abnormal repeat numbers is very small, it is difficult to determine the threshold for the number of abnormal CAG repeats in SCA17.

The clinical case described in the work

confirms that SCA 17 is a clinically and genetically heterogeneous disease. In this case, we do not observe an inverse correlation between the degree of expansion and the age of manifestation of symptoms of the disease, as well as a direct relationship between the degree of expansion of repeats and the severity of clinical manifestations. We also observe differences in the clinical picture of SCA 17 in this case and SCA 1, which is widespread in Yakutia, which is also important for differential diagnosis between these two hereditary ataxias to determine the prognosis of the disease and the degree of risk of gene mutation transmission to offspring.

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