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BONE METABOLISM MARKERS AND *ENOS* GENE POLYMORPHISM IN CHILD POPULATION OF EASTERN SIBERIA WITH DISEASES OF THE MUSCULOSKELETAL SYSTEM

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At present, it seems quite relevant to study genetic predictors of all classes of diseases in children including diseases of the musculoskeletal system.

The aim of this study was to examine markers of musculoskeletal diseases associated with specific indicators of bone metabolism and the G894T polymorphism of the *eNOS* gene in children from Eastern Siberia.

Materials and methods. We examined two groups of children aged 3-7 years from Eastern Siberia. The main group was made of 69 children with diseases of the musculoskeletal system. The comparison group included 47 children without such diseases. The study involved investigating biochemical (N-osteocalcin, ionized calcium, and alkaline phosphatase) and molecular genetic (the G894T polymorphism of the *eNOS* gene) indicators; they were identified by using relevant methods (PCR in real time and ELISA tests).

Results. We comparatively analyzed the result of bone remodeling in both groups and established that the children from the main group had up to 2.6 times higher levels of N-osteocalcin, ionized calcium, and alkaline phosphatase than their counterparts from the comparison group ($p < 0.05$). These indicators have a pathogenetic association with bone metabolism disorders. Our analysis of the G894T polymorphism of the *eNOS* gene revealed that frequency of the allele T equaled 27.5% in the main group and was almost 2 times higher than in the comparison group ($p < 0.05$).

Conclusion. We established significantly excessive levels of N-osteocalcin, ionized calcium, and alkaline phosphatase in children with diseases of the musculoskeletal system as well as more frequent G894T polymorphism of the *eNOS* gene (T-allele) associated with such diseases (OR=2.37; 95% CI 1.18-4.74; $p=0.01$). This indicates developing demineralization of bone tissue in this group and allows considering the analyzed markers to be predictors of musculoskeletal diseases in children from Eastern Siberia.

Keywords: diseases of the musculoskeletal system, the G894T polymorphism of the *eNOS* gene, N-osteocalcin, ionized calcium.

Introduction. The health of the child population is one of the most sensitive indicators of the state of society. At the moment, there is a high level of childhood morbidity in all groups of nosologies [1]. There are high growth rates of the pathology of the musculoskeletal system in the preschool children - 2.6 times [12].

Violation of the formation of bone mass in childhood creates the prerequisites for the development of osteoporosis in adulthood. Among a number of reasons for the formation of osteopenic conditions in children, genetic factors, disorders of mineral and vitamin metabolism, malnutrition,

various pathological processes, etc., are of the greatest importance [6].

The use of some biochemical indicators allows assessing the state of bone tissue metabolism, identifying its violations, leading to the development of damage to the musculoskeletal system [9].

One of the promising and frequently studied markers of bone formation is the protein osteocalcin. It is the main protein of the bone matrix (molecular weight 5800 Da) and is synthesized mainly by osteoblasts. The content of this protein in blood serum reflects the state of bone metabolism in general [3, 10]. Ionized calcium is calcium that circulates freely in the blood and is not bound to proteins. The level of ionized calcium better reflects calcium metabolism than the level of total calcium [4]. Alkaline phosphatase is an enzyme involved in the metabolism of phosphoric acid. Its activity increases with the development of pathological processes in bone tissue [8]. To date, of particular relevance are studies to identify associations of genetic predisposition to the development of pathological processes in bone tissue and markers of its phenotypic implementation, that is, the identification of genetic and metabolomic predictors of pathological conditions.

The aim of this study was to examine markers of musculoskeletal diseases associated with specific indicators of bone

metabolism and the G894T polymorphism of the *eNOS* gene in children from Eastern Siberia.

Materials and methods. 116 children living in the territory of one of the regions of Eastern Siberia were selected for the study. The observation group included 69 children aged 4-7 years (34 boys, 35 girls) with pathology of the musculoskeletal system (mainly dorsopathy and dorsalgia). The comparison group included 47 children aged 3-7 years (21 boys, 26 girls) without pathologies of the musculoskeletal system.

In the course of the study, a complex of biochemical and molecular genetic methods was used. To do this, the following biological media were taken from the examined children after signing the informed voluntary consent to medical intervention by the parents: blood to determine the levels of N-osteocalcin by ELISA using monoclonal antibodies to osteoprotein, ionized calcium by the ion-selective method and alkaline phosphatase by the kinetic colorimetric method; buccal epithelium to determine the genotype by polymorphism G894T (Glu298Asp) of the *eNOS* gene by polymerase chain reaction with further allelic discrimination of amplification curves.

Statistical processing of the obtained quantitative data was performed in the Statistica 6.0 program and presented

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as $X \pm SE$ - the arithmetic mean (X) and the standard error of the mean (SE). Differences were considered significant if the corresponding p values were less than 0.05. Calculation of the frequency distribution of genotypes and alleles and observance of the Hardy-Weinberg equilibrium in the observation and comparison groups was carried out using the Gen-Expert online program. The association strength of the analyzed traits was determined using the odds ratio (OR). For OR , a confidence interval (CI) was calculated at the 95% significance level.

Results. The state of bone metabolism was assessed by the level of the main markers of bone tissue metabolism – N-osteocalcin, ionized calcium and alkaline phosphatase. When comparing these indicators, it was found that in the main group, the level of N-osteocalcin was statistically significantly higher than similar indicators in the comparison group by 2.6 times ($p < 0.05$), while 50% of children in the observation group exceeded the reference level. The concentration of ionized calcium was increased in all children of the observation group relative to the reference level and 1.2 times relative to the comparison group. At the same time, the values of alkaline phosphatase were in the range of the physiological norm in 90% of children, but 1.2 times higher than in the comparison group (table 1).

Genetic analysis for the establishment of the genotype according to the G894T polymorphism of the *eNOS* gene revealed that the distribution of genotype frequencies in groups corresponds to the Hardy-Weinberg equilibrium, so the data can be analyzed using the general and multiplicative models of inheritance (for the observation group, $p = 1.00$; for the comparison group, $p = 0.16$).

The study of the occurrence of genotypes established the following frequencies in the group of children with diseases of the musculoskeletal system: G/G - 52.2%; G/T - 40.6%; T/T - 7.2%. At the same time, the presence of the G/T and T/T genotypes increased the association with diseases of the musculoskeletal system by 3.9 and 1.15 times relative to the comparison group ($p < 0.05$) (Table 2).

The analysis of alleles according to the multiplicative inheritance model showed that the occurrence of the T allele in the observation group is 27.5%, which is almost 2 times higher than in the comparison group ($p < 0.05$) (table 2).

Discussion. Currently, dorsalgia and dorsopathy of childhood and adolescence are becoming more common [11]. In our study, dorsopathy occurred in 93%

Table 1

Comparative analysis of indicators of bone metabolism in children with pathology of the musculoskeletal system and healthy children

Indicator	Reference level	Observation group (n=69)	Comparison group (n=47)
N-osteocalcin. ng/cm ³	27.92-67.95	72.93±11.30*/**	27.70±2.79
Ionized calcium. mmol/dm ³	1.03-1.10	1.26±0.01*/**	1.06±0.02
Alkaline phosphatase. U/dm ³	71.00-645.00	501.21±32.24**	405.94±26.88

* – differences are significant relative to the reference level ($p < 0.05$);

** – differences are significant relative to the comparison group ($p < 0.05$).

Table 2

Frequencies of genotypes and alleles of the *eNOS* gene in subjects with pathologies of the musculoskeletal system and healthy children

Gene	Genotypes/ alleles	Observation group (n=69)	Comparison group (n=47)	OR (95% CI)	p
<i>eNOS</i> (rs1799983)	G/G	0.522	0.787	0.29 (0.13-0.69)	0.01
	G/T	0.406	0.149	3.90 (1.53-9.95)	
	T/T	0.072	0.064	1.15 (0.26-5.04)	
	G	0.725	0.862	0.42 (0.21-0.85)	0.01
	T	0.275	0.138	2.37 (1.18-4.74)	

of children 4-7 years old with pathology of the musculoskeletal system.

Both high and low levels of osteocalcin in the blood can indicate bone loss. Animal studies suggest that high levels of osteocalcin are due to its reabsorption from bone tissue, which releases it, increasing blood levels [16]. Also, an increase in the level of osteocalcin in the blood is associated with fractures [13], osteoporosis [17], and bone softening [14].

One of the pathogenetically important criteria for calcium and phosphorus metabolism is the level of ionized calcium and the content of alkaline phosphatase. Previous studies have shown that in children with diseases of the musculoskeletal system, excess concentrations of ionized calcium in the blood serum reached 1.17 ± 0.01 mmol/dm³ [5] and alkaline phosphatase in patients of the orthopedic-traumatology department - 486.25 U/l with a reference level of $129-417$ U/l [2].

Nitric oxide, the formation of which is responsible for NO-synthase, is involved in cellular processes responsible for the renewal of bone tissue. It promotes the proliferation and differentiation of osteoblasts, and also plays a key role in osteoclast activity, as a decrease in NO levels has been shown to enhance osteoclastogenesis and associated bone resorption [15].

When studying the distribution of G894T polymorphic genotypes and alleles of the *eNOS* gene, significant differences were obtained among children with undifferentiated connective tissue dysplasia and healthy children in St. Petersburg. So, in all groups of children with undifferentiated connective tissue dysplasia, characterized by defects in fibrous structures and the main substance involved in the construction of cartilage and bone tissue, the T/T genotype and the T allele were more common [7].

Conclusion. Thus, it has been established that in children with pathology of the musculoskeletal system, significantly increased blood levels of N-osteocalcin, ionized calcium and alkaline phosphatase should be attributed to the features of bone tissue metabolism, which indicates a lower mineralization of the bones of the skeleton in this category of patients according to compared with the group of children without pathology ($p < 0.05$). One of the etiological factors in the development of this condition is the factor of genetic determination of the G894T polymorphism of the candidate endothelial NO synthase (*eNOS*) gene. According to the results of this study, the occurrence of the T allele of the *eNOS* gene in the observation group was significantly higher than in the comparison group ($OR = 2.37$; 95% CI 1.18-4.74; $p = 0.01$).

Conflict of interest. The authors of the article report that there is no conflict of interest.

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