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RELATIONSHIP BETWEEN THE *RS6265* POLYMORPHISM OF THE *BDNF* GENE AND SERUM CONCENTRATIONS OF BRAIN-DERIVED NEUROTROPHIC FACTOR IN PATIENTS WITH VIBRATION DISEASE

Vibration syndrome is an occupational disease characterized by polymorphic clinical symptoms and involving disorders of the nervous, vascular, immune, and musculoskeletal systems of the upper and lower extremities. The versatility of brain-derived neurotrophic factor (BDNF) is emphasized by its contribution to a range of adaptive neuronal responses encompassing long-term potentiation and depression, some forms of short-term synaptic plasticity, and homeostatic regulation of intrinsic neuronal excitability. Many stressful and harmful working conditions are known to be associated with decreased BDNF expression in the CNS. In recent decades, a number of studies have emphasized the contribution of the *rs6265 BDNF* gene polymorphism to impaired post-transcriptional processing and secretion of BDNF. At the same time, there is no information in the literature about the effect of the *rs6265 BDNF* gene single nucleotide polymorphism on neurotrophin serum concentration in subjects with VS.

The aim of the study is to determine the effect of the *rs6265 BDNF* gene polymorphism on the serum level of brain-derived neurotrophic factor in patients with VS.

Material and methods. BDNF serum concentrations were determined by solid-phase enzyme-linked immunoassay. DNA was extracted from the whole blood. The *rs6265* polymorphic locus of the *BDNF* gene was typed by real-time polymerase chain reaction.

Results. The highest level of neurotrophic factor in the group of patients with VS was registered in persons with G/G genotype, the lowest protein content was found in carriers of G/A and A/A genotypes of the *rs6265 BDNF* gene polymorphism. During the analysis of interrelations between the polymorphic variant of the *BDNF* gene and the content of neurotrophin in the blood serum of patients with VS, it was found that carriage of the A allele is associated with lower protein concentrations and 4.79 times reduces the risk of its hyperproduction, having a protective effect (dominant model). In addition, each copy of the rare allele reduces the risk of increased neurotrophin concentrations (log-additive model).

Conclusion. As a result of the study, it was found that there is some association of the *rs6265 BDNF* gene polymorphism with the BDNF serum level in patients with VS.

Limitations of this study include the number of the persons who were examined in all groups, especially in the comparison group.

Keywords: vibration syndrome, brain-derived neurotrophic factor, single nucleotide polymorphism *rs6265*, *BDNF* gene.

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Introduction. Vibration syndrome (VS) has been one of the leading positions among occupational diseases. The clinical picture of VS is characterized by polymorphic clinical symptoms and includes disorders of nervous, vascular, immune systems and musculoskeletal apparatus of upper and lower extremities. In this regard, the urgent task is to improve methods of early diagnosis of the disease.

Brain-derived neurotrophic factor (BDNF) is among the universal key proteins of the nervous system that is involved in many vital processes of neuronal adaptation and regulation of intrinsic excitability. The universality of BDNF is emphasized by its contribution to a number of adaptive responses of neurons, including long-term potentiation and depression, some forms of short-term synaptic plasticity, and regulation of intrinsic neuronal excitability. Many stressful and harmful conditions are known to be associated with decreased BDNF expression in the CNS and increased free radicals [16]. It should be noted that we have previously shown that in patients with VS the BDNF content varied depending on the stage of the disease. The increase of the neurotrophin concentration is parallel to the worsening of the severity of the pathological process that indicates the progression of the disease [1].

Recent decades studies have emphasized the contribution of the *rs6265 BDNF* gene polymorphism to impaired posttranscriptional processing and secretion of BDNF [8, 12, 14]. This mutation results in the substitution of valine (Val) for methionine (Met) in the 66th codon of the pro-domain encoding region. The mutation is thought to disrupt the function and distribution of the protein by interrupting its folding, dimerization, and intracellular transport, as well as interfering with activity-dependent release of BDNF [10]. The interaction between BDNF and sortilin is impaired, which subsequently leads to a deficiency of the mature form of the protein in carriers of the A allele of the *rs6265 BDNF* gene polymorphism. Also, this allele can affect intercellular transport and secretion of neurotrophin, forming homo- and heterodimers that are less efficiently sorted and secreted from

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neurons [3]. As a consequence, carriage of the minor allele is associated with reduced synaptic plasticity, impaired memory and learning ability, and increased susceptibility to neurodegenerative and psychiatric diseases [13].

At the same time, there is no information in the literature about the effect of the *rs6265* single nucleotide polymorphism of the *BDNF* gene on the serum concentration of the neurotrophin in individuals with VS. The present study is relevant due to the fact that the mechanisms of genetic regulation of BDNF production have not been sufficiently investigated. All this will further provide an opportunity to identify individuals at increased risk of developing disorders in the nervous system in workers with VS.

The aim of the study is to determine the effect of the *rs6265 BDNF* gene polymorphism on the serum level of brain-derived neurotrophic factor in patients with VS.

Material and methods. The study included 100 men with VS (mean age 51.56±0.67 years, mean work experience in conditions of vibration exposure 25.11±0.74 years) who were examined in the clinic of the Institute in 2023. The diagnosis of occupational disease was established by occupational pathologists in accordance with the International Classification of Diseases 10th revision. The occupations of persons with VS are represented by assemblers, assembly fitters, tunnel sinkers, heavy truck drivers, crane drivers, tractor drivers, excavator and bulldozer operators. The criteria to include the participants into the study were the presence of the diagnosis of VS established during the work, absence of comorbid pathology, and the exclusion criteria were concomitant acute and chronic diseases. The comparison group consisted of 40 conditionally healthy men who were 53,15±1,87 years old (according to the data of complex examination), and who were not in contact with harmful production factors. All the persons taking part in the study were Russians living in the Irkutsk Region. Data on ethnicity were obtained with the help of a specially designed questionnaire.

Venous fasting blood was drawn in the morning into Improvacuter vacuum tubes with coagulation activator SiO_2 (Improve Medical, China) for immunoenzymatic analysis and Improvacuter tubes with anticoagulant K_3-EDTA (Improve Medical, China) for molecular genetic studies. Then it was centrifuged at 1500 rpm for 15 min on a CM-6MT centrifuge (ELMI, Latvia). The serum was selected for enzyme immunoassay and the cell fraction was selected for molecular genetic studies, then they were aliquoted and the samples were stored at –70°C.

Serum concentrations of BDNF were determined by solid-phase enzyme-linked immunoassay ("sandwich" variant) by using the test system "Human Free BDNF Quantikine ELISA Kit" (Cat. No. DBD00, R&D Systems, USA) according to the manufacturer's instructions. The results were read with the help of an ELx800 automatic photometer (Bio-Tek Instruments, USA).

DNA extraction was performed by using DNA-Extran-1 reagent kit (Cat. No. EX-509-100, Syntol, Russia) according to the manufacturer's methodology. The *rs6265* polymorphic locus of the *BDNF* gene was typed by polymerase chain reaction with real-time detection of results on a CFX96 amplifier (Bio-Rad, USA). The test system (Cat. No. NP-623-100) produced by Syntol (Russia) was used to determine the genotypes. The parameters of the thermal cycle were performed in accordance with the reagent kit instructions.

Statistical processing of the results was performed by using Statistica 10.0 program (StatSoft, USA). The following parameters were used to describe quantitative features: arithmetic mean (M) and a standard error of mean (m) for age and work experience under vibration exposure conditions, the median (Me) and interquartile range (Q25–Q75) for BDNF concentration (pg/mL). The Mann-Whitney U-criterion was used to evaluate differences in BDNF content between groups. While analyzing the frequency distribution of the *rs6265 BDNF* gene polymorphism, the observed frequency of genotypes was checked against the expected one by using an online calculator (https://wpcalc.com/en/equilibrium-hardy-weinberg/). The significance of differences in the distribution of genotype and allele frequencies was assessed by using Fisher's exact test (df=1). The search of the *rs6265 BDNF* gene polymorphism associations with the disease, and serum concentration of neurotrophin was done in the online program SNPstats (https://www.snpstats.net/). The odds ratio (OR) with 95% confidence interval (95% CI) was calculated by using the logistic regression method according to 5 probable inheritance models (codominant, dominant, recessive, overdominant, and log-additive). The found differences between the groups were considered statistically significant at $p<0.05$.

The study was performed in accordance with the ethical standards of the Declaration of Helsinki (as amended in 2000), the "Rules of Good Clinical Practice in the Russian Federation" approved by the order of the Ministry of Health of the Russian Federation №200n from 01.04.2016, and the voluntary informed consent signed by the participants. The work was approved by the local ethical committee of the Institute (Minutes No. 5 of 21.03.2023).

Results and discussion. While estimating genotype and allele frequencies of the *rs6265 BDNF* gene polymorphism in the group of patients with VS, the following values were obtained: G/G (abs. 60) – 60.0%; G/A (abs. 37) – 37.0%; A/A (abs. 3) – 3.0%; G (abs. 157) – 78.5%; A (abs. 43) – 21.5%. The frequencies of genotypes and alleles were also determined in the comparison group: G/G (abs. 24) –60.0%; G/A (abs. 15) – 37.5%; A/A (abs. 1) – 2.5%; G (abs. 63) – 78.7%; A (abs. 17) – 21.3%. The analysis of genotype frequency distribution of *rs6265 BDNF* gene polymorphism in the group of patients with VS and the comparison group for their compliance with the Hardy-Weinberg equilibrium showed that the observed genotype frequencies of the studied polymorphic variant of the gene corresponded to the expected frequencies ($\chi^2=0.65$, $p=0.420$; $\chi^2=0.50$, $p=0.480$, respectively). It is necessary to note that the allele frequencies of the studied polymorphic locus of the *BDNF* gene in individuals with VS and the com-

Table 1

Serum concentration depending on the genotypes. Me (Q25–Q75)

BDNF concentration (pg/mL)			Significance level p
Grops	G/G	G/A+A/A	
Patients with VS	395.49 (304.18-977.70)	309.25 (252.09-446.87)	**0.008**
Comparison group	354.14 (347.23-381.31)	234.99 (197.84-267.72)	**0.032**

Note: p is the level of significance for Mann-Whitney U-criterion. differences are significant at $p<0.05$.

parison group are comparable with the data obtained for European and Russian populations [2, 11]. No statistically significant differences were found between the compared groups with respect to genotype (G/G p=0.573; G/A p=0.552; A/A p=0.678) and allele frequencies (G, A p=0.551). When calculating the odds ratio, no association of genotypes with the disease was found.

While comparing BDNF serum concentrations between the groups, statistically significant differences were established: a higher neurotrophin level was registered in individuals with VS (366.20 (281.89–617.63) pg/mL) relatively to the protein concentration values in the comparison group (272.89 (234.99–353.14) pg/mL; p=0.048).

When dividing individuals of both groups into subgroups depending on the genotypes of the *rs6265 BDNF* gene polymorphism, BDNF level significantly differed. Individuals with G/A and A/A genotypes were combined into one subgroup to reveal the role of the minor allele. The highest level of neurotrophic factor was registered in individuals with G/G genotype (395.49 (304.18–977.70) pg/mL) in the group of patients with VS, the lowest protein content was found in carriers of G/A and A/A genotypes (309.25 (252.09–446.87) pg/mL). The concentration of neurotrophin in the comparison group also differed depending on genotypes: in individuals with G/G genotype it amounted to 354.14 (347.23–381.31) pg/mL, in individuals with G/A and A/A genotypes had 234.99 (197.84–267.72) pg/mL, respectively (Table 1).

Individuals with VS were divided into two groups basing on the BDNF median concentration obtained in the comparison group: patients with concentration <273.0 and ≥273.0 pg/mL (Table 2). According to the results of the regression analysis, the dominant and log-additive inheritance models are the most correct for the *rs6265 BDNF* gene polymorphism, as they are characterized by the lowest value of the Akaike information criterion (AIC=113.4). The analysis of interrelations between the polymorphic variant of the *BDNF* gene and neurotrophin content in serum of patients with VS showed that carriage of the A-allele is associated with lower protein concentrations and it decreases the risk of its hyperproduction by 4.79 times, having a protective effect (OR=4.79; 95% CI 1.87–12.24; p=0.0007; dominant model). In addition, each copy of the rare allele reduced the risk of increased neurotrophin concentration (OR=4.0; 95% CI 1.72–9.33; p=0.0007; log-additive model).

The results of our study are in agreement with the literature results [4, 5], whose authors made the following conclusions: homozygous or heterozygous individuals with allele A have lower plasma BDNF levels compared to carriers of the normal G/G genotype, which can be explained by impaired intracellular transport of pro-BDNF, which leads to decreased production of mature BDNF in cells of allele A carriers. Also Gallinat et al. reported that in healthy individuals, the rare A allele is associated with lower serum BDNF concentration [6]. The G allele that is associated with increased BDNF secretion may be a risk allele for the development of some neuropsychiatric diseases [15]. In patients with Parkinson's disease, the maximum protein level was found in carriers of G/G and G/A genotypes of the *rs6265* polymorphic locus of the *BDNF* gene, while the minimum concentration was observed in carriers of the A/A genotype [9]. Carrying the A allele increases the risk of BDNF level reduction almost 10 times in patients with thyroid pathology, while the presence of the G allele, on the contrary, prevents its concentration reduction; however, a decreased level of the neurotrophin in serum of individuals with hypothyroidism of different genesis was observed in carriers of all three genotypes compared to the control group [7].

At the same time, there are opposite opinions in the literature: Terracciano et al., Li et al. found that the *rs6265* polymorphic variant of the *BDNF* gene does not directly affect the level of BDNF in serum [8; 14]. BDNF expression can also be reduced as a result of posttranscriptional processes or epigenetic mechanisms, such as DNA methylation or histone acetylation [10].

Conclusion. As a result of this study, it was found that there is some associa-

Table 2

Associations between genotypes of the rs6265 BDNF gene polymorphism and BDNF serum level in patients with VS

Genetic model	Genotype	Patients with BDNF concentration		OR (95%CI)	p	AIC
		<273.0 pg/mL, abs. (%)	≥273.0 pg/mL, abs. (%)			
Codominant	G/G	10 (34.5)	50 (70.4)	1.00	**0.0027**	115.1
	G/A	17 (58.6)	20 (28.2)	**4.53 (1.74–11.79)**		
	A/A	2 (6.9)	1 (1.4)	9.25 (0.76–113.24)		
Dominant	G/G	10 (34.5)	50 (70.4)	1.00	**0.0007**	113.4
	G/A–A/A	19 (65.5)	21 (29.6)	**4.79 (1.87–12.24)**		
Recessive	G/G–G/A	27 (93.1)	70 (98.6)	1.00	0.2000	123.2
	A/A	2 (6.9)	1 (1.4)	4.76 (0.41–55.16)		
Overdominant	G/G–A/A	12 (41.4)	51 (71.8)	1.00	**0.0033**	116.3
	G/A	17 (58.6)	20 (28.2)	**3.87 (1.54–9.74)**		
Log-additive	G/G–G/A–A/A	–	–	**4.00 (1.72–9.33)**	**0.0007**	113.4

Notes. Table 2 shows odds ratio and 95% confidence interval OR (95% CI); p – value of the level of statistically significant difference; AIC – Akaike information criterion value.

tion of the *rs6265 BDNF* gene polymorphism with BDNF serum level in patients with VS. Individuals with normal G/G genotype had a higher level of neurotrophin, while carriers of G/A and A/A genotypes had a lower BDNF content. The results of regression analysis indicate that carriage of the G allele prevents a decrease in BDNF concentration, while the presence of the A allele reduces the risk of neurotrophin hyperproduction. The risk of protein concentration reduction was increased by 4.79 times in carriers of the A-allele. The obtained results expand our ideas about genetic features that may predetermine the sensitivity of patients with VS to the production of brain-derived neurotrophic factor.

The results we obtained are preliminary and require further study due to the low frequency of certain genotypes and insufficient number of subjects in the groups.

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