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PREVALENCE OF 4a/4b POLYMORPHIC VARIANTS OF THE *eNOS* GENE INTRONE IN PATIENTS WITH DIFFERENT TYPES OF ENCEPHALOPATHIES

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Questions related to the disruption of vasoregulatory processes as essential factor in neurological pathology require further study. The leading role in the vasoregulatory mechanisms is played by endothelial NO synthase which gene has 15 allelic variants. Recent data indicated a probable association between eNOS gene polymorphism and cerebrovascular diseases. The aim of the present work was to study the prevalence of the 4a/4b introne polymorphism of the eNOS gene in patients with various types of encephalopathies and to evaluate the influence of a particular genotype of the studied gene on the occurrence and/or progression of encephalopathy. A total of 96 patients with encephalopathies of various genesis: chronic traumatic encephalopathy, chronic alcohol-induced encephalopathy, chronic vascular encephalopathy, post-infectious encephalopathy were involved in the study. The patients received inpatient treatment in the neurological department of "Ternopil Regional Clinical Psychoneurological Hospital". Molecular and genetic differentiation of the studied gene variants was carried out by allele-specific PCR or PCR-restriction fragment length polymorphism analysis by standard operational protocols. Analysis of the frequency distribution of eNOS gene 4a/4b polymorphic variant showed that 4b allele prevailed among patients with all types of encephalopathies carriers. Relative to practically healthy individuals, the difference was found only in patients with chronic vascular encephalopathy (CVE), among whom about 39% were carriers of the 4a allele. The presence of the 4a allele was shown to increase the risk of CVE occurrence and/or progression by 4.5 times. The results obtained suggest the reasonability to include the 4a/b intron polymorphism of the eNOS gene in a genetic panel to monitor patients CVE.

K e y w o r d s: encephalopathies, endothelial NO-synthase, 4a/4b introne, eNOS gene polymorphism.

Questions related to the disruption of vasoregulatory processes as essential factors in the occurrence of neurological pathology are controversial and require further study. There is currently no information regarding the nature of changes in vasoregulation in patients with various types of encephalopathies, except those of vascular origin. It is believed that the leading role in the violation of vasoregulatory mechanisms is played by the nitrogen (II) oxide (NO) – NOS synthase enzyme system [1]. Furthermore, studies have shown a link between NO levels and dementia [2]. Corzo L. also demonstrated that levels of serum NO are significantly lower in patients with dementia, especially in patients with vascular dementia [3]. NOS generates NO through sequential oxidation steps that convert the amino acid L-arginine to L-citrulline and have neurodegenerative and neuroprotective properties. There are three distinct forms of NOS, of which the first two isoforms, neuronal NOS (nNOS or NOS1) and inducible NOS (iNOS or NOS2), mediate early and late neurotoxic effects, respectively. At the same time, endothelial NOS (eNOS or NOS3) is mainly expressed in vascular endothelial cells and can prevent neuronal damage by producing small amounts of NO to dilate blood vessels, maintain cerebral circulation, inhibit platelet aggregation, and prevent the development of oxidative stress [4-6].

The human *eNOS* gene is located on chromosome 7q35–36, has 26 exons, and 25 introns and encodes a protein with a molecular weight of 135 kDa [7]. Today, 15 allelic variants are known in the *eNOS* gene, and polymorphisms are found in the promoter (regulatory regions), exons (informative regions) and introns (non-informative regions). The most studied are T-786C polymorphism of the promoter, G894T

(Glu298Asp) polymorphism of the 7th exon and 4a/b of the 4th intron of the *eNOS* gene [8]. 4a/4b polymorphism of the *eNOS* gene is due to the presence of 5- or 4-fold tandem repeats of 27 bp in intron 4. The standard version contains five repeats (denoted as 4b), the mutant– 4 (marked as 4a). Carriers of the 4b/4b genotype are homozygotes for the major allele, 4b/4a are heterozygotes, and carriers of the 4a/4a genotype are homozygotes for the minor allele.

It is believed that the 4a/4a variant of the *eNOS* gene polymorphism is associated with a violation of its expression and a decrease in NO synthesis. Still, the significance of this polymorphism remains unclear today and requires further study [9]. In particular, there are data that the microsatellite polymorphism of the 4th intron affected the level of nitrates and nitrites (NO_x levels) in the blood plasma – in carriers of the 4a/4a genotype, this indicator was significantly higher than in carriers of the normal genotype (4b/4b). The researchers note that this may not be due to increased activity of eNOS at all, but of other NO-producing systems, particularly iNOS [10]. V. E. Dosenko et al. studied the expression activity of the *eNOS* gene and the NOS enzyme in platelets isolated from individuals with arterial hypertension [11]. Enzyme activity was 1.7 times lower in carriers of the 4a/4a genotype compared to normal homozygotes ($P > 0.05$) and 1.9 times lower than in heterozygotes ($P > 0.05$). A positive correlation between the *eNOS* gene's expression and the eNOS enzyme's activity was not found. On the contrary, lower activity of the enzyme was recorded against the background of increased expression in individuals with the 4a/4a genotype. The researchers note that the reading of the eNOS gene with this intron variant is indeed more active. Still, the resulting RNA is somewhat defective, and a more significant number of eNOS protein molecules are not synthesized, or this protein has lower catalytic activity.

The aim of the present work was to study the prevalence of the 4a/4b polymorphism of the *eNOS* gene in patients with various types of encephalopathies and to evaluate the influence of the presence of a particular genotype of the studied gene on the occurrence and/or progression of encephalopathy.

Materials and Methods

Patients. A total of 96 patients with encephalopathies of various genesis were included in the study. The patients were receiving inpatient treatment in the neurological departments of the Communal Non-commercial Enterprise "Ternopil Regional Clinical Psychoneurological Hospital" of Ternopil Regional Council, Ternopil, Ukraine, during 2021–2022.

The examined patients were divided based on the genesis of encephalopathy. Thus, the distribution by type of encephalopathy was the following: chronic traumatic encephalopathy (CTE) $n = 26$, chronic alcohol-induced encephalopathy (AIE) $n = 26$, chronic vascular encephalopathy (CVE) (or microvascular ischemic disease of the brain or cerebral small vessel disease) $n = 18$ and postinfectious encephalopathy (PIE) $n = 26$. The control group consisted of 12 people, who were representative in age and gender.

Taking into consideration the fact that currently there is no unified classification of encephalopathies and their grades of severity, which would take into account the genesis and clinic of each type, the verification of various types of encephalopathies was carried out according to the criteria proposed by several authors [12-14]. Numerous factors, in particular, determine the course of each of the studied subtypes of encephalopathies: the cause of encephalopathy, its impact on the rate of development and progression of brain tissue damage and clinical presentation, and the effect of concomitant diseases. Each type of encephalopathy, depending on the severity and course of the disease, is characterized by a particular spectrum of neurological symptoms: behavioral disorders, apathy, changes in memory and attention, cognitive impairment up to dementia, extrapyramidal insufficiency, pyramidal insufficiency, moderate neurological deficit.

Patient inclusion criteria were the following: age from 18 to 75 years; compliance with diagnosis criteria; and availability of the patient's informed consent. Exclusion criteria: the presence of oncopathology; concomitant pathology in the stage of decompensation; use of psychoactive substances; the presence of other diseases that could cause psychoneurological disorders, behavioral and mental disorders.

The performed study is a single-moment clinical study of the "case-control" type. The study protocol included screening of patients to determine compliance with inclusion and exclusion criteria, carrying out laboratory determinations, genetic research, and statistical analysis of the obtained data. All patients were informed about the purpose of the clinical study and gave written informed consent for

their participation in it. Confidentiality about the patient's identity and state of health was preserved.

Ethical approval. The patient's informed consent form, examination card, and all stages of the research were approved by the bioethics commission of the I. Horbachevsky Ternopil National Medical University, Ternopil (protocol No. 74 dated September 1, 2023).

Molecular genetic study of polymorphic variant 4a/4b of the eNOS gene. Its first stage was isolating DNA from whole peripheral blood on a paper blank using the commercial kit "Quick-DNA Miniprep Plus Kit" (Zymo Research, USA) according to the instructions. Molecular and genetic differentiation of the studied gene variants was carried out by allele-specific PCR or PDRF PCR (restriction fragment length polymorphism) by standard operational protocols developed in the molecular genetics laboratory of the State Institution "Reference center for molecular diagnostics of the Ministry of Health of Ukraine" Kyiv.

Electrophoretic separation was carried out in the System for horizontal electrophoresis multi Sub Midi (Cleaver Scientific, Great Britain). The size of amplified and restriction fragments was estimated by comparison with the molecular weight marker GeneRuler DNA Ladder (Thermo Scientific, USA) in an ethidium bromide-stained 3% agarose gel (Cleaver Scientific, UK). In the visualization process, the formed fragments for each sample were evaluated and photofixation of the obtained images was carried out. The genotypes of the pieces were determined according to the SOPs approved by the institution by evaluating the molecular weight of the restriction/amplified fragments compared to the molecular weight and corresponding positive control samples (Table 1).

Statistics. The Hardy-Weinberg law was used to assess the correspondence between the genotypes of the selected sample and the general population.

Table 1. Molecular weight of restriction/amplified fragments

Gene and polymorphism, rs	The size of the restriction/ amplified fragments and the corresponding genotype
eNOS 4a/4b, rs61722009	Genotype *4b/4b*: 421bp
	Genotype *4b/4a*: 421 and 394 bp
	Genotype *4a/4a*: 394 bp

Comparison of observed frequencies and expected frequencies (Pearson Chi-Square, χ^2), calculated using Pearson's formula: $p^2 + 2pq + q^2 = 1$ (Hardy-Weinberg equilibrium), was carried out using Pearson's χ^2-square. When obtaining values of the reliability coefficient $P > 0.05$, we accepted the "null" hypothesis about the equality of the samples, that is, the correspondence between the selected model and the general population. Comparative analysis of frequency tables was performed using Pearson Chi-square (χ^2) and Fisher exact p, two-tailed (in those cases when the values of expected frequencies (expected frequencies) of individual indicators did not exceed 5). To assess the influence of the factor (the presence of a particular gene genotype) on the investigated feature (occurrence and progression of the disease), the odds ratio (OR) and its 95% confidence interval (95% CI) were calculated. The influence was considered statistically probable at $P < 0.05$ for the OR.

Results

Analysis of the frequency distribution of genotypes 4a/b–4 of the intron (4b/4a) of the *eNOS* gene according to the Hardy-Weinberg law and assessment of compliance with population balance was performed in patients with CTE, CVE, AIE and PIE and the control group. It was established that the frequency of the genotype responsible for the 4b/4a polymorphism of the *eNOS* gene both in patients with the studied types of encephalopathies and in the group of control did not significantly deviate from the Hardy-Weinberg equilibrium ($P > 0.05$) (Table 2).

The results of the frequency distribution of the genotypes of the *eNOS* gene showed that the 4b/4b genotype prevailed in patients with the studied types of encephalopathies and the control group (Table 3). Genotype 4a/4a was detected most rarely in patients with the studied types of encephalopathies. In contrast, this genotype was not detected both in the control group and in patients with CTE and PIE.

No statistically significant differences were found in comparing the distribution of *eNOS* gene genotypes in patients with the studied types of encephalopathies and the control group. At the same time, in the group of patients with CTE, the frequency distribution of genotypes of the *eNOS* gene probably differed from the data of patients with CTE and PIE ($\chi^2 = 19.45$; $P = 0.013$). It should be noted that genotype 4a/4a was detected only in patients with CVE (27.78%) and patients with AIE (15.38%).

Table 2. 4a/b−4 polymorphism of the intron of the eNOS gene according to the Hardy-Weinberg law in patients with various types of encephalopathies

Genotype	CTE		CVE		AIE		PIE		Control	
	expected	available	expected	available	expected	available	expected	available	expected	available
Homozygotes that occur frequently 4b/4b	20.35	20	9.31	9	12.46	14	22.16	22	9.19	9
Heterozygotes 4a/4b	5.31	6	3.38	4	11.08	8	3.69	4	2.63	3
Homozygotes, which are rare 4a/4a	0.35	0	0.31	0	2.46	4	0.15	0	0.19	0
χ^2, *P*	$\chi^2 = 0.44$; *P* > 0.05		$\chi^2 = 0.43$; *P* > 0.05		$\chi^2 = 2.00$; *P* > 0.05		$\chi^2 = 0.18$; *P* > 0.05		$\chi^2 = 0.24$; *P* > 0.05	

Note. χ^2 – Pearson Chi-square test; *P* – level of its significance. *Statistically significant result (*P* < 0.05)

Table 3. Polymorphism 4a/b−4 of the intron of the eNOS gene between the studied groups

Genotype	CTE		CVE		AIE		PIE		Control	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%
4b/4b	20	76.92	9	50.00	14	53.85	22	84.62	9	75.00
4a/4b	6	23.08	4	22.22	8	30.77	4	15.38	3	25.00
4a/4a	0	0.00	5	27.78	4	15.38	0	0.00	0	0.00
P (EP/CG)	$\chi^2 = 0.02$; *P* = 0.897		$\chi^2 = 4.11$; *P* = 0.128		$\chi^2 = 2.55$; *P* = 0.280		$\chi^2 = 0.51$; *P* = 0.478		–	
χ^2, *P*	$\chi^2 = 19.45$; *P* = 0.013*; $P_{1\text{-}2,2\text{-}4} < 0.05$*									

Note. χ^2 – Pearson Chi-square test; *P* – level of its significance. *Statistically significant result *P* < 0.05).

Analyzing the frequency distribution of *eNOS* gene alleles, it was established that among patients with CTE, CVE, AIE, and PIE, carriers of the 4b allele predominated (Table 4). Comparing the frequencies of alleles of the *eNOS* gene among patients with the studied types of encephalopathies, probable differences were found in the CVE group compared to the group of control (frequency of allele 4b – 61.11% vs. 87.50%; frequency of allele 4a – 38.89% vs. 12,50%).

It is worth noting a similar trend in the frequency distribution of *eNOS* gene alleles in patients with AIE compared to the control group (4b allele frequency – 69.23% vs. 87.50%; 4a allele frequency – 30.77% vs. 12.50%), however, these changes were statistically not significant.

Analyzing the OR and its 95% CI for genotypes 4a/b−4 of the intron of the *eNOS* gene in patients with the studied types of encephalopathies, no statistically significant relationship was established between carrying genotypes 4b/4b, 4a/4b, 4a/4a and the risk of encephalopathies (Table 5).

Analyzing the OR and its 95% CI for alleles of the *eNOS* gene in patients with the studied types of encephalopathies, it was established that there is a statistically significant relationship between the carriage of alleles 4b and 4a and the occurrence of encephalopathy only in patients with CVE (Table 6). Thus, the presence of the 4a allele increases the risk of encephalopathy in this cohort of patients by 4.5 times.

Table 4. The frequency of alleles 4a/b−4 of the intron of the eNOS gene in patients with various types of encephalopathies

Frequency of alleles	CTE		CVE		AIE		PIE		Control	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%
Allele 4b	46	88.46	22	61.11	36	69.23	48	92.31	21	87.50
Allele 4a	6	11.54	14	38.89	16	30.77	4	7.69	3	12.50
P (EP/CG)	*P* = 0.999		*P* = 0.040*		*P* = 0.153		*P* = 0.672		–	

Note. *Statistically significant difference ($P < 0.05$)

Table 5. Odds ratio for eNOS gene intron 4a/b−4 genotypes in patients with different types of encephalopathies

Type of encephalopathy	Polymorphism of the *eNOS* gene					
	4b/4b		4a/4b		4a/4a	
	OR	95% CI	OR	95% CI	OR	95% CI
CTE	1.11	0.23−5.47	0.90	0.18−4.43	0.47	0.01−25.18
CVE	0.33	0.07−1.65	0.86	0.15−4,76	10.19	0.51−203.67
AIE	0.39	0.09−1.77	1.33	0.28−6.28	5.00	0.25−100.68
PIE	1.83	0.34−9.90	0.55	0.10−2.94	0.47	0.01−25.18

Note. OR − odds ratio, 95% CI − 95% confidence interval. *Statistically significant difference ($P < 0.05$)

Table 6. Odds ratio for alleles 4a/b−4 of the intron of the eNOS gene in patients with different types of encephalopathies

Allele	CTE		CVE		AIE		PIE	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
4b	1.10	0.25−4.80	0.22*	0.06−0.89	0.32	0.08−1.23	1.71	0.35−8.34
4a	0.91	0.21−4.01	4.46*	1.12−17.76	3.50	0.91−13.51	0.58	0.12−2.84

Note. OR − odds ratio, 95% CI − 95% confidence interval. *Statistically significant difference ($P < 0.05$)

Discussion

A key role in cerebral blood flow is played by the NO signaling pathway, which is synthesized by eNOS and nNOS and can be altered or blocked by hypoxia and ischemia. An increase in the activity of nNOS and eNOS contributes to the activation of NO generation by endothelial and neuronal brain cells. Exactly in the endothelial cells of the brain, the enhanced regulatory activity of eNOS occurs, which contributes to its accumulation in these cells, leading to their further dysfunction [1]. eNOS-derived NO is very important for the maintenance of microvascular stability and vascular tone under physiological conditions [15]. Cardenas H. L. et al. noted that endothelial NO is an important mediator of proper cerebrovascular function [16] and Liao F. F. et al. note that eNOS-derived NO plays a key role in the regulation of basal cerebral blood flow via vasodilation of cerebral vessels, and aberrant biogenesis of NO is associated with cerebral small-vessel disease, cerebral hypoperfusion, and the impairment of the blood-brain barrier [17]. Lenz I. J. et al. demonstrated that eNOS-deficient mice have significantly increased morbidity and mortality after experimental subarachnoid hemorrhage. Researchers also found that eNOS-deficient mice have already a reduced microvessel density and a significant number of microvasospasms before subarachnoid hemorrhage [15].

Analyzing the frequency distribution of alleles of the polymorphic variant 4a/4b of the *eNOS* gene, we found that among patients with all types of encephalopathies studied, carriers of the 4b allele prevailed. At the same time, significant differences in the frequency distribution of alleles relative to prac-

tically healthy individuals were found only in patients with CVE, among whom 38.89% were carriers of the 4a allele (in the group of practically healthy individuals, only 12.5% were carriers of the 4a allele). Analyzing the odds ratio and its confidence interval for *eNOS* gene alleles in patients with CTE, CVE, AIE, and PIE, it was established that there is a statistically significant relationship between carrying the 4a allele and the risk of occurrence and/or progression of encephalopathy only in patients with CVE.

eNOS gene polymorphisms are known to be associated with development of vasospasm and increased chances of rupture of aneurysm [18]. There is data that genetic polymorphisms decreasing eNOS activity are linked to increased susceptibility for cerebrovascular disease [19, 20]. Moreover, 4a/4b (rs61722009) polymorphism has been reported to be involved in the atherosclerotic process. Significant associations were detected between the allelic and dominant models of the eNOS 4a/4b polymorphism, and carotid atherosclerosis risk in an Asian subgroup (allelic: $P = 0.02$; OR, 95% CI = 1.49 [1.07, 2.07]; dominant: $P = 0.01$; OR, 95% CI = 1.50 [1.09, 2.05]), but not in a Caucasian subgroup [21]. In the study of Z. Farbood et al., the potential association between *eNOS* 4a/4b gene polymorphisms and essential hypertension as an individual risk factor was examined in the southern population of Iran. Researchers demonstrated statistically significant associations between 4a/4a polymorphism of the *eNOS* gene and essential hypertension ($P < 0.05$) [22].

There is data that 4a eNOS polymorphism is associated with reduced NO production, in particular T. Tsukada et al. [23] showed that the plasma NO level of healthy subjects with the 'a' allele was significantly lower than in those without the 'a' allele ($P < 0.05$). Sivri N. et al. observed that eNOS 4a/b expression was increased in the coronary artery disease (CAD) group compared to the control group. The results also exhibited that eNOS intron 4a/b 27 VNTR polymorphism was associated with CAD, and homozygote 4a4a genotype frequency was significantly higher in the CAD group compared with the control group. Furthermore, the results stated that heterozygote 4a/4b genotype carriers have increased the level of eNOS expression compared with the same genotype in the control group, which might contribute to reducing the risk of CAD [24]. Probably, the increased risk of developing and/or progression of CVE in carriers of the 4a allele polymorphism of the 4th intron of the *eNOS* gene in the present study is associated with a decrease in the generation of NO in the vascular endothelium, which contributes to the development of atherosclerotic changes, thrombosis, vasospasm, and arterial hypertension.

Hassan A. et al. determined the role of 3 potentially functional eNOS polymorphisms (T-786C, intron 4ab, G894T) as risk factors for cerebral small-vessel disease and its different subtypes: isolated lacunar infarction ($n = 137$) and ischemic leukoaraiosis ($n = 160$). They found that the intron 4a allele of the *eNOS* gene was protective against cerebral small-vessel disease, an effect confined to isolated symptomatic lacunar infarction [25]. Researchers suggested that there are several possible explanations for the association between the 4a allele and disease. The intron 4 locus could act simply as a marker for another functional polymorphism in linkage disequilibrium. Another possibility is that the intron 4a allele has intrinsic functional significance because there was a weak association with plasma NO_x levels in their study. Although this variant lies within an intron, an insertion/deletion polymorphism could affect mRNA stability and enzyme levels. A third possibility is that the intron 4 locus modulates the effects of a variant in linkage disequilibrium. Because the combination of -786C and intron 4a was protective in our study, this haplotype could have a particular functional role. Consistent with this hypothesis, the intron 4a allele led to a significant increase in NO_x levels associated with the -786CC genotype and increased levels across the different T-786C genotypes. One potential explanation is that the intron 4 27-bp repeat element has a cis-regulatory role enhancing transcription activity at the -786 locus.

It is worth pointing out that some researchers did not find a probable association between 4a/4b (rs61722009) polymorphism of the *eNOS* gene and cerebrovascular diseases. In particular, V. E. Dosenko et al. demonstrated that allelic polymorphism in the promoter (T→C), but not in exon 7 (G→T) or the variable number tandem repeat in intron 4, of the *eNOS* gene was positively associated with acute coronary syndrome in the Ukrainian population. It was shown that the percentages of normal homozygotes, heterozygotes, and pathological homozygotes for the 4a/4b polymorphism in intron 4, were 64.7, 31.2 and 4.1%, respectively (controls: 62.7, 32.5 and 4.8%; $P > 0.05$) [26]. Ben Ali M. and co-authors investigated the relationship of the 894G>T (rs1799983) and 4a/4b (rs61722009) polymorphisms of the *eNOS*

gene with the presence of coronary artery disease in the Tunisian population and found that 4a/4b polymorphism was not associated with coronary artery disease under any of the genetic models tested [27]. Therefore, the question of the functional implementation of the polymorphism of the 4th intron of the *eNOS* gene remains open and requires further research.

Conclusions. For the first time in the Ukrainian population, an analysis of the frequency distribution of genotypes and alleles of the polymorphic variant 4a/b–4 of the intron of the *eNOS* gene in patients with encephalopathies of various genesis was performed and statistically significant differences were found only in patients with CVE compared to the healthy individuals. At the same time, the presence of the 4a allele of the *eNOS* gene increases the risk of CVE occurrence and/or progression by 4.5 times, which indicates the expediency of including the corresponding single-nucleotide polymorphism in the genetic panel of CVE patients.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

ПОШИРЕНІСТЬ ПОЛІМОРФНИХ ВАРІАНТІВ 4a/4b ІНТРОНУ ГЕНА *eNOS* У ХВОРИХ ІЗ РІЗНИМИ ТИПАМИ ЕНЦЕФАЛОПАТІЙ

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Порушення вазорегуляторних процесів, як суттєвого фактору у розвитку неврологічної патології, потребує подальшого вивчення. Провідну роль у вазорегуляторних механізмах відіграє ендотеліальна NO-синтаза, ген якої має 15 алельних варіантів. Результати нещодавніх досліджень вказують на те, що є ймовірний зв'язок між поліморфізмом гену *eNOS* та цереброваскулярними захворюваннями. Метою дослідження було вивчити поширеність інтрону 4a/4b поліморфізму гена *eNOS* у пацієнтів із різними типами енцефалопатій та оцінити вплив наявності певного генотипу досліджуваного гена на виникнення та/або прогресування енцефалопатії. У дослідженні взяли участь 96 пацієнтів із енцефалопатіями різного генезу: хронічна травматична енцефалопатія, хронічна алкоголь-індукована енцефалопатія, хронічна судинна енцефалопатія, післяінфекційна енцефалопатія. Пацієнти знаходились на стаціонарному лікуванні в неврологічних відділеннях Тернопільської обласної клінічної психоневрологічної лікарні. Молекулярно-генетичну диференціацію досліджуваних варіантів генів здійснювали методами алель-специфічної ПЛР або ПЛР ПДРФ (поліморфізм довжини рестрикційних фрагментів) згідно зі стандартними операційними протоколами. Аналіз частотного розподілу алелей поліморфного варіанту 4a/4b гена *eNOS* показав, що серед пацієнтів з усіма досліджуваними типами енцефалопатій переважали носії алелі 4b. Вірогідні розбіжності у частотному розподілі алелей відносно практично здорових осіб виявлено лише у пацієнтів з ХСЕ, серед яких 39% виявилися носіями алелі 4a. При цьому наявність алелі 4a гена *eNOS* підвищує ризик виникнення та/або прогресування ХСЕ у 4,5 рази. Це обумовлює доцільність включення поліморфізму 4a/b–4 інтрону гена *eNOS* до генетичної панелі дослідження пацієнтів із ХСЕ.

Ключові слова: енцефалопатія, ендотеліальна NO-синтаза, 4a/4b інтрон, поліморфізм *eNOS* гена.

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