ABSTRACT

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ASSOCIATION BETWEEN MALAT1 RS4102217-POLYMORPHIC VARIANT AND ISHEMIC ATHEROTHROMBOTIC STROKE DEVELOPMENT IN PEOPLE WITH INCREASED BODY MASS INDEX

Background. The recent discovery of a group of mediators known as long non-coding RNAs (lncRNAs) is the basis for research that will reduce the risk of cardiovascular disease in the long run. lncRNAs are expressed depending on conditions, and there is ample evidence of their involvement in a variety of biological processes. Indeed, lncRNA abnormalities are directly related to human diseases, including cardiovascular pathology and other diseases. LncRNA MALAT1 is one of the numerous factors causing functional changes in ischemic atherothrombotic stroke (IATS), in particular, it affects the functioning of endothelial cells and is involved in the implementation of inflammatory processes and regulation of autophagy. All those conditions play a role in the development of atherosclerosis, which underlies the pathogenesis of IATS. The effects of rs4102217-polimorphism of MALAT1 on IATS were poorly explored. This research aimed to find out, whether MALAT1 was associated with the susceptibility to IATS in patients with overweight.

Materials and Methods. A total of 200 ischemic atherothrombotic stroke patients and 234 controls without acute cardiovascular pathology were enrolled in this study. The rs4102217-polymorphisms in the promoter of MALAT1 were genotyped by using Real-Time PCR. Calculations were made using Statistical Package for the Social Sciences software (SPSS, version 17.0). A value of $P < 0.05$ was considered as statistically significant.

Results. The SNP rs4102217 in the promoter of MALAT1 was associated with the risk of ischemic atherothrombotic stroke in people with increased body mass index (BMI $\geq$ 25 kg/m^2) (Dominant model: adjusted OR = 1.66, 95% CI, 1,024–2,700, P = 0.040)

Conclusions. The results showed that c-carriers with elevated BMI were 1.66 times more likely to develop ischemic atherothrombotic stroke.

Keywords: SNP, ischemic atherothrombotic stroke, MALAT1, Polymerase chain reaction, long non-coding RNA.

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АСОЦІАТИВНЕ ДОСЛІДЖЕННЯ МІЖ МАЛАТ1 RS4102217-ПОЛІМОРФНИМ ВАРІАНТОМ ТА РОЗВИТКОМ ІШЕМІЧНОГО АТЕРОТРОМБОТИЧНОГО ІНСУЛЬТУ СЕРЕД ЛЮДЕЙ З ПІДВИЩЕНИМ ІНДЕКСОМ МАСИ ТІЛА

Вступ. Нещодавнє відкриття групи медіаторів, відомих як довгі некодучі РНК (lncRNAs), є основою для досліджень, які знизять ризик розвитку серцево-судинних захворювань у довгостроковій перспективі. lncRNA експресуються залежно від умов, і є багато доказів їхньої участі в різноманітних біологічних процесах. Дійсно, аномалії lncRNA безпосередньо пов'язані із захворюваннями людини, включаючи серцево-судинні та інші захворювання. Довга некодуюча РНК MALAT1 є одним із численних факторів, що викликають функціональні зміни при ішемічному атеротромботичному інсульті (IATI), зокрема впливає на функціонування ендотеліальних клітин та бере участь у здійсненні запальних процесів, регуляції аутофагії. Усі ці умови відіграють певну роль у розвитку атеросклерозу, що лежить в основі патогенезу IATI. Вплив поліморфізму rs4102217 MALAT1 на IATI мало досліджений. Метою цього дослідження було з'ясувати, чи пов'язаний MALAT1 зі сприйнятливістю до IATI у пацієнтів із надлишковою вагою.

Матеріали та методи. У дослідженні було залучено 200 пацієнтів з ішемічним атеротромботичним інсультом та 234 особи групи контролю без гострої серцево-судинної патології. Поліморфізм rs4102217, що знаходиться в промоторній ділянці гена MALAT1, був генотипований за допомогою ПЛР у реальному часі. Розрахунки проводилися за допомогою Statistical Package for the Social Sciences (SPSS, версія 17.0). Значення P < 0,05 вважалося значущим.

Результати. SNP rs4102217 у промоторі MALAT1 був пов'язаний з ризиком IATS у людей із підвищеним індексом маси тіла (IMT > 25 кг/м^2) (домінантна модель: після поправки OR = 1.66, 95% CI, 1,024–2,700, P = 0.040)

Висновки. Результати показали, що у носіїв мінорного алеля із підвищеним IMT в 1,66 рази більша ймовірність розвитку ішемічного атеротромботичного інсульту.

Ключові слова. Однонуклеотидний поліморфізм, ішемічний атеротромботичний інсульт, MALAT1, полімеразна ланцюгова реакція, довга некодуюча РНК.

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INTRODUCTION/ВСТУП

Ischemic atherothrombotic stroke (IATS) is one of the leading causes of mortality and disability worldwide. Discovering the cellular and molecular pathophysiological processes in IATS has been a top priority. In recent year, increasing evidence suggests that noncoding RNAs play important roles in the regulation of tissue homeostasis and pathophysiological conditions [1]. Long non-coding RNAs (lncRNAs) are transcripts > 200 nucleotides

long with little protein-coding function [2] that are characterized by low levels of sequence conservation and expression; lncRNAs modulate various biological functions at epigenetic, transcriptional and post-transcriptional levels, or directly regulate protein activity [3]. Nine specific lncRNAs, antisense non-coding RNA in the INK4 locus (ANRIL), metastasis-associate lung adenocarcinoma transcript 1 (MALAT1), N1LR, maternally expressed gene 3 (MEG3), H19, CaMK2D-associated transcript 1 (C2dat1), Fos downstream transcript (FosDT), small nucleolar RNA host gene 14 (SNHG14), and taurine-upregulated gene 1 (TUG1), were found increased in cerebral ischemic animals and/or oxygen-glucose deprived (OGD) cells [4]. Recently, MALAT1 expression was found in vascular endothelial cells, skeletal muscle, cardiomyocytes, and was suggested to participate in the pathological myogenesis and angiogenesis [5, 6, 7].

The aim. The purpose of the study was to analyze the relation between rs4102217 SNP and risk of development IATS among overweight people.

Materials and methods. In the study, venous blood samples of IATS patients and 234 normal controls (without acute cardiovascular pathology) were used. 200 patients with IATS were included in study group, mean age (66.72 ± 10.1) years. Patients of this group were on the outpatient follow-up in Sumy Clinical Hospital No. 5, inpatient treatment at the Sumy Regional Clinical Hospital. The diagnosis of IATS was based on anamnestic data, clinical manifestations of store and MRI of the brain.

The patients who were included in the control were treated at the Sumy Regional Clinical Hospital for War Veterans, their mean age (66.0 ± 14.53) years. In the control group, the absence of acute cardiovascular pathology was confirmed by ECG recording, blood pressure monitoring and generally accepted neurological examination.

The work was performed in accordance with the principles of Helsinki Declaration of the World Medical Association "Ethical principles of medical research with human participation as an object of study" and approved by the Commission on Bioethics of the Medical Institute of Sumy State University. All participants gave written informed consent for the use of blood in genetic research.

Determination of genotypes of time was performed by real-time polymerase chain reaction (Real-PCR).

Statistical analysis. The association between the rs4102217 SNP of the MALAT1 gene and the development of IATS was assessed using a logistic regression method adjusted for age, sex, body mass index (BMI), diabetes mellitus, and smoking habits. Continuous variables were showed as the mean $\pm$ SD, categorical – like absolute and percentage values. Categorical indicators were compared using the Chi square (χ^2) criterion. Two-tailed Student's t-test was used to compare the mean values in two groups, for comparison between the three groups using one-way analysis of variance (ANOVA). (Previously, the normal distribution of quantitative data was established using the Kolmogorov-Smirnov test). Values of $P < 0.05$ were considered reliable. Mathematical analysis of the results was performed in the SPSS complex (version 17.0, IBM, USA).

Results. In the study groups, the calculation of BMI (kg/m^2) was performed on the basis of the obtained indicators of height (cm), weight (kg).

One-way analysis of variance in the comparison groups for both women and men showed no association of different genotypes with values of height, weight and BMI (Table 1).

Sorting of individuals in both study groups depending on BMI $(< 25 \ kg/m^2$ and $\geq 25 \ kg/m^2)$ and subsequent analysis of genotype distribution by rs410227-polymorphism of the MALAT1 gene showed a significant difference $(P = 0.045)$ among individuals in control and examine groups with a body mass index more than $25 \ kg/m^2$, as shown in Table 2. There was no difference in the distribution $(P = 0.643)$ in the group of persons with a body mass index less than $25 \ kg/m^2$ between the group of control and the group of patients with IATS.

The use of one- and multifactor regression analysis within the four models of inheritance also did not show a significant difference among the studied groups in people with normal BMI. Regression analysis is shown in Table 3, among control and examine groups with elevated BMI, carriers of the minor allele had a 1.76 times higher risk of stroke than dominant homozygotes (according to the dominant model $R_{obs} = 0.021$). For heterozygotes compared to homozygotes for the main allele, the risk is 1.64 times higher (according to the additive model $RS_{obs} = 0.054$). After adjusting for age, gender, diabetes, and smoking habits, reliability remained only in the dominant model $(R_{adj} = 0.040)$, and the risk remained almost unchanged. The results of the analysis within the recessive and over-dominant models were not significant.

Table 1 – Anthropometric indicators in persons of different sexes in groups of comparison depending on the genotype of rs4102217-polymorphism of the MALAT1 gene, (M±m)

		Genotype			F	P
		G/G	G/C	C/C		
Women						
Hight, cm	Control	158,5 ± 7,90	158,6 ± 12,05	156,0 ± 3,61	0,365	0,695
	IATS	164,1 ± 6,35	166,1 ± 6,63	162,2 ± 3,49	1,391	0,254
Weight, kg	Control	68,8 ± 12,2	74,8 ± 10,16	71,3 ± 12,86	1,710	0,188
	IATS	76,7 ± 12,46	77,5 ± 9,31	76,0 ± 12,43	0,064	0,938
BMI kg/m^2	Control	28,1 ± 5,01	30,1 ± 5,52	29,4 ± 5,59	0,970	0,384
	IATS	28,5 ± 5,10	28,0 ± 3,39	28,8 ± 4,211	0,170	0,844
Men						
Hight, cm	Control	167,9 ± 8,43	168,8 ± 9,42	163,5 ± 2,12	0,415	0,661
	IATS	172,4 ± 7,53	173,1 ± 8,12	173,5 ± 6,98	0,127	0,881
Weight, kg	Control	75,8 ± 12,94	75,8 ± 15,78	67,5 ± 3,54	0,373	0,689
	IATS	81,6 ± 10,94	83,5 ± 17,57	87,5 ± 12,01	0,718	0,490
BMI kg/m^2	Control	26,9 ± 4,01	26,7 ± 5,28	25,3 ± 2,00	0,141	0,868
	IATS	27,5 ± 3,80	27,7 ± 4,27	29,1 ± 3,30	0,463	0,631

Note: F – Fisher's test; P – the significance of differences between genotypes according to one-way analysis of variance

Table 2 – Distribution of genotypes rs410227-polymorphism of the MALAT1 gene in patients with IATS and in the control group depending on BMI

Group	Генотип			P	X^2
	G/G	G/C	C/C		
BMI < 25 kg/m^2					
IATS, n (%)	38 (77,6)	9 (18,3)	2 (4,1)	0,643	0,884
Control, n (%)	57 (81,4)	12 (17,2)	1 (1,4)		
BMI ≥ 25 kg/m^2					
IATS, n (%)	93 (61,6)	49 (32,4)	9 (6,0)	0,045	6,197
Control, n (%)	121 (73,8)	39 (23,8)	4 (2,4)		

Note: n - number of patients; P is the statistical significance of differences in Pearson's χ^2 test

Discussion. To date, it is known that long non-coding RNA changing the expression of genes involved in the regulation of apoptosis, necrosis, inflammation and angiogenesis, they affect both the severity of the pathological process and the resistance of nerve cells to ischemia [4, 8, 9]. Metastasis-associated lung adenocarcinoma transcript (MALAT)-1 (also known as hepcarcin [HCN], nuclear paraspeckle assembly transcript [NEAT]2, PRO2853, and NCRNA00047) is one of the first lncRNAs to be identified and studied [10].

MALAT-1 is located on chromosome 11q13 and is approximately 8.7 kb in length [11], it is broadly expressed in mammalian tissue and cancers. Numerous studies show that the MALAT1 gene can regulate several stages in tumor development. MALAT1 has been demonstrated in cancers of breast, cervix, gallbladder, lung, ovary, pancreas, prostate, glioma, hepatocellular carcinoma and multiple myeloma [12]. There are also isolated studies on the role of long non-coding RNA Malat1 in the development of pathology of vessels,

including lupus erythematosus [13], coronary artery disease [14] and the development of atherosclerosis, which is a predictor of stroke. It is reported that MALAT1 involved in the protection of cerebral microvessels after ischemic insult from a RNA sequencing study. Thus, Zhang et al. showed that silencing of Malat1 in cultured BMECs and *in vivo* in mice increases the levels of pro-apoptotic factor Bim and proinflammatory cytokines MCP-1, IL-6 and E-selectin following ischemia [15]. Confirmation of protective properties is also reflected in the work of Wang et al. [16] who demonstrated that the rs1194338 (C > A) variant of the MALAT1 gene promoter is associated with a reduced risk of IATI development in the southwest Chinese population (OR = 0.596; P = 0.008 for the dominant inheritance model). Moreover, individuals with the rs600231A-rs1194338C-rs4102217G-rs591291C haplotype are 1.3 times more likely to develop IATS (P = 0.027). In Qi Gao and Yanfeng Wang study provides the evidence of MALAT1 as a regulator of endothelial cell apoptosis during ischemic stroke. MALAT1 may have anti-apoptotic effect in ischemic stroke and function as a ceRNA for miR-205-3p to modulate PTEN expression. Thus, MALAT1 can be a protect factor in OGD-induced endothelial injury [17].

Table 3 – Association analysis between rs4102217-polymorphism of the MALAT1 gene and IATS in individuals with BMI $\geq$ 25 kg/m^2

Regression model	Covariate	P_{obs}	OR_{obs} (95% CI)	P_{agj}	OR_{adj} (95% CI)
Dominant	GC + CC vs GG	0,021	1,76 (1,088 – 2,831)	0,040	1,66 (1,024 – 2,700)
Recessive	CC vs GC + GG	0,128	2,54 (0,764 – 8,411)	0,251	2,05 (0,601 – 6,994)
Over-dominant	GC vs GG + CC	0,088	1,54 (0,938 – 2,527)	0,177	1,42 (0,853 – 2,375)
Additive	GC vs GG	0,054	1,64 (0,992 – 2,695)	0,127	1,50 (0,892 – 2,506)
	CC vs GG	0,081	2,93 (0,874 – 9,801)	0,182	2,32 (0,675 – 8,005)

Note: 95% CI – 95% confidence interval; P_{obs} – observed value of P (without correction for covariates); OR_{obs} – observed ratio of odds; Corrections – corrections for age, gender, smoking habits and diabetes in BMI subgroups; OR_{adj} – ratio of odds after the correction for covariates

The opposite results were obtained by Nevine Fathy et al., according to their study MALAT1 rs619586 AA and rs3200401 CT, TT was associated with an increased risk of cerebral ischemic stroke [18]. The results of our study confirm the conclusions of other scientists on the important role of polymorphisms in the gene of long non-coding RNA MALAT1. Thus, in a study of individuals with elevated BMI, we found that carriers of the minor C-allele at the rs4102217-polymorphic locus have in a 1.66-fold higher risk of developing IATIS compared with carriers of GG-genotype.

CONCLUSIONS/ВИСНОВКИ

The results showed that the rs4102217 locus was associated with the onset of IATS. Individuals with the GG genotype have a lower risk of developing the disease compared to individuals with elevated BMI and C-allele carriers.

PROSPECTS FOR FUTURE RESEARCH/ПЕРСПЕКТИВИ ПОДАЛЬШИХ ДОСЛІДЖЕНЬ

Further studies with extended groups of comparison are needed for the confirmation of results. Moreover, it will be informative to study the association between other MALAT1 SNPs and IATS development.

CONFLICT OF INTEREST/КОНФЛІКТ ІНТЕРЕСІВ

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS/ВКЛАД АВТОРІВ

All authors substantively contributed to the drafting of the initial and revised versions of this paper. They take full responsibility for the integrity of all aspects of the work.

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