Sydorchuk Andrii, Sydorchuk Larysa. The severity of essential hypertension in terms of blood pressure values does not depend on *NOS3* (rs2070744) and *GNB3* (rs5443) genes polymorphisms in the West-Ukrainian population. Journal of Education, Health and Sport. 2021;11(10): 332-341. eISSN 2391-8306. DOI http://dx.doi.org/10.12775/JEHS.2021.11.10.032
https://apcz.umk.pl/JEHS/article/view/JEHS.2021.11.10.032
https://zenodo.org/record/5794084

The journal has had 5 points in Ministry of Science and Higher Education parametric evaluation. § 8. 2) and § 12. 1. 2) 22.02.2019.

Received: 30.09.2021. Revised: 12.10.2021. Accepted: 29.10.2021.

THE SEVERITY OF ESSENTIAL HYPERTENSION IN TERMS OF BLOOD PRESSURE VALUES DOES NOT DEPEND ON *NOS3* (rs2070744) AND *GNB3* (rs5443) GENES POLYMORPHISMS IN THE WEST-UKRAINIAN POPULATION

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Abstract

Objective: to evaluate the association of essential arterial hypertension (EAH) and its severity with genes polymorphism of *NOS3* (rs2070744) and *GNB3* (rs5443) in West-Ukrainian population.

Materials and methods. One-hundred EAH patients (48 – healthy control) participated in the cohort case-control study. Blood pressure (BP), Creatinine, glucose, lipids panel were studied. *GNB3* (rs5443) and *NOS3* (rs2070744) genotyping performed by TaqMan probes (CFX96™Real-Time PCR). Risk assessed by Relative Risk, Odds Ratio and 95% Confidential intervals.

Results. A mutation of the *NOS3* gene (*786T>C*, rs2070744) and the *GNB3* gene (*825C>T*, rs5443) in the homozygous state in the West-Ukrainian population suffers from EAH occurs with a frequency of 16.67% and 8.33%, with no differences with the control subjects (p>0.05). In both groups dominate the *T*-allele of the *NOS3* gene and the *C*-allele of the *GNB3* gene: in patients by 12.5% (χ^2=4.50; p=0.034) and 41.66% (χ^2=50.0; p<0.001), in the control – by 25.0% (χ^2=12.0; p<0.001) and 40.0% (χ^2=33.33; p<0.001), respectively. The results of the binary logistic regression analysis did not confirm the prediction of the EAH appearance by polymorphic variants of the *NOS3* (rs2070744) and *GNB3* (rs5443) genes. However, the *TT* genotype of the GNB3 gene (rs5443) increases unreliably the EAH risk almost twice as likely [OR=2.0; OR 95%CI:0.40-10.82; p>0.05]. Epidemiological analysis did not confirm the association of the *NOS3* gene with the EAH severity. But *T*-allele of the *GNB3* gene increases the probability of high normal BP almost 5 times [OR=4.86; OR 95%CI:0.99-24.75; p=0.042].

Conclusions: *NOS3* (rs2070744) and *GNB3* (rs5443) genes polymorphisms are not associated with blood pressure values and EAH severity as well.

Key words: NOS3 (rs2070744) and GNB3 (rs5443) genes; Arterial Hypertension; Risk.

Introduction. Essential arterial hypertension (EAH) is one of the most common cardiovascular disease worldwide (4.3 million / person / year). The risk of complications and death from EAH increases according to the number of concomitant risk factors [1-3]. Therefore, early detection and correction of risk factors is important for effective secondary prevention of EAH. The same applies to regular monitoring of blood pressure (BP). Thus, according to official statistics in the analysis of the EAH structure by BP, in 50% of patients have EAH 1st degree of BP elevation, every third EAH person – EAH 2nd degree, every fifth – EAH 3rd degree [2, 3]. But, the study of genetic and epigenetic risk factors for EAH, the severity of its course and the occurrence of complications requires further research [1].

The first organ affected by EAH is the vascular endothelium. In cardiovascular pathology and carbohydrate and lipid metabolism disorders (acute myocardial infarction, hypertension, stroke, acute / chronic heart failure, diabetes mellitus, metabolic syndrome, etc) endothelium undergoes functional and structural changes, loses its protective role, becoming a proatherosclerotic structure [4, 5]. Since the renin-angiotensin-aldosterone system (RAAS) is a multicomponent mechanism for the vascular tone regulation, blood vessels and myocardium remodelling, hydro and electrolyte balance, metabolic and hormonal homeostasis, study its

components, including the genetic markers of vascular endothelial activity – endothelial nitric oxide synthase (eNOS / NOS3) gene (rs2070744) and smooth muscle cell proliferation marker – guanine nucleotide-binding protein beta-3 (GNB3) gene (rs5443) is important in the pathogenesis of EAH development and its progression. Moreover, the identification of unmodifiable novel risk factors, including genetics, has been heavily investigated over the past few decades. In addition, some genetics predictors are of considerable interest since might allow identification of EAH subjects who could benefit from targeted genetically predisposed interventions.

Therefore, **the objective** of our study was to evaluate the association of EAH and its severity with genes polymorphism of NOS3 (rs2070744) and GNB3 (rs5443) in West-Ukrainian population (Bukovina region).

Material and methods

Study was performed in compliance with the Council of Europe Convention on Human Rights and Biomedicine and recommendations of the Committee on Bioethics of the Ministry of Health of Ukraine. Patients' Examination Cards and Patients' Informed Consent Forms were approved by the Biomedical Ethics Commission of Bukovinian State Medical University, Ministry of Health of Ukraine (Chernivtsi, Ukraine). After screening (matching inclusion/exclusion criteria) 100 EAH patients with hypertensive-mediated organs damaging (2^{nd} stage), the 1-3 degrees of BP elevation, moderate, high or very high cardio-vascular risk were involved in the cohort case-control study. There were 75% women, 25% men, mean age 59.87±7,98yo. The genetic examination was performed for 72 EAH patients. The control group consisted of 48 practically healthy who were not relatives of the patients and without reliable differences of gender distribution (62.5% females, 37.5% males) and mean age (49.13±6.28 yo) with a study group. All enrolled subjects signed a consent form to participate in the study. The inclusion and exclusion criteria had been presented in our former research [6-18].

All enrolled patients underwent a complex of examinations according European recommendations (ESC 2018, 2021): general clinical examinations, complete blood count, creatinine, uric acid, glucose, total cholesterol (TC) level, triglycerides (TG) and low/high density level cholesterol (LDL-C, HDL-C), body mass index (BMI, kg/m2), Waist circumference and Waist-to-Hip ratio (WHR) for evaluation of overweight and abdominal obesity, office measurement of systolic and diastolic BP (SBP, DBP), heart rate (HR), ECG in 12 leads, EchoCG; consultations of ophthalmologist and neurologist on demand.

The genes *NOS3* (rs2070744) and *GNB3* (rs5443) genotyping was performed with specific TaqMan signal probe by Real-Time Polymerase Chain Reaction (RT-PCR) using CFX96 RT-PCR Detection System. DNA was extracted from the lymphocyte's nuclei according to the Thermo Scientific GeneJET Genomic DNA Purification Kit manufacturer's Protocol (Thermo Fisher Scientific, USA), as it was described in our former publications [6-9, 11, 13, 14]. Allele's discrimination of *NOS3* (rs2070744) and *GNB3* (rs5443) genes polymorphisms was provided by licensed computer Software Bio-Rad RealTime (USA).

Statistical analysis was performed using StatSoft Statistica v. 7.0 (USA) software. Pearson's criterion ($\chi 2$) was used for the genotype's distribution comparison. Analysis of qualitative data (categorical variables), risk of pathology development was assessed by a binary logistic regression model using relative risk (RelR); risk ratio (RR) was estimated by odds ratio (OR) with 95% confidence interval [95% CI] using a chi-square test ($\chi 2$) (df=1). Differences were regarded as significant at $p<0.05$.

Results and discussion

Out of 144 isolated alleles in EAH patients and 96 alleles in the control group, the *T*-allele of the *NOS3* gene (*786 T> C*, rs2070744) dominated over the *C*-allele (Table 1): among patients – by 12.5% (χ^2=4.50; p=0.034), in the control group – by 25.0% (χ^2=12.0; p<0.001). The relative frequency of wild and mutational alleles, as well as individual genotypes, between patients and practically healthy people did not differ significantly.

Table 1 – Allele and genotypes distribution of *NOS3* gene *786 T>C* polymorphism in hypertensive patients and control

Polymorphic variants of *NOS3* gene		Patients, n=72 (%)	Control, n=48 (%)	χ^2	p
NOS3 (*786T>C*), n (%)	*TT*	21 (29.17)	20 (41.67)	2.0	>0.05
	TC	39 (54.17)	20 (41.67)	1.80	>0.05
	CC	12 (16.67)	8 (16.67)	-	>0.05
χ^2 ; p		χ^2=2,23; p>0,05		-	-
NOS3 (*786T>C*), n (%)	*T*-allele	81 (56.25)	60 (62.50)	<1.0	>0.05
	C-allele	63 (43.75)	36 (37.50)	<1.0	>0.05
χ^2 ; p		χ^2=4.50; p=0.034	χ^2=12.0; p<0.001	-	-

The alleles and genotypes distribution of the *GNB3* gene *825C>T* polymorphism (rs5443) between both groups did not differ significantly (Table 2). In both groups, the wild *C*-allele dominated over the *T*-allele: in EAH patients – by 41.66% (χ^2=50.0; p<0.001), in the control group – by 40.0% (χ^2=33.33; p<0.001), respectively.

The allelic distributions for both genes correspond to those for Caucasian populations and is fully consistent with *Hardy-Weinberg's* law equilibrium.

Table 2 – Allele and genotypes distribution of *GNB3* (rs5443) gene *825 C>T* polymorphism in hypertensive patients and control

Polymorphic variants of *GNB3* gene		Patients, n=72 (%)	Control, n=48 (%)	χ^2	p
GNB3 (*825C>T*), n (%)	*CC*	36 (50.0)	22 (45.83)	<1.0	>0.05
	CT	30 (41.67)	24 (50.0)	<1.0	>0.05
	TT	6 (8.33)	2 (4.17)	<1.0	>0.05
χ^2 ; p		χ^2<1,0; p>0,05		-	-
GNB3 (*825C>T*), n (%)	*C*-allele	102 (70.83)	68 (70.83)	-	>0.05
	T-allele	42 (29.17)	28 (29.17)	-	
χ^2 ; p		χ^2=50.0; p<0.001	χ^2=33.33; p<0.001		

The results of the epidemiological analysis did not confirm the prediction of polymorphic variants of the *NOS3* (rs2070744) and *GNB3* (rs5443) genes regarding the EAH appearance in the examined population (Table 3). However, the presence of the *TT* genotype of the *GNB3* gene (rs5443) almost doubles the EAH risk, but not significantly [OR=2.0; OR95% CI:0.40-10.82; p>0.05].

Table 3 – Polymorphic variants of *NOS3* (rs2070744) and *GNB3* (rs5443) genes as a prediction of essential arterial hypertension in the observed population

Potential predictors	RR	RR 95%CI	OR	OR 95%CI	P
Alleles and genotypes of the *NOS3* (rs2070744) gene					
TT-genotype	0.70	0.43-1.14	0.58	0.27-1.24	>0.05
TC- genotype	1.30	0.87-1.93	1.65	0.79-3.46	>0.05
CC- genotype	1.0	0.44-2.26	1.0	0.38-2.66	>0.05
TC+CC- genotypes	1.21	0.92-1.61	1.73	0.81-3.73	>0.05
T-allele	0.90	0.73-1.11	0.77	0.45-1.31	>0.05
C-allele	1.17	0.85-1.60	1.30	0.76-2.20	
Alleles and genotypes of the *GNB3* (rs5443) gene					
CC- genotype	1.09	0.72-1.60	1.18	0.57-2.46	>0.05
CT-r genotype	0.83	0.56-1.23	0.71	0.34-1.49	>0.05
TT- genotype	2.0	0.42-9.50	2.09	0.40-10.82	>0.05
CT+TT- genotypes	0.92	0.65-1.31	0.85	0.41-1.76	>0.05
C-allele	1.0	0.85-1.18	1.0	0.57-1.76	>0.05
T-allele	1.0	0.67-1.50	1.0	0.56-1.77	

The EAH severity according to the BP values depending on the *NOS3* (rs2070744) and *GNB3* (rs5443) genes polymorphisms are presented in tables 4 and 5. Significant differences of BP values have not been established between the genotypes' carriers of the *NOS3* gene (rs2070744). Whereas the *T*-allele's carriers of the *GNB3* gene (rs5443) in EAH patients with high normal SBP and DBP (130-139 / 80-89 mmHg) were more often registered than those with *CC*-genotype - by 16.67% (χ^2=4.18; p=0.041).

Table 4 – Arterial hypertension severity depending on polymorphic variants of *NOS3* (rs2070744) gene

Blood pressure values SBP/ DBP, mmHg		Genotypes of *NOS3* (*786T>C*) gene, n=72 (%)		χ^2	p
		TT-genotype, n=21	*TC*-, CC-genotypes, n=51		
SBP/ DBP, n (%)	130-139 / 80-89	4 (19.05)	6 (11.76)	<1.0	>0.05
	1st degree BP elevation 140-159 / 90-99	11 (52.38)	21 (41.18)	<1.0	>0.05
	2nd, 3rd degree BP elevation, ≥160 / ≥100	6 (28.57)	24 (47.06)	2.09	0.148

Table 5 – Arterial hypertension severity depending on polymorphic variants of *GNB3* (rs5443) gene

Blood pressure values SBP/ DBP, mmHg		Genotypes of *GNB3* (*825C>T*) gene, n=72 (%)		χ^2	p
		CC-genotype, n=36	*CT*-, *TT*-genotypes, n=36		
SBP/ DBP, n (%)	130-139 / 80-89	2 (5.55)	8 (22.22)	4.18	0.041
	140-159/ 90-99	19 (52.78)	13 (36.11)	2.03	0.154
	≥160 / ≥100	15 (41.67)	15 (41.67)	0	1.0

Epidemiological analysis did not confirm the prediction of the *NOS3* gene (rs2070744) regarding the EAH severity (Table 6). Whereas *T*-allele of the *GNB3* gene (rs5443) elevates the probability of high normal BP almost 5 times [OR=4.86; OR 95%CI:0.99-24.75; p=0.042].

Therefore, numerous genetic variations involving *NOS3* (rs2070744) and *GNB3* (rs5443) genes might influence the RAAS, endothelium function, vessels wall remodeling process, adipocytes activities, kidney failure development induce, worsening some metabolic disorders and hypertension course via diminishing of endothelial nitric oxide synthase activity, elevation of smooth muscle cell proliferation marker – guanine nucleotide-binding protein beta-3 secretion [19-21], as we have suggested in our research. The research

perspective of the current study wuold be supplemented by the linkage evaluation between metabolic disorders and *NOS3* (rs2070744) and *GNB3* (rs5443) genes' polymorphic variants, as well as some peculiarities of endothelium dysfunction in hypertensive patients.

Table 6 – Genes *NOS3* (rs2070744) and *GNB3* (rs5443) as a predictors of essential arterial hypertension severity

Genotypes	SBP/DBP, mmHg	RR	RR 95%CI	OR	OR 95%CI	P
NOS3 (786*T*>*C*, rs2070744) gene						
TT	<160/100	1.35	0.93-1.96	2.21	0.75-6.63	>0.05
	≥160/100	0.61	0.29-1.27	0.44	0.16-1.32	>0.05
TC, CC	<160/100	0.74	0.51-1.08	0.45	0.15-1.34	>0.05
	≥160/100	1.65	0.79-3.44	2.22	0.74-6.64	>0.05
GNB3 (825*C*>*T*, rs5443) gene						
CC	<140/90	0.25	0.06-1.09	0.21	0.04-1.02	0.041
CT, TT	<140/90	4.0	0.91-17.55	4.86	0.99-24.75	0.042
	<160/100	1.0	0.68-1.48	1.0	0.39-2.55	>0.05
	≥160/100	1.0	0.58-1.73	1.0	0.39-2.55	>0.05

Conclusions: A mutation of the *NOS3* gene (*786T>C*, rs2070744) and the *GNB3* gene (*825C>T*, rs5443) in the homozygous state in the West-Ukrainian population suffers from EAH occurs with a frequency of 16.67% and 8.33%, respectively, with no differences with the control subjects (p>0.05). In both groups dominate the *T*-allele of the *NOS3* gene and the *C*-allele of the *GNB3* gene: in patients by 12.5% and 41.66% (p<0.05), in the control – by 25.0% and 40.0% (p<0.001), respectively.

The results of the binary logistic regression analysis did not confirm the prediction of the EAH appearance by polymorphic variants of the *NOS3* (rs2070744) and *GNB3* (rs5443) genes. However, the *TT* genotype of the GNB3 gene (rs5443) increases unreliably the EAH risk almost twice as likely [OR=2.0; OR 95%CI:0.40-10.82; p>0.05]. Epidemiological analysis did not confirm the association of the *NOS3* gene (rs2070744) with the EAH severity. But in *T*-allele carriers of the *GNB3* gene (rs5443) increases the probability of high normal BP almost 5 times [OR=4.86; OR 95%CI:0.99-24.75; p=0.042].

Conflict of Interest

The authors declare no conflict of interest.

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