Linkage of Metabolic Disorders, Endothelial Dysfunction and NOS3 (rs2070744) and GNB3 (rs5443) Genes Polymorphisms in Hypertensive Patients

Andrii Sydorchuk 1, Larysa Sydorchuk 2*, Adelina Gutnitska 2, Anna Margaryan 3, Valentina Dzhuryak 2, Ruslan Sydorchuk 2*, Oksana Iftoda 2

1 Emergency and Trauma Surgery Department, Donauklinik, Neu Ulm, Germany
2 Department of Family Medicine, Bukovinian State Medical University, Ukraine
3 Department of First Aid, Emergency and Civil Protection, Yerevan State University, Yerevan, Armenia
* Correspondence: rsydorchuk@bsmu.edu.ua (L.S.); Scopus Author ID 8428375700

Received: 18.12.2021; Accepted: 15.01.2022; Published: 25.03.2022

Abstract: The vascular endothelium is one of the earliest damage-targeted hypertensive-mediated organs. The study aims to analyze the link of metabolic disorders with endothelial dysfunction (ED) and NOS3 (rs2070744) and GNB3 (rs5443) genes polymorphism in essential arterial hypertension (EAH). One hundred EAH patients (48 – healthy control) participated in the case-control study. Creatinine, glucose, triglycerides, total cholesterol (TC), low/high-density lipoproteins values (LDL-C, HDL-C), Atherogenicity Index (AI), Soluble Vascular Cell Adhesion Molecule, total NO metabolites (NO2-/NO3-) were studied. GNB3 (rs5443) and NOS3 (rs2070744) genotyping performed by TaqMan probes (CFX96™Real-Time PCR). Moderate-severe ED in EAH patients associated with higher blood pressure – by 6.90% and 4.69% (P<0.05), creatinine blood increase – by 10.08% (P=0.037), profound dysmetabolic changes but only in men: hyperglycemia by 46.46% (P=0.004), hypercholesterolemia and lower HDL-C content by 15.79% (P=0.024), AI elevation by 33.93% (P=0.029). TT-genotype of GNB3 gene patients has a higher TC blood content by 13.97% (P=0.035). The one-way ANOVA analysis did not confirm the linkage of the NOS3 (rs2070744) and GNB3 (rs5443) genes polymorphic site with metabolic disorders. Meanwhile, the risk of obesity increases in EAH patients with C-allele of NOS3 gene and T-allele of GNB3 gene almost 6 and 10 times [OR95%CI:2.11-14.82; P<0.001 and OR 95%CI:2.25-45.44; P<0.001], respectively.

Keywords: NOS3 (rs2070744) and GNB3 (rs5443) genes; arterial hypertension; endothelial dysfunction; metabolic disorders; obesity; risk.

© 2022 by the authors. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Essential Arterial Hypertension (EAH) remains the most widespread cardiovascular disease worldwide and is often associated with metabolic disorders. EAH enchains to metabolic syndrome and is followed by visceral obesity, hyperglycemia, dyslipidaemia, and blood pressure (BP) elevation [1,2]. Each component, independently or in combination, may affect vascular endothelium. Moreover, vascular endothelium is one of the earliest damage-targeted hypertensive-mediated organs. On the other hand, the primary loss or change of physiological endothelial functions with vascular relaxation insufficiency can lead to atherosclerosis, hypertension, diabetes, hyperhomocysteinemia, etc. [3-5]. Healthy endothelium maintains the vascular tone and proper smooth muscle vascular dilation through the endothelium-derived...
hyperpolarizing factors release, monoxide nitrogen (NO) synthesis, inhibition of leukocyte adhesion and migration with antioxidant and anti-inflammatory effects, suppression of Endothelin-1 production, angiotensin II influence, which prevent smooth muscle cell proliferation and migration and vascular wall remodeling afterward. The endothelial dysfunction (ED) is associated with the loss of physiological functions that are often caused by such metabolic disorders as hypercholesterolemia, dyslipidaemia, hyperglycemia, hyperhomocysteinemia, etc. [5] and may result in thrombosis, atherosclerosis, acute or chronic vascular catastrophes [6]. Endothelium responds to mentioned above pathological conditions by increasing the cyclooxygenases (COX-2) release, vascular reactive oxygen species (ROS), and Nox, nicotinamide adenine dinucleotide phosphate (NADPH) oxidases formation, and consequently the oxidative stress evoke and augment driving vascular growth and remodeling [7-10]. Therefore, metabolic syndrome can switch normal endothelium performance to a dysfunctional state, promoting ROS production and low/high-grade systemic inflammation via NADPH and adipocytokines synthesis increase and protective superoxide dismutase (SOD) attenuation. COX-2, ROS, and NADPH pathways activation results in diminished endothelial nitric oxide synthase (eNOS) production through the two key mechanisms: depressed soluble guanylate cyclase (sGC) activity and reduced L-arginine transformation. Furthermore, inactivation of 5′-AMP-activated protein kinase (AMPK) and tetrahydrobiopterin (BH4) enhance the reduced activity of eNOS. ROS, COX-2, and NADPH drive the synthesis of numerous endothelium-derived contracting factors (EDCF) like prostaglandin F2α, thromboxane A2, endothelin-1, phenylephrine, and pro-inflammatory adipokines release, deepening further endothelium imbalance and ED.

Various studies demonstrate that metabolic disorders and ED-related hypertension pathways might be partially genetically determined by genes regulating RAAS or NO activity or code the enzymatic synthesis or expression, or associate with vascular smooth muscles remodeling and hypertrophy [11-19]. However, the mechanisms that couple metabolic disorders with ED and genetic factors in hypertensive patients still have to be determined.

Therefore, the study aims to analyze the link of metabolic disorders with ED and polymorphic variants of NOS3 (rs2070744) and GNB3 (rs5443) genes in EAH patients.

2. Materials and Methods

2.1. Compliance with bioethics.

The study fully adhered to European Convention on Human Rights and Biomedicine, GCP, GLP principles, EUC directive #609, and other EU and international legislation on bioethics. The Study Protocol was approved by the Ethics’ Committee of the Bukovinian State Medical University (Protocol №2 from 14.10.2021). The research is defined as a prospective, cohort, case-control study.

2.2. Diagnosis of arterial hypertension.

Hypertension was defined as office systolic BP (SBP) values ≥140 mmHg and/or diastolic BP (DBP) values ≥90 mmHg at least for three measurements during a month, according to European Societies of Hypertension and Cardiology (ESH/ESC) recommendations [20, 21].
All enrolled patients underwent a complex of examinations: general clinical examinations, complete blood count, creatinine, glucose, total cholesterol (TC) level, triglycerides (TG) and low/high-density lipoproteins level cholesterol (LDL-C, HDL-C), Atherogenicity Index (AI = (TC – HDL-C) / HDL-C, Unit), body mass index (BMI, kg/m2) for evaluation of overweight and abdominal obesity (AO), Waist circumference (WC), Waist-to-Hip ratio (WHR), office measurement of SBP, DBP, heart rate (HR), GFR calculation (according to Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation with Creatinine level), ECG in 12 leads, EchoCG, consultations of ophthalmologist and neurologist according to European recommendations (ESC 2018, 2021).

2.3. Inclusion/exclusion criteria.

The study included EAH patients with hypertensive-mediated organ damage (HMOD) estimated according to European Societies of Hypertension and Cardiology recommendations (ESH/ESC 2018, 2021) [20, 21]: target-organ damage – 2nd stage (asymptomatic EAH), from the 1st through to the 3rd grade of blood pressure (BP) elevation; moderate-high cardiovascular (CV) risk; aged from 45 to 65 years.

Exclusion criteria have been described in previous publications [11, 17, 18, 22-25]: we excluded patients with EAH stage 3 (established CV disease, chronic kidney diseases (CKD) – with estimated glomerular filtration rate (eGFR) decline <30 ml/min/1.73 m2); secondary arterial hypertension; EAH patients with complications of HMOD, chronic heart failure (CHF) higher than II functional class (NYHA III-IV); diabetes mellitus type I (DM 1), sub- and decompensated diabetes mellitus (DM) type 2 (with diabetic target-organ damage); malignant or uncontrolled arterial hypertension; sub- and decompensated liver diseases (triple growth over the normal level of aspartate aminotransferase, alanine aminotransferase); bronchial asthma, chronic obstructive pulmonary disease of III-IV stage with C or D risk value (GOLD 2019); acute or exacerbated infectious diseases, or during unstable remission; psychological disorders; malignancies of any location; administration of oral corticosteroids or contraceptives; pregnancy or lactation.

After screening of matching inclusion and exclusion criteria, 100 patients were selected for further examination (75% women, 25% men, mean age 59.87±7.98 yo). The genetic examination was performed in 72 patients. The control group included 48 practically healthy individuals who were not relatives of the patients and without reliable differences in gender distribution (62.5% females, 37.5% males) and mean age (49.13±6.28 yo) with the study group. All enrolled subjects signed a consent form to participate in the study.

2.4. Genotyping of the endothelial nitric oxide synthase (NOS3, rs2070744) and guanine nucleotide-binding protein beta-3 (GNB3, rs5443) genes polymorphisms. DNA isolation, amplification and genotyping.

Venous blood was collected in a sterile vacutainer, stabilized by K2-EDTA. DNA was isolated from the whole venous blood lymphocytes’ nuclei and purified according to GeneJET Genomic DNA Purification Kit Manufacturer’s Guidance (Thermo Fisher Scientific, USA). DNA fragments of analyzed genes amplified by Quantitative Real-Time PCR (qRT-PCR) with specific for each gene TaqMan probes and genotyping with TaqMan Genotyping Master Mix on CFX96 Touch™ RT-PCR Detection System (Bio-Rad Laboratories, Inc., USA). The genotyping protocol was described in our previous publications [11, 17, 18, 22-25]. Alleles’
discrimination of NOS3 (rs2070744) and GNB3 (rs5443) genes polymorphisms were analyzed by licensed CFX96 RT-PCR Detection System Software (Microsoft, USA).

2.5. Markers of endothelial function.

Soluble Vascular Cell Adhesion Molecule (sVCAM-1; CD 106) level in serum was determined by the Enzyme Linked-Immuno-Sorbent Assay (ELISA) according to the Manufacturer’s Guidelines with highly sensitive sVCAM-1 ELISA KIT® (Diaclon SAS, France) on a “StatFax 303” equipment (USA). The sVCAM-1 assay has a sensitivity of 0.6 ng/mL.

The Monoxide Nitrogen metabolites (NO2-/NO3-) concentration was evaluated in serum, stabilized with EDTA (1 mg/ml), by colorimetric method with Total NO/NO2-/NO3-Assay Kit (RDS, UK) on a 550 nm Spectrophotometer (TS8210, China). Detection principle: after recovery of nitrate to nitrite by nitrate-reductase enzyme, nitrite reacts with chromogenic agent producing light red azo-compound, and the content of nitrite can be calculated by measuring the OD value at 550 nm.

2.6. Carotid Intima-Media Thickness and Endothelium-Dependent Flow-Mediated Dilation of the Brachial Artery Ultrasound Assessment

Endothelial-Dependent Flow-Mediated Dilation of the Brachial Artery (FMD BA) measurement according to the FMD assessment Guidelines [26-29] using ultrasonographic complex “ACCUVIX A30” (Samsung Medison, South Korea) with duplex scanning of brachial arteries (BA), high-frequency vascular transducer, color, and spectral Doppler and internal ECG monitor. A blood pressure cuff was applied to the forearm and inflated to a pressure that was 50 mm Hg above the baseline SBP for 5 min. From 30 s before to 2 min after cuff deflation, the BA diameter was recorded on ultrasound. Cuff deflation induces reactive hyperemia – a brief high-flow through the brachial artery to accommodate the dilated resistance vessels. An increase of internal BA diameter was expressed in the percentage of the baseline BA diameter. The diameter increase of less than 10% was determined as Endothelial dysfunction (ED) or FMD insufficiency.

2.7. Statistical analysis.

Statistical analysis performed using StatSoft Statistica v.7.0 software (StatSoft Inc., USA). To verify the differences between groups, we applied the Student’s t-test (two-tail distribution and equal variances between the two samples), ANOVA, Pearson’s χ2 test, or the Wilcoxon-Mann-Whitney U-test (in case of uneven data distribution according to W-Shapiro-Wilk or Kolmogorov-Smirnov test results). The risk of pathology was assessed by a binary logistic regression model using relative risk (ReIR); risk ratio (RR) was estimated by odds ratio (OR) with 95% confidence interval [95% CI] using a chi-square (χ2) test (df=1). Differences were regarded as significant at P<0.05 values.
3. Results and Discussion

3.1. Metabolic disorders, Endothelial Dysfunction, and genes polymorphism of NOS3 (rs2070744) and GNBP3 (rs5443) in hypertensive patients.

The cohort of patients (n=100) was divided into groups depending on ED severity considering FMD BA deviations, total NO metabolites decrease, and sVCAM-1 concentration elevation (Table 1). Mild ED grade (1st degree) was regarded by reduced FMD BA below the upper quartile (percentile) of the patient’s group (10.0-8.0%), and total NO metabolites (25-21 µmol/l) as well, with normal sVCAM-1 level (<1050 ng/ml); Moderate ED grade (2nd degree) – a decrease of the examined variables within the interquartile range (Q2): FMD BA – 7.9-7.0%, NO2/NO3 – 20.9-18.0 µmol/l, with increased sVCAM-1 value of 1050-1390 ng/ml; Severe ED grade (3rd degree) was extrapolated by FMD BA, and total NO metabolites decrease below the lower quartile (Q1): FMD BA <7.0%, NO2/NO3 <18.0 µmol/l, and an increase of sVCAM-1 >1390 ng/ml.

Eighteen EAH patients had a mild ED (1st degree), 82 individuals – had moderate and severe ED (2nd and 3rd degree). Clinical and biochemical parameters considering ED severity are presented in Table 1. SBP and DBP in moderate and severe ED patients are higher than those with mild ED – by 6.90% (P=0.001) and 4.69% (P=0.024), respectively. On the contrary, the WHR in males with mild ED is higher than in moderate-severe ED cases – by 14.43% (P=0.005). It should be noted that WHR and GFR (CKD-EPI by creatinine) in males are higher than those in females depending on ED severity: with WHR – by 26.14% (P=0.002) and 7.78% (P=0.001), with GFR – by 17.01% (P=0.01) and 20.46% (P<0.001), respectively. The EAH patients with the 2nd_3rd stages ED had higher Creatinine levels. On the contrary, GFR (CKD-EPI) was lower irrespective of gender than with mild ED: after blood Creatinine – by 10.08% (P=0.037), GFR in males – by 8.41% (P=0.004), in females – by 11.03% (P=0.027), respectively. There were no significant differences found, emphasizing the rest of the parameters.

Table 1. Clinical and biochemical parameters depending on the endothelial dysfunction severity and gender, M±SD.

| Parameters                      | Control              | Mild ED (1st degree) | Moderate, severe ED (2nd, 3rd degree) |
|---------------------------------|----------------------|----------------------|---------------------------------------|
| SBP, mmHg                       | 117.0±2.32           | 152.0±3.80           | P<0.001                               |
|                                |                      | 162.49±2.54          | P<0.001 P1=0.001                      |
| DBP, mmHg                       | 76.05±2.79           | 93.33±2.10           | P<0.001                               |
|                                |                      | 97.71±1.65           | P<0.001 P1=0.024                      |
| BMI, kg/m²                      | 24.82±1.25           | 30.83±1.58           | P<0.001                               |
|                                |                      | 31.39±1.32           | P<0.001                               |
| WC, sm                          | M 96.10±2.24         | 117.0±2.83           | P=0.001                               |
|                                | F 78.79±2.93         | 95.67±4.38           | P=0.001 Pm=0.02                       |
|                                |                      | 101.0±4.36           | P<0.001 Pm=0.001                      |
| WHR, U                          | M 0.93±0.03          | 1.11±0.03            | P=0.001                               |
|                                | F 0.78±0.02          | 0.88±0.02            | P=0.001 Pm=0.001                      |
|                                |                      | 0.90±0.01            | P=0.001 Pm=0.001                      |
| Plasma Creatinine, µmol/l       | 71.60±3.22           | 73.23±2.55           | P=0.045 P=0.037                       |
|                                |                      | 80.61±3.09           | P=0.045 P=0.037                       |
| GFR by Creatinine CKD-EPI, ml/min/1.73m² | M 129.1±6.95     | 95.26±1.13           | P=0.003                               |
|                                | F 91.12±6.45         | 81.41±4.02           | P=0.001 Pm=0.001                      |

ED – endothelial dysfunction; SBP / DBP – systolic/diastolic blood pressure; BMI – body mass index; M – males; F – females; WC – waist circumference; WHR – Waist-Hip ratio; GFR – glomerular filtration rate by Creatinine (CKD-EPI); P – significance of differences with a control group; P1 – significance of differences with patients’ group with Mild ED; Pm – significance of differences between genders (with males).
Analysis of the carbohydrate-lipid metabolism values depending on ED severity showed that the 2nd-3rd degrees of ED was associated with higher glucose level and AI – by 46.46% (P₁=0.004) and 33.93% (P₁=0.029) with lower HDL-C content than in mild ED patients – by 15.79% (P₁=0.024), though it concerns only males (Table 2).

Table 2. Lipids’ panel and glucose value depending on the endothelial dysfunction severity and gender, M±SD.

| Parameters          | Control         | Mild ED (1st degree) | Moderate, severe ED (2nd, 3rd degree) |
|---------------------|-----------------|----------------------|-------------------------------------|
| Glucose, mmol/L     | 5.08±0.17       | 6.81±0.66 P<0.001    | 7.5±0.86 P<0.001                    |
| Glucose, mmol/L     | F: 5.0±0.16     | M: 5.28±0.13         | 5.73±0.23 P=0.004                   |
| Glucose, mmol/L     |                 | T: 5.28±0.13         | 5.62±0.31                           |
| TC, mmol/L          | 5.57±0.22       | 5.62±0.31            | 5.6±0.3                             |
| TG, mmol/L          | 1.64±0.16       | 2.12±0.18 P<0.01     | 1.94±0.25                           |
| LDL-C, mmol/L       | 3.90±0.24       | 4.32±0.30            | 4.14±0.26                           |
| HDL-C, mmol/L       | 1.40±0.09       | 1.28±0.07 P<0.01     | 1.17±0.06 P<0.006                   |
| AI, U               |                 | T: 1.53±0.07         | 1.32±0.07 P<0.003                   |
| AI, U               |                 | F: 1.53±0.07         | 1.32±0.07 P<0.003                   |
| AI, U               |                 | M: 1.24±0.09         | 1.14±0.06 P<0.005                   |
| AI, U               |                 | P=0.015              | 0.96±0.05 P<0.002                   |
| AI, U               |                 |                      | P=0.02                             |
| HDL-C, mmol/L       |                 |                      |                                    |
| Glucose, mmol/L     | 2.99±0.34       | 3.54±0.27            | 3.95±0.32 P<0.042                   |
| Glucose, mmol/L     | F: 2.72±0.26    | M: 2.2±0.033         | 3.44±0.23 P<0.004                   |
| Glucose, mmol/L     |                 |                      | 3.68±0.25 P<0.009                   |
| TC – Total cholesterol; TG – Triglycerides; LDL-C / HDL-C – low / high-density lipoproteins cholesterol; AI – atherogenicity index; P – the significance of differences with the control group; P₁ – the significance of differences with patients’ group with Mild ED; P_F – the significance of differences between genders (with females).

Table 3. Lipids’ panel and glucose value depend on the polymorphic variants of NOS3 gene (rs2070744), M±SD.

| Parameters          | Genotypes of NOS3 gene in the control | Genotypes of NOS3 gene in patients |
|---------------------|--------------------------------------|----------------------------------|
| Glucose, mmol/L     | TT- 5.07±0.12                        | 7.84±1.05 P=0.006               |
| Glucose, mmol/L     | TC- 5.08±0.23                       | 6.72±0.64 P=0.008               |
| Glucose, mmol/L     | CC- 5.17±0.25                       | 7.63±1.07 P<0.039               |
| TG, mmol/L          | TT- 1.72±0.27                       | 1.87±0.19                       |
| TG, mmol/L          | TC- 1.62±0.14                       | 2.02±0.30                       |
| TG, mmol/L          | CC- 1.57±0.20                       | 2.12±0.46                       |
| TC, mmol/L          | TT- 5.57±0.28                       | 5.82±0.35                       |
| TC, mmol/L          | TC- 5.49±0.22                       | 5.72±0.39                       |
| TC, mmol/L          | CC- 5.59±0.20                       | 5.65±0.30                       |
| HDL-C, mmol/L       | TT- 1.56±0.12                       | 1.26±0.09 P=0.015               |
| HDL-C, mmol/L       | TC- 1.31±0.15                       | 1.25±0.08                       |
| HDL-C, mmol/L       | CC- 1.39±0.14                       | 1.23±0.06                       |
| LDL-C, mmol/L       | TT- 3.78±0.24                       | 4.40±0.32                       |
| LDL-C, mmol/L       | TC- 4.05±0.27                       | 4.26±0.38                       |
| LDL-C, mmol/L       | CC- 4.04±0.25                       | 4.12±0.29                       |
| AI, U               | TT- 3.43±0.40                       | 3.7±0.034                       |
| AI, U               | TC- 3.43±0.40                       | 3.7±0.034                       |
| AI, U               | CC- 3.26±0.42                       | 3.7±0.034                       |
| P – significance of differences with control group.
The TC blood concentration was higher in EAH patients with TT-genotype of GNB3 (rs5443) gene than in the CC-genotype carriers – by 13.97% (P=0.035) (Table 4). In the control TG blood content prevailed among homozygous C-allele carriers over those with TT-genotype by 48.82% (P=0.019). But the one-way ANOVA analysis did not confirm the linkage of GNB3 (rs5443) gene with the lipid parameters and glucose metabolism values either in EAH patients or the control group. At the same time, the relative incidence of obese patients was almost in every third EAH patients with the mutated T-allele of the GNB3 gene (30.56%), in the absence of such among the healthy. The epidemiological analysis confirmed more than 10 times [OR=10.12; OR 95%CI: 2.25–45.44; P<0.001] increased risk of obesity in EAH patients with T-allele of the GNB3 (rs5443) gene.

| Parameters | Genotypes of GNB3 gene in the control | Genotypes of GNB3 gene in patients |
|------------|----------------------------------------|----------------------------------|
| Glucose, mmol/L | CC- 5.14±0.18 | 7.38±0.91 P=0.007 |
| | CT- 5.19±0.20 | 7.16±0.81 P=0.019 |
| | TT- 4.90±0.22 | 6.32±0.40 P=0.014 |
| TG, mmol/L | CC- 1.89±0.17 | 2.10±0.34 |
| | CT- 1.54±0.20 | 1.77±0.22 |
| | TT- 1.27±0.25 | 2.40±0.39 P=0.043 |
| TC, mmol/L | CC- 5.45±0.35 | 5.51±0.32 |
| | CT- 5.69±0.26 | 5.90±0.44 |
| | TT- 5.46±0.22 | 6.28±0.27 P=0.05 |
| HDL-C, mmol/L | CC- 1.34±0.13 | 1.22±0.10 |
| | CT- 1.46±0.10 | 1.27±0.07 P=0.033 |
| | TT- 1.48±0.07 | 1.29±0.04 P=0.028 |
| LDL-C, mmol/L | CC- 3.78±0.24 | 4.40±0.32 |
| | CT- 4.05±0.27 | 4.26±0.38 |
| | TT- 4.02±0.25 | 4.12±0.29 |
| Al, U | CC- 3.45±0.47 | 3.66±0.33 |
| | CT- 3.14±0.39 | 3.76±0.37 |
| | TT- 2.82±0.31 | 3.87±0.24 P=0.026 |

P – significance of differences with control group; PCC, PCT - significance of differences with CC-genotype and CT-genotype carriers in the corresponding group.

3.2. Discussion.

Many reviews regarding the physiological endothelial function [30,31]. However, only a few focus on the ED mechanisms coupled with some metabolic disorders like hyperglycemia, dyslipidemia, obesity, diabetes mellitus, insulin resistance, etc. [30,32].

Some studies proved that glucose loads and high-fat meals might impair endothelium-dependent vasodilation via increasing free fatty acids circulation and ROS production induction [33,34]. There is major evidence of the interaction between insulin and the NO system. In healthy conditions, insulin induces a dose-dependent increase of blood flow in lower limbs by mitigating skeletal muscle vascular resistance [35] through the two key pathways: activation of the insulin receptor substrate 1 and phosphatidylinositol 3-kinase-dependent/Akt signaling pathway and increased eNOS expression with enhanced NO synthesis [36,37]. Other researchers did not detect a direct trace of insulin on vasodilation [38,39], switching a potentiating effect of insulin on acetylcholine-mediated vasodilation mechanism. Therefore, the pathways of insulin reciprocity via the eNOS/NO system and other molecular-receptors, intra-(extra)cellular mechanisms, are involved in the vascular tone reactivity and regulation. Apart from that, insulin resistance is associated with ED not only in obese and diabetic patients.
but in polycystic ovary syndrome [40], hypertension and other cardiovascular diseases, chronic kidney failure, peripheral artery disease, cancer, thrombosis, etc. [41]. Hyperinsulinemia in insulin-resistant conditions correlates directly with endotelin-1 secretion [42]. Some studies (human and animal models) demonstrated that endothelial function improves by reducing insulin resistance, whereas ameliorating insulin sensitivity diminishes ED [42]. To sum up, epidemiological, clinical, physiological, and cellular studies firmly support a reciprocal linkage between insulin resistance and ED that helps to bind cardiovascular diseases and metabolic disorders.

On the other hand, few studies witnessed the dependence of metabolic disorders on genetic NOS3 (rs2070744) and GNB3 (rs5443) factors, but they are conflicting and inconclusive. Hsiao et al. [43] found that in a general Taiwanese population (n=983) GNB3 (rs5443) SNP polymorphic site did not significantly associate with obesity, overweight, or obesity-related metabolic traits. Still, the triglycerides and total cholesterol values were higher in CC-genotype carriers than in T-allele subjects (P<0.05). Contrary, in a meta-analysis of 15 case-control studies with a total of 10,396 subjects (3171 cases of overweight/obesity and 7225 controls) was proven the association between GNB3 825C>T polymorphism and the risk of overweight and obesity: the presence of TT homozygote became one of the genetic factors susceptible to overweight/obesity, especially in males under 30 years [44]. The other systematic review and meta-analysis also were suggested a significant association between the TT genotype of the GNB3 gene polymorphism and obesity risk [OR=1.237, 95%CI: 1.040-1.472, P=0.016] [45]. This data corresponds to our obtained results: in EAH patients with TT-genotype (GNB3, rs5443), we observed higher TC blood content by 13.97% (P=0.035) and higher risk of obesity in T-allele subjects as well [OR= 10.12; OR 95%CI: 2.25-45.44; P<0.001].

Fattakhov et al. [46] revealed elevation of TC value in CC-genotype of NOS3 (T-786C) gene and association with metabolic syndrome. Another study also found significantly higher TC level and intima-media thickness of carotid arteries in 786CC homozygotes [47]. In other publications were not demonstrated associations of NOS3 (T-786C) gene polymorphism with glucose and lipids metabolism, but some of them confirmed a linkage with obesity [48,49], as we suggested in our research. Our study provides evidence that the C-allele of NOS3 gene (rs2070744) increases the probability of obesity six times compared to control, suggesting its significance in EAH. Thus, numerous publication biases and controversial results in genetic association studies in various populations demand further research.

4. Conclusions

Moderate-severe ED (2nd-3rd degrees) in EAH patients associated with higher SBP and DBP values – by 6.90% and 4.69% (p<0.05), creatinine blood level increase – by 10.08% (p=0.037), GFR (CKD-EPI by creatinine) decrease both in males and females as well – by 8.41% (p=0.004) and 11.03% (p=0.027), respectively. ED of 2nd-3rd degrees is associated with more pronounced dysmetabolic changes but only in men: by 46.46% glucose concentration increase (p=0.004) coupled with hypercholesterolemia and lower HDL-C content by 15.79% (p=0.024), that caused Atherogenity index elevation by 33.93% (p=0.029).

The EAH patients with TT-genotype of GNB3 (rs5443) gene have a higher TC blood content by 13.97% (P=0.035). However, the one-way ANOVA analysis did not confirm the linkage of the polymorphic site of NOS3 (rs2070744) and GNB3 (rs5443) genes with metabolic disorders in hypertensive patients.
The risk of obesity increases almost 6-fold [OR 95%-CI: 2.11-14.82; P<0.001] in C-allele carriers of the NOS3 gene (rs2070744) and more than 10 times [OR 95%-CI: 2.25-45.44; P<0.001] in T-allele carriers of the GNB3 (rs5443) gene. The relative incidence of obese EAH patients is higher among the mutated C-allele carriers of the NOS3 gene by 31.94% (χ²=13.58; P<0.001) and in the T-allele patients of the GNB3 gene (30.56%).

**Funding**

This research received no external funding.

**Acknowledgments**

We would like to acknowledge the personnel of the Scientific Laboratory Centre of Bukovinian State Medical University for technical and laboratory support.

**Conflicts of Interest**

The authors declare no conflict of interest.

**References**

1. Saklayen, M.G. The global epidemic of the metabolic syndrome. *Curr Hypertens Rep* 2018, 20, 1–8, https://doi.org/10.1007/s11906-018-0812-z.
2. Tran, V.; De Silva, T.M.; Sobey, C.G.; Lim, K.; Drummond, G.R.; Vinh, A.; Jelinic, M. The Vascular Consequences of Metabolic Syndrome: Rodent Models, Endothelial Dysfunction, and Current Therapies. *Front Pharmacol* 2020, 11, https://doi.org/10.3389/fphar.2020.00148.
3. Citi, V.; Martelli, A.; Gorica, E.; Brogi, S.; Testai, L.; Calderone, V. Role of hydrogen sulfide in endothelial dysfunction: Pathophysiology and therapeutic approaches. *J Advanced Res* 2021, 27, 99-113, https://doi.org/10.1016/j.jare.2020.05.015.
4. Kruger-Genre, A.; Blocki, A.; Franke, R.P.; Jung, F. Vascular Endothelial Cell Biology: An Update. *Int J Mol Sci* 2019, 20, https://doi.org/10.3390/ijms20184411.
5. Charbonier, F.W.; Zamani, M.; Huang, N.F. Endothelial cell mechanotransduction in the dynamic vascular environment. *Adv Biosyst* 2019, 3, https://doi.org/10.1002/adb.201800252.
6. Favero, G.; Paganelli, C.; Buffoli, B.; Rodella, L.F.; Rezzani, R. Endothelium and its alterations in cardiovascular diseases: life style intervention. *Biomed Res Int* 2014, 2014, https://doi.org/10.1155/2014/801896.
7. Jiang, X.; Liu, X.; Liu, X.; Wu, X.; Jose, P.A.; Liu, M.; Yang, Z. Low-Dose Aspirin Treatment Attenuates Male Rat Salt-Sensitive Hypertension via Platelet Cyclooxygenase 1 and Complement Cascade Pathway. *J Am Heart Assoc* 2020, 9, https://doi.org/10.1161/JAHA.119.013470.
8. Bruder-Nascimento, T.; Kress, T.C.; Kennard, S.; Belin de Chantemèl, E.J. HIV Protease Inhibitor Ritonavir Impairs Endothelial Function Via Reduction in Adipose Mass and Endothelial Leptin Receptor-Dependent Increases in NADPH Oxidase 1 (Nox1), C-C Chemokine Receptor Type 5 (CCR5), and Inflammation. *J Am Heart Assoc* 2020, 9, https://doi.org/10.1161/JAHA.120.018074.
9. Takac, I.; Schröder, K.; Brandes, R.P. The Nox family of NADPH oxidases: friend or foe of the vascular system? *Curr Hypertens Rep* 2012, 14, 70-8, https://doi.org/10.1007/s11906-011-0238-3.
10. Widlansky, M.E.; Gutterman, D.D. Regulation of endothelial function by mitochondrial reactive oxygen species. *Antioxid Redox Signal* 2011, 15, 1517-1530, https://doi.org/10.1089/ars.2010.3642.
11. Dzhuryak, V.S.; Sydorchuk, L.P.; Sydorchuk, A.R.; Kamyshnyi, O.M.; Kishanovska, A.; Levytska, S.A.; Knut, R.P.; Sheremet, M.I.; Ivashchuk, S.I.; Petrynch, O.A.; Kazantseva, T.V.; Nikiyor, L.V.; Melnychuk, L.V.; Sokolenko, A.A.; Yarynych, Y.M.; Semianiv, M.M.; Repchuk, Y.V.; Voroniuk, K.O.; Sydorchuk, R.I.; Sokolenko, L.; Ifoda, O.M.; Kushnir, O.V. The cytochrome 11B2 aldosterone synthase gene CYP11B2 (RS1799998) polymorphism associates with chronic kidney disease in hypertensive patients. *Biointerface Res Appl Chem* 2020, 10, 5406-5411, https://doi.org/10.33263/BRIAC103.406411.
12. Ji, L.D.; Li, J.Y.; Yao, B.B.; Cai, X.B.; Shen, Q.J.; Xu, J. Are genetic polymorphisms in the renin-angiotensin-aldosterone system associated with essential hypertension? Evidence from genome-wide association studies. *J Hum. Hypertens* 2017, 31, 695-8, https://doi.org/10.1038/jhh.2017.29.
13. Sydorchuk, L.P.; Gaborets, I.Y.; Sydorchuk A.R.; Ursuliak, Y.V.; Gaborets, I.Y.; Sokolenko, A.A.; Ivashchuk, S.I.; Bi-Ryuk, I.G.; Kostenko, V.V. Combined Effects of ACE (I/D) and eNOS (894T>G) Genes
Polymorphism in Patients with Arterial Hypertension in the Realization of Molecular Mechanisms of Left Ventricular Hypertrophy. New Armenian Med J 2013, 7, 32-42.

14. Sydorchuk, L.P.; Ursuliak, Y.V. Genes allele status of angiotensinconverting enzyme (I/D) and endothelial nitric oxide synthase (894 G > T) in patients with acute coronary syndrome. Lik Sprava 2015, 5-6, 24-34.

15. Tegegne, B.S.; Man, T.; van Roon, A.M.; Asea, N.G.; Riese, H.; Nolte, I.; Snieder, H. Heritability and the Genetic Correlation of Heart Rate Variability and Blood Pressure in >29 000 Families: The Lifelines Cohort Study. Hypertension 2020, 76, 1256-1262, https://doi.org/10.1161/HYPERTENSIONAHA.120.15227.

16. Charoen, P.; Eu-Ahsanthornwattana, J.; Thongmung, N.; Jose, P.A.; Sritara, P.; Vathesatagkit, P.; Kittyakara, C. Contribution of Four Polymorphisms in Renin-Angiotensin-Aldosterone-Related Genes to Hypertension in a Thai Population. Int J Hypertens 2019, 2019, https://doi.org/10.1155/2019/4861081.

17. Repchuk, Y.; Sydorchuk, L.; Fedonyuk, I.; Nebesna, Z.; Vasiuk, V.; Sydorchuk, A.; Iftoda, O. Association of Lipids’ Metabolism with Vitamin D Receptor (rs10735810, rs222857) and Angiotensinogen (rs699) Genes Polymorphism in Essential Hypertensive Patients. Open Access Maged J Med Sci 2021, 9, 1052-6, https://doi.org/10.3889/oamjms.2021.6975.

18. Semianiv, M.M.; Sydorchuk, L.; Fedonyuk, I.; Nebesna, Z.; Kamysnyhny, O.; Sydorchuk, A.; Vasiuk, V.; Dzhuryak, V.; Semianiv, I.; Sydorchuk, R. Metabolic and Hormonal Prognostic Markers of Essential Arterial Hypertension Considering the Genes Polymorphism AGTR1 (rs5186) and VDR (rs2228570). Rom J Diabetes Nutr Metabol Dis 2021, 28, 284-91.

19. Smyth, L.J.; Cañadas-Garre, M.; Cappa, R.C.; Maxwell, A.P.; McKnight, A.J. Genetic associations between genes in the renin-angiotensin-aldosterone system and renal disease: a systematic review and meta-analysis. BMJ Open 2019, 9, https://doi.org/10.1136/bmjopen-2018-026777.

20. Visseren, F.J.L.; Mach, F.; Smulders, Y.M.; Carballo, D.; Koskinas, K.C.; Bäck, M.; Benetos, A.; Biffi, A.; Boavida, J.M.; Capodanno, D.; Consyns, B.; Crawford, C.; Davos, C.H.; Desormais, I.; Di Angelantonio, E.; Franco, O.H.; Halvorsen, S.; Hobbs, F.D.R.; Hollander, M.; Jankowska, E.A.; Michal, M.; Sacco, S.; Sattar, N.; Tokgozoglu, L.; Tonstad, S.; Tsioufis, K.P.; van Dis, I.; van Gelder, I.C.; Wanner, C.; Williams, B.; ESC National Cardiac Societies; ESC Scientific Document Group. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. Eur Heart J 2021, 42, 3227-3337, http://doi.org/10.1093/eurheartj/ehab484.

21. Bryan, W.; Mancia, G.; Spiering, W.; Agabiti, R.E.; Azizi, M.; Burnier, M.; Clement, D.L.; Coca, A.; de Simone, G.; Dominiczak, A.; Kahan, T.; Mahfoud, F.; Redon, J.; Ruijope, L.; Zanchetti, A.; Smyth, L.J.; Cañadas-Garre, M.; Cappa, R.C.; Maxwell, A.P.; McKnight, A.J. Genetic associations between genes in the renin-angiotensin-aldosterone system and renal disease: a systematic review and meta-analysis. BMJ Open 2019, 9, https://doi.org/10.1136/bmjopen-2018-026777.

22. Semianiv, M.M.; Sydorchuk, L.P.; Dzhuryak, V.S.; Gerush, O.V.; Palamar, A.O.; Muzyka, N.Y.; Korovenkova, O.M.; Blazhievskva, O.M.; Sydor, V.V.; Sydorchuk, A.R.; Semianiv, I.O.; Sydorchuk, R.I. Association of AGTR1 (rs5186), VDR (rs2228570) genes polymorphism with blood pressure elevation in patients with essential hypertension in Ukrainian population. J Med Life 2021, 14, November – December, in press.

23. Repchuk, Y.; Sydorchuk, L.P.; Fedonyuk, L.Y.; Kamysnyhny, O.; Korovenkova, O.; Plehutsa, I.M.; Dzhuryak, V.S.; Myshkovskii, Y.M.; Iftoda, O.M.; Sydorchuk, R.I. Linkage of blood pressure, obesity and diabetes mellitus with angiotensinogen gene (AGT 704T>C;rs699) polymorphism in hypertensive patients. Bratisl Lek Listy 2021, 122, 715-720, https://doi.org/10.4149/BLL_2021_114.

24. Sydorchuk, L.; Dzhuryak, V.; Sydorchuk, A.; Levtyska, S.; Petrynych, V.; Knut, R.; Kshanosvka, A.; Iftoda, O.; Tkachuk, O.; Kyfiak, P.; Popovich, A.; Khomko, O.; Sydorchuk, R. The cytochrome 1B2 aldosterone synthase gene rs1799998 single nucleotide polymorphism determines elevated aldosterone, higher blood pressure, and reduced glomerular filtration, especially in diabetic female patients. Endocr Regul 2020, 54, 217-226, https://doi.org/10.2478/enr-2020-0024.

25. Sydorchuk, L.P.; Dzhuryak, V.S.; Sydorchuk, A.R.; Levtyska, S.A.; Knut, R.P.; Sokolenko, M.O.; Iftoda, O.M.; Kushnir, O.V.; Popovich, A.I.; Sydorchuk, R.I. Association of lipids’ metabolism disorders with aldosterone synthase CYPI1B2 (-344C/T) gene polymorphism in hypertensive patients depending on glomerular filtration rate. Pharmacology OnLine 2020, 2, 230-242.

26. Corretti, M.C.; Anderson, T.J.; Benjamin, E.J.; Celermajer, D.; Charbonneau, F.; Creager, M.A.; Deanfield, J.; Drexler, H.; Gerhard-Herman, M.; Herrington, D.; Vaitkus, P.; Vita, J.; Vogel, R.; International Brachial Artery Reactivity Task Force. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 2002, 39, 257-265, https://doi.org/10.1016/s0735-1097(01)01746-6.

27. Holder, S.M.; Bruno, R.M.; Shkredova, D.A.; Dawson, E.A.; Jones, H.; Hopkins, N.D.; Hopman, M.T.E.; Bailey, T.G.; Coombes, J.S.; Askew, C.D.; Naylor, L.; Maiorana, A.; Ghiadoni, L.; Thompson, A.; Green, D.J.; Thijssen, D.H. J. Reference Intervals for Brachial Artery Flow-Mediated Dilation and the Relation with Cardiovascular Risk Factors. Hypertension 2021, 77, 1469-1480, https://doi.org/10.1161/HYPERTENSIONAHA.120.15754.
28. Maruhashi, T.; Kajikawa, M.; Kishimoto, S.; Hashimoto, H.; Takaeko, Y.; Yamaji, T.; Harada, T.; Han, Y.; Aibara, Y.; Yusoff, F.M.; Hidaka, T.; Kihara, Y.; Chayama, K.; Nakashima, A.; Goto, C.; Tomiyama, H.; Takase, B.; Kohro, T.; Suzuki, T.; Ishizu, T.; Ueda, S.; Yamazaki, T.; Furumoto, T.; Kario, K.; Inoue, T.; Koba, S.; Watanabe, K.; Takemoto, Y.; Hano, T.; Sata, M.; Ishibashi, Y.; Node, K.; Maemura, K.; Ohya, Y.; Furukawa, T.; Ito, H.; Ikeda, H.; Yamashina, A.; Higashi, Y. Diagnostic Criteria of Flow-Mediated Vasodilation for Normal Endothelial Function and Nitroglycerin-Induced Vasodilation for Normal Vascular Smooth Muscle Function of the Brachial Artery. *J Am Heart Assoc* 2020, 9, https://doi.org/10.1161/JAHA.119.013915.

29. Thijssen, D.H.J.; Bruno, R.M.; van Mil, A.C.C.M.; Holder, S.M.; Faita, F.; Greyling, A.; Zock, P.L.; Taddei, S.; Deanfield, J.E.; Luscher, T.; Green, D.J.; Ghiadoni, L. Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans. *Eur Heart J* 2019, 40, 2534-2547, https://doi.org/10.1093/eurheartj/ehz350.

30. Rajendran, P.; Rengarajan, T.; Thangavel, J.; Nishigaki, Y.; Sakthisekaran, D.; Sethi, G.; Nishigaki, I. The vascular endothelium and human diseases. *Int J Biol Sci* 2013, 9, 1057-69, https://doi.org/10.7150/ijbs.7502.

31. Park, J.B.; Charbonneau, F.; Schiffrin, E.L. Correlation of endothelial function in large and small arteries in human essential hypertension. *J Hypertens* 2001, 19, 415-20, https://doi.org/10.1097/00004872-200103000-00009.

32. Genders, A.J.; Frison, V.; Abramson, S.R.; Barrett, E.J. Endothelial cells actively concentrate insulin during its transendothelial transport. *Microcirculation* 2013, 20, 434-9, https://doi.org/10.1111/mic.12044.

33. Mohanty, P.; Hamouda, W.; Garg, R.; Aljada, A.; Ghanim, H.; Dandona, P. Glucose challenge stimulates reactive oxygen species (ROS) generation by leucocytes. *J Clin Endocrinol Metab* 2000, 85, 2970-3, https://doi.org/10.1210/jcem.85.8.6854.

34. Tripathy, D.; Mohanty, P.; Dhindsa, S.; Syed, T.; Ghanim, H.; Aljada, A.; Dandona, P. Elevation of free fatty acids induces inflammation and impairs vascular reactivity in healthy subjects. *Diabetes* 2003, 52, 2882-7, https://doi.org/10.2337/diabetes.52.12.2882.

35. Calles-Escandon, J.; Cipolla, M. Diabetes and endothelial dysfunction: a clinical perspective. *Endocr Rev* 2001, 22, 36-52, https://doi.org/10.1210/edrv.22.1.0417.

36. Muniyappa, R.; Iantorno, M.; Quon, M.J. An integrated view of insulin resistance and endothelial dysfunction. *Endocrinol Metab Clin North Am* 2008, 37, 685-711, https://doi.org/10.1016/j.ecm.2008.06.001.

37. Mather, K.; Laakso, M.; Edelman, S.; Hook, G.; Baron, A. Evidence for physiological coupling of insulin-mediated glucose metabolism and limb blood flow. *Am J Physiol Endocrinol Metab* 2000, 279, E1264-70, https://doi.org/10.1152/ajpendo.2000.279.6.E1264.

38. Taddei, S.; Virdis, A.; Mattei, P.; Natali, A.; Ferrannini, E.; Salvetti, A. Effect of insulin on acetylcholine-induced vasodilation in normotensive subjects and patients with essential hypertension. *Circulation* 1995, 92, 2911-8, https://doi.org/10.1161/01.cir.92.10.2911.

39. Usselman, C.W.; Yarovsky, T.O.; Steele, F.E.; Leone, C.A.; Taylor, H.S.; Rachenfeld, N.S. Androgens drive microvascular endothelial dysfunction in women with polycystic ovary syndrome: role of the endothelin B receptor. *J Physiol* 2019, 597, 2853-2865, https://doi.org/10.1113/JP277756.

40. Li, R.; Zhang, H.; Wang, W.; Wang, X.; Huang, Y.; Huang, C.; Gao, F. Vascular insulin resistance in prehypertensive rats: role of PI3-kinase/Akt/eNOS signaling. *Eur J Pharmacol* 2010, 628, 140-7, https://doi.org/10.1016/j.ejphar.2009.11.038.

41. Liu, Q.; Cui, H.; Ma, Y.; Han, X.; Cao, Z.; Wu, Y. Triglyceride-glucose index associated with the risk of cardiovascular disease: the Kaibulan study. *Endocrine* 2021, https://doi.org/10.1007/s12020-021-02862-3.

42. Kim, J.A.; Montagnani, M.; Koh, K.K.; Quon, M.J. Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. *Circulation* 2006, 113, 1888-904, https://doi.org/10.1161/CIRCULATIONAHA.105.563213.

43. Hsiao, T.J.; Hwang, Y.; Liu, C.H.; Chang, H.M.; Lin, E. Association of the C825T polymorphism in the GNB3 gene with obesity and metabolic phenotypes in a Taiwanese population. *Genes Nutr* 2013, 8, 137–44, https://doi.org/10.1007/s12263-012-0304-8.

44. Li, H.L.; Zhang, Y.J.; Chen, X.P.; Luo, J.Q.; Liu, S.Y.; Zhang, Z.L. Association between GNB3 c.825C>T polymorphism and the risk of overweight and obesity: A meta-analysis. *Meta Gen* 2016, 9, 18–25, https://doi.org/10.1016/j.mgene.2016.03.002.

45. Faramarziyan, N.; Rahmati, M.; Mirnasouri, R.; Bahrami, M. The Association between GNB3 Gene Polymorphism and Obesity: A Systematic Review and Meta-Analysis. *Iranian Journal of Diabetes and Obesity* 2017, 9, 69-81.

46. Fattakhov, N.; Smirnova, L.; Atotech, D.; Parshukova, D.; Skuratovskaiia, D.; Painter, Q.; Zatolokin, P.; Semke, A.; Litvinova, L.; Ivanova, S. Haplotype analysis of endothelial nitric oxide synthase (NOS3) genetic variants and metabolic syndrome in healthy subjects and schizophrenia patients. *Int J Obes (Lond)* 2018, 42, 2036–2046, https://doi.org/10.1038/s41366-018-0124-z.

47. Misiaik, B.; Kroluk, M.; Kukowka, A.; Lewera, A.; Leszcynsky, P.; Stankiewicz-Olczyk, J.; Slezak, R. The Role of −786T/C Polymorphism in the Endothelial Nitric Oxide Synthase Gene in Males with Clinical and Biochemical Features of the Metabolic Syndrome. *Inter J Endocrinology* 2011, 2011, https://doi.org/10.1155/2011/458750.
48. Gaunt, T.R.; Smith, G.D. eNOS and coronary artery disease: Publication bias and the eclipse of hypothesis-driven meta-analysis in genetic association studies. *Gene* **2015**, 556, 257-258, https://doi.org/10.1016/j.gene.2014.11.052.

49. Pawlik, A.; Błaszczyk, H.; Rać, M.; Maciejewska-Skrendo, A.; Safranow, K.; Dziedziejko, V. NOS3 Gene rs1799983 and rs2070744 Polymorphisms in Patients with Unstable Angina. *J Vasc Res* **2020**, 57, 136-142, https://doi.org/10.1159/000506160.