Positive Association between $EDN1$ rs5370 (Lys198Asn) Polymorphism and Large Artery Stroke in a Ukrainian Population

Yevhen I. Dubovyk, Tetyana B. Oleshko, Viktoriia Yu. Harbuzova, and Alexander V. Ataman

1Department of Physiology, Pathophysiology and Medical Biology, Sumy State University, Sumy 40007, Ukraine
2Scientific Laboratory of Molecular Genetic Research, Sumy State University, Sumy 40007, Ukraine

Correspondence should be addressed to Yevhen I. Dubovyk; janitor@ukr.net

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There are a lot of convincing evidences about the involvement of endothelin pathway proteins in the pathogenesis of atherosclerosis and its fatal complications. In this study, the analysis of a possible association between $EDN1$ rs5370 and $EDNRA$ rs5335 gene polymorphisms and the risk of large artery stroke (LAS) in a Ukrainian population was conducted. 200 LAS patients and 200 unrelated controls were enrolled in a case-control study. The polymerase chain reaction-restriction fragment length polymorphism method (PCR-RFLP) was used for SNP genotyping. Our results revealed that $EDN1$ rs5370 polymorphism was associated with LAS development both before and after adjustment for atherosclerosis risk factors (sex, age, body mass index, arterial hypertension, type 2 diabetes mellitus, and smoking). The risk for a LAS incident in rs5370-T allele carriers was 1.6 times higher (CI = 1.066–2.403; $P = 0.020$) than in subjects with the GG genotype. No link between $EDNRA$ rs5335 and LAS risk in a Ukrainian population was found. The present study indicated that $EDN1$ rs5370, but not $EDNRA$ rs5335, can be the strong genetic predictor for LAS development in a Ukrainian population.

1. Introduction

As it is well known, endothelial dysfunction plays an important role in the development of common cardiovascular diseases and their complications [1]. One of the main pathogenetic pathways of endothelial dysfunction development is an increased formation and biological activity of the powerful vasoconstrictor and proinflammatory peptide endothelin (ET-1) [2], which mediates own effects via two pharmacologically distinguishable receptor subtypes, endothelin A (ETA) and endothelin B (ETB) receptors, respectively [3]. There are several lines of evidence indicating that the ET-1-induced endothelial dysfunction is realized through decreasing production and increasing degradation of NO, through enhancement of Von Willebrand factor and reactive oxygen species formation, and also through the activation of proinflammatory metabolic pathways in the endotheliocytes [4].

In recent years, a wide range of case-control studies to test the association between various single nucleotide polymorphisms (SNPs) of the ET-1 ($EDN1$) and its receptor ($EDNRA$ and $EDNRB$) genes and development of arterial hypertension (AH) [5, 6], pulmonary hypertension [7], myocardial infarction [8], diabetic retinopathy [9] and nephropathy [10], metabolic syndrome [11], and hemorrhagic stroke [12] have been carried out. There are several works that concern the investigation of the effect of $EDN1$, $EDNRA$, and $EDNRB$ genetic polymorphisms on ischemic stroke (IS) development. Zhang and Sui showed that rs5370-T allele ($EDN1$ gene) increased the IS incidence risk in Northern Han men, whereas the rs5335-CC genotype ($EDNRA$ gene) had a protective effect in the same population [13]. Yamaguchi et al. revealed a significant association between $EDN1$ rs5370 polymorphism and high risk for IS only among Japanese women [14], whereas Aslan et al. and Gormley et al. did not find any relation between the
mentioned SNP and stroke morbidity among the Turkish [15] and English [16] population, respectively. Thus, the data obtained in different populations are contradictory, which requires further study about the role of polymorphic variants of the endothelin family genes in the IS development.

The aim of the present case-control study was to investigate the possible association between EDN1 rs5370 and EDNRA rs5335 polymorphisms and large artery stroke (LAS) in representatives of the Ukrainian population.

### 2. Materials and Methods

#### 2.1. Study Population

Venous blood of 200 unrelated Caucasians (Ukrainians) with LAS (89 females and 111 males; mean age 66.7 ± 10.1) and 200 control subjects (75 females and 125 males; mean age 68.1 ± 13.9) was used for the study. Each stroke patient had been under dispensary observation in the 5th Sumy Clinical Hospital since April 2009 to December 2017. Computed tomography and (or) magnetic resonance imaging investigations of the head as well as electrocardiographic, biochemical, and coagulation tests and carotid ultrasonography were used for final LAS diagnosis establishment. The pathogenic variant of IS was determined according to the TOAST criteria [17]. Individuals with cardioembolic, lacunar, and hemorrhagic strokes, traumatic brain injury, and brain tumors were excluded. The clinical characteristics of LAS patients included systolic, diastolic, pulse and mean arterial blood pressure (BP), body mass index (BMI), lipid profile parameters, and coagulogram indices.

Only participants without the history of IS or other acute cerebrovascular pathologies, myocardial infarction, and other atherosclerosis complications were enrolled to the control group. Subjects of the comparison groups were divided into subgroups defined by sex and the presence or absence of AH (systolic BP > 140 mmHg, diastolic BP > 90 mmHg, or both). The study protocol complied with the Declaration of Helsinki and was approved by the Ethic Committee of the Medical Institute of Sumy State University (number 2/02.17.09). An appropriate written informed consent was obtained from all individuals.

#### 2.2. Genotyping of SNPs

Genomic DNA was isolated from peripheral leukocytes using GeneJET Whole Blood Genomic DNA Purification Mini Kit (Thermo Fisher Scientific, USA). Polymerase chain reaction-restriction fragment length polymorphism analysis (PCR-RFLP) was used for genotyping EDN1 rs5370 and EDNRA rs5335 SNPs. The reaction mixture for PCR (total volume 25 μL) included 2 mM MgSO4, 0.2 mM dNTPs (Thermo Fisher Scientific, USA), 5 μL 5 × PCR buffer, 1 U Taq DNA polymerase (Thermo Fisher Scientific, USA), and 75–100 ng DNA. The nucleotide sequence of the primers and PCR conditions, which were used for each polymorphism investigation, are shown in Table 1. PCR was carried out in Thermocycler GeneAmp PCR System 2700 (Thermo Fisher Scientific, USA).

2 U of Cac81 (Thermo Fisher Scientific, USA) was used for restriction analysis of EDN1 rs5370 polymorphism (incubation at 37°C for 17 h). The presence of guanine at the 5665th position of the EDN1 gene led to the cleavage of the amplicon (262 bp) by Cac81 into two parts of 189 and 73 bp. In the case of guanine to thymine replacement, we had only one 262 bp fragment due to the loss of the Cac81 catalytic site (Figure 1). Restriction analysis of EDNRA rs5335 SNP required using 3 U of NmuCI (Tsp45I) (Thermo Fisher Scientific, USA) (incubation at 37°C for 19 h). The presence of cytosine at the 70th position of the EDNRA 3′-untranslated region (3′-UTR) allowed NmuCI to cut the primary amplicon (174 bp) into two fragments of 116 and 58 bp. Cytosine to guanine substitution resulted in preventing the restriction and preservation of the original 174 bp fragment (Figure 2). Horizontal electrophoresis

| Table 1: PCR conditions for EDN1 rs5370 and EDNRA rs5335 genotyping. |
|----------------------|----------------------|-----------------|----------------------|----------------------|
| **Gene** | **SNP** | **Primer nucleotide sequence** | **Thermocycling conditions** | **PCR amplicon size** |
|----------------------|----------------------|-----------------|----------------------|----------------------|
| **EDN1** | rs5370 | F: 5′-TCTTGCTTTATTAGGTCCGAGACC-3′ | 94°C (60 s) 61°C (60 s) 72°C (45 s) | 262 bp |
| | | R: 5′-TTTGAACGAGGACGTGGTC-3′ | | |
| **EDNRA** | rs5335 | F: 5′-TAGAAGCCTCCTCGGTACTCC-3′ | 94°C (50 s) 60°C (40 s) 72°C (60 s) | 174 bp |
| | | R: 5′-TCG TAGATGTTGGGTGGATA-3′ | | |

Annotation: F: forward; R: reverse; D: denaturation; H: hybridization; E: elongation; bp: base pairs.

![Figure 1: Results of EDN1 rs5370 polymorphism restriction analysis. M—molecular marker (bp—base pairs); lanes 3, 4, 5, and 8—GG genotype; lanes 6, 7, 10, and 11—GT genotype; lanes 1, 2, and 9—TT genotype.](image-url)
(10 V/cm) in 2.5% agarose gel (10 mg/mL ethidium bromide) with subsequent ultraviolet visualization was used for restriction fragment detection.

2.3. Statistical Analysis. The Statistical Package for Social Science software (SPSS, version 17.0, Chicago, IL, USA) was used for most statistical analyses. Continuous variables are presented as the mean ± SD (checking the normality of distribution was performed using Shapiro-Wilk test); categorical variables are presented as absolute number and percentage value. Two-tailed Student’s t-test and ANOVA with subsequent Bonferroni post hoc test were used for comparison of the mean values between two or more different patient groups. In order to control type 1 error, multiple adjustment using false discovery rate (FDR) method was performed. Statistical power analysis was done using Quanto. Hardy-Weinberg equilibrium testing was carried out using Online Encyclopedia for Genetic Epidemiology Studies (http://www.oege.org/software/hardy-weinberg.html). Chi square (χ²) test was used to compare the frequency of EDN1 rs5370 and EDNRA rs5335 alleles and genotypes as well as other categorical variables between the control and case groups. An odds ratio (OR) and 95% confidence interval (CI) were obtained from logistic regression for dominant, recessive, and additive models of inheritance. Multivariable logistic regression was used to exclude the effect of other atherosclerosis risk factors including sex, age, BMI, AH, type 2 diabetes mellitus (T2DM), and smoking status. All statistical tests were based on a two-tailed probability; a value of P < 0.05 was considered as significant.

2.4. Prediction Analysis. In order to uncover the functional effects of EDN1 rs5370 and EDNRA rs5335 polymorphic sites, web available consensus classifiers PredictSNP2 [18] and SNPinfo were used [19]. Herewith to predict the effect of rs5370 SNP of the EDN1 5’ exon on protein function PredictSNP was used [20]. We also used SpliceAid2 tool to check if SNP 5370 is located in splicing regulatory sequence [21]. Online miRDB resource [22] was used for microRNA target prediction in the framework of the functional analysis of rs5335 SNP of EDNRA 3’-UTR.

3. Results

The general characteristics of the study groups are summarized in Table 2. Their detailed description was presented in our previous article [23].

The distribution of EDN1 rs5370 and EDNRA rs5335 alleles and genotypes in comparison groups is shown in Table 3. Obtained genotype frequencies for each SNP did not significantly deviate from Hardy-Weinberg equilibrium expectations (P_{HWE} > 0.05). The frequency of EDN1 rs5370 genotypes and alleles in LAS patients significantly differed from the control group (P = 0.006 and P = 0.002, resp.), while the distribution of EDNRA rs5335 genotypes and alleles was similar between case and control individuals (P = 0.391 and P = 0.521, resp.). Statistical power analysis indicated that the rs5370 locus had strong power (0.689—for dominant model; 0.956—for recessive model; and 0.991—for additive model). At the same time, rs5335 SNP had poor power (0.252—for dominant model; 0.059—for recessive model; and 0.654—for additive model).

The results of two investigated SNP genotypes’ association with LAS are presented in Table 4. Significant association between EDN1 rs5370 and stroke was revealed under dominant (P_c = 0.012; OR_c = 1.657, 95% CI = 1.115–2.462), recessive (P_c = 0.007; OR_c = 2.839, 95% CI = 1.331–6.057), and additive (P_c = 0.003; OR_c = 3.291, 95% CI = 1.512–

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**Figure 2:** Results of EDNRA rs5335 polymorphism restriction analysis. M—molecular marker (bp—base pairs); lanes 1, 2, 3, 4, 5, 6, and 11—CG genotype; lanes 7 and 9—GG genotype.

**Table 2:** General characteristics of the study population.

| Parameter                  | Cases (n = 200) | Controls (n = 200) | P      |
|----------------------------|-----------------|--------------------|--------|
| Age, years                 |                 |                    |        |
|                           | 66.7 ± 10.1     | 68.1 ± 13.9        | 0.261  |
| Sex, female/male           | 89/111          | 75/125             | 0.155  |
| Body mass index, kg/m²     | 27.9 ± 3.7      | 27.3 ± 4.6         | 0.105  |
| Systolic BPs, mmHg         | 167.9 ± 28.7    | 151.7 ± 22.6       | <0.001 |
| Diastolic BP, mmHg         | 96.0 ± 15.5     | 86.5 ± 11.7        | <0.001 |
| Pulse BP, mmHg             | 71.9 ± 22.4     | 65.2 ± 17.2        | 0.002  |
| Mean BPs, mmHg             | 119.9 ± 17.9    | 108.3 ± 14.0       | <0.001 |
| Fasting glucose, mmol/L    | 6.05 ± 1.5      | 5.25 ± 0.7         | <0.001 |
| Total cholesterol, mmol/L  | 4.98 ± 1.46     | 4.75 ± 1.52        | 0.124  |
| HDL cholesterol, mmol/L    | 1.01 ± 0.29     | 1.09 ± 0.38        | 0.018  |
| LDL cholesterol, mmol/L    | 3.16 ± 1.39     | 2.94 ± 1.17        | 0.087  |
| Triglyceride, mmol/L       | 1.67 ± 0.78     | 1.54 ± 0.66        | 0.073  |
| Current smokers, n (%)     | 60 (30.0)       | 55 (27.5)          | 0.581  |
| T2DM, n (%)                | 50 (25.0)       | 19 (9.5)           | <0.001 |
| Arterial hypertension, n (%)| 150 (75.0)      | 116 (58.0)         | <0.001 |

Categorical variables were compared by χ² test, continuous variables by t-test.
Table 3: Distributions of genotypes and alleles in case and control groups.

| Gene  | SNP   | LAS (n = 200) | Control (n = 200) | $P_{HWE}$ | $P$ |
|-------|-------|---------------|------------------|----------|-----|
|       | $n$   | $\%$          | $n$              | $\%$     |     |
|       | GG    | 94            | 47.0             | 118       | 59.0|
|       | GT    | 80            | 40.0             | 72        | 36.0|
| $EDN1$ | rs5370 | TT            | 26               | 13.0      | 10  |
|       | Alleles |              |                  |          |     |
|       | G     | 268           | 67.0             | 308       | 77.0|
|       | T     | 132           | 33.0             | 92        | 23.0|
|       | $OR_a = 2$ |              |                  | $P = 0.006$ |     |
|       | $OR_c = 1.377$ |              |                  | $95\% CI = 1.011-2.689$ |     |
| $EDNRA$ | rs5335 | GG            | 33               | 16.5      | 35  |
|       | Alleles |              |                  |          |     |
|       | C     | 220           | 55.0             | 229       | 57.3|
|       | G     | 180           | 45.0             | 171       | 42.7|
|       | $OR_a = 3.903$ |              |                  | $P = 0.002$ |     |

7.165—for TT genotype) models of inheritance. After adjusting for covariates of age, sex, BMI, AH, T2DM, and smoking status, genotypic association of rs5370 SNP remained under dominant ($P_a = 0.020$; $OR_a = 1.601$, $95\% CI = 1.066-2.403$), recessive ($P_a = 0.003$; $OR_a = 3.251$, $95\% CI = 1.492-7.084$), and additive ($P = 0.002$; $OR = 3.637$, $95\% CI = 1.639-8.073$) for TT genotype models. Logistic regression analysis for $EDNRA$ rs5335 did not show any significant link with LAS development neither before nor after adjustment for other risk factors ($P > 0.05$).

The analysis of rs5370 and rs5335 genotypic association with LAS risk in female and male subjects is presented in Table 5. In women, significant difference for rs5370 locus was revealed in the crude dominant ($P_a = 0.021$; $OR_a = 2.090$, $95\% CI = 1.119-3.903$) and additive ($P = 0.034$; $OR = 2.008$, $95\% CI = 1.055-3.823$) for TT genotype models, as well as in adjusted dominant ($P_a = 0.014$; $OR_a = 2.437$, $95\% CI = 1.202-4.940$) and additive ($P = 0.042$; $OR = 2.135$, $95\% CI = 1.107-4.393$) for TT genotype; $P_a = 0.018$; $OR = 5.634$, $95\% CI = 1.157-27.436$ for TT genotype models. $EDNRA$ rs5335 SNP was not associated with stroke development in the female subgroup ($P > 0.05$).

In male subjects, association of rs5370-TT genotype was revealed regardless of adjustment under recessive ($P_a = 0.007$, $P = 0.007$; $OR = 3.512$, $95\% CI = 1.401-8.806$) and additive models ($P_a = 0.008$, $P = 0.009$; $OR = 3.535$, $95\% CI = 1.377-9.076$). The significant link between rs5335 polymorphism and LAS development in the mentioned subgroup was absent ($P > 0.05$).

Due to influential role of the endothelin pathway in hypertension development, we also investigated the association between $EDN1$ and $EDNRA$ gene polymorphisms and ischemic stroke development in patients with and without AH (Table 6). In nonhypertensive subjects, both SNPs were not associated with LAS either before or after adjustment for age, sex, BMI, T2DM, and smoking ($P > 0.05$). In the hypertensive cohort, the minor T allele for the rs5370 locus was found to be significantly more prevalent in stroke patients. Before adjusting for covariates, positive association was revealed under dominant ($P_a = 0.045$; $OR_a = 1.649$, $95\% CI = 1.011-2.689$), recessive ($P_a = 0.024$; $OR_a = 3.220$, $95\% CI = 1.164-8.903$), and additive ($P_a = 0.013$; $OR_a = 3.744$, $95\% CI = 1.322-10.607$) for TT genotype models. After adjusting for the covariates, a significant link between $EDN1$ rs5370 and LAS remained under dominant ($P_a = 0.036$; $OR_a = 1.711$, $95\% CI = 1.035-2.829$), recessive ($P_a = 0.008$; $OR_a = 4.102$, $95\% CI = 1.448-11.617$), and additive ($P_a = 0.004$; $OR_a = 4.743$, $95\% CI = 11.633-13.776$) for TT genotype models.

Table 7 indicates the clinical characteristics of LAS patients stratified by $EDN1$ rs5370 genotypes. Using the ANOVA test, significant difference was found for diastolic (GG—93.4 ± 14.9 mmHg, GT—99.3 ± 16.2 mmHg, TT—91.7 ± 13.3 mmHg; $P = 0.034$) BP. Nevertheless, Bonferroni post hoc test revealed no significant difference between patients with different genotypes ($P = 0.103$ for GG versus GT; $P = 0.087$ for GT versus TT). Moreover, FDR multiple adjustment revealed that none of the parameters are associated with rs5370 genotypes. No link between $EDNRA$ rs5335 genotypes and BMI, BP indices, coagulogram parameters, fasting glucose, and blood plasma lipid profile in stroke patients was found (Table 8).

The bioinformatical analysis of $EDN1$ rs5370 by the SNPInfo tool showed that the mentioned polymorphic locus might be located in splicing regulatory sequences recognized by exonic splicing enhancers or exonic splicing silencers (score—2.56). SpliceAId2 tool demonstrated that rs5370 lies between recognized sites for SFRS9 and hnRNP H1 splicing factors, but this SNP has no influence on their structure. Finally, prediction analysis by PredictSNP and PredictSNP2 allowed classifying $EDN1$ rs5370 (Lys198Asn mutation) as neutral (PredictSNP—neutral with 83% expected accuracy (EA); MAPP—neutral with 68% EA; PhD-DNA—neutral with 78% EA; PolyPhen1—neutral with 67% EA; PolyPhen2—neutral with 61% EA; SIFT—neutral with 67% EA; SNAP—neutral with 50% EA; PredictSNP2—neutral with 89% EA; CADD—neutral with 90% EA; DANN—neutral with 73% EA; FATHMM—neutral with 84% EA; and FusedSeq2—detrimental with 62% EA).

Prediction analysis of $EDNRA$ rs5335 by the SNPInfo tool demonstrated that this SNP is possibly located in hsa-miR-27a-3p and hsa-miR-27b-3p binding sites (score—153.0). Analysis using miRDB confirmed that the $EDNRA$ gene is in the list of mentioned miRNA targets (target score—50). However, PredictSNP2 results classified rs5335 mutation as neutral (PredictSNP2—neutral with 88% EA; CADD—neutral with 86%; DANN—neutral with 79% EA; FATHMM—neutral with 93% EA; FunSeq2—neutral with 62% EA; and GWAWA—detrimental with 64% EA).

4. Discussion

The essence of rs5370 polymorphism is the replacement of guanine by thymine at the 5665th position (5 exon) of the
EDN1 gene, which in turn leads to replacement of lysine by asparagine in the 198th position of the preproendothelin-1 molecule. Several studies have shown that endothelin-1 blood plasma concentration in T (Asn) allele carriers is higher than in subjects with GG (Lys/Lys) genotype [24, 25]. Considering the localization of this SNP, it can be assumed that its functional effect is due to the effect on the quality or speed of preproendothelin-1 posttranslational modification. However, Tanaka et al. showed that the amount of ET-1 and big ET-1 in the supernatant of Asn-type and Lys-type transfected cells was similar [26]. In addition, the plasma endothelin-1 level in patients with essential hypertension was not different in individuals with the Asn allele and Lys/Lys genotype. The conclusion that another SNP in strong LD with rs5370 may provide its clinical effects was made. Our prediction analysis of rs5370 also did not confirm the role of this polymorphic locus in preproendothelin-1 posttranslation modification. Applying bioinformatic tools did not allow highlighting the possible functional effects of the mentioned SNP. Taking together experimental and prediction data, it seems more likely that rs5370 is in strong LD with another influential SNP.

The polymorphic site rs5335 is located within the 3′-UTR of the EDNRA gene and leads to cytosine/guanine conversion at position 61,772. The functional studies of this SNP do not exist, while several clinical studies have demonstrated the association of this locus with increased risk of AH development [27, 28] and level of plasma endothelin-1 [29]. On the one hand, it can be assumed that changes in the nucleotide sequence of 3′-UTR may affect the stability of mRNA and thus affect the amount of the receptor protein. On the other hand, this SNP may change the structure of the mRNA and thus affect the amount of the receptor protein.

See Table 4; \( P_c \): P value adjusted for age, sex, body mass index, arterial hypertension, type 2 diabetes mellitus, and smoking.

See Table 4; \( P_c \): P value adjusted for age, sex, body mass index, arterial hypertension, type 2 diabetes mellitus, and smoking.
Disease Markers

Table 6: Analysis of EDN1 rs5370 and EDNRA rs5335 genotypic association with LAS in subjects with and without arterial hypertension.

| Model     | Parameter | Genotype | OR (95% CI) | Pc | ORa (95% CI) | Pa |
|-----------|-----------|----------|-------------|----|-------------|----|
| Dominant  | EDN1 rs5370 | Nonhypertensive | 1.500 (0.739–3.046) | 0.262 | 0.201 | 1.624 (0.773–3.411) |
|          |           | Hypertensive | 2.572 (0.770–8.593) | 0.125 | 0.118 | 2.685 (0.778–9.269) |
|          |           | Dominant    | 1.259 (0.584–2.714) | 0.556 | 0.470 | 1.348 (0.600–3.029) |
|          |           | Additive    | 2.800 (0.810–9.680) | 0.104 | 0.093 | 2.980 (0.834–10.650) |
| Recessive |           | Nonhypertensive | 1.649 (1.011–2.689) | 0.045 | 0.036 | 1.711 (1.035–2.829) |
|          |           | Hypertensive | 3.220 (1.164–8.903) | 0.024 | 0.008 | 4.102 (1.448–11.617) |
| Additive  |           | Nonhypertensive | 1.411 (0.845–2.354) | 0.188 | 0.212 | 1.400 (0.825–2.374) |
|          |           | Hypertensive | 3.744 (1.322–10.607) | 0.013 | 0.004 | 4.743 (1.633–13.776) |

See Table 4; Pc: P value adjusted for age, sex, body mass index, type 2 diabetes mellitus, and smoking.

Table 7: Characteristics of the LAS subjects stratified by EDN1 rs5370 genotype.

| Parameter          | GG (n = 94) | Genotype | TT (n = 26) | P value | P_FDR  |
|--------------------|-------------|----------|-------------|---------|--------|
| Body mass index, kg/m² | 28.1 ± 3.6 | 27.6 ± 3.8 | 28.4 ± 3.9 | 0.448 | 0.647 |
| Systolic BP, mmHg    | 165.1 ± 29.3 | 171.3 ± 28.8 | 167.5 ± 25.9 | 0.372 | 0.642 |
| Diastolic BP, mmHg   | 94.4 ± 14.9 | 99.3 ± 16.2 | 91.7 ± 13.3 | 0.034 | 0.429 |
| Pulse BP, mmHg       | 70.7 ± 20.2 | 71.9 ± 24.8 | 75.8 ± 22.5 | 0.600 | 0.720 |
| Mean BP, mmHg        | 117.9 ± 18.5 | 123.3 ± 17.8 | 116.9 ± 15.2 | 0.099 | 0.429 |
| Total cholesterol, mmol/L | 4.91 ± 1.4 | 5.15 ± 1.6 | 4.74 ± 1.3 | 0.374 | 0.641 |
| HDL cholesterol, mmol/L | 1.02 ± 0.31 | 1.01 ± 0.31 | 0.96 ± 0.25 | 0.610 | 0.720 |
| LDL cholesterol, mmol/L | 3.10 ± 1.3 | 3.33 ± 1.5 | 2.89 ± 1.3 | 0.347 | 0.642 |
| Triglyceride, mmol/L  | 1.65 ± 0.8 | 1.71 ± 0.8 | 1.58 ± 0.7 | 0.750 | 0.812 |
| Prothrombin time, s   | 9.47 ± 2.0 | 9.38 ± 2.0 | 9.29 ± 2.0 | 0.900 | 0.900 |
| Thrombin time, s       | 16.71 ± 3.7 | 16.49 ± 3.5 | 17.60 ± 4.0 | 0.395 | 0.642 |
| Fibrinogen, g/L        | 4.21 ± 1.3 | 3.93 ± 1.2 | 3.64 ± 1.3 | 0.089 | 0.429 |
| Fasting glucose, mmol/L | 6.17 ± 1.7 | 6.06 ± 1.4 | 5.59 ± 1.0 | 0.236 | 0.642 |

n: number of cases; BP: blood pressure; HDL: high-density lipoprotein; LDL: low-density lipoprotein; P_FDR: P value after false discovery rate adjustment.

EDN1 3′-UTR [31]. In order to test these hypotheses, we used bioinformatic prediction. Our results revealed that rs5335 might change the structure of hsa-miR-27a-3p and hsa-miR-27b-3p binding sites, which are the possible regulators of EDNRA expression. Future functional analysis to confirm these results is required.

The results obtained in the present study about the link of rs5370 (EDN1 gene) and rs5335 (EDNRA gene) polymorphisms with LAS showed that in a Ukrainian population, only the rs5370 locus is associated with the development of the mentioned disease. Regardless of adjustment for other atherosclerosis risk factors, it was found that the risk of ischemic stroke development in individuals with GT and TT genotypes is higher than in GG genotype carriers. However, a similar study performed by Aslan et al. among the Turkish population did not show any correlation between EDN1 rs5370 and rs10478694 polymorphisms and ischemic cerebrovascular disease [15]. Herewith, Gormley et al. demonstrated no association between EDN1 rs5370 and rs10478694 polymorphisms and lacunar infarction development among English patients as well [16]. The authors of both
Authors demonstrated that cerebral infarction (ACI) in a large-scale Japanese cohort of 202 SNPs of 152 candidate genes with atherothrombotic Yamaguchi et al. [14], who assessed a possible association models). Such data were consistent with results obtained by increased risk of LAS both among women (under dominant rs5335 (EDNRA di pathogenesis undoubtedly manifested in a complex set of other important risk factors. Further studies using complex analysis to draw definitive conclusion are necessary.

To date, there are number of studies about the association between endothelin system gene polymorphisms and risk of AH and pulmonary hypertension, as well as BP indices [5, 6, 11, 24, 34–36]. The present study revealed a high risk of LAS development in rs5370-T allele carriers with AH, which is inconsistent with data obtained by Yamaguchi et al. in subgroups stratified by the presence of AH in a Japanese population [14]. Our results also demonstrated a possible correlation between rs5370 polymorphism and indices of diastolic arterial blood pressure in stroke patients. Similar results were obtained for patients with preeclampsia [24], AH [34], and healthy individuals [37]. However, the association of EDN1 rs5370 locus with increased risk of AH development, as well as with different parameters of blood pressure among African-American patients with ischemic heart disease, was not found [11].

A significant limitation of our study is the relatively small number of patients enrolled into the case and control groups. Therefore, some associations between rs5370 and rs5335 SNPs and the risk of LAS development, indices of blood pressure, blood plasma lipoprotein level, and coagulation parameters might have been missed due to a small statistical power. Another limitation is the investigation of the link between SNPs and phenotype without an assessment of their effects on the EDN1 and EDNRA mRNA level and ET-1 concentration in the blood plasma. Therefore, future case-control studies involving more patients and functional studies of endothelin pathway polymorphisms effects are required.

5. Conclusion

In conclusion, this is the first evidence about the association between endothelin system genetic polymorphisms and
LAS development in a Ukrainian population. Our data demonstrated that EDN1 rs5370 polymorphism is related to increased risk of LAS development regardless of other atherosclerosis risk factors. No link between EDNRA rs5335 and risk of LAS in a Ukrainian population was found. At present, a small number of case-control studies about the role of rs5370 and rs5335 SNPs in ischemic stroke development have been performed. Increasing the number of such studies with subsequent meta-analysis is necessary in order to draw a firm conclusion about the association between the mentioned polymorphisms and risk of ischemic stroke.

**Conflicts of Interest**

The authors declare that there is no conflict of interest regarding the publication of this paper.

**Authors’ Contributions**

Yevhen I. Dubovyk performed the clinical research and wrote the manuscript. Tetyana B. Oleshko performed genotyping. Viktoria Yu. Harbuza performed genotyping and biostatistics. Alexander V. Ataman designed the research plan and organized the study.

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