Akopyan A. A.[1], Kirillova K.I.[1], Strazhesko I. D.[2, 1], Samokhodskaya L.M.[1], Orlova I.A.[1]

[1] Medical Research and Educational Center of the M. V. Lomonosov Moscow State University, Moscow, Russia

[2] Russian Clinical and Research Center of Gerontology,
Pirogov Russian National Research Medical University, Moscow, Russia

Association of *AGT, ACE, NOS3, TNF, MMP9, CYBA* polymorphism with subclinical arterial wall changes

Aim	Activation of the renin-angiotensin-aldosterone system, decreased nitric oxide production, chronic inflammation, and oxidative stress result in subclinical changes in the arterial wall, which favor the development of cardiovascular diseases (CVD). The effect of allelic gene variants that encode the proteins participating in pathogenetic pathways of age-associated diseases with subclinical changes in the arterial wall [increased pulse wave velocity (PWV), increased intima-media thickness, endothelial dysfunction (ED), presence of atherosclerotic plaques (ASP)] are understudied. This study analyzed the relationship between *AGT, ACE, NOS3 TNF, MMP9*, and *CYBA* gene polymorphism and the presence of subclinical changes in the arterial wall, including the dependence on risk factors for CVD, in arbitrarily healthy people of various age.
Material and methods	The relationship of polymorphisms c.521C>T of *AGT* gene, Ins>Del of *ACE* gene, c.894G>T of *NOS3* gene, – 238G>A of *TNF* gene, – 1562C>T of *MMP9* gene, and c.214T>C of *CYBA* gene with indexes of changes in the arterial wall and risk factors for CVD was studied in 160 arbitrarily healthy people by building models of multiple logistic regression and also by analyzing frequencies of co-emergence of two signs with the Pearson chi-squared test (χ^2) and Fisher exact test.
Results	The DD-genotype of Ins>Del *ACE* gene polymorphism was correlated with increased PWV (p=0.006; odds ratio (OR) =3.41, 95% confidence interval (CI): 1.48–8.67) and ED (p=0.014; OR=2.60, 95% CI: 1.22–5.68). The GG genotype of c.894G>T *NOS3* gene polymorphism was correlated with ED (p=0.0087; OR=2.65, 95% CI: 1.26–5.72); the TT-genotype of c.894G>T *NOS3* gene polymorphism was correlated with ASP (p=0.033; OR=0.034, 95% CI: 0.001–0.549).
Conclusion	Polymorphic variants of *ACE* and *NOS3* genes correlated with ED, increased arterial wall stiffness, and the presence of subclinical changes in the arterial wall.

Keywords	Arterial stiffness; endothelial dysfunction; risk factors of cardiovascular diseases; *ACE* gene polymorphism; *NOS3* gene polymorphism; intima-media thickness, atherosclerotic plaques
For citation	Akopyan A. A., Kirillova K.I., Strazhesko I. D., Samokhodskaya L.M., Orlova Ya.A. Association of *AGT, ACE, NOS3, TNF, MMP9, CYBA* polymorphism with subclinical arterial wall changes. Kardiologiia. 2020;61(3):57–65. [Russian: Акопян А.А., Кириллова К.И., Стражеско И.Д., Самоходская Л.М., Орлова Я.А. Связь полиморфизма генов *AGT, ACE, NOS3, TNF, MMP9, CYBA* с субклиническими изменениями артериальной стенки. Кардиология. 2020;61(3):57–65]

Corresponding author Akopyan A. A. E-mail: a.alexandrova18@gmail.com

Introduction

CCardiovascular diseases (CVDs) are the leading cause of death worldwide. Subclinical changes in the arterial wall, such as increased arterial stiffness, wall thickening, atherosclerotic plaques, and endothelial dysfunction (ED), contribute to the development of CVDs. They are associated with traditional cardiovascular risk factors (male sex, age, arterial hypertension [AH], diabetes mellitus [DM], and obesity) and new risk factors (insulin resistance [IR], leukocyte telomere length [LTL]) [1–3].

Genetic predisposition is also a cardiovascular risk factor. Despite more and more works investigating the effects of gene alleles on cardiovascular risks, their relation to subclinical changes in the arteries has not been sufficiently studied. Establishing such relations can help to find more effective ways to prevent CVDs at the preclinical stage. We selected gene alleles encoding proteins engaged in pathophysiological pathways of age-associated changes in the arterial wall. The genes encoding angiotensinogen (*AGT*) and angiotensin-converting enzyme (*ACE*) are associated with the renin-angiotensin-aldosterone system (RAAS). The endothelial NO-synthase (*NOS3*) gene is associated with the production of nitrogen oxide (NO). The genes encoding tumor necrosis factor-alpha (*TNF*), matrix metalloproteinase 9 (MMP 9), and cytochrome b light chain (*CYBA*) are associated with the development of chronic inflammation and oxidative stress. There is evidence in the literature that polymorphisms of the genes of interest are associated with AH [4, 5], coronary artery disease (CAD) [6–8], and ischemic stroke (IS) [9–11]. The objective of

our study was to identify the relation between subclinical changes in the arteries and the gene alleles, including given traditional and new cardiovascular risk factors.

Material and methods

The study included 160 (55 male and 105 female) patients from 25 to 82 years who visited the Medical Research and Educational Center of the Lomonosov Moscow State University for preventive examination in 2018–2019. Any known chronic non-communicable diseases, the regular use of any medication, pregnancy, lactation, and refusal to sign the informed consent were the exclusion criteria. All included patients signed the informed consent to participate in the study. The study protocol was approved by the Ethics Committee of the Medical Research and Educational Center of the Lomonosov Moscow State University.

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using a calibrated device with an inflatable cuff (HEM-7200 M3, Omron Healthcare, Kyoto, Japan). AH was diagnosed if SBP was ≥ 140 mmHg and/or DBP was ≥ 90 mm. The pulse wave velocity (PWV), which is a marker of arterial wall stiffness, was measured using the applanation tonometry technique (SphygmoCor 9.0 hardware, Atcor, Sydney). PWV > 10 m/sec was considered increased. Carotid ultrasound was performed using a PHILIPS EPIQ 5 system (Netherlands). The presence of atherosclerotic plaque (AP) was defined as focal thickening of the vessel wall by more than 50% compared to the surrounding areas or as a focal increase in the intima-media thickness by more than 1.5 mm protruding into the vessel lumen. Intima-media thickness (IMT) ≥ 0.9 mm was considered elevated. Endothelium-dependent vasodilation (EDV) was estimated using a reactive hyperemia test in the PHILIPS EPIQ 5 system (Netherlands). Endothelium-dependent vasodilatation < 10% was treated as ED.

Biochemical parameters of blood (total cholesterol [TC], high-density lipoprotein cholesterol [HDL–C], triglycerides [TG], fasting glucose) were evaluated by routine methods. The level of low-density lipoprotein cholesterol (LDL–C) was calculated using the formula:

$$LDL–C = TC – (TG/2.2+HDL–C).$$

DM type 2 was diagnosed if fasting glucose was ≥ 7.0 mmol/L. Immunoreactive blood insulin was determined by chemiluminescence. The HOMA IR index was calculated using the formula: fasting glucose (mmol/L) × fasting immunoreactive insulin (μU/L)/22.5. IR was diagnosed with HOMA ≥2.5.

LTL was determined on the genomic DNA by real-time PCR [12]. The polymorphisms of *AGT* (rs4762), *ACE* (rs1799752), *NOS3* (rs1799983), *TNF* (rs361525), and *MMP9* (rs391824) were determined by real-time PCR using the DNA Technology kits (Russia), and the *CYBA* (rs4673) polymorphism was studied using the Applied Biosystems kits (USA). DNA was isolated from whole blood using the Qiagen DNA Blood Mini Kit (Germany) according to the instructions. The Hardy-Weinberg equilibrium of the genotype frequencies was verified using the χ^2 test and the level of significance $\alpha = 0.05$. The distribution of genotype frequencies of the six genes fits with the Hardy – Weinberg equilibrium.

Statistical Analysis

Statistical analysis of the data was carried out using the R language (version 3.6.1). The mean (M) and standard deviation (DM) are used for normally distributed parameters, and the median (Me) and lower (Q1) and upper (Q3) quartiles are provided for non-normally distributed data. The normality of distributions was tested using Pearson's χ^2 test. The frequencies were analyzed to find the correlation between genetic polymorphism and the arterial wall parameters of interest using Pearson's χ^2 test or Fischer's exact test with the significance level $\alpha = 0.05$. The Fischer's test was used if the frequency of the co-occurrence of two factors was less than 5. Next, correlation analysis was performed by constructing multivariate logistic regression models assessing the presence of a sign of vascular wall lesion, according to the genetic polymorphism of interest. Gene alleles under study were forcibly included as variables in the model of dependence of the signs of vascular wall lesion on the genetic polymorphism.

Multivariate logistical regression models with cardiovascular risk factors used as independent variables were also studied. The best model was selected for each sign of vascular wall lesion by adding cardiovascular risk factors. The Akaike information criterion (AIC) was used to search the model and include/exclude variables.

Results and Discussion

The clinical profile of the 160 patients included is provided in the additional materials published on the journal's website (Table 9).

Statistically significant results of the univariate analysis of the association of PWV with *ACE* Ins>Del polymorphism are given in Table 1. The distribution of polymorphism alleles and genotypes of all genes of interest in the groups with and without increased arterial stiffness is provided in the additional materials posted on the journal's website (Table 10).

Thus, the D allele and DD genotype presence increased the risk of arterial stiffness 1.89-fold (95% CI: 1.16–3.12) and 3.42-fold (95% CI 1.39–9.36), respectively.

ISSN 0022-9040. Kardiologiia. 2021;61(3). DOI: 10.18087/cardio.2021.3.n1212

The multivariate logistic model showed a 3.41-fold increase in arterial stiffness independent of other mutant genotypes (95% CI: 1.48–8.67) in carriers of the *ACE* Ins>Del polymorphism DD genotype (Table 2).

PWV predicts cardiovascular events and all-cause mortality [13, 14]. RAAS is a key factor in the development of arterial stiffness and remodeling of the arterial wall. In response to blood pressure reduction and the activation of the sympathetic nervous system, the kidneys produce renin, a protein involved in the transformation of angiotensinogen into angiotensin I. Under the influence of angiotensin-converting enzyme (*ACE*) in the lungs, the latter turns into angiotensin II, a powerful vasoconstrictor [15]. The 50% variation in blood *ACE* levels is associated with *ACE* Ins>Del polymorphism. The levels of *ACE* are higher in carriers of the D allele of this polymorphism [16]. The elevated levels of angiotensin II increase the vascular wall tonus and contribute to the development of IR [17], systemic inflammation, oxidative stress [18–21], and ED, [19, 20] which have a pathogenetic effect in the increase of arterial stiffness.

A multivariate logistic model was constructed to study the association of PWV with the mutant *ACE* genotype, taking into account the cardiovascular risk factors. Table 3 presents the factors that had a statistically significant association with PWV.

In the multivariate logistic model of mutant genotypes with cardiovascular risk factors, the risk of arterial wall stiffness was increased by the mutant *ACE* genotype, male sex, and age; there was a trend toward the statistical significance of the association between arterial wall stiffness and AH. Our findings are consistent with the published works. Male sex and age increase the risk of developing arterial stiffness [22, 23]. Arterial stiffness is associated with AH. Increased PWV was shown to cause the development of AH, as well as AH leading to an increase in PWV [24].

In the review by Logan et al., the data are provided on the association of D allele with arterial stiffness in the general population [25]. The DD genotype of the *ACE* polymorphism increased the risk of IS [26], CAD [27], and AH [5, 28]. The presence of the D allele was associated with the worse functional outcome of IS [29] and essential AH [30].

Statistically significant results of the univariate analysis of the association of ED with *ACE* Ins>Del polymorphism genotypes and the *NOS3* c.894G>T polymorphism are provided in Table 4. All results of the univariate analysis of the association of ED with the polymorphisms of the genes of interest are presented in the additional materials (Table 11).

The univariate analysis found an association between ED and the *NOS3* c.894G>T polymorphism. The presence of the GG genotype increased the risk of ED 2.65-fold (95% CI: 1.26–5.72), and the GT genotype had a protective effect against endothelial dysfunction (OR: 0.4, 95% CI: 0.18–0.86).

Table 1. Distribution of alleles and genotypes of *ACE* Ins>Del polymorphism in patients with and without increased arterial stiffness

Gene	Allele/genotype	PWV >10 m/s, n (%)	PWV ≤ 10 m/s, n (%)	p	OR	95% CI
ACE Ins>Del	I	85 (45.21)	72 (61.02)	0.010	0.53	(0.32–0.86)
	D	103 (54.79)	46 (38.98)		1.89	(1.16–3.12)
	II	24 (25.53)	21 (35.59)	0.013	0.62	(0.29–1.34)
	ID	37 (39.36)	30 (50.85)		1.12	(0.56–2.24)
	DD	33 (35.11)	8 (13.56)		3.42	(1.39–9.36)
	II+ID	61 (64.89)	51 (86.44)	0.006	0.29	(0.11–0.72)
	ID+DD	70 (74.47)	38 (64.41)	0.25	1.61	(0.75–3.46)

PWV, pulse wave velocity; OR, odds ratio; CI, confidence interval.

Table 2. Association of PWV > 10 m/s with mutant genotypes of polymorphisms of the genes of interest

Genotype	Estimate (coefficient)	Std. error	z, value	p	OR	95% CI
AGT TT genotype	–0.228	1.437	–0.159	0.874	0.796	(0.031–20.714)
ACE DD genotype	1.226	0.447	2.745	0.006	3.407	(1.478–8.672)
NOS3 TT genotype	–0.107	0.804	–0.134	0.894	0.898	(0.188–4.888)
TNF AA genotype	12.896	882.744	0.015	0.988	N/A1	N/A[1]
MMP9 TT genotype	–0.337	1.031	–0.327	0.744	0.714	(0.081–6.235)
CYBA CC genotype	0.432	0.357	1.210	0.226	1.54	(0.77–3.13)
Constant	0.013	0.25	0.05	0.96	-	-

[1] – N/A, not applicable due to small sample size.

Table 3. Association of PWV > 10 m/s with cardiovascular risk factors and the mutant genotypes of polymorphisms of the genes of interest

Risk factors	Estimate (coefficient)	Std. error	z, value	p	OR	95% CI
ACE DD genotype	1.247	0.519	2.402	0.016	3.481	(1.315–10.269)
Male	1.232	0.468	2.632	0.008	3.429	(1.408–8.921)
Age	0.093	0.019	4.790	< 0.001	1.097	(1.059–1.143)
AH	0.858	0.505	1.698	0.09	2.359	(0.904–6.72)
Constant	–5.012	1.039	–4.825	< 0.001	–	–

AH, arterial hypertension; OR, odds ratio, CI, confidence interval.

Table 4. . Distribution of alleles and genotypes of *ACE* Ins>Del polymorphism and *NOS3* c.894G>T polymorphism in a patient with and without ED

Gene	Allele/genotype	ED+, n (%)	ED-, n (%)	p	OR	95% CI
ACE Ins >Del	I	49 (42.24)	98 (55.68)	0.033	0.58	(0.35–0.96)
	D	67 (57.76)	78 (44.32)		1.71	(1.04–2.84)
	II	13 (22.41)	29 (32.95)	0.08	0.59	(0.25–1.33)
	ID	23 (39.66)	40 (45.45)		0.79	(0.38–1.63)
	DD	22 (37.93)	19 (21.59)		2.21	(0.997–4.94)
	II+ID	36 (62.07)	69 (78.41)	0.0498	0.45	(0.2–1.003)
	ID+DD	45 (77.59)	59 (67.04)	0.23	1.7	(0.75–3.98)
NOS3 c.894G >T	G	96 (82.76)	123 (69.89)	0.019	2.06	(1.12–3.9)
	T	20 (17.24)	53 (30.11)		0.48	(0.26–0.89)
	GG	40 (68.97)	40 (45.45)	0.016	2.65	(1.26–5.72)
	GT	16 (27.59)	43 (48.86)		0.4	(0.18–0.86)
	TT	2 (3.45)	5 (5.68)		0.59	(0.05–3.79)
	GG+GT	56 (96.55)	83 (94.32)	0.7	1.68	(0.26–18.23)
	GT+TT	18 (31.03)	48 (54.55)	0.0087	0.38	(0.17–0.79)

ED+, patients with endothelial dysfunction;
ED -, patients without endothelial dysfunction, OR, odds ratio; CI, confidence interval.

ED is another important manifestation of subclinical changes in the arterial wall. The recovery of endothelium subjected to oxidative stress and chronic inflammation worsens with age, contributing to the onset or progression of vascular diseases [31]. The endothelium produces NO, a molecule with an important role in vasodilation. NO has an anti-inflammatory effect, reduces platelet aggregation, leukocyte adhesion, and cytokine synthesis [32]. Endothelial NO-synthase (NOS-3) is the main substance that produces NO and regulates the vascular wall tonus [33]. NOS-3 dysregulation reduces the production of NO and active forms of oxygen [34].

The published data on the *NOS3* c.894G>T polymorphism are controversial. The *NOS3* polymorphism TT genotype was associated with the risk of atherosclerosis in patients with type 2 DM [35]. TT and GT genotypes were associated with the risks of CAD [36, 37] and AH [38]. The results of our study are consistent with the findings of Campedelli et al., who showed that the presence of GG genotype increased the predisposition to atherosclerosis [39].

The *ACE* Ins>Del polymorphism is also associated with ED; the presence of the D allele increased the risk of ED 1.71-fold (95% CI: 1.04–2.84), and the I allele had a protective effect (OR: 0.58, 95% CI: 0.35–0.96).

In the multivariate logistic model including mutant genotypes of six genes, the mutant *ACE* genotype increased the risk of ED 2.6-fold (95% CI: 1.22–5.68). The relevant data are given in Table 5.

The activation of tissue RAAS in the vascular wall is an important factor causing oxidative stress and associated ED [19]. Its element angiotensin II leads to the development of ED through the activation of chronic inflammation [40].

Table 6 presents the results of the multivariate analysis of the association of ED with mutant genotypes and cardiovascular risk factors. Only the indicators that have a significant association with ED are presented.

According to the multiple logistic regression, age, AH, and TC are the most significant predictors of ED, which is consistent with the published data [41, 42]. There is a trend toward the statistical significance of the association of ED with the mutant genotype of *ACE* Ins>Del polymorphism.

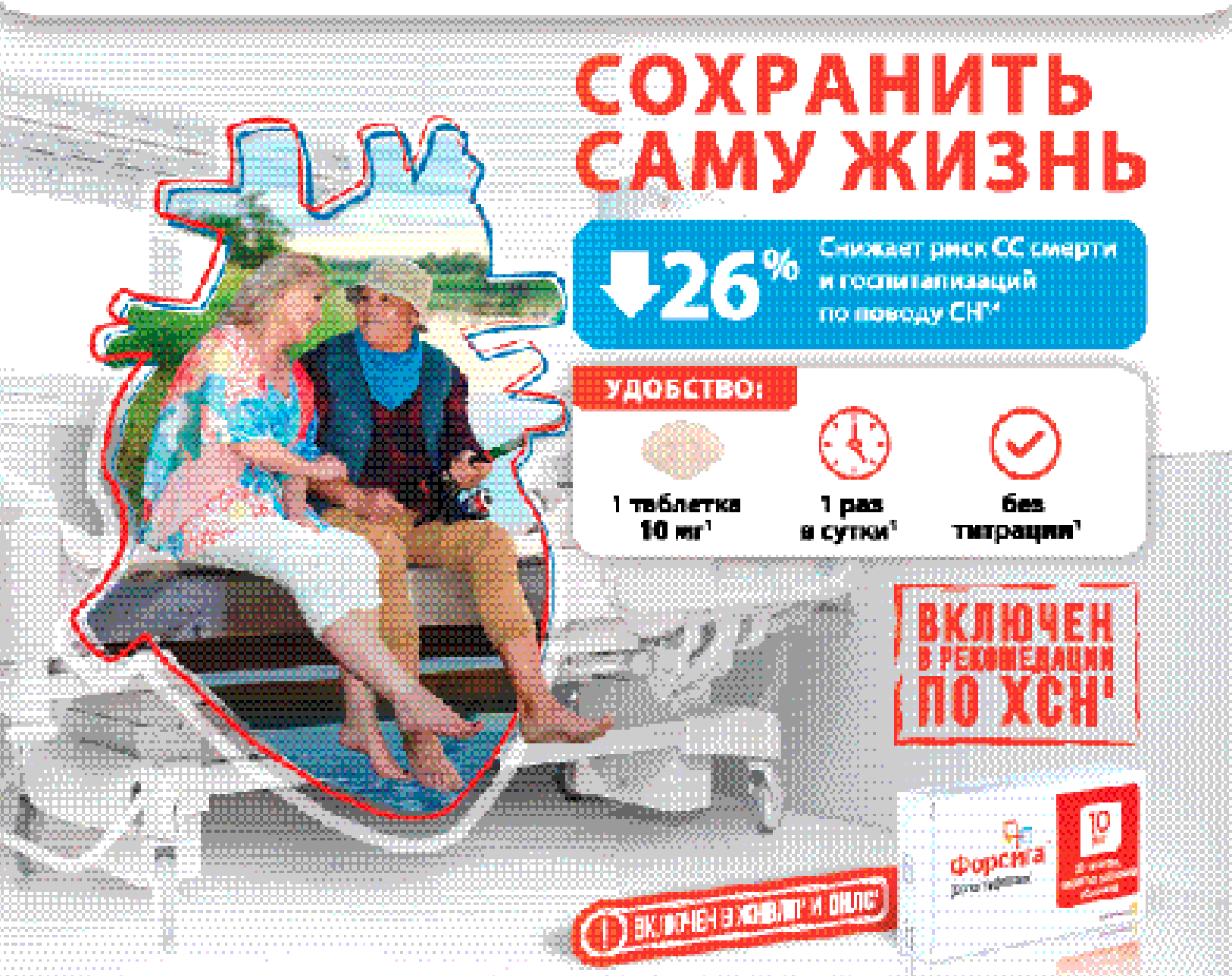
Despite the known association of IMT with RAAS activation [43], chronic inflammation, and oxidative stress [44, 45], we did not detect an association of IMT with polymorphisms of the genes of interest and mutant

ISSN 0022-9040. Kardiologiia. 2021;61(3). DOI: 10.18087/cardio.2021.3.n1212

форсига

ФОРСИГА® — НОВЫЙ ЖИЗНЕСПАСАЮЩИЙ ПРЕПАРАТ ДЛЯ ПАЦИЕНТОВ С ХСНнФВ[1,2]

СОХРАНИТЬ САМУ ЖИЗНЬ



↓26% Снижает риск СС смерти и госпитализаций по поводу СН[1,4]

УДОБСТВО:

- 1 таблетка 10 мг[1]
- 1 раз в сутки[1]
- Без титрации[1]

ВКЛЮЧЕН В РЕКОМЕНДАЦИИ ПО ХСН[1]

ВКЛЮЧЕН В ЖНВЛП® И ОНЛС

genotypes in the univariate and multivariate analyses (additional materials, Table 12–13).

Table 7 provides the results of the multivariate logistic regression analysis of the association of IMT with cardiovascular risk factors and mutant genotypes. Only factors that demonstrated statistical significance are provided.

Age and the presence of type 2 DM increased the risk of intima-media thickening, which is consistent with the published data [46–48]. IMT is a marker of early vascular aging and is often used in large studies, including genetic ones, as a marker of early atherosclerosis, due to the relative simplicity, accessibility, and noninvasive nature of the measurement technique [49]. A 0.1-mm increase in IMT is associated with an 18% increase in IS risk and a 15% increase in MI risk [50].

The results of the univariate analysis of the association of the presence of plaques with the polymorphisms of the genes of interest are presented in the additional materials (Table 14). There is a trend toward the association of the C allele of the *AGT* polymorphism of interest with the risk of atherosclerotic plaques (OR: 2.08, 95% CI: 0.99–4.55; p = 0.053). Our results contradict the published evidence of the association between the T allele and the development of cardiovascular diseases: CAD [51] and IS [11].

Association of atherosclerotic plaques with mutant genotypes of the genes of interest was not shown in the multivariate analysis. The results are given in the additional materials (Table 15).

The results of the multivariate analysis of the association of the presence of plaques with risk factors and mutant genotypes are presented in Table 8.

Association of the presence of plaques with age and AH was found, which corresponds to the published data. Plaques, like IMT, are markers of systemic atherosclerosis. Carotid plaques mainly occur at the site of turbulent blood flow and predict the presence of coronary atherosclerosis and CAD with high sensitivity [52]. Plaques are associated with traditional RFs, vascular wall inflammation [53], oxidative stress, and RAAS activation [54]. The identified

Table 5. Association of ED with mutant genotypes of polymorphisms of the genes of interest

Genotype	Estimate (coefficient)	Std. error	z, value	p	OR	95% CI
AGT TT genotype	0.668	1.431	0.467	0.640	1.951	(0.076–50.421)
ACE DD genotype	0.957	0.391	2.448	0.014	2.603	(1.218–5.678)
NOS3 TT genotype	–0.818	0.893	–0.916	0.359	0.441	(0.058–2.292)
TNF AA genotype	–14.893	882.743	–0.017	0.987	N/A1	N/A[1]
MMP9 TT genotype	0.649	1.028	0.631	0.528	1.913	(0.22–16.672)
CYBA CC genotype	0.078	0.353	0.222	0.824	1.082	(0.54–2.163)
Constant	–0.708	0.269	–2.630	0.009	–	–

[1] N/A, not applicable due to small sample size, OR, odds ratio; CI, confidence interval.

Table 6. Association of ED with cardiovascular risk factors and the mutant genotypes of polymorphisms of the genes of interest

Risk factors	Estimate (coefficient)	Std. error	z, value	p	OR	95% CI
ACE DD genotype	0.851	0.44	1.934	0.053	2.342	(0.995–5.644)
Age	0.049	0.017	2.916	0.004	1.05	(1.017–1.087)
TC > 5 mmol/L, %	0.988	0.472	2.090	0.037	2.684	(1.089–7.053)
AH	1.545	0.454	3.406	< 0.001	4.689	(1.966–11.781)
IR	0.371	0.444	0.836	0.403	1.449	(0.601–3.458)
Constant	–4.477	1.005	–4.454	< 0.001	–	–

TC, total cholesterol; AH, arterial hypertension; IR, insulin resistance; OR, odds ratio, CI, confidence interval.

Table 7. Association of IMT ≥ 0.9 mm with cardiovascular risk factors and the mutant genotypes of polymorphisms of the genes of interest

Risk factors	Estimate (coefficient)	Std. error	z, value	p	OR	95% CI
DM 2	2.314	0.627	3.693	< 0.001	10.110	(3.068–36.9)
Sex	1.26	0.700	1.799	0.072	3.525	(0.903–14.701)
Age	0.150	0.039	3.896	< 0.001	1.162	(1.086–1.265)
Constant	–11.421	2.538	–4.5	< 0.001	–	–

DM, diabetes mellitus; OR, odds ratio; CI, confidence interval.

ISSN 0022-9040. Kardiologiia. 2021;61(3). DOI: 10.18087/cardio.2021.3.n1212

Table 8. Association of the presence of plaques with cardiovascular risk factors and the mutant genotypes of polymorphisms of the genes of interest

Risk factors	Estimate (coefficient)	Std. error	z, value	p	OR	95% CI
NOS3 TT genotype	<0.001	1.581	–2.131	0.033	0.034	(0.001–0.549)
DM 2	1.327	0.729	1.820	0.069	3.768	(0.994–18.863)
Age	0.115	0.023	5.076	< 0.001	1.122	(1.076–1.177)
LTL	–0.814	0.538	–1.513	0.13	0.443	(0.142–1.166)
AH	1.617	0.534	3.030	0.002	5.037	(1.838–15.184)
Constant	1.401	5.453	0.257	0.797	-	-

DM, diabetes mellitus; LTL, leukocyte telomere length, AH, arterial hypertension; OR, odds ratio; CI, confidence interval.

protective effect of the TT genotype of *NOS3* c.894G>T polymorphism does not fully correspond to the published data: a predisposition to atherosclerosis in GG genotype carriers was found in one study [39].

Conclusion

Of the six gene alleles of interest, polymorphisms of *ACE* (rs1799752) and *NOS3* (rs1799983) were associated with subclinical changes in the arterial wall. The DD genotype of *ACE* Ins>Del polymorphism and the GG genotype of *NOS3* c.894G>T polymorphism may serve as genetic markers, regardless of other risk factors, of the development of subclinical changes in the arterial wall, and their carriers are at risk of early vascular aging and in need of early prevention of CVDs. The small sample size limits this study. The findings may give rise to further, larger studies.

Funding

This study was performed as a part of the State Assignment of the Medical Research and Educational Center at the M. V. Lomonosov Moscow State University.

No conflict of interest is reported.

The article was received on 02/06/2020

REFERENCES

1. Nilsson P. Early vascular aging (EVA): consequences and prevention. Vascular Health and Risk Management. 2008;4(3):547–52. DOI: 10.2147/VHRM.S1094
2. Strazhesko ID, Tkacheva ON, Akasheva DU, Dudinskaya EN, Plokhova EV, Pykhtina VS et al. Growth Hormone, Insulin-Like Growth Factor-1, Insulin Resistance, and Leukocyte Telomere Length as Determinants of Arterial Aging in Subjects Free of Cardiovascular Diseases. Frontiers in Genetics. 2017; 8:198. DOI: 10.3389/fgene.2017.00198
3. Gomez-Sanchez M, Gomez-Sanchez L, Patino-Alonso MC, Cunha PG, Recio-Rodriguez JI, Alonso-Dominguez R et al. Vascular aging and its relationship with lifestyles and other risk factors in the general Spanish population: Early Vascular Ageing Study. Journal of Hypertension. 2020;38(6):1110–22. DOI: 10.1097/HJH.0000000000002373
4. Yang W, Lu J, Yang L, Zhang J. Association of Matrix Metalloproteinase-9 Gene -1562C/T Polymorphism with Essential Hypertension: A Systematic Review and Meta-Analysis Article. Iranian Journal of Public Health. 2015;44(11):1445–52. PMID: 26744701
5. Abouelfath R, Habbal R, Laaraj A, Khay K, Harraka M, Nadifi S. *ACE* insertion/deletion polymorphism is positively associated with resistant hypertension in Morocco. Gene. 2018; 658:178–83. DOI: 10.1016/j.gene.2018.03.028
6. Luo J-Q, Wen J-G, Zhou H-H, Chen X-P, Zhang W. Endothelial Nitric Oxide Synthase Gene G894T Polymorphism and Myocardial Infarction: A Meta-Analysis of 34 Studies Involving 21068 Subjects. PLoS ONE. 2014;9(1): e87196. DOI: 10.1371/journal.pone.0087196
7. Cheng Y, An B, Jiang M, Xin Y, Xuan S. Association of Tumor Necrosis Factor-alpha Polymorphisms and Risk of Coronary Artery Disease in Patients with Non-alcoholic Fatty Liver Disease. Hepatitis Monthly. 2015;15(3): e26818. DOI: 10.5812/hepatmon.26818
8. Xu Q, Yuan F, Shen X, Wen H, Li W, Cheng B et al. Polymorphisms of C242T and A640G in *CYBA* Gene and the Risk of Coronary Artery Disease: A Meta-Analysis. PLoS ONE. 2014;9(1):e84251. DOI: 10.1371/journal.pone.0084251
9. Rai H, Parveen F, Kumar S, Kapoor A, Sinha N. Association of Endothelial Nitric Oxide Synthase Gene Polymorphisms with Coronary Artery Disease: An Updated Meta-Analysis and Systematic Review. PLoS ONE. 2014;9(11):e113363. DOI: 10.1371/journal.pone.0113363
10. Buraczynska K, Kurzepa J, Ksiazek A, Buraczynska M, Rejdak K. Matrix Metalloproteinase-9 (MMP-9) Gene Polymorphism in Stroke Patients. NeuroMolecular Medicine. 2015;17(4):385–90. DOI: 10.1007/s12017-015-8367-5
11. Isordia-Salas I, Santiago-Germán D, Cerda-Mancillas MC, Hernández-Juárez J, Bernabe-García M, Leaños-Miranda A et al. Gene polymorphisms of angiotensin-converting enzyme and angiotensinogen and risk of idiopathic ischemic stroke. Gene. 2019;688: 163–70. DOI: 10.1016/j.gene.2018.11.080
12. Cawthon RM. Telomere measurement by quantitative PCR. Nucleic Acids Research. 2002;30(10):e47. DOI: 10.1093/nar/30.10.e47
13. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of Cardiovascular Events and All-Cause Mortality with Arterial Stiffness: a systematic review and meta-analysis. Journal of the American College of Cardiology. 2010;55(13):1318–27. DOI: 10.1016/j.jacc.2009.10.061
14. Zhong Q, Hu M-J, Cui Y-J, Liang L, Zhou M-M, Yang Y-W et al. Carotid–Femoral Pulse Wave Velocity in the Prediction of Cardiovascular Events and Mortality: An Updated Systematic Review and Meta-Analysis. Angiology. 2018;69(7):617–29. DOI: 10.1177/0003319717742544
15. Patel S, Rauf A, Khan H, Abu-Izneid T. Renin-angiotensin-aldosterone (RAAS): The ubiquitous system for homeostasis and pathologies.

Biomedicine & Pharmacotherapy. 2017; 94:317–25. DOI: 10.1016/j.biopha.2017.07.091
16. Rigat B, Hubert C, Corvol P, Soubrier R. PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidyl carboxypeptidase 1). Nucleic Acids Research. 1992;20(6):1433. DOI: 10.1093/nar/20.6.1433-a
17. Marcus Y, Shefer G, Stern N. Adipose tissue renin–angiotensin–aldosterone system (RAAS) and progression of insulin resistance. Molecular and Cellular Endocrinology. 2013;378(1–2):1–14. DOI: 10.1016/j.mce.2012.06.021
18. Manrique C, Lastra G, Gardner M, Sowers JR. The Renin Angiotensin Aldosterone System in Hypertension: Roles of Insulin Resistance and Oxidative Stress. Medical Clinics of North America. 2009;93(3):569–82. DOI: 10.1016/j.mcna.2009.02.014
19. Aroor AR, DeMarco VG, Jia G, Sun Z, Nistala R, Meininger GA et al. The Role of Tissue Renin-Angiotensin-Aldosterone System in the Development of Endothelial Dysfunction and Arterial Stiffness. Frontiers in Endocrinology. 2013;4:161. DOI: 10.3389/fendo.2013.00161
20. Zieman SJ, Melenovsky V, Kass DA. Mechanisms, Pathophysiology, and Therapy of Arterial Stiffness. Arteriosclerosis, Thrombosis, and Vascular Biology. 2005;25(5):932–43. DOI: 10.1161/01.ATV.0000160548.78317.29
21. Barhoumi T, Kasal DA, Li MW, Shbat L, Laurant P, Neves MF et al. T Regulatory Lymphocytes Prevent Angiotensin II–Induced Hypertension and Vascular Injury. Hypertension. 2011;57(3):469–76. DOI: 10.1161/HYPERTENSIONAHA.110.162941
22. Strazhesko I.D., Akasheva D.U., Dudinskaya E.N., Tkacheva O.N. Vascular ageing: main symptoms and mechanisms. Cardiovascular Therapy and Prevention. 2012;11(4):93–100. [Russian: Стражеско И.Д., Акашева Д.У., Дудинская Е.Н., Ткачева О.Н. Старение сосудов: основные признаки и механизмы. Кардиоваскулярная терапия и профилактика. 2012;11(4):93-100]. DOI: 10.15829/1728-8800-2012-4-93-100
23. Cheng H-M, Park S, Huang Q, Hoshide S, Wang J-G, Kario K et al. Vascular aging and hypertension: Implications for the clinical application of central blood pressure. International Journal of Cardiology. 2017; 230:209–13. DOI: 10.1016/j.ijcard.2016.12.170
24. Nilsson PM. Early Vascular Aging in Hypertension. Frontiers in Cardiovascular Medicine. 2020; 7:6. DOI: 10.3389/fcvm.2020.00006
25. Logan JG, Engler MB, Kim H. Genetic Determinants of Arterial Stiffness. Journal of Cardiovascular Translational Research. 2015;8(1):23–43. DOI: 10.1007/s12265-014-9597-x
26. Yuan H, Wang X, Xia Q, Ge P, Wang X, Cao X. Angiotensin converting enzyme (I/D) gene polymorphism contributes to ischemic stroke risk in Caucasian individuals: a meta-analysis based on 22 case-control studies. International Journal of Neuroscience. 2015;126(6):488–98. DOI: 10.3109/00207454.2015.1036421
27. Bhatti GK, Bhatti JS, Vijayvergiya R, Singh B. Implications of *ACE* (I/D) Gene Variants to the Genetic Susceptibility of Coronary Artery Disease in Asian Indians. Indian Journal of Clinical Biochemistry. 2017;32(2):163–70. DOI: 10.1007/s12291-016-0588-3
28. Hussain M, Awan FR, Gujjar A, Hafeez S, Islam M. A case control association study of *ACE* gene polymorphism (I/D) with hypertension in Punjabi population from Faisalabad, Pakistan. Clinical and Experimental Hypertension. 2018;40(2):186–91. DOI: 10.1080/10641963.2017.1356842
29. Malueka RG, Dwianingsih EK, Sutarni S, Bawono RG, Bayuangga HF, Gofir A et al. The D allele of the angiotensin-converting enzyme (*ACE*) insertion/deletion (I/D) polymorphism is associated with worse functional outcome of ischaemic stroke. International Journal of Neuroscience. 2018;128(8):697–704. DOI: 10.1080/00207454.2017.1412962
30. Krishnan R, Sekar D, karunanithy S, Subramanium S. Association of angiotensin converting enzyme gene insertion/deletion polymorphism with essential hypertension in south Indian population. Genes & Diseases. 2016;3(2):159–63. DOI: 10.1016/j.gendis.2016.03.001
31. Strazhesko I.D., Tkacheva O.N. Cellular mechanisms of morphological and functional changes of the arterial wall with age and the role of statin therapy in the prevention. Kardiologiia. 2015;55(7):89–96. [Russian: Стражеско И.Д., Ткачева О.Н. Клеточные механизмы морфологических и функциональных изменений артериальной стенки с возрастом и роль терапии статинами в их профилактике. Кардиология. 2015;55(7):89-96]
32. Tousoulis D, Kampoli A-M, Tentolouris Nikolaos Papageorgiou C, Stefanadis C. The Role of Nitric Oxide on Endothelial Function. Current Vascular Pharmacology. 2012;10(1):4–18. DOI: 10.2174/157016112798829760
33. Zhao Y, Vanhoutte PM, Leung SWS. Vascular nitric oxide: Beyond eNOS. Journal of Pharmacological Sciences. 2015;129(2):83–94. DOI: 10.1016/j.jphs.2015.09.002
34. Meza CA, La Favor JD, Kim D-H, Hickner RC. Endothelial Dysfunction: Is There a Hyperglycemia-Induced Imbalance of NOX and NOS? International Journal of Molecular Sciences. 2019;20(15):3775. DOI: 10.3390/ijms20153775
35. El Dayem SM, Battah AA, El Bohy AEM, Ahmed S, Hamed M, El Fattah SNA. Nitric Oxide Gene Polymorphism is a Risk Factor for Diabetic Nephropathy and Atherosclerosis in Type 1 Diabetic Patients. Macedonian Journal of Medical Sciences. 2019;7(19):3132–8. DOI: 10.3889/oamjms.2019.831
36. Corapcioglu D, Sahin M, Emral R, Celebi ZK, Sener O, Gedik VT. Association of the G894T Polymorphism of the Endothelial Nitric Oxide Synthase Gene with Diabetic Foot Syndrome Foot Ulcer, Diabetic Complications, and Comorbid Vascular Diseases: A Turkish Case–Control Study. Genetic Testing and Molecular Biomarkers. 2010;14(4):483–8. DOI: 10.1089/gtmb.2010.0023
37. Johns R, Chen Z-F, Young L, Delacruz F, Chang N-T, Yu C et al. Meta-Analysis of *NOS3* G894T Polymorphisms with Air Pollution on the Risk of Ischemic Heart Disease Worldwide. Toxics. 2018;6(3):44. DOI: 10.3390/toxics6030044
38. ALrefai AA, Habib MSE, Yaseen RI, Gabr MK, Habeeb RM. Association of endothelial nitric oxide synthase (eNOS) gene G894T polymorphism with hypertension risk and complications. Molecular and Cellular Biochemistry. 2016;421(1–2):103–10. DOI: 10.1007/s11010-016-2790-2
39. Campedelli FL, e Silva KSF, Rodrigues DA, Martins JVM, Costa IR, Lagares MH et al. Polymorphism of the gene eNOS G894T (Glu298Asp) in symptomatic patients with aterosclerosis. Genetics and Molecular Research. 2017;16(2). DOI: 10.4238/gmr16029550
40. Srivastava P, Badhwar S, Chandran DS, Jaryal AK, Jyotsna VP, Deepak KK. Imbalance between Angiotensin II - Angiotensin (1-7) system is associated with vascular endothelial dysfunction and inflammation in type 2 diabetes with newly diagnosed hypertension. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2019;13(3):2061–8. DOI: 10.1016/j.dsx.2019.04.042
41. Konukoglu D, Uzun H. Endothelial Dysfunction and Hypertension. Advances in Experimental Medicine and Biology. 2017; 956:511–40. DOI: 10.1007/5584_2016_90
42. Gimbrone MA, García-Cardeña G. Endothelial Cell Dysfunction and the Pathobiology of Atherosclerosis. Circulation Research. 2016;118(4):620–36. DOI: 10.1161/CIRCRESAHA.115.306301
43. Johnson CT, Brewster LP. Carotid Artery Intima-Media Thickness and the Renin-Angiotensin System. Hospital Practice. 2013;41(2):54–61. DOI: 10.3810/hp.2013.04.1026
44. Wu S-S, Kor C-T, Chen T-Y, Liu K-H, Shih K-L, Su W-W et al. Relationships between Serum Uric Acid, Malondialdehyde Levels, and Carotid Intima-Media Thickness in the Patients with Metabolic Syndrome. Oxidative Medicine and Cellular Longevity. 2019; 2019:6859757. DOI: 10.1155/2019/6859757
45. Seyedsadjadi N, Berg J, Bilgin AA, Grant R. A Pilot Study Providing Evidence for a Relationship between a Composite Lifestyle Score and Risk of Higher Carotid Intima-Media Thickness: Is There a Link to Oxidative Stress? Oxidative Medicine and Cellular Longevity. 2018; 2018:4504079. DOI: 10.1155/2018/4504079
46. Dudinskaya E.N., Tkacheva O.N., Shestakova M.V., Brailova N.V., Strazhesko I.D., Akasheva D.U. et al. Telomere length and vascular wall in patients with Type 2 Diabetes Mellitus. Diabetes melli-

ISSN 0022-9040. Kardiologiia. 2021;61(3). DOI: 10.18087/cardio.2021.3.n1212

tus. 2014;17(3):31–8. [Russian: Дудинская Е.Н., Ткачева О.Н., Шестакова М.В., Браилова Н.В., Стражеско И.Д., Акашева Д.У. и др. Длина теломер и состояние сосудистой стенки у пациентов с сахарным диабетом 2 типа. Сахарный диабет. 2014;17(3):31-8]. DOI: 10.14341/DM2014331-38
47. Zhou Y-Y, Qiu H-M, Yang Y, Han Y-Y. Analysis of risk factors for carotid intima-media thickness in patients with type 2 diabetes mellitus in Western China assessed by logistic regression combined with a decision tree model. Diabetology & Metabolic Syndrome. 2020;12(1):8. DOI: 10.1186/s13098-020-0517-8
48. Watanabe K, Ouchi M, Ohara M, Kameda W, Susa S, Oizumi T et al. Change of carotid intima-media thickness is associated with age in elderly Japanese patients without a history of cardiovascular disease. Geriatrics & Gerontology International. 2015;15(8):1023–30. DOI: 10.1111/ggi.12402
49. Paternoster L, Martinez-Gonzalez NA, Charleton R, Chung M, Lewis S, Sudlow CLM. Genetic Effects on Carotid Intima-Media Thickness: Systematic Assessment and Meta-Analyses of Candidate Gene Polymorphisms Studied in More Than 5000 Subjects. Circulation: Cardiovascular Genetics. 2010;3(1):15–21. DOI: 10.1161/CIRCGENETICS.108.834366
50. Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of Clinical Cardiovascular Events with Carotid Intima-Media Thickness: A Systematic Review and Meta-Analysis. Circulation. 2007;115(4):459–67. DOI: 10.1161/CIRCULATIONAHA.106.628875
51. Wang W-Z. Association between T174M polymorphism in the angiotensinogen gene and risk of coronary artery disease: a meta-analysis. Journal of geriatric cardiology: JGC. 2013;10(1):59–65. DOI: 10.3969/j.issn.1671-5411.2013.01.010
52. Inaba Y, Chen JA, Bergmann SR. Carotid plaque, compared with carotid intima-media thickness, more accurately predicts coronary artery disease events: A meta-analysis. Atherosclerosis. 2012;220(1):128–33. DOI: 10.1016/j.atherosclerosis.2011.06.044
53. Fernández-Friera L, Fuster V, López-Melgar B, Oliva B, Sánchez-González J, Macías A et al. Vascular Inflammation in Subclinical Atherosclerosis Detected by Hybrid PET/MRI. Journal of the American College of Cardiology. 2019;73(12):1371–82. DOI: 10.1016/j.jacc.2018.12.075
54. Husain K. Inflammation, oxidative stress and renin angiotensin system in atherosclerosis. World Journal of Biological Chemistry. 2015;6(3):209–17. DOI: 10.4331/wjbc.v6.i3.209