https://doi.org/10.25557/2073-7998.2024.06.11-19

Пилотное исследование ассоциации распространенных вариантов NOS3 с риском развития преэклампсии у российских беременных женщин из Ростовской области

Алаяса Н.Н.[1], Шкурат Т.П.[1,2]

1 – Южный федеральный университет, Академия биологии и биотехнологии имени Д.И. Ивановского
344090, г. Ростов-на-Дону, пр. Стачки, д. 194/1

2 – Медицинский центр «Наука»
344034, г. Ростов-на-Дону, ул. Загорская, д. 23А

Преэклампсия (ПЭ) – тяжелое заболевание, поражающее беременных женщин, часто развивается после 20 недель гестации, и является наиболее распространенным серьезным расстройством, составляя 2-8% осложнений, связанных с беременностью, во всем мире. Цель исследования: изучить ассоциацию двух вариантов в гене *NOS3* (-786T>C; rs2070744) и (894G>T; rs1799983) с риском развития ПЭ у российских беременных женщин из Ростовской области. В исследование типа «случай-контроль» были включены 106 беременных женщин (46 с ПЭ и 60 здоровых, составивших контрольную группу). Генотипирование выбранных генетических вариантов *NOS3* (-786T>C) и (894G>T) проводилось методом аллель-специфической RT-PCR. Для оценки взаимодействия SNP-SNP с риском развития ПЭ использовали многофакторное снижение размерности (MDR). Кроме того, мы проанализировали неравновесие по сцеплению (LD) и ассоциацию гаплотипов выбранных SNP в исследуемой популяции. Согласно нашим результатам, оба полиморфизма *NOS3* были статистически значимы (p=0,004 и p=0,045, соответственно). Генотип *NOS3* -786(TT) (OR=0,25 95% CI [0,11- 0,58]) и генотип *NOS3* 894(GG) (OR=0,38 95% CI [0,17- 0,84]) были ассоциированы с низким риском развития ПЭ. Генотипы -786(TC) (OR=4,17 95% CI [1,76-9,85]), 894(GT) (OR=2,48 95% CI [1,10-5,63]) и доминантная модель *NOS3* (-786T>C) TC+CC (OR=3. 97 95% CI [1,72-9,14]), а также доминантная модель GT+TT *NOS3* (894G>T) (OR=2,64 95% CI [1,20-5,82]) были связаны с повышенным риском развития ПЭ. Кроме того, аллели -786(C) (OR=2,21 95% CI [1,23-3,98]) и 894(T) (OR=2,14 95% CI [1,15-4,01]) были ассоциированы с повышенным риском ПЭ. Взаимодействие SNP-SNP было статистически значимым (p=0,0003). Кроме того, было показано, что два гаплотипа вариантов гена *NOS3* -786C*894G (OR=3,01 95% CI [1,15-7,86]; p=0,027) и -786C*894T (OR=3,03 95% CI [1,27-7,23]; p=0,014) статистически значимо ассоциированы с повышенным риском ПЭ.

Ключевые слова: преэклампсия; полиморфизм; *eNOS*; *NOS3*.

Для цитирования: Алаяса Н.Н., Шкурат Т.П. Пилотное исследование ассоциации распространенных вариантов *NOS3* с риском развития преэклампсии у российских беременных женщин из Ростовской области. *Медицинская генетика* 2024; 23(6): 11-19.

Автор для корреспонденции: Алаяса Н.Н.; **e-mail:** alayasa.nadeim@gmail.com
Финансирование. Исследование выполнено при финансовой поддержке гранта Министерства науки и высшего образования РФ № FENW-2023-0018.
Конфликт интересов. Авторы заявляют об отсутствии конфликта интересов.
Поступила: 18.06.2024

Pilot association of common NOS3 variants with Preeclampsia risk in Russian pregnant women from Rostov region

Alayasa N.N.[1], Shkurat T.P.[1,2]

1 – Academy of Biology and Biotechnology named after D I Ivanovsky, Southern Federal University,
194/1 Stachki Avenue, Rostov on Don 344090, Russian Federation

2 – Medical Center "Nauka"
23A Zagorskaya, Rostov-on-Don 344034, Russian Federation

Preeclampsia (PE) is a severe medical condition affecting pregnant women, often developing after 20 weeks, and is the most common serious disorder during pregnancy, contributing to 2%-8% of pregnancy-related complications worldwide. The goal of this study is to investigate the association of two genetic variants in *NOS3* (-786T>C; rs2070744) and (894G>T; rs1799983) with the risk of developing PE in Russian pregnant women from Rostov region. The case–control study involved 106 pregnant women (46 pregnant women with PE and 60 healthy controls). Genotyping of the selected *NOS3* genetic variants (-786T>C) and (894G>T) was done using allele specific RT-PCR. Multifactor dimensionality reduction (MDR) was used to assess the relationship between the SNP-SNP interaction and risk of developing PE. In addition, we analyzed the linkage disequilibrium (LD), and haplotypes association for the selected SNPs in the studied population. According to our results, both *NOS3* polymorphisms were statistically significant (P=0.004 and P=0.045, respectively). The *NOS3* -786(TT) genotype (OR=0.25 95% CI [0.11- 0.58]) and the *NOS3* 894(GG) genotype (OR=0.38 95% CI [0.17- 0.84]) were associated with low risk of

developing PE. While -786(TC) genotype (OR=4.17 95% CI [1.76-9.85]), 894(GT) (OR=2.48 95% CI [1.10-5.63]) and dominant model of *NOS3* (-786T>C) TC+CC (OR=3.97 95% CI [1.72-9.14]) as well as the dominant model GT+TT of *NOS3* (894G>T) (OR=2.64 95% CI [1.20-5.82]) were associated with increased risk of PE. In addition, the polymorphism -786(C) allele (OR=2.21 95% CI [1.23-3.98]) and polymorphism 894(T) allele (OR=2.14 95% CI [1.15-4.01]) were both associated with an increased risk of PE. According to the MDR results, the SNP-SNP interaction was statistically significant (P=0.0003). Moreover, two haplotypes of *NOS3* gene variants -786C*894G (OR=3.01 95% CI [1.15-7.86]; P=0.027) and -786C*894T (OR=3.03 95% CI [1.27-7.23]; P=0.014) were shown to be statistically related with an elevated risk of PE.

Keywords: Preeclampsia; polymorphism; *eNOS*; *NOS3*.

For citation: Alayasa N.N., Shkurat T.P. Pilot association of common *NOS3* variants with Preeclampsia risk in Russian pregnant women from Rostov region. *Medical genetics [Medicinskaya genetika]* 2024; 23(6): 11-19.

Corresponding author: Alayasa N.N.; **e-mail:** alayasa.nadeim@gmail.com
Funding. This study was funded by the Ministry of Science and Higher Education of the Russian Federation grant № FENW-2023-0018.
Conflict of Interest. The authors declare no conflict of interest.
Accepted: 18.06.2024

Introduction

Preeclampsia (PE), the predominant hypertension condition during pregnancy, is a significant contributor to both fetal and mother morbidity and death on a global scale [1]. Nevertheless, the extent of this condition in many regions, particularly in low- and middle-income nations, is still not well understood. Estimations indicate that around 10% of women have high blood pressures throughout their pregnancies [2]. Nevertheless, the cellular and molecular processes responsible for its etiopathogenesis are still a subject of discussion. Research conducted on both animals and people has shown that insufficient invasion of trophoblast cells, inadequate remodeling of spiral arteries, reduced blood flow to the uterus and placenta, and consequent oxidative stress contribute to endothelial and immunological dysfunction, which are key factors in the development of PE [3-5].

Endothelial nitric oxide (*eNOS*/*NOS3*), produced by nitric oxide synthase in endothelial cells, plays a crucial role in controlling blood flow and vasomotor tone by suppressing smooth muscle contraction [6]. According to the findings, endothelial NO has the potential to augment blood volume, boost cardiac output, and reduce blood pressure [7]. Hence, it is evidently crucial for the preservation of maternal systemic vasodilation and the decrease in vascular reactivity throughout a normal pregnancy [8]. The development of PE is primarily influenced by the *NOS3* synthase gene [9], which is located in the chromosome 7 (q35-q36) area. This gene plays a crucial role in regulating endothelial dysfunction in PE, among other mediators. According to recent studies, preeclamptic women had a much lower plasma nitric oxide (NO) level than normotensive pregnant women [10,11]. Moreover, the polymorphisms in the *NOS3* gene were associated with the production and availability of nitric oxide. Two variants in the *NOS3* gene have been thoroughly studied: 1) (894G>T; Glu298Asp; rs1799983) variant with substitution of guanine-thymine on exon 7 at position 894, resulting in substitution from glutamate amino acid to aspartate amino acid at position 298. 2) (-786T>C; rs2070744) variant with substitution of thymine-cytosine in the 5'-flanking region of promoter at position -786. Over the past few decades, a number of studies have centered on studying the genetic association between these two *NOS3* variants and PE [12-15]. In Russia, the association between *NOS3* polymorphisms and PE also studied in different regions of Russian federation [16-21]. According to our knowledge, the 894G>T variant of *NOS3* gene is more common in Russians and is associated with PE [22,23]. Nevertheless, the findings have been inconsistent and conflicting across several studies. In this regard, the aim of this study is to investigate the association of these genetic variations with PE risk by conducting case-control research including pregnant women with PE from the Rostov region of Russia. Such studies provide a more precise evaluation of the genetic contribution to the development of PE, since each demographic has its own set of genes and, therefore, varying frequencies of "bad" alleles. By having knowledge of these frequencies, it becomes feasible to determine the composition of the gene pool, carry out further association studies, and pinpoint the most noteworthy genetic risk factors for different diseases within the population.

Methods

Participants

The present study was conducted at Southern Federal University in collaboration with the "Nauka" medical center (Rostov-on-Don, Russian federation) in the period between 2019 and 2022. The genetic analysis was performed using blood samples of 106 pregnant women aged between 25-43 years old, from Rostov-on-Don region, Russia. The women were divided into two groups to study *NOS3* gene

variants: the first group consist of pregnant women with PE (n=46), and the second group consist of pregnant women with normal pregnancy (n=60). The inclusion criteria for the main group include a clinical blood pressure reading of 140/90 mmHg or above and daily proteinuria over 0.3 g/l, both of which must be first seen after 20 weeks of gestation. The exclusion criteria included: Conditions including hypertension or proteinuria during pregnancy, labor, the postpartum period, chronic liver, kidney, or endocrine diseases, abnormalities in the amount of amniotic fluid, disorders of metabolism or bleeding, Rh incompatibility, systemic lupus erythematosus, or any other systemic disease were not eligible for inclusion in the study. All participants' ages, parities, and gestational ages were carefully matched in the study. The mean age in the groups was 35.2 ± 5.1 and 32.4 ± 6.4 years. According to daily monitoring data, the mean systolic BP in the main group was 133.69 ± 3.25, in the control group – 112.37 ± 1.82 mmHg ($p = 0.032$), mean diastolic BP – 83.51 ± 2.71 and 73.92 ± 1.24 mmHg, respectively ($p = 0.027$). Definition of PE based on the American College of Obstetricians and Gynecologist (ACOG) standards. This study was approved by Southern Federal University's Academy of Biology and Biotechnology's Local Ethics Committee. All participants gave informed permission for the study.

SNP selection, DNA extraction and genotyping

Venous blood samples were collected and stored at-20°C. Genomic DNA was isolated from peripheral blood leukocytes using «PROBA-NK» extraction kit («DNA-Technology», Russia) according to the manufacturer's protocol. The isolated DNA was quantitatively assessed using NanoDrop (Thermo Fisher Scientific, USA). Genotyping of *NOS3* (-786T>C) (rs2070744) and *NOS3* (894G>T) (rs1799983) variants was conducted using SNP-express kits with a SYBR green qPCR reagent (Lytech. Co. Ltd., Russian Federation). These variants were selected due to their role in maintaining normal vascular function during pregnancy. The genotyping was performed on QuantStudio™5 Real-Time PCR instrument (Thermo Fisher Scientific, USA). The PCR protocol involved an initial denaturation at 95 °C for 1 min, followed by 35 cycles of denaturation at 93 °C for 10 s, annealing at 60 °C for 10 s, with a final extension step at 72 °C for 20 s. For targeted groups of polymorphisms, fluorescent labels, and results were automatically registered with DT-96 detecting amplifier («DNA Technology», Russia) following the manufacturer's instructions. Amplification plots and melt curve analysis was performed to confirm specificity and accuracy of the used kit after running qPCR.

Statistical analysis

The statistical analysis was conducted using the free online platform "Medical Statistics" based in Kazan, Russia. The platform may be accessed at https://www.medstatistic.ru/. The qualitative characteristics of PE pregnancies were compared to those of the control group using an independent t-test. The findings were reported as the mean value ± the standard deviation. The disparity in those parameters between the study groups was assessed by computing the P value (where a value of <0.05 was deemed statistically significant). The genotypes were assessed for Hardy-Weinberg equilibrium using a Chi-square test using an internet calculator found at https://genecalc.pl/hardy-weinberg-page. Chi-squared tests were used to analyze the disparities in allele frequency and genotype distribution of each examined polymorphism in both PE and control pregnancies. The association with PE risk in both groups was measured by calculating odds ratios (OR) with 95% confidence intervals (CI). A positive association (risk effect) was regarded when odds ratios (OR) were more than 1, whereas a negative association (protective effect) was indicated by OR values less than 1. A P value of less than 0.05 was used as the threshold for statistical significance. In order to examine the relationships between our genetic variations, we used the multifactor dimensionality reduction (MDR) method v3.0.2 software developed by the Computational Genetics Laboratory at the Institute for Quantitative Biomedical Sciences in Dartmouth, NH, USA. Furthermore, to address the issue of a limited number of samples, the Fisher's exact test was chosen to be used in MDR. Given the close proximity of the genes on the same chromosome, we conducted linkage disequilibrium (LD) analysis using SNPStats, which may be accessed at https://www.snpstats.net/start.htm. The Lewontin (D′) coefficient was computed to assess the likelihood of recombination. A value of $D' = 1$ implies a state of full linkage disequilibrium, suggesting that there is no evidence of recombination between the two sites. Conversely, a value of $D' = 0$ shows the absence of any linkage disequilibrium. In order to analyze the connection between haplotypes and the risk of PE, the most often occurring haplotype was chosen as the reference. The SNPStats software was used to determine the odds ratio (OR) and 95% confidence interval (CI) in order to assess the level of relationship between haplotypes and the risk of PE.

Results

Association between NOS3 (-786T>C) and (894G>T) variants with PE

The genotype frequency distributions were in accordance with the Hardy-Weinberg equilibrium (HWE). The

results suggested that for both variants of *NOS3* gene did not deviate from HWE (-786T>C (P=0.371); 894G>T (P=0.862)). The genotype and alleles distribution of *NOS3* (894G>T) and *NOS3* (-786T>C) variants between controls and PE is demonstrated in **table 1.**

According to the results obtained from the both variants (-786T>C) and (894G>T) with susceptibility to PE, we found an association between the two studied variants and PE risk. The heterozygote genotype of both variants -786(TC) and 894(GT) was significant (OR=4.17 95% CI [1.76-9.85]; P=0.003 and OR=2.48 95% CI [1.10-5.63]; P=0.044, respectively). Moreover, the dominant model of both variants (-786TC+CC) and (894GT+TT) was also significant (OR=3.97 95% CI [1.72-9.14]; P<0.001 and OR=2.64 95% CI [1.20-5.82]; P=0.016, respectively). The studying of alleles distribution also showed a significant association of minor alleles of both variants (-786C) and (894T) with PE risk (OR=2.21 95% CI [1.23-3.98]; P=0.008 and OR=2.14 95% CI [1.15-4.01]; P=0.017, respectively).

When analyzing the distribution of genotypes and alleles of the two polymorphic variants (-786T>C) and (894G>T) in the two groups analyzed, statistically significant differences were found: the frequency of dominant (TT) of (-786T>C) and (GG) of (894G>T) genotypes were more frequent in control groups (58.3% and 65%) compered to preeclamptic groups (26.1% and 41.3%), respectively. While the genotype frequency of heterozygote (TC) of (-786T>C) and (GT) of (894G>T) were more frequent in preeclamptic groups (65.2%, 50%, respectively) compared to control groups (35%, 31.7%, respectively), and this suggested to be associated with more risk of developing PE. In addition, the studying of allele frequency of both variants of *NOS3* gene

Таблица 1. Частоты генотипов и аллелей полиморфизмов *NOS3* (-786T>C) и (894G>T)
Table 1. Genotype and Allele frequencies of *NOS3* (-786T>C) and (894G>T) polymorphisms

Genotype/allele	Control n (%) (n = 60)	PE n (%) (n = 46)	**P* value	OR (95 % CI)
NOS3 -786T>C				
TT	35 (58.3%)	12 (26.1%)		**0.25 (0.11- 0.58)**
TC	21 (35.0%)	30 (65.2%)	**P=0.003**	**4.17 (1.76-9.85**)
CC	4 (6.7%)	4 (8.7%)		2.92 (0.63-13.51)
Dominant model TT TC+CC	 35 (58.3%) 25 (41.7%)	 12 (26.1%) 34 (73.9%)	**P<0.001**	 **0.25 (0.11- 0.58)** **3.97 (1.72-9.14)**
Recessive model TT+TC CC	 56 (93.3%) 4 (6.7%)	 42 (91.3%) 4 (8.7%)	P=0.713	 0.75 (0.18-3.17) 2.92 (0.63-13.51)
T	91 (75.8%)	54 (58.7%)	P=0.008	0.45 (0.25-0.82)
C	29 (24.2%)	38 (41.3%)		**2.21 (1.23-3.98)**
NOS3 894G>T				
GG	39 (65.0%)	19 (41.3%)		**0.38 (0.17- 0.84)**
GT	19 (31.7%)	23 (50.0%)	**P=0.044**	2.48 (1.10-5.63)
TT	2 (3.3%)	4 (8.7%)		4.11 (0.69-24.44)
Dominant model GG GT+TT	 39 (65.0%) 21 (35.0%)	 19 (41.3%) 27 (58.7%)	**P=0.016**	 **0.38 (0.17- 0.84)** **2.64 (1.20-5.82)**
Recessive model GG+GT TT	 58 (96.7%) 2 (3.3%)	 42 (91.3%) 4 (8.7%)	P=0.241	 0.37 (0.06-2.07) 4.11 (0.69-24.44)
G	97 (80.8%)	61 (66.3%)	**P=0.017**	0.47 (0.25-0.87)
T	23 (19.2%)	31 (33.7%)		**2.14 (1.15-4.01)**

Примечание: HWE – равновесие Харди-Вайнберга; OR – отношение шансов; CI – доверительный интервал; *p < 0,05 считается статистически значимым.
Note. HWE: Hardy-Weinberg equilibrium; OR: odds ratio; CI: confidence interval; *p < 0.05 is considered statistically significant.

showed that the minor alleles (C) of (-786T>C) variant and (T) of (894G>T) variant were more frequent in preeclamptic groups (41.3% and 33.7%, respectively) compared to control groups (24.2% and 19.2%, respectively) and this also suggested to be associated with increased risk of PE.

Multiple-locus interactions for NOS3 variants

We used the MDR algorithm to study gene-gene interactions between the selected polymorphisms (-786T>C) and (894G>T) with the susceptibility to developing PE in pregnant women from the Rostov area of Russia. The resulted interaction model is statistically significant (P=0.0003, OR = 4.38 CI 95% [1.91-10.74]), with accuracy of 66.9% and cross-validation consistency of 10/10. **Table 2** presents high-risk and low-risk interactive genotypes (-786T>C) and (894G>T), indicating their individual contribution to PE. The combinations of TT/GG between (-786T>C) and (894G>T) polymorphisms showed a protective factor of developing PE, while TC/GT showed a high-risk of developing PE. Furthermore, data-analysis indicated that individuals with heterozygous TC/GT genotype have a significant 1.7-fold higher risk of developing PE. Data presented in **(Fig. 1A)**. According to the Fruchterman-Rheingold graph **(Fig. 1C)**, a high level of redundancy (− 3.12 %) was noticed. The independent effect of *NOS3* (-786T>C) variant was higher than that of *NOS3* (894G>T) (7.82 % and 4.25 %, respectively).

The main effect of each polymorphism is represented by values in nodes. On the other hand, the value between nodes shows the interaction effects. Red color represents the positive entropy (epistasis), whereas blue color (negative entropy) indicates redundancy. Yellow color represents independence.

Linkage disequilibrium (LD) analysis

Pairwise linkage disequilibrium (LD) analysis was conducted between the *NOS3* (-786T>C) and (894G>T) variants due to their chromosomal position on chromosome 7. The SNPStats study revealed that the D' value for the -786T>C and 894G>T variants was 0.515, indicating the absence of linkage disequilibrium (LD).

Haplotypes association with PE risk

A pairwise haplotype association analysis was performed for all investigated variants using SNPStats software. **Table 3** displays the haplotypes results. Two Haplotypes of *NOS3* gene variants -786C*894G (OR=3.01 95% CI [1.15 – 7.86]; P=0.027) and -786C*894T (OR=3.03 95% CI [1.27 – 7.23]; P=0.014) were shown to be statistically related with an elevated risk of PE.

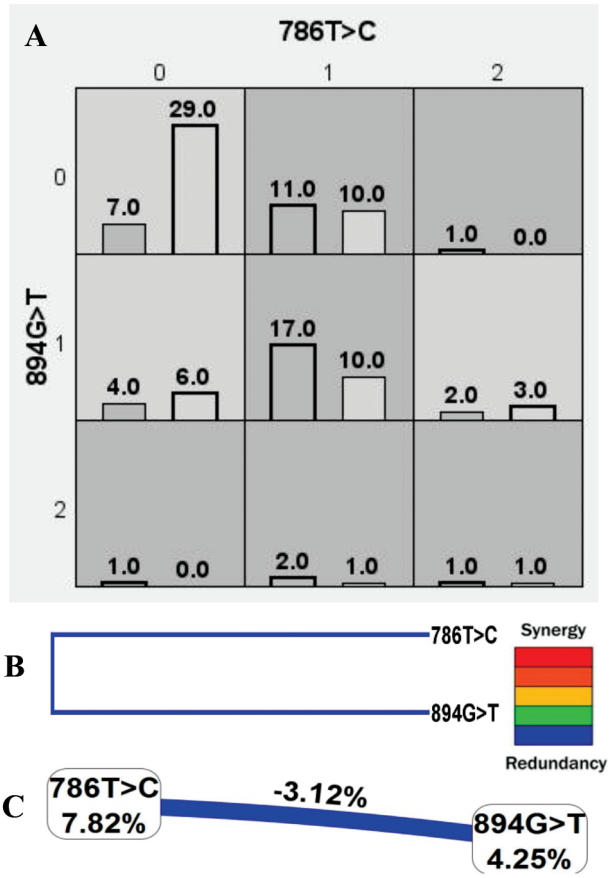

Рис. 1. Модель взаимодействия MDR *NOS3* (-786T>C) и (894G>T). Для каждой комбинации мультилокусных генотипов показано распределение пациентов с ПЭ (левые столбцы) и контрольной группы (правые столбцы). Неизвестная информация представлена белыми ячейками, светло-серые ячейки обозначают «низкий риск», а темно-серые – «высокий риск». (B) Дендрограмма взаимодействия. (C) Схема Фрухтермана – Рейнгольда. Граф взаимодействия с узлами, соединенными друг с другом попарно. Основной эффект каждого полиморфизма представлен значениями в узлах. Значение между узлами показывает эффекты взаимодействия. Красный цвет представляет положительную энтропию (эпистаз), тогда как синий цвет (отрицательная энтропия) указывает на избыточность. Желтый цвет указывает на независимость.

Fig. 1. *NOS3* (-786T>C) and (894G>T) MDR interaction model. For each multilocus genotype combination, the distributions of cases (shown by the left bars) and controls (shown by the right bars) were shown. Unknown information is represented by white cells, while light gray cells indicate «low-risk» and dark gray cells indicate «high-risk». (B) interaction dendrogram. (C) Fruchterman–Rheingold scheme. Graph of interaction with nodes that are connected to one another in a pairwise connection.

Discussion

Endothelial dysfunction, characterized by reduced nitric oxide (NO) production, plays a crucial role in the development of PE [24], a serious pregnancy complication associated with maternal and fetal morbidity and mortality. This dysfunction is mediated by variants in the *NOS3* gene, which located at the q35-q36 of chromosome 7, particularly the -786T>C and 894G>T variants. These variants alter the transcription and activity of *NOS3*, leading to decreased NO production and vascular dysfunction [25-28]. Reduced NO levels contribute to endothelial dysfunction, which in turn promotes the development of PE [29,30]. Epidemiologic and experimental studies have demonstrated a link between endothelial NO production and the occurrence of PE [9]. Therefore, *NOS3* polymorphisms, particularly the -786T>C and 894G>T variants, are considered to be significant risk factors for PE.

In our study, the associations of both variants of *NOS3* gene (-786T>C) and (894G>T) with risk of development PE were investigated. According to the results obtained from -786T>C variant, the recessive homozygote CC was not associated with PE risk, while the dominant homozygote TT has a protective effect against PE development. In addition, both the heterozygote TC genotypes and dominant model TC+CC were significantly associated with high risk of developing PE. Moreover, we found an increased prevalence of the minor allele -786(C) among preeclamptic women compared to control group. The presence of the minor -798C allele in the *NOS3* promoter region has been linked to decreased mRNA expression and lower levels of nitrite/nitrate in the bloodstream. Resulting in the absence of NO. Also, as it is known, the *NOS3* -786T>C variant is found in the promoter region of chromosome 7 at position 786, resulting in a 50% decrease in promoter activity. Consequently, this results in impaired *NOS3* activity, leading to a lower production of NO. As for *NOS3* (894G>T) variant, our results showed that only the dominant model GT+TT and the minor T allele were associated with increased risk of developing PE. While the dominant homozygote GG was associated with lower-risk of developing PE, which means that it has a protective effect against PE risk. Moreover, recessive homozygote TT was not associated with PE risk.

Comparing our results with other Russian studies in other regions, we found that our data are consistent with the results of a number of studies including a study by Fetisova et. found that the heterogenetic genotype 786TC are associated with increasing risk of developing PE in pregnant women from Ivanovo region [16]. Another study by Kovalev et al. on pregnant women from Ekaterinburg region, found a statistically significant association between the 786T>C variant of the *NOS3* gene and PE, with women in the study group having a higher frequency of the genotype containing the C allele (786TC + CC) compared to the control group [17], while we contradicting his findings in another study which found that genotypes containing the 786C allele and 894T allele showing a protective effect against the development of PE [18]. Movreover, a recent meta analysis involved 19 case-control studies [31], were conducted to

Таблица 2. Лучшие прогностические модели межгенного взаимодействия, выявленные при MDR-анализе
Table 2. Best predictive gene–gene interaction models identified by MDR analysis

Locus model	Accuracy	CVC consistency	Sensitivity	Specificity	χ2 (P value)	OR (95% CI)
NOS3 -786(TC) + 894(GT)	66.9%	10/10	71.7%	63.3%	12.82 (p = 0.0003)	4.38 (1.91-10.74)

Таблица 3. Анализ ассоциации гаплотипов вариантов NOS3 (-786T>C) и (894G>T) с риском ПЭ
Table 3. Haplotype analysis on association of NOS3 (-786T>C) and (894G>T) variants with PE risk

Haplotype (Alleles)	Frequencies			P value	OR (95%CI)
	Total	Control	PE		
NOS3 -786T*894G	0.5995	0.6935	0.4626	Ref	1.00
NOS3 -786C*894G	0.1458	0.1148	0.2004	**0.027***	**3.01 (1.15 – 7.86)**
NOS3 -786T*894T	0.0845	0.0648	0.1243	0.076	2.84 (0.91 – 8.89)
NOS3 -786C*894T	0.1702	0.1268	0.2126	**0.014***	**3.03 (1.27 – 7.23)**

Примечание. OR – отношение шансов, CI – доверительный интервал, * Пповышенный риск ПЭ (p <0,05).
Note. OR = Odds Ratio, CI = Confidence Interval, * Increased PE risk (p < 0.05).

evaluate the association between *NOS3* -786T>C variant and PE risk, revealed that 12 studies of which have shown non-significant association between the -786T>C variant and PE risk. While 7 studies have shown the significant association. For the 894G>T variant, our findings are consistent with a previous study by Radkov et al., which revealed that the 894G>T variant, has been associated with PE in pregnant women from Tver region. The presence of the 894T allele increases the risk of PE, while the 894G allele or G/G genotype is associated with a reduced risk of gestational hypertension [19]. However, our findings differ from those of some previous studies that found significant associations between the 894G>T (TT) homozygote and PE [12,15,32]. These discrepancies may be explained by differences in participant populations, sample sizes, and study designs. The identification of *NOS3* polymorphisms as a potential risk factor for PE has important implications for diagnostic and treatment strategies. Further research is needed to confirm these findings and to develop targeted interventions for individuals at risk for PE based on their genotype.

The performed MDR analysis confirmed the results of genotype association, by showing that the carriers of *NOS3* -786TT * *NOS3* 894GG had a significantly lower risk of developing PE. Furthermore, *NOS3* -786TC * *NOS3* 894GT interaction was associated with a higher risk of developing PE, in accordance with genotyping data presented in Table 1.

Haplotype association analysis indicated that -786C * 894G and -786C * 894T haplotypes were significantly associated with the risk of developing PE. This suggests that the studied gene variations could be risk factors for PE developing.

One limitation of our study is its relatively small sample size, which may limit the generalizability of our findings. Additionally, our study was conducted on a single population, which may limit the ability to generalize these results to other populations. Other factors, such as environmental and lifestyle factors, could also have influenced the results. Future studies with larger sample sizes and more diverse populations are necessary to confirm our findings and further explore the relationship between *NOS3* polymorphisms and PE.

Conclusion

We concluded that both of *NOS3* variants (-786T>C) and (894G>T) were significantly associated with increased risk of developing PE in Russian pregnant women from Rostov region. The findings will enhance our understanding of genetic aspects of PE, helping in the development of new prenatal prognostic markers, improving diagnosis, prognosis, and prevention, but further investigations with larger sample size and different ethnic populations are needed to understand the mechanisms and interaction between genes with PE susceptibility.

Литература

1. Akaba G.O., Anyang U.I., Ekele B.A. Prevalence and materno-fetal outcomes of preeclampsia/eclampsia amongst pregnant women at a teaching hospital in north-central Nigeria: a retrospective cross-sectional study. Clinical Hypertension. 2021 Dec;27:1-0. doi: 10.1186/S40885-021-00178-Y/TABLES/5
2. Bello N.A., Woolley J.J., Cleary K.L., Falzon L., Alpert B.S., Oparil S., Cutter G., Wapner R., Muntner P., Tita A.T., Shimbo D. Accuracy of blood pressure measurement devices in pregnancy: a systematic review of validation studies. Hypertension. 2018 Feb;71(2):326-35. doi: 10.1161/HYPERTENSIONAHA.117.10295/-/DC1
3. Mendes S., Timóteo-Ferreira F., Almeida H., Silva E. New insights into the process of placentation and the role of oxidative uterine microenvironment. Oxidative medicine and cellular longevity. 2019 Jun 25;2019. doi: 10.1155/2019/9174521
4. San Juan-Reyes S., Gómez-Oliván L.M., Islas-Flores H., Dublán-García O. Oxidative stress in pregnancy complicated by preeclampsia. Archives of biochemistry and biophysics. 2020 Mar 15;681:108255. doi: 10.1016/J.ABB.2020.108255
5. Wu F., Tian F.J., Lin Y., Xu W.M. Oxidative stress: placenta function and dysfunction. American journal of reproductive immunology. 2016 Oct;76(4):258-71. doi: 10.1111/AJI.12454
6. Ally A., Powell I., Ally M.M., Chaitoff K., Nauli S.M. Role of neuronal nitric oxide synthase on cardiovascular functions in physiological and pathophysiological states. Nitric Oxide. 2020 Sep 1;102:52-73. doi: 10.1016/J.NIOX.2020.06.004
7. Tran N., Garcia T., Aniqa M., Ali S., Ally A., Nauli S.M. Endothelial nitric oxide synthase (eNOS) and the cardiovascular system: in physiology and in disease states. American journal of biomedical science & research. 2022;15(2):153. doi: 10.34297/ajbsr.2022.15.002087
8. Spradley F.T. Sympathetic nervous system control of vascular function and blood pressure during pregnancy and preeclampsia. Journal of hypertension. 2019 Mar 1;37(3):476-87. doi: 10.1097/HJH.0000000000001901
9. Ssengonzi R., Wang Y., Maeda-Smithies N., Li F. Endothelial Nitric Oxide synthase (eNOS) in Preeclampsia: An Update. Journal of pregnancy and child health. 2024;6. doi: 10.29011/JPCH-121.100021
10. Darkwa E.O., Djagbletey R., Essuman R., Sottie D., Dankwah G.B., Aryee G. Nitric oxide and pre-eclampsia: a comparative study in Ghana. Open Access Macedonian Journal of Medical Sciences. 2018 Jun 6;6(6):1023. doi: 10.3889/OAMJMS.2018.252
11. Deniz R., Baykus Y., Ustebay S., Ugur K., Yavuzkir Ş., Aydin S. Evaluation of elabela, apelin and nitric oxide findings in maternal blood of normal pregnant women, pregnant women with pre-eclampsia, severe pre-eclampsia and umbilical arteries and venules of newborns. Journal of Obstetrics and Gynaecology. 2019 Oct 3;39(7):907-12. doi: 10.1080/01443615.2019.1572727
12. Abbasi H., Dastgheib S.A., Hadadan A., Karimi-Zarchi M., Javaheri A., Meibodi B., Zanbagh L., Tabatabaei R.S., Neamatzadeh H. Association of endothelial nitric oxide synthase 894G> T polymorphism with preeclampsia risk: a systematic review and meta-analysis

based on 35 studies. Fetal and pediatric pathology. 2021 Oct 6;40(5):455-70. doi: 10.1080/15513815.2019.1710880
13. Kurbanov B. Study of C-786T polymorphism in the *NOS3* gene in the development of preeclampsia. Journal of Hypertension. 2023 Jun 1;41(Suppl 3):e286. doi: 10.1097/01.HJH.0000941980.64629.E2
14. Sljivancanin Jakovljevic T., Kontic-Vucinic O., Nikolic N., Carkic J., Stamenkovic J., Soldatovic I., Milasin J. Association Between Endothelial Nitric Oxide Synthase (eNOS)− 786 T/C and 27-bp VNTR 4b/a Polymorphisms and Preeclampsia Development. Reproductive Sciences. 2021 Dec;28(12):3529-39. doi: 10.1007/S43032-021-00632-0/METRICS
15. Zeng F., Zhu S., Wong M.C., Yang Z., Tang J., Li K., Su X. Associations between nitric oxide synthase 3 gene polymorphisms and preeclampsia risk: a meta-analysis. Scientific reports. 2016 Mar 21;6(1):23407. doi: 10.1038/srep23407
16. Фетисова И.Н., Панова И.А., Малышкина А.И., Рокотянская Е.А., Ратникова С.Ю., Смирнова Е.В., Фетисов Н.С., Назарова А.О. Генетические аспекты преэклампсии. Современные проблемы науки и образования. 2014(6):1040-.1040.
17. Ковалев В.В., Третьякова Т.Б., Дерябина Е.Г., Путилова Т.А., Мазуров, А.Д. Гипертензивные осложнения беременности у пациенток с гестационным сахарным диабетом на основе анализа полиморфизмов генов-регуляторов артериального давления. Уральский медицинский журнал. 2012; 98(6):49-53.
18. Ковалев В.В., Путилова Т.А., Третьякова Т.Б., Дерябина Е.Г., Мазуров. А.Д. Генетические предикторы преэклампсии у пациенток с гестационным сахарным диабетом. Уральский медицинский журнал. 2012; 103(11): 49-53.
19. Радьков О.В., Заварин В.В., Калинкин М.Н. Анализ ассоциации полиморфизма вазоактивных генов с преэклампсией. Acta Biomedica Scientifica. 2011(5):109-12.
20. Глотов А.С., Вашукова Е.С., Насыхова Ю.А., Глотов О.С., Мазур А.М., Курилов Р.В., Пехов В.М., Храмеева Е.Е., Иващенко Т.Э., Баранов В.С. Исследование популяционных частот полиморфизма генов, ассоциированныхс гестозом. Экологическая генетика. 2013;11(1):91-100.
21. Буштырева И.О., Курочка М.П. Роль полиморфизма гена эндотелиальной синтазы в развитии гестоза. Кубанский научный медицинский вестник. 2009(7):26-9.
22. Малышева О.В., Мозговая Е.В., Демин Г.С. и др. Ассоциация полиморфных аллелей генов ACE и eNOS с развитием гестозов. Медицинская генетика. 2003; 2:78–82
23. Демин Г.С. Анализ ассоциации полиморфизма генов «сосудистой системы» и «эндотелиальной дисфункции» с развитием преэклампсии : автореф. дис. ... канд. биол. наук. – СПб., 2008. – 25 с.
24. Theofilis P., Sagris M., Oikonomou E., Antonopoulos A.S., Siasos G., Tsioufis C., Tousoulis D. Inflammatory mechanisms contributing to endothelial dysfunction. Biomedicines. 2021 Jul 6;9(7):781. doi: 10.3390/BIOMEDICINES9070781
25. Gamil S., Erdmann J., Abdalrahman I.B., Mohamed A.O. Association of NOS3 gene polymorphisms with essential hypertension in Sudanese patients: a case control study. BMC medical genetics. 2017 Dec;18:1-9. doi: 10.1186/S12881-017-0491-7/TABLES/5
26. Joshaghani H.R., Salehi A., Samadian E., Gharaei R., Ahmadi A.R. Association between NOS3 G894T, T-786C and 4a/4b Variants and coronary artery diseases in iranian population. Iranian journal of public health. 2018 Dec;47(12):1891.
27. Oliveira-Paula G.H., Lacchini R., Tanus-Santos J.E. Endothelial nitric oxide synthase: From biochemistry and gene structure to clinical implications of NOS3 polymorphisms. Gene. 2016 Jan 10;575(2):584-99. doi: 10.1016/J.GENE.2015.09.061
28. Zhao G.L., Li Q.J., Lu H.Y. Association between NOS3 genetic variants and coronary artery disease in the Han population. Genet Mol Res. 2016 Jun 3;15(2):1-4. doi: 10.4238/gmr.15028044
29. Boeldt D.S., Bird I.M. Vascular adaptation in pregnancy and endothelial dysfunction in preeclampsia. The Journal of Endocrinology. 2016 Oct 11;232(1):R27-44. doi: 10.1530/JOE-16-0340
30. Osol G., Ko N.L., Mandalà M. Altered endothelial nitric oxide signaling as a paradigm for maternal vascular maladaptation in preeclampsia. Current hypertension reports. 2017 Oct;19:1-2. doi: 10.1007/S11906-017-0774-6/METRICS
31. Tesfa E., Munshea A., Nibret E., Tebeje Gizaw S. Association of endothelial nitric oxide synthase gene variants in pre-eclampsia: an updated systematic review and meta-analysis. The Journal of Maternal-Fetal & Neonatal Medicine. 2023 Dec 15;36(2):2290918. doi: 10.1080/14767058.2023.2290918
32. Qi H.P., Fraser W.D., Luo Z.C., Julien P., Audibert F., Wei S.Q. Endothelial nitric oxide synthase gene polymorphisms and risk of preeclampsia. American journal of perinatology. 2013 Jan 17:795-804. doi: 10.1055/S-0032-1333406

References

1. Akaba G.O., Anyang U.I., Ekele B.A. Prevalence and materno-fetal outcomes of preeclampsia/eclampsia amongst pregnant women at a teaching hospital in north-central Nigeria: a retrospective cross-sectional study. Clinical Hypertension. 2021 Dec;27:1-0. doi: 10.1186/S40885-021-00178-Y/TABLES/5
2. Bello N.A., Woolley J.J., Cleary K.L., Falzon L., Alpert B.S., Oparil S., Cutter G., Wapner R., Muntner P., Tita A.T., Shimbo D. Accuracy of blood pressure measurement devices in pregnancy: a systematic review of validation studies. Hypertension. 2018 Feb;71(2):326-35. doi: 10.1161/HYPERTENSIONAHA.117.10295/-/DC1
3. Mendes S., Timóteo-Ferreira F., Almeida H., Silva E. New insights into the process of placentation and the role of oxidative uterine microenvironment. Oxidative medicine and cellular longevity. 2019 Jun 25;2019. doi: 10.1155/2019/9174521
4. San Juan-Reyes S., Gómez-Oliván L.M., Islas-Flores H., Dublán-García O. Oxidative stress in pregnancy complicated by preeclampsia. Archives of biochemistry and biophysics. 2020 Mar 15;681:108255. doi: 10.1016/J.ABB.2020.108255
5. Wu F., Tian F.J., Lin Y., Xu W.M. Oxidative stress: placenta function and dysfunction. American journal of reproductive immunology. 2016 Oct;76(4):258-71. doi: 10.1111/AJI.12454
6. Ally A., Powell I., Ally M.M., Chaitoff K., Nauli S.M. Role of neuronal nitric oxide synthase on cardiovascular functions in physiological and pathophysiological states. Nitric Oxide. 2020 Sep 1;102:52-73. doi: 10.1016/J.NIOX.2020.06.004
7. Tran N., Garcia T., Aniqa M., Ali S., Ally A., Nauli S.M. Endothelial nitric oxide synthase (eNOS) and the cardiovascular system: in physiology and in disease states. American journal of biomedical science & research. 2022;15(2):153. doi: 10.34297/ajbsr.2022.15.002087
8. Spradley F.T. Sympathetic nervous system control of vascular function and blood pressure during pregnancy and preeclampsia. Journal of hypertension. 2019 Mar 1;37(3):476-87. doi: 10.1097/HJH.0000000000001901
9. Ssengonzi R., Wang Y., Maeda-Smithies N., Li F. Endothelial Nitric Oxide synthase (eNOS) in Preeclampsia: An Update. Journal of pregnancy and child health. 2024;6. doi: 10.29011/JPCH-121.100021
10. Darkwa E.O., Djagbletey R., Essuman R., Sottie D., Dankwah G.B., Aryee G. Nitric oxide and pre-eclampsia: a comparative study in

Ghana. Open Access Macedonian Journal of Medical Sciences. 2018 Jun 6;6(6):1023. doi: 10.3889/OAMJMS.2018.252
11. Deniz R., Baykus Y., Ustebay S., Ugur K., Yavuzkir Ş., Aydin S. Evaluation of elabela, apelin and nitric oxide findings in maternal blood of normal pregnant women, pregnant women with pre-eclampsia, severe pre-eclampsia and umbilical arteries and venules of newborns. Journal of Obstetrics and Gynaecology. 2019 Oct 3;39(7):907-12. doi: 10.1080/01443615.2019.1572727
12. Abbasi H., Dastgheib S.A., Hadadan A., Karimi-Zarchi M., Javaheri A., Meibodi B., Zanbagh L., Tabatabaei R.S., Neamatzadeh H. Association of endothelial nitric oxide synthase 894G> T polymorphism with preeclampsia risk: a systematic review and meta-analysis based on 35 studies. Fetal and pediatric pathology. 2021 Oct 6;40(5):455-70. doi: 10.1080/15513815.2019.1710880
13. Kurbanov B. Study of C-786T polymorphism in the NOS3 gene in the development of preeclampsia. Journal of Hypertension. 2023 Jun 1;41(Suppl 3):e286. doi: 10.1097/01.HJH.0000941980.64629.E2
14. Sljivancanin Jakovljevic T., Kontic-Vucinic O., Nikolic N., Carkic J., Stamenkovic J., Soldatovic I., Milasin J. Association Between Endothelial Nitric Oxide Synthase (eNOS)− 786 T/C and 27-bp VNTR 4b/a Polymorphisms and Preeclampsia Development. Reproductive Sciences. 2021 Dec;28(12):3529-39. doi: 10.1007/S43032-021-00632-0/METRICS
15. Zeng F., Zhu S., Wong M.C., Yang Z., Tang J., Li K., Su X. Associations between nitric oxide synthase 3 gene polymorphisms and preeclampsia risk: a meta-analysis. Scientific reports. 2016 Mar 21;6(1):23407. doi: 10.1038/srep23407
16. Fetisova I.N., Panova I.A., Malyshkina A.I., Rokotyanskaya E.A., Ratnikova S.Yu., Smirnova E.V., Fetisov N.S., Nazarova A.O. Geneticheskiye aspekty preeklampsii. [Genetic aspects of preeclampsia]. Sovremennyye problemy nauki i obrazovaniya. [Modern problems of science and education]. 2014(6):1040-.1040. (In Russ.)
17. Kovalev V.V., Tretyakova T.B., Deryabina E.G., Putilova T.A., Mazurov, A.D. Gipertenzivnyye oslozhneniya beremennosti u patsiyentok s gestatsionnym sakharnym diabetom na osnove analiza polimorfizmov genov-regulyatorov arterial'nogo davleniya [The hypertension disorders in women with diabetes mellitus association of genes polymorphisms]. Ural'skiy meditsinskiy zhurnal [Ural Medical Journal]. 2012; 98(6):49-53. (In Russ.)
18. Kovalev V.V., Putilova T.A., Tretyakova T.B., Deryabina E.G., Mazurov, A.D. Geneticheskiye prediktory preeklampsii u patsiyentok s gestatsionnym sakharnym diabetom [Genetic predictors of preeclampsia in women with gestational diabetes mellitus]. Ural'skiy meditsinskiy zhurnal [Ural Medical Journal]. 2012; 103(11): 49-53. (In Russ.)
19. Radkov O.V., Zavarin V.V., Kalinkin M.N. Analiz assotsiatsii polimorfizma vazoaktivnykh genov s preeklampsiyey [Analysis of the association of vasoactive gene polymorphisms with preeclampsia]. Acta Biomedica Scientifica. 2011(5):109-12. (In Russ.)
20. Glotov A.S., Vashukova Y.S., Nasykhova Y.A., Glotov O.S., Mazur A.M., Kurilov R.V., Pekhov V.M., Khrameyeva Y.Y., Ivashchenko T.E., Baranov V.S. Issledovaniye populyatsionnykh chastot polimorfizma genov, assotsiirovannykhs gestozom [Study of population frequencies of genes polymorphism associated with preeclampsia-associated genes polymorphism]. Ekologicheskaya genetika [Ecological genetics]. 2013;11(1): 91-100. doi: 10.17816/ecogen11191-100 (In Russ.)
21. Bushtyreva I.O., Kurochka M.P. Rol' polimorfizma gena endotelial'noy sintazy v razvitii gestoza [The role of gene of endothelial NO-synthase polymorphism in preeclampsia development]. Kubanskiy nauchnyy meditsinskiy vestnik [Kuban Scientific Medical Bulletin]. 2009(7):26-9. (In Russ.)
22. Malysheva O.V., Mozgovaya E.V., Demin G.S. et al. Assotsiatsiya polimorfnykh alleley genov ASE i eNOS s razvitiyem gestozov [Association of polymorphic alleles of the ACE and eNOS genes with the development of preeclampsia]. Meditsinskaya genetika [Medical genetics]. 2003; 2: 78–82. (In Russ.)
23. Demin G.S. Analiz assotsiatsii polimorfizma genov «sosudistoy sistemy» i «endotelial'noy disfunktsii» s razvitiyem preeklampsii : avtoref. dis. ... kand. biol. Nauk [Analysis of the association of gene polymorphisms of the "vascular system" and "endothelial dysfunction" with the development of preeclampsia: abstract. dis. ...cand. biol. Sci.]. – SPb., 2008. – 25 p. (In Russ.)
24. Theofilis P., Sagris M., Oikonomou E., Antonopoulos A.S., Siasos G., Tsioufis C., Tousoulis D. Inflammatory mechanisms contributing to endothelial dysfunction. Biomedicines. 2021 Jul 6;9(7):781. doi: 10.3390/BIOMEDICINES9070781
25. Gamil S., Erdmann J., Abdalrahman I.B., Mohamed A.O. Association of NOS3 gene polymorphisms with essential hypertension in Sudanese patients: a case control study. BMC medical genetics. 2017 Dec;18:1-9. doi: 10.1186/S12881-017-0491-7/TABLES/5
26. Joshaghani H.R., Salehi A., Samadian E., Gharaei R., Ahmadi A.R. Association between NOS3 G894T, T-786C and 4a/4b Variants and coronary artery diseases in iranian population. Iranian journal of public health. 2018 Dec;47(12):1891.
27. Oliveira-Paula G.H., Lacchini R., Tanus-Santos J.E. Endothelial nitric oxide synthase: From biochemistry and gene structure to clinical implications of NOS3 polymorphisms. Gene. 2016 Jan 10;575(2):584-99. doi: 10.1016/J.GENE.2015.09.061
28. Zhao G.L., Li Q.J., Lu H.Y. Association between NOS3 genetic variants and coronary artery disease in the Han population. Genet Mol Res. 2016 Jun 3;15(2):1-4. doi: 10.4238/gmr.15028044
29. Boeldt D.S., Bird I.M. Vascular adaptation in pregnancy and endothelial dysfunction in preeclampsia. The Journal of Endocrinology. 2016 Oct 11;232(1):R27-44. doi: 10.1530/JOE-16-0340
30. Osol G., Ko N.L., Mandalà M. Altered endothelial nitric oxide signaling as a paradigm for maternal vascular maladaptation in preeclampsia. Current hypertension reports. 2017 Oct;19:1-2. doi: 10.1007/S11906-017-0774-6/METRICS
31. Tesfa E., Munshea A., Nibret E., Tebeje Gizaw S. Association of endothelial nitric oxide synthase gene variants in pre-eclampsia: an updated systematic review and meta-analysis. The Journal of Maternal-Fetal & Neonatal Medicine. 2023 Dec 15;36(2):2290918. doi: 10.1080/14767058.2023.2290918
32. Qi H.P., Fraser W.D., Luo Z.C., Julien P., Audibert F., Wei S.Q. Endothelial nitric oxide synthase gene polymorphisms and risk of preeclampsia. American journal of perinatology. 2013 Jan 17:795-804. doi: 10.1055/S-0032-1333406