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Pokhylko V.I., Cherniavska Y.I., Yakovenko O.V., Davydenko A.V., Rossokha Z.I.[1]
Poltava State Medical University, Poltava
[1]Expert and Analytical Center of Molecular Genetics, P.L. Shupyk National Health Care University of Ukraine, Kyiv

INFLUENCE OF VDR (RS731236) AND NOS3 (RS1143634) GENE VARIANTS ON CARDIOMETABOLIC PROFILE INDICATORS IN NEWBORNS

e-mail: v.i.pokhylko@gmail.com

The study of genetic factors that influence the development of hypoxic-ischemic lesions in newborns is a relevant area of modern neonatology. The study analyzed the associations between NOS3 and VDR gene polymorphisms and clinical and cardiohemodynamic indicators in newborns, taking into account the mother's condition. Multivariate regression analysis showed that the CC VDR genotype was associated with a decrease in blood pressure (β=−8.17; p=0.016). The most significant predictor of blood pressure was venous blood pH (OR=0.61, p=0.004). The TT genotype of the NOS3 gene was associated with a significant increase in the risk of hypotension in newborns with hypoxic-ischemic CNS injury (OR=11.7; p=0.043). The identified trends in changes in cardiac function and vascular tone suggest a possible role for genetic mechanisms in shaping individual responses to hypoxia. The results obtained expand understanding of the pathogenesis of neonatal disorders and can inform risk stratification and treatment personalization.

Key words: VDR, NOS3, newborns, cardiometabolic profile, Hypoxic-ischemic central nervous system injury, gene variants, blood pressure.

Похилько В.І., Чернявська Ю.І., Яковенко О.В., Давиденко А.В., Россоха З.І.

ВПЛИВ ВАРІАНТІВ ГЕНІВ VDR (RS731236) ТА NOS3 (RS1143634) НА ПОКАЗНИКИ КАРДІОМЕТАБОЛІЧНОГО ПРОФІЛЮ У НОВОНАРОДЖЕНИХ

Дослідження генетичних факторів, що впливають на розвиток гіпоксично-ішемічних уражень у новонароджених, є актуальним напрямком сучасної неонатології. У роботі проаналізовано асоціації варіантів генів NOS3 та VDR з клінічними та кардіогемодинамічними показниками у новонароджених, з урахуванням стану здоров'я матерів. При багатофакторному регресійному аналізі встановлено, що генотип CC VDR асоціювався зі зниженням артеріального тиску (β=−8,17; p=0,016). Найбільш значущим предиктором рівня артеріального тиску був pH венозної крові (ВШ=0,61, p=0,004). Генотип TT гена NOS3 асоціювався зі значним підвищенням ризику гіпотензії у новонароджених з ГІУ ЦНС (ВШ=11,7; p=0,043), Виявлені тенденції до змін показників серцевої функції та судинного тонусу свідчать про генетичну детермінованість у формуванні індивідуальної реакції на гіпоксію. Отримані результати розширюють уявлення про патогенез неонатальних порушень та можуть бути використані для стратифікації ризику і персоналізації лікування.

Ключові слова: VDR, NOS3, новонароджені, кардіометаболічний профіль, гіпоксично-ішемічне ураження центральної нервової системи, варіанти генів, артеріальний тиск.

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Many experimental studies have been conducted to explain the phenotypic consequences of intrauterine changes leading to the development of metabolic syndrome, obesity, diabetes, hyperinsulinemia, hypertension, and cardiovascular disease in adulthood [15]. Elucidation of the mechanisms involved in this process in newborns and young children has become particularly relevant. Vitamin D is actively involved in the renin-angiotensin-aldosterone system and upregulation of endothelial nitric oxide synthase, which produces nitric oxide (NO), leading to increased arteriolar tone and endothelial dysfunction [8]. VDR acts as a receptor for the active vitamin D metabolite 1α,25-dihydroxyvitamin D3 and mediates its biological functions. Single-nucleotide variants located in the VDR gene affect its expression and the structure of the synthesized protein product, the vitamin D receptor (VDR) [9]. The most clinically characterized and known variants are: rs10735810 (FokI), rs1544410 (BsmI), rs7975232 (ApaI), and rs731236 (TaqI) [12, 13]. VDR gene polymorphisms may be associated with cardiovascular diseases due to their presence in cardiomyocytes, vascular smooth endothelial cells, platelets, and various immune cells [1]. Vitamin D also reduces inflammation and oxidative stress associated with cardiovascular diseases [3]. Observational studies have shown an inverse correlation between vitamin D levels and cardiovascular events [9], linking deficiency to hypertension, cardiac hypertrophy, arterial stiffness, and myocardial infarction [7]. Hypovitaminosis D has been reported to be associated with increased risk of microbial infections and sepsis in children and infants [10]. Alterations in the structure or expression of the VDR gene result in downregulation of target genes involved in calcium-phosphorus metabolism, immune response, and cardiometabolic processes,

including regulation of insulin sensitivity, lipid profile, and endothelial function [13, 14].

The impact and interaction of genetic markers associated with vitamin D deficiency and endothelial dysfunction on cardiometabolic changes in newborns is still unknown. Severe vitamin D deficiency is known to be a reversible cause of dilated cardiomyopathy in infants and cardiogenic shock. Hypocalcemia due to vitamin D deficiency can provoke cardiac arrhythmias, seizures, and sudden cardiac arrest [5]. However, the question of the genetic determination of cardiometabolic changes in newborns at risk of cardiovascular diseases, namely those born to mothers with metabolic syndrome, is debatable.

The NOS3 gene, which encodes endothelial NO synthase, plays a key role in regulating vascular tone by producing nitric oxide, which promotes vasodilation and helps maintain adequate blood pressure. tissue perfusion. The GT polymorphism of the NOS3 gene (rs1143634) is associated with variations in endothelial NO synthase expression and nitric oxide production. The presence of the T allele may be associated with reduced NO synthesis, increased cardiovascular disease risk, and endothelial dysfunction, thereby increasing the risk of cardiovascular and metabolic disorders [11]. In newborns, especially with hypoxic-ischemic injury, impaired NOS3 function is associated with hemodynamic instability, including a tendency to hypotension and impaired microcirculation. Studies also indicate that genetic variants of NOS3 may affect the adaptive mechanisms of the cardiovascular system in the early neonatal period and modulate the risk of cardiometabolic complications [6]

The purpose of the study was to examine the effects of genetic variants in the VDR (TaqI) and NOS3 (rs1143634) genes on cardiometabolic features in newborns with hypoxic-ischemic CNS injury whose mothers had metabolic syndrome**.**

Materials and methods. A retrospective cohort study was conducted. The main group included 45 newborns with hypoxic-ischemic CNS injury, born to mothers with metabolic syndrome; the control group consisted of 20 relatively healthy newborns. All of them were treated during December 2023 – December 2024 at the Poltava Regional Clinical Hospital named after M.V. Sklifosovsky. The criteria for including newborns in the study were: a diagnosis of metabolic syndrome in the mother; the presence of clinical signs and laboratory-confirmed criteria for hypoxic-ischemic CNS injury, in accordance with the regulatory documents of the health care system; and completeness of medical documentation of the demographic, anamnestic, and clinical characteristics of each child. The exclusion criteria were the presence of congenital anomalies in the child, genetic, chromosomal, and other severe decompensated diseases, and the refusal of the parents to participate in the study. The information base of the study was formed from specially developed questionnaires, which were filled out by copying data from primary documents: “History of childbirth” (file No. 96/o), “Exchange card of the maternity hospital, maternity ward of the hospital” (file No. 113/o), “Newborn development card” (file No. 097/o). According to the decision of the Bioethics Commission of Poltava State Medical University No. 217 dated 06/12/2023, the materials of the scientific study meet the ethical requirements of humane treatment of patients, defined by the Tokyo Declaration of the World Medical Association, the Helsinki International Recommendations, the Universal Declaration of Human Rights, the Council of Europe Convention on Human Rights and Biomedicine, as well as the current legislation of Ukraine, orders of the Ministry of Health and the provisions of the Code of Ethics of a Doctor of Ukraine. All parents gave their consent to the examination.

Characteristics of the clinical group. The study included newborns with gestational ages of 32.8±4.49 weeks in the main group and 39.1±1.04 weeks in the control group, and weights of 2164.9±154.3 g and 3407.6±105.5 g, respectively. The distribution in the groups by gender had no statistically significant difference: among infants with hypoxic-ischemic CNS injury, 62.2 % were boys, and among healthy children, 71.4 %.

Genetic studies. DNA for molecular genetic studies was extracted from buccal epithelial cells. Sample collection was carried out using disposable sterile brushes, after which the material was stored and transported in tubes containing the preservative “DNA/RNA Shield” (Zymo Research, USA). DNA isolation was carried out using a commercial kit “Quick-DNA Mini Prep Plus Kit” (Zymo Research, USA). Variants in the NOS3 (G894T, rs1799983) and VDR (TaqI, rs731236**)** genes were identified by polymerase chain reaction followed by restriction fragment length polymorphism analysis.

Statistical analysis was performed using Stata version 14.0. Categorical variables were presented as absolute numbers and percentages. Continuous variables were expressed as mean ± standard deviation or median (interquartile range), depending on the distribution. The associations between VDR CC and NOS3 TT genotypes and neonatal outcomes were assessed using logistic regression models adjusted for maternal metabolic syndrome (group variable). Odds ratios (ORs) with 95 % confidence intervals (CIs) were calculated. A p-value < 0.05 was considered statistically significant. Due to the small number of events for some outcomes (cardiac massage and adrenaline administration), the estimates were interpreted with caution.

Results of the study and their discussion. Investigating the distribution of genetic variants in neonates is essential for understanding individual

susceptibility to hypoxic-ischemic CNS injury. Identification of potential genetic contributors may improve early risk stratification and support the development of personalized management strategies. Moreover, elucidating the role of NOS3 and VDR polymorphisms may provide insights into the underlying pathophysiological mechanisms, particularly those related to vascular regulation and tissue perfusion. Therefore, at the beginning of the study, the genotype and allele frequencies among newborns with hypoxic-ischemic CNS injury and healthy children were determined (Table 1).

Table 1

Frequency of genotypes and alleles distribution for the NOS3 and VDR genes in the study groups

Gene (variant)	Genotype/ allele	Neonates without hypoxic-ischemic CNS damage (n=20)	Neonates with hypoxic-ischemic CNS damage (n=45)	p; OR (95 % CI)
NOS3 (G894T, rs1799983)	GG	12(63.2 %)	17 (37.8 %)	0.171
	TG	6 (31.6 %)	25 (55.6 %)	
	TT	1 (5.3 %)	3 (6.7 %)	
	G	30 (0.79)	59 (0.66)	0.13; 1.97 (0.82–4.73)
	T	8 (0.21)	31(0.34)	0.729
VDR (Taq1, rs731236)	TT	7 (35.0 %)	20(44.4 %)	
	TC	11(55.0 %)	19 (42.2 %)	
	CC	2 (10.0 %)	6 (13.3 %)	
	T	25 (0.63)	59 (0.66)	0.89; 1.06 (0.51–2.20)
	C	15(0.37)	31(0.34)	

The results did not reveal statistically significant differences in the distribution of NOS3 genotypes and alleles between the main and control groups. At the same time, there was a tendency toward increased T-allele frequency in newborns with pathology (0.34 versus 0.21 in the control), which was accompanied by an increased risk of disease (OR=1.97), but without achieving statistical significance (p=0.13). This may indicate a potential role of this polymorphism in the development of vascular dysfunction, but requires confirmation in larger sample sizes. After all, the NOS3 gene encodes endothelial NO synthase, which plays a key role in maintaining vasodilation and adequate tissue perfusion. A violation of its function can lead to decreased nitric oxide production, which, in turn, contributes to the development of ischemic damage. Regarding the VDR gene polymorphism (rs731236), our study did not reveal any differences between groups by genotype or allele (p=0.89; OR=1.06). This indicates that this genetic variant has no significant effect on the development of hypoxic-ischemic CNS damage in newborns in the studied sample.

Since cardiometabolic disorders in a child after birth can lead to deterioration and the need for resuscitation measures, we analyzed the potential impact of polymorphic variants in the VDR and NOS3 genes on the main resuscitation interventions performed immediately after birth. Logistic regression analysis, adjusted for maternal metabolic syndrome, did not reveal a significant association between the VDR CC genotype and neonatal resuscitation interventions. The presence of the CC genotype was not associated with an increased risk of intubation with oxygen therapy (OR=1.12, 95 % CI 0.21–5.91, p=0.89) or cardiac massage (OR=0.96, 95 % CI 0.05–18.1, p=0.98). No statistically significant association was found between the TT NOS3 genotype and the need for resuscitation in newborns (p=0.718). Due to the low number of resuscitation events, logistic regression analysis was not feasible, and the analysis was performed using contingency tables. The distribution of resuscitation did not differ significantly across genotype groups.

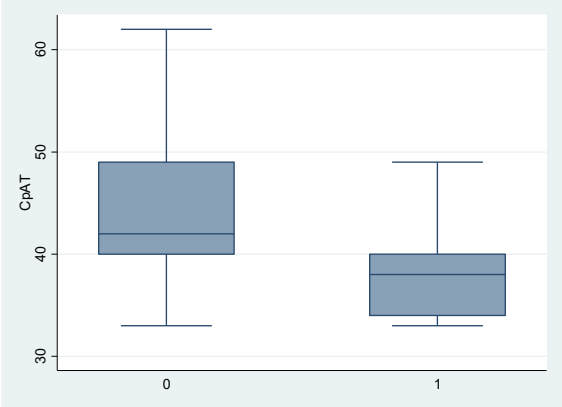

Fig. 1. Systolic blood pressure levels (mmHg) in newborns with hypoxic-ischemic CNS injury who are carriers of the CC VDR (1) and CT&TT (0) genotypes, respectively.

The next step of this study was to analyze the associations between VDR and NOS3 genotype variants and hemodynamic parameters and biochemical indicators. The NOS3 polymorphism was not associated with hemodynamic parameters such as heart rate (p=0.058), systolic blood pressure (p=0.135), diastolic blood pressure (p=0.069), or mean arterial pressure (p=0.856). Echocardiographic parameters, including ejection fraction (p=0.657), fractional shortening (p=0.111), and ventricular dimensions (p=0.064), also showed no significant relationship with NOS3 variants of genotype after adjustment for gestational age. Logistic regression analysis showed a trend toward an increased risk of hypotension in newborns with the TT genotype of the NOS3 gene (β=2.06; OR=7.85; 95 % CI 0.95–64.8; p=0.056), but this association did not reach statistical

significance. However, a statistically significant association was found between the CC VDR genotype and the level of systolic blood pressure (SBP) (β=−8.17; p=0.016). Carriers of the CC genotype had a decrease in SBP (Fig. 1).

For other hemodynamic parameters (heart rate, diastolic blood pressure (BP), mean BP), no significant associations were found (p>0.05), although a trend towards a decrease was observed for mean BP (p=0.059). Multivariate regression analysis found that the CC VDR genotype was associated with a decrease in SBP (OR 3.21; p=0.034) after correction for metabolic parameters, acid-base status, and inotropic support (Table 2).

Table 2

Odds ratios and predictive coefficients of the predictive model for low blood pressure in newborns with hypoxic-ischemic CNS injury

Variables	OR	95 % CI	p
Model 1			
CC VDR	3.21	1.08–9.54	0.036
Obesity in the mother	2.88	1.02–8.11	0.045
pH (venous blood)	0.61	0.44–0.85	0.004
Dopamine prescription	2.75	1.01–7.49	0.047
Adrenaline prescription	3.92	1.12–13.7	0.032
CRP	1.05	0.98–1.12	0.16
Model 2			
TT NOS3	11.7	0.87–155.9	0.043
Level of LDG1	1.0	0.83–0.97	0.02
Ejection fraction	1.1	0.86–0.99	0.031

Notes: CRP – C-reactive protein; LDG1 – lactate dehydrogenase type 1.

The most significant predictor of blood pressure in the first model was pH (OR=0.61; p=0.004). The CC VDR genotype is an independent factor associated with decreased systolic blood pressure in newborns, which may indicate a role for vitamin D receptor-dependent mechanisms in the regulation of vascular tone.

The TT genotype of the NOS3 gene was associated with a significantly increased risk of hypotension in newborns with hypoxic-ischemic CNS injury (OR=11.7; p=0.043), but the confidence interval was wide, indicating limited precision of the estimate. LDG1 level was also an independent predictor (p=0.02), while ejection fraction showed a statistically significant but unstable association with outcome (p=0.031).

The results demonstrate that the CC VDR genotype is associated with a decrease in systolic blood pressure in newborns even after correction for key clinical factors, including acid-base status, inflammatory markers, and the use of inotropic support. This indicates a potentially independent role of the VDR polymorphism in regulating hemodynamics in the early neonatal period, which may be mediated by different pathways. Accumulated data indicate that women with metabolic syndrome often have vitamin D deficiency during pregnancy, which is associated with insulin resistance, chronic inflammation, and endothelial dysfunction [13]. This condition can affect intrauterine fetal development, particularly the formation of the central nervous system, due to impaired neurogenesis, angiogenesis, and oxidative stress regulation. It is important to understand that the effects of vitamin D deficiency, as well as the normal level in mothers, can be modified by genetic factors in the fetus due to variants of the VDR gene, which can affect receptor expression and functional activity [9]. In the context of maternal vitamin D deficiency in metabolic syndrome and low-function variants of the VDR gene, a dual effect in the fetus is observed, which potentiates disorders of calcium-phosphorus metabolism, neuroinflammatory processes, and antioxidant protection. This may contribute to increased vulnerability of the fetal brain to hypoxic-ischemic injury, impaired myelination, and the subsequent development of neuropsychiatric disorders. Thus, the interaction between maternal metabolic status and fetal genotype, particularly VDR gene variants, can be considered an important component of the pathogenesis of central nervous system damage in children. According to the previously published study [2], the rs731236 (TaqI) gene variant can predict the VDR haplotype and assess the risk of developing vitamin D deficiency and impaired metabolism. In the presence of the CC and TC genotypes of the TaqI (rs731236) gene variant, in the aforementioned study, serum vitamin D levels were lower, and pronounced progressive liver damage was observed. The clinical significance of the results may lie in the personalization of therapy (the VDR genotype may be a risk marker for hemodynamic instability and a prognostic factor) and in the role of vitamin D (its potential effects on vascular tone and adaptation in the newborn), which opens up prospects for further research and targeted correction.

Therefore, the risk of developing cardiometabolic pathology is likely associated with the VDR gene variant (Taq1) and will clearly progress in the presence of vitamin deficiency. A promising approach for such a polymorphic gene variant is personalized therapy, including dosage selection and the use of various vitamin D preparations, with constant monitoring of its level in blood serum. Polymorphisms in the NOS3 gene, which encodes endothelial NO synthase, and the VDR gene, which mediates the effects of vitamin

D, may play an important role in regulating vascular tone and adaptive mechanisms in newborns. Impaired nitric oxide production and altered sensitivity to vitamin D can lead to endothelial dysfunction [4], a key pathogenetic mechanism in the development of hypoxic-ischemic lesions. A possible gene-gene interaction between NOS3 and VDR is that vitamin D signaling pathways may affect NOS3 expression and nitric oxide bioavailability, thereby modulating vascular reactivity. In turn, NOS3 polymorphisms may alter the efficiency of these mechanisms, enhancing or weakening the effect of VDR gene variants.

Limitations. The main limitations of this study include the relatively small sample size and the limited number of clinical events, which may have reduced the statistical power and precision of the estimates. In addition, the short follow-up period and single-center design may limit the generalizability of the findings and the assessment of long-term outcomes.

Conclusions

1. A statistically significant association was found between the CC VDR genotype and systolic blood pressure (β=−8.17; p=0.016).

2. The most significant predictors of low blood pressure level were pH (OR=0.61; p=0.004), CC variant of the VDR gene (OR=3.21, p=0.036).

3. The TT NOS3 genotype was also associated with a decrease in blood pressure in newborns (OR=11.7, p=0.043), which may indicate a role for vitamin D receptor-dependent mechanisms in the regulation of vascular tone.

4. Combined analysis of NOS3 and VDR genes is a promising approach for identifying genetic risk factors for cardiometabolic disorders in newborns and may contribute to a better understanding of individual variability in clinical manifestations. Further studies in a larger sample of patients are needed.

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Conflict of interest. The authors have no conflicts of interest to declare.

ORCID: Pokhylko V.I. http://orcid.org/0000-0002-1848-0490, Cherniavska Y.I. https://orcid.org/0000-0002-4522-7538, Yakovenko O.V. https://orcid.org/0009-0001-1062-9035, Davydenko A.V. https://orcid.org/0000-0002-4042-644X, Rossokha Z.I. https://orcid.org/0000-0002-4767-7364.

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