ARCTIC MEDICINE

N.V. Zaitseva, O.V. Dolgikh, N.A. Nikonoshina, V.B. Alekseev

POLYMORPHISM OF CANDIDATE GENES FOR THE FORMATION OF "POLAR STRESS SYNDROME" IN CHILDREN LIVING IN THE CIRCUMPOLAR TERRITORY (BY THE EXAMPLE OF *ANKK1/DRD2* (RS18004976) AND *TNF* (RS1800629))

DOI 10.25789/YMJ.2024.85.18

UDC [613.11+612.017.2+612.392.69]:616-06(571.121)

"Polar stress syndrome" is the result of a negative extreme climatic and geographical conditions impact in the circumpolar territories with the subsequent formation of maladaptation disorders of immune and nervous regulation. There is no doubt the relevance of studying the SNP features of candidate *ANKK1*/*DRD2* (rs18004976) and *TNF* (rs1800629) genes in the aspect of identifying probable predisposition markers to the "polar stress syndrome" development in the circumpolar territory population. **The aim** is to study the features of polymorphism of immune and nervous systems regulatory genes as possible markers of predisposition to the "polar stress syndrome" formation in the children population of circumpolar territory by the example of the dopamine receptor *ANKK1*/*DRD2* (rs18004976) and the tumor necrosis factor *TNF* (rs1800629) genes**. Materials and methods**. 717 children aged 7-13 years were examined. 136 people live in conditions conducive to the formation of "polar stress syndrome" (circumpolar territory); 581 people live in the middle latitude territories. The identification of apoptosis receptors $CD3^+CD95^+$ and TNFR was carried out by flow cytofluorometry, dopamine production was analyzed by ELISA. The SNP of *ANKK1*/*DRD2* (rs18004976) and *TNF* (rs1800629) genes were identified by real-time PCR. **Results.** The results of children genotyping established the reliable association of the T-allele and TT-genotype of the *ANKK1*/*DRD2* gene (rs18004976) with dopamine hyperproduction and the similar relation of the G-allele and GG-genotype of the *TNF* (rs1800629) gene with an excessive content of TNFR and $CD3^+CD95^+$ lymphocytes ($p<0.05$). Overexpression of TNFR, $CD3^+CD95^+$ and dopamine in children was significantly associated with T-allele and TT-genotype of the *ANKK1*/*DRD2* gene (rs18004976) and G-allele and GG-genotype of the *TNF* gene (rs1800629) relative to the CC-genotype of the *ANKK1*/*DRD2* (rs1800497) gene and AA-genotype of *TNF* (rs1800629) gene independently of the analyzed sample ($p<0.05$) and reached the maximum values in carriers of the TT-genotype of the *ANKK1*/*DRD2* (rs18004976) gene and GG-genotypes of *TNF* (rs1800629) gene in the observation group. Moreover, the difference in the allele and genotype frequency distribution of candidate genes between analyzed groups was significant (TT-genotype of the *ANKK1*/*DRD2* gene (rs18004976) (OR=2.43; 95% CI=1.43-4.15; p=0.04); GG-genotype of the *TNF* gene (rs1800629) (OR=1.66; 95% CI=1.02-2.70; p=0.03)), which verified the contribution of genetic predisposition to the development of "polar stress syndrome" in children in extreme climatic and geographic conditions of the circumpolar territory.

Keywords: "polar stress syndrome", children, dopamine, apoptosis, genetic polymorphism, candidate genes.

Introduction. "Polar stress syndrome" or "northern stress" is a complex of specific changes in regulatory and metabolic processes in the body by the reason of adaptive reserves depletion in extreme climatic and geographic conditions of a circumpolar territory. The phenomenon of "polar stress syndrome" and its consequences are considered as a polysyndrome characterized by metabolic disorders; detoxification inhibition; oxidative stress, cellular and tissue hypoxia; increased blood clotting, regenerative-plastic insufficiency, immunosuppression, endocrine disorders, biological rhythm desynchronization, meteopathy, functional asymmetry of hemispheric interactions and the formation of chronic psycho-emotional stress [3, 4]. School-age children are more vulnerable to the stressful action of external factors because of active morphofunctional internal organs and systems restructuring in a growing and developing organism and as a consequence of intensive educational process. The nature of "polar stress syndrome" manifestations and their severity is largely determined by the hereditary adaptive potential of an individual organism, including by the polymorphic variants of immune and nervous regulation genes [1, 2]. Therefore, the study of the polymorphism of dopamine receptor *ANKK1/DRD2* (rs18004976) and tumor necrosis factor *TNF* (rs1800629) genes as possible neuroimmune predisposition markers to the development of "polar stress syndrome" in school-age children (7-13 years) living in a circumpolar territory is particularly relevant in the terms of prompt diagnostics and effective prevention on the early stages of its formation.

The aim is to study the features of *ANKK1*/*DRD2* (rs18004976) and *TNF* (rs1800629) polymorphism as candidate predisposition genes to the formation of "polar stress syndrome" in children living in a circumpolar territory.

Material and methods. 717 children aged 7-13 years were examined. The observation group consisted of 136 children living in the circumpolar territory (69° n.l.). The comparison group consisted of children (581 people) living in the middle latitude (56° n.l.). According to the results of the neuropsychological testing (STROOP test) the observation group significantly differed from the comparison group with a decrease in the indicators of figurative and numerical memory, atten-

ZAITSEVA Nina Vladimirovna – MD, Professor, Academician of the Russian Academy of Sciences, Scientific Director of FBSI Federal Scientific Center for Medical and Preventive Health Risk Management Technologies, e-mail: znv@fcrisk.ru, ORCID: 0000-0003-2356-1145; **DOLGIKH Oleg Vladimirovich** – MD, Head of the Department of Immunobiological Diagnostics of FBSI Federal Scientific Center for Medical and Preventive Health Risk Management Technologies, e-mail: oleg@fcrisk.ru, ORCID: 0000- 0003-4860-3145; **NIKONOSHINA Natalya Alekseevna** – junior research assistant of Department of Immunobiological Diagnostics, postgraduate student of the FBSI Federal Scientific Center for Medical and Preventive Health Risk Management Technologies, e-mail: nat08.11@yandex.ru; ORCID: 0000-0001-7271-9477; **ALEKSEEV Vadim Borisovich** – MD, Professor, Director of FBSI Federal Scientific Center for Medical and Preventive Health Risk Management Technologies, e-mail: root@fcrisk.ru; ORCID: 0000-0001-5850-7232

tion level, signs of emotional instability, frequent headaches and sleep disorders, as possible manifestations of the "polar stress syndrome".

The study was carried out in accordance with the Helsinki Declaration of the WMA "Ethical principles of conducting medical research involving people as subjects" and the National Standard of the Russian Federation GOST-R 52379-2005 "Good Clinical Practice" (ICH E6 GCP). Parents or other legal representatives of the children have signed a voluntary informed consent to conduct a medical examination.

The content of TNFR and CD3+CD95+ lymphocytes was determined by flow cytofluorimetry on the FACSCalibur device ("Becton Dickinson", USA) using the universal program "CellQuestPrO". The dopamine production level was determined by the ELISA method on the Infinite F50 analyzer ("Tecan Austria GmbH", Austria).

Statistical processing of the results is implemented in the Statistica 10 application software package (StatSoft, USA). The Shapiro-Wilk criterion was used to determine the data distribution nature in the surveyed samples. The Student's parametric criterion was used to assess the level of reliability of the obtained data, taking into account the normal distribution of variables in the compared groups. The nonparametric Mann-Whitney U-test was used to compare the data in the case of deviation from the normal distribution. The study results are presented in the form of the arithmetic mean (X) and its standard error (SE) of the studied indicators. The differences between the groups were considered statistically significant at $p<0.05$.

The SNP of dopamine receptor *ANKK1*/*DRD2* (rs1800497) and tumor necrosis factor *TNF* (rs1800629) genes were analyzed using real-time PCR on the CFX96 Real Time System C1000 Thermal Cycler ("BioRad", Singapour). The genetic material was extracted from buccal scraping using a set of reagents for DNA extraction "AmpliPrime DNA-sorbB Form 2 Variant 100" ("NextBio" LLC, Russia) by the sorbent method. The test systems - reagent kits for SNP (C2137T *ANKK1*/*DRD2* (rs1800497) and G308A *TNF* (rs1800629) ("Syntol", Russia)) identification were used to determine the genetic polymorphism of the studied genes. The allelic discrimination method was used to establish the human genotype in the TaqMan program. The calculation of allele and genotype frequency distribution according to the Hardy-Weinberg equilibrium, the odds ratio (OR) and its 95% confidence interval (CI) was carried out using the online "SNPStat" and "Gen-Expert" programs.

Results and discussion. The results of genotyping of the examined children revealed polymorphic variants of the dopamine receptor *ANKK1*/*DRD2* (rs1800497) and tumor necrosis factor *TNF* (rs1800629) genes that characterize signs of a possible genetic predisposition to the development of "polar stress syndrome" in the circumpolar territory children population associated with the maladaptation disorders of immune and neurohumoral regulation. The frequency allele and genotype distribution of these genes corresponds to the Hardy-Weinberg equilibrium ($p<0.05$) and is described by multiplicative (test $\chi 2$, df = 1) and additive (Cochrane-Armitage test for linear trends, xi = [0,1,2], df = 1) inheritance models (Table 1).

The polymorphism of the dopamine receptor *ANKK1*/*DRD2* (rs1800497) gene in children with the signs of "polar stress syndrome" living in the circumpolar territory is characterized by the significant increase in the T-allele and the corresponding homozygous TT-genotype frequency relative to the comparison group ($p<0.05$). It is associated with reduced expression of this gene and, as a consequence, a decrease in the D2-dopamine receptors density. In turn, a significant increase in the production of their dopamine ligand in the observation group relative to the comparison group and the reference level ($p<0.05$) is a response to acute stress conditions, where genome–mediated inhibition of reception enhances the effects associated with the unrelated ligand persistence. It has been shown that it is the acute impact of unpredictable and uncontrolled stress factors such as sharp and sudden fluctuations in the climatic and geographical parameters of the circumpolar territories leads to an increase in the extracellular dopamine content in the mesocortical system and striatum due to the pulsed phasic dopaminergic neurons activation, whereas chronic moderate stress inhibits the production of this neurotransmitter, causing a depression development in the future [5, 6].

At the same time, an increased G-allele and GG-genotype frequencies of the *TNF* gene (rs1800629) were found in the observation group ($p<0.05$). It is related with the overexpression $CD3^+CD95^+$-lymphocytes as marker of cell death - in relation to the comparison group and excess

Table 1

The allele and genotype frequency distribution of *ANKK1/DRD2* (rs1800497) and *TNF* (rs1800629) genes in children with the manifestations of "polar stress syndrome"

SNP	Genotype / Allele	Observation group (n=136)	Comparison group (n=581)	OR	
					95% CI
ANKK1/DRD2 (rs1800497)	CC	0.611	0.644	0.87	0.59 – 1.27
	CT	0.213	0.275	0.71	0.46 – 1.12
	TT	0.176	0.081	2.43	1.43 – 4.15
	C	0.717	0.781	0.71	0.53 – 0.95
	T	0.283	0.219	1.41	1.05 – 1.90
TNF (rs1800629)	GG	0.831	0.747	1.66	1.02 – 2.70
	GA	0.162	0.225	0.66	0.40 – 1.09
	AA	0.007	0.028	0.26	0.03 – 1.99
	G	0.912	0.860	1.68	1.07 – 2.64
	A	0.088	0.140	0.59	0.38 – 0.93

Note. $\chi[T(ANKK1/DRD2)]^2 = 5{,}10$, $p = 0{,}02$; $\chi[TT(ANKK1/DRD2)]^2 = 4{,}03$, $p=0{,}04$; $\chi[G(TNF)]^2 = 5{,}22$, $p = 0{,}02$; $\chi[GG(TNF]^2 = 4{,}93$, $p = 0{,}03$.

Table 2

The content of TNFR, CD3+CD95+ and dopamine in children with the manifestations of "polar stress syndrome"

Indicator	Reference interval	Observation group (n=136)	Comparison group	p
TNFR, %	1 – 3	5.703±0.683*	3.842±0.586	0.001
CD3+CD95+-lymphocytes, %	15 - 25	25.755±0.967	22.900±0.916	0.032
Dopamine, pg/cm^3	5.6 - 44	59.358±2.133*	36.945±5.899	0.001

Note: * - differences with the reference level are significantly significant ($p<0.05$).

of tumor necrosis factor receptor TNFR in relation to the comparison group and the reference interval ($p<0.05$). The physiological meaning of the lymphocyte apoptosis excess in extreme climato-geographic conditions in the circumpolar territory is associated with cell life cycle acceleration and cellular phenotypes changes, reflecting the need to compensate for energy losses and maintain the cytokine profiles stability, which is accompanied by immunoregulatory stress in the form of immunosuppression [9, 10].

The results of a comparative analysis of the TNFR, $CD3^+CD95^+$ and dopamine production levels in children with different polymorphic variants of the *ANKK1*/*DRD2* (rs1800497) and *TNF* (rs1800629) genes demonstrate similar trends in these indicators for the identified polymorphic markers both in the general sample (n=717) and in isolation in the observation group (n=136) and in the comparison group (n=581) (Fig. 1, 2). In children with TT-genotype of the *ANKK1*/*DRD2* (rs1800497) gene and GG-genotype of the *TNF* (rs1800629) gene in the general sample and in the compared groups, a significant increase in the content of dopamine, TNFR and $CD3^+CD95^+$-lymphocytes was found in relation to the carriers of CC-genotype of *ANKK1*/*DRD2* (rs1800497) gene and AA-genotype of TNF (rs1800629) gene in the same groups ($p<0.05$). It shows signs of a possible genetic predisposition to the neuroimmune profile changes characterizing the northern stress development, including in the population of 56 ° n.l., but to a lesser extent. It was found that TNFR, $CD3^+CD95^+$ and dopamine production levels in children with TT-genotype of the *ANKK1*/*DRD2* (rs1800497) gene and the GG-genotype of the *TNF* (rs1800629) gene in the observation group significantly exceeds similar values shown in children with the same genotypes in the comparison group and in the general sample ($p<0.05$). The revealed maximum values of TNFR, $CD3^+CD95^+$ and dopamine content in the carriers of TT-genotype of *ANKK1*/*DRD2* (rs18004976) gene and GG-genotype of *TNF* (rs1800629) gene in the observation group confirm that the living conditions extremity implements a genetically determined neuroimmune mechanism for the "polar stress syndrome" formation in children.

The immune and nervous systems work in constant interaction, providing a body homeostasis in changing environmental conditions. Thus, dopamine specifically bound to receptors acts as an immune coregulator, ensuring the relationship not only between neurons, but

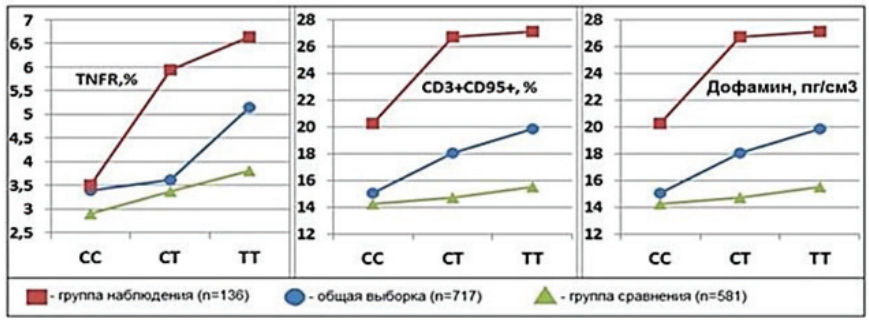

Fig. 1. Changes in the production of TNFR, CD3+CD95+ and dopamine in children with different genotypes of the *ANKK1/DRD2* gene (rs1800497)

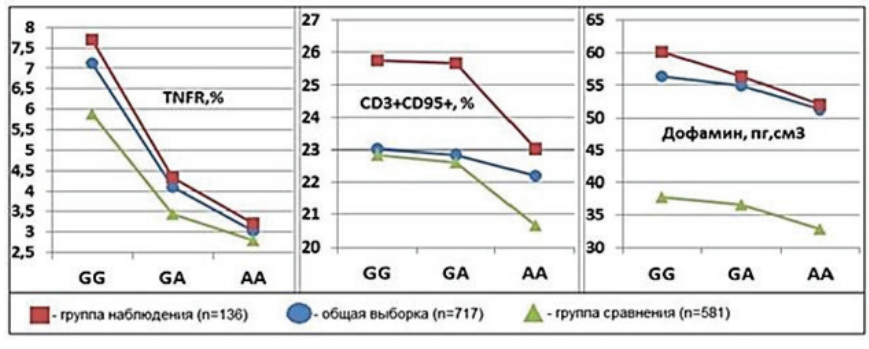

Fig. 2. Changes in the production of TNFR, CD3+CD95+ and dopamine in children with different genotypes of the *TNF* gene (rs1800629)

also between immunocytes. Human peripheral blood lymphocytes express dopamine receptors and transport proteins, synthesize endogenous dopamine and related catecholamines – epinephrine and norepinephrine. When dopamine binds to specific D2 and D3-receptors, it leads to an integrin-dependent chemotaxis activation, selective adhesion to the intercellular matrix and increased cytotoxic $CD8^+$-lymphocytes migration, as well as signs of proinflammatory cytokines (TNFα and IFNγ) hyperproduction [8, 11]. It is believed that dopamine-induced cytotoxicity is not associated with receptor activation and it manifests itself through spontaneous oxidation, leading to programmed lymphocyte death caused by oxidative stress, which signs are observed in examined children with "polar stress syndrome" [7].

Conclusion. The genetic profile of children with the "polar stress syndrome" living in the circumpolar territory is characterized by an increased frequencies of the T-allele and TT-genotype of the *ANKK1*/DRD2 (rs18004976) gene, as well as the G-allele and GG-genotype of the *TNF* (rs1800629) gene associated with an increase in TNFR, $CD3^+CD95^+$-lymphocytes and dopamine relative to the reference level and the comparison group ($p<0.05$). It indicates the signs of excessive cell death and chronic inflammation as manifestations of the immune mechanism for the formation of acute stress reactions associated with dopamine-induced hypersympathicotonia. TNFR, $CD3^+CD95^+$ and dopamine expression variability in the general sample, in the observation group and in the comparison group is characterized by a significant increase in the level of their production in the TT-genotype carriers of *ANKK1*/*DRD2* gene (rs18004976) and in the GG-genotype carriers of the *TNF* (rs1800629) gene relative to the CC-genotypes of the *ANKK1*/*DRD2* (rs1800497) gene and AA-genotypes of *TNF* (rs1800629) gene in all analyzed samples ($p<0.05$). It demonstrates the contribution of genetic polymorphism to the formation of "polar stress syndrome". The revealed maximum values of TNFR, CD3+CD95+ and dopamine in the TT-genotype carriers of *ANKK1*/*DRD2* (rs18004976) gene and GG-genotype carriers of TNF (rs1800629) gene in the observation group confirm the combined contribution of genetic predisposition and living conditions in the circumpolar territory to the formation of "polar stress syndrome" in children.

References

1. Devrishov R.D., Kolomin V.V., Filyaev V.N., Kudryasheva I.A. Gigienicheskie aspekty vozde-

jstviya faktorov sredy obitaniya na formirovanie zdorov'ya uchashchihsya [Hygienic aspects of influence of environmental factors on formation of schoolchildren’s health]. Rossijskij mediko-biologicheskij vestnik imeni akademika I.P. Pavlova [Pavlov’s Russian Medical Biological Herald. 2019; 27(4):530-535 (In Russ.).] DOI: 10.23888/PAVLOVJ2019274530-535

2. Nikonoshina N.A., Dolgikh O.V. Osobennosti immunnoj i endokrinnoj regulyacii detskogo naseleniya severa Sibiri, associirovannye s polimorfizmom gena MTNR1A (rs34532313) [Characteristics of immune and endocrine regulation associated with MTNR1A gene polymorphism (rs34532313) in the child population in the north of Siberia]. Zdorov'e naseleniya i sreda obitaniya [Public Health and Life Environment. 2020; 5:25-28 (In Russ.).] DOI: 10.35627/2219-5238/2020-326-5-25-28

3. Solonin Yu.G., Boyko E.R. Mediko-fiziologicheskie problemy v Arktike [Medical and physiological problems of the Arctic]. Izvestiya Komi NC UrO RAN [Proceedings of the Komi Science Center of the Ural Division of the Russian Academy of Sciences. 2017; 4(32):33 – 40 (In Russ.).]

4. Khasnulin V.I., Khasnulin P.V. Sovremennye predstavleniya o mekhanizmah formirovaniya severnogo stressa u cheloveka v vysokih shirotah [Modern concepts of the mechanisms forming northern stress in humans in high lattitudes]. Ekologiya cheloveka [Human ecology. 2012; 19(1):3–11 (In Russ.).] DOI: 10.17816/humeco17512

5. Baik J-H. Stress and the dopaminergic reward system. Exp Mol Med. 2020; 52(12):1879-1890. DOI: 10.1038/s12276-020-00532-4

6. Cui W, Aida T, Ito H, Kobayashi K, Wada Y, Kato S, Nakano T, Zhu M, Isa K, Kobayashi K, Isa T, Tanaka K, Aizawa H. Dopaminergic Signaling in the Nucleus Accumbens Modulates Stress-Coping Strategies during Inescapable Stress. J Neurosci. 2020; 40(38):7241-7254. DOI: 10.1523/JNEUROSCI.0444-20.2020.

7. Matt SM, Gaskill PJ. Where is dopamine and how do immune cells see it? Dopamine-mediated immune cell function in health and disease. J. Neuroimmune Pharmacol. 2020; 15:114–164.

8. Penedo MA, Rivera-Baltanás T, Pérez-Rodríguez D, Allen J, Borrajo A, Alonso-Crespo D, Fernández-Pereira C, Nieto-Araujo M, Ramos-García S, Barreiro-Villar C, Caruncho HJ, Olivares JM, Agís-Balboa RC. The role of dopamine receptors in lymphocytes and their changes in schizophrenia. Brain, Behavior, & Immunity – Health. 2021; 12:100199 DOI: 10.1016/j.bbih.2021.100199

9. Tseng W-Y, Huang Y-S, Lin H-H, Luo S-F, McCann F, McNamee K, Clanchy F, Williams R. TNFR signalling and its clinical implications. Cytokine. 2018; 101:19-25. DOI: 10.1016/j.cyto.2016.08.02

10. Risso V, Lafont E, Le Gallo M. Therapeutic approaches targeting CD95L/CD95 signaling in cancer and autoimmune diseases. Cell Death Dis. 2022; 13:248

11. Vidal PM, Pacheco R. The Cross-Talk Between the Dopaminergic and the Immune System Involved in Schizophrenia. Front Pharmacol. 2020; 11:394. DOI: 10.3389/fphar.2020.00394