Personalized Psychiatry and Neurology

Case Report

Genetic Profiling of the Nitric Oxide Synthases' System in a 55-Year-Old Woman with the Tension-Type Headache and Arterial Hypertension Phenotype: Case Report

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Abstract: The tension-type headache (TTH) and arterial hypertension (AH) phenotype is a common overlap syndrome in adult patients. A genetically determined disturbance of the nitric oxide (NO) synthesis system is actively considered as one of the important possible pathogenetic mechanisms for the development of this phenotype. Neuronal NO-synthase is expressed both in the brain, skeletal muscles, and in the vascular endothelium; therefore, single-nucleotide variants of the *NOS1* gene, encoding this enzyme, are the most interesting, but insufficiently studied genetic biomarkers of the TTH and AH phenotype. The aim of the case report is to present the experience of using genetic profiling of the nitric oxide synthases' system in a 55-year-old patient with treatment-resistant TTH and AH phenotype.

Keywords: *tension-type headache; arterial hypertension; comorbidity; nitric oxide; nNOS; iNOS; eNOS; NOS1; NOS2; NOS3; genetic biomarker; rs3782218; rs7314935; rs2779249; rs2297518; rs1799983; rs2070744*

Introduction

The tension-type headache (TTH) is the most common type of primary headache, and arterial hypertension (AH) is a prevalent condition worldwide [1]. Many studies support the hypothesis that TTH patients have a higher risk of developing AH, while hypertensive patients have a higher risk of developing TTH than in the general population [2]. The relationship between TTH and AH is potentially of great pathophysiological and clinical interest as a clinical phenotype, but it is poorly understood [3, 4].

Nitric oxide (NO) is an important autocrine and paracrine signaling molecule that plays a crucial role in cardiovascular and cerebrovascular disorders [5]. Reduced bioavailability of NO in the vascular endothelium is an important predictor for impaired vasodilation and AH. Furthermore, NO is involved in nociceptive processing. A NO-induced biphasic response with immediate and a delayed headache is typical for chronic TTH in humans [3].

As we know, NO-synthases (NOSs) are expressed in three isoforms: neuronal (nNOS), inducible (iNOS), and endothelial (eNOS) [6]. The functional activity of these isoenzymes depends on the carriage of wild, highly functional, low functional, and nonfunctional alleles of single nucleotide variants (SNVs) of *NOS1*, *NOS2*, and *NOS3* genes encoding the enzyme isoforms [7].

We have identified 9 most promising single nucleotide variants (SNVs) that can be potential biomarkers of the TTH and AH phenotype development (Figure 1 [4]).

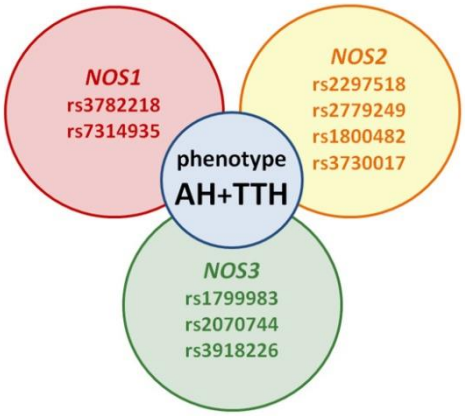

Figure 1. Potential SNVs of *NOS1, NOS2, NOS3* genes predisposed to the tension-type headache and arterial hypertension phenotype development (modification by authors from [4]).

Objective

The aim of the case report is to present the experience of using genetic profiling of the nitric oxide synthases' system in a 55-year-old patient with treatment-resistant TTH and AH phenotype.

Materials and Methods

3.1. Procedure

Genetic profiling was used based on the profile of patient (homo- and heterozygous carriers of risk alleles of low-functional and non-functional SNVs of the *NOS1*, *NOS2*, *NOS3* genes encoding nNOS, iNOS, eNOS.

TaqMan® quantitative Real-Time Polymerase Chain Reaction (RT-PCR) (Applied Biosystems, Foster City, CA, USA) was used to determine the genotype of the 6 SNVs [8]: rs3782218 (chr12:117771511 C>T) and rs7314935 (chr12:117718837 G>A) of the *NOS1* gene; rs2779249 (chr17:26128581 C>A) and rs2297518 (chr17:26096597 G>A) of the *NOS2* gene; rs1799983 (chr7:150696111 G>T) and rs2070744 (chr7:150690079 T>C) of the *NOS3* gene using diagnostic equipment Rotor-Gene 6000 (Corbett Life Science, Australia) and technology allelic discrimination of TaqMan and fluorescent probes (Applied Biosystems, USA).

3.2. Patient Observation

The study was a part of a comprehensive study on the topic "Clinical and genetic predictors of the tension-type headache and arterial hypertension phenotype", registration No. 122030300108-6 dated March 03, 2022.

TTH was diagnosed with by a neurologist according to the International Classification of Headache Disorders (ICHD-III beta) criteria [9]. AH was diagnosed by a cardiologist according to the European Society of Cardiology and the European Society of Hypertension (2018) criteria [10] and the Russian Society of Cardiology (2020) criteria [11].

At visit 1, biological material (venous blood) was collected from the patient. Then the genetic analysis of the sample was carried out. Genetic profiling revealed SNVs of the candidate genes (*NOS1, NOS2, NOS3*), and assessed the risk of TTH development in patient with AH. At visit 2, correction of antihypertensive and headache therapy was carried out taking into account the results of genetic profiling.

3.3. Ethical Aspects

The study was performed in accordance with the standards of good clinical practice and the principles of the Declaration of Helsinki. Study material was obtained after

approval of the local ethics committee of the V.F. Voino-Yasenetsky Krasnoyarsk State Medical University (KrasSMU), protocol No. 101/2020 dated October 31, 2020.

The study was supported by the intra-university grant to support the research of young scientists of the KrasSMU (No. 462-base dated July 12, 2021).

The participant signed a voluntary informed consent. The patient did not receive any remuneration for participating in the clinical trial. Researchers did not receive any remuneration for conducting clinical trials.

Results

4.1. Medical History

Caucasian patient A., 55-years-old, resident of Krasnoyarsk city (Eastern Siberia), economist, has been suffering from infrequent episodic TTH since the age of 22, and frequent episodic TTH since the age of 38. The patient did not consult a neurologist, she was self-medicated, took various non-steroidal anti-inflammatory drugs (NSAIDs).

At the age of 50, the patient began to notice an increase in blood pressure. AH was diagnosed at 52 years old. Enalapril 10 mg was prescribed at a single appointment without dynamics control. Also, during these 5 years from the onset of AH, the patient established an association between headaches and increased blood pressure.

So, patient A. went to the family doctor and neurologist of our clinic due to the increase in headache episodes and their resistance to both antihypertensive and NSAIDs during the last two months. 24-hour blood pressure monitoring was performed, a non-dipper profile was revealed, however, episodes of increased blood pressure did not correlate with episodes of AH (secondary headache attributed to disorder of homoeostasis was excluded) or the use of antihypertensive drugs and NSAIDs (medication-overuse headache was excluded) [9]. Thus, the phenotype TTH and AH was first diagnosed by a neurologist in this patient at the age of 55 years. The patient was recommended genetic profiling of the NO synthesis system, including SNVs of genes *NOS1, NOS2, NOS3*.

4.2. Results of Genetic Profiling

The results of the genetic profiling performed are presented in **Table 1**.

Table 1. Results of Genetic Profiling in a patient with tension-type headache and arterial hypertension phenotype (clinically significant deviations are shown).

Gene: OMIM (Cytogenetic locations)	Protein	Single-nucleotide variant	Normal	Result
NOS1: 163731 (12q24.22)	nNOS	rs3782218 (117771511 C>T)	CC	**TT**
		rs7314935 (117718837 G>A)	GG	GG
NOS2: 163730 (17q11.2)	iNOS	rs2779249 (26128581 C>A)	CC	CC
		rs2297518 (26096597 G>A)	GG	GG
NOS3: 163729 (7q36.1)	eNOS	rs1799983 (150696111 G>T)	GG	GG
		rs2070744 (150690079 T>C)	TT	TC

Thus, the patient was found to have a homozygous TT genotype for the minor allele T rs3782218 of the *NOS1* gene encoding the nNOS enzyme. This explains the genetically determined decrease in the functional activity of the nNOS and the decrease in NO synthesis in cerebrovascular and peripheral vessels, skeletal muscles, which predisposes to vasospasm and muscle spasm. The results of genetic profiling correlated with the

phenotype TTH and AH in the patient herself and her aggravated maternal family history (TTH and AH phenotype was also detected in the 79-year-old mother of the patient).

4.3. Recommendations for the Patient

1) Keeping a blood pressure diary and headache diary.

After two weeks of keeping a diary at a follow-up consultation, the patient herself drew attention to the fact that episodes of increased blood pressure and episodes of headache do not always coincide, although she previously considered this relationship to be direct.

2) Regular intake of antihypertensive therapy.

It is recommended to change Enalapril with Perindopril. Because Enalapril has headache in a list of its side effects of and is not recommended for patients with a clinical phenotype of TTH and AH [2].

3) Psychotherapist consultation and lifestyle modification.

Cancellation of the regular intake of NSAIDs in order to avoid the development of medication-overuse headache.

Discussion

As is known, the presented case report is the first description of the use of the genetic profiling of the NO synthesis system in patient with TTH and AH phenotype. Therefore, this case report may be of scientific and clinical interest.

The genetic profiling results are consistent with our previously published study [12] showing that the minor allele T rs3782218 (117771511 C>T) of the *NOS1* gene was statistically significantly associated with a high risk of developing the TTH and AH phenotype compared with the controls – healthy volunteers (odds ratio (OR) = 22.2 (95% confidential interval (CI): 2.8–173.5)) and compared with the hypertensive patients without headache (OR = 4.0 (95% CI: 1.4–11.8)) in Caucasian Siberian population of Russian Federation [12].

To our knowledge, the role of this SNV in TTH development has not been previously investigated, either in the general population or in hypertensive patients. No associations were found in patients with other primary headache, including migraines [13] in Turkish (Alaşehirli B. et al. (2012) [14]), Japanese (Ishii M. et al. (2014) [15]), and Spanish (García-Martín E. et al. (2015) [16]) populations. This SNV also has previously been investigated as a genetic biomarker for AH. So, Levinsson A. et al. (2014) found that the carriage of the T allele rs3782218 of the NOS1 gene reduces the risk of developing AH (OR = 0.81, 95% CI = 0.67–0.97, p-value = 0.02) in the Swedish population [17].

Thus, our data supplement our previous studies of the role of the SNV-marker rs3782218 of the *NOS1* gene in the Russian population of Eastern Siberia, but do not agree with the results of Levinson's (2014) [17] study performed in the Swedish population (Northern Europe). It should be noted that the sample size and the absence of other ethnic control groups (for example, Asians living in Eastern Siberia), which could supplement the existing picture of the allele distribution, do not allow for general conclusions to be drawn at this stage regarding the strength of the association of this marker with a genetic predisposition to TTH and AH overlap syndrome in Eastern Siberia [12]. Nevertheless, the obtained data, by way of dozens of samples, determine the feasibility of further studies, while it is necessary to take into account the difference in the distribution of genetic biomarkers in different populations (population stratification factor). Thus, the frequency of allelic variants of SNV rs3782218 of the *NOS1* gene, differently presented in the population of the European and Asian regions [18], may differ both within different populations of Western and Eastern Europe, and within the population system of Russia. According to the available data, the gene pool of the studied population living in Eastern Siberia is less affected by the "gene influx" from the countries of Western Europe, and, on the contrary, more affected by the "gene influx" from the countries of the Asian region, according to the central and European parts of Russia [12]. This has been demonstrated in

associated genetic studies of other neurological diseases [33]. So, the studied SNV-marker rs3782218 of the *NOS1* gene can be useful for predicting the risk of TTH and AH overlap syndrome in the consideration area. At the same time, the risk of this syndrome developing increases with the heterozygous (CT) genotype. It is possible that an increase in the sample size in further population studies may provide new information on the frequency of the rare homozygous (TT) genotype in Caucasians of Eastern Siberia [12].

This is important from a scientific and clinical point of view, because a new class of drugs that inhibit nNOS has been proposed in recent years, both for the treatment of TTH and AH. A new strategy for predicting and disease-modifying therapy of the common TTH and AH overlap syndrome can increase the effectiveness and safety of treatment, improve patient quality of life, and reduce the risk of life-threatening cardiovascular complications [12].

Conclusions

Thus, the performed genetic profiling showed a high risk of developing the phenotype TTH and AH in Caucasian Siberian patient A., 55-years-old, which was a homozygous carrier of the TT genotype rs3782218 of the *NOS1* gene encoding nNOS.

Funding: The study was supported by the intra-university grant to support the research of young scientists of the KrasSMU (No. 462-base dated July 12, 2021).

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of V.F. Voino-Yasenetsky Krasnoyarsk State Medical University (protocol code No. 101/2020 dated October 31, 2020).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

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Conflicts of Interest: The author declares no conflict of interest.

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