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THE IMPORTANCE OF CALCA AND ACE GENES IN THE PATHOGENESIS OF MIGRAINE

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Abstract. Until now, the diagnosis of "migraine" is exclusively clinical, and any diagnostic tests are aimed only at excluding other causes of headache. Despite the availability of a large number of specific anti-migraine drugs, the therapy of patients with migraine is still not effective enough. A significant clinical problem is the chronification of migraine attacks and the development of chronic daily headache, which occurs in 1% of patients per year. At the same time, about 10% of patients with migraine in the population and 40-60% of patients seeking specialized headache centers are resistant to standard therapy. The article presents the results of our research on this issue.

Keywords: CALCA, ACE, drug, patient, method, treatment.

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INTRODUCTION

Currently, the role of the hereditary factor in the development of migraine is beyond doubt. Relatives of patients with migraine suffer from this disease significantly more often than in the general population. Population studies of families with migraine have revealed a 1.5-fold increase in the risk of developing the disease in close relatives. A study of the genes of monogenic forms of migraine in the population of patients with classical migraine with aura and migraine without aura has not yielded results. One approach to studying migraine is to conduct association studies of candidate genes that can increase the risk of the disease or determine the characteristics of its course. An association with migraine has been shown for many genes, in particular those regulating the activity of the serotonergic and dopaminergic systems, the level of female sex hormones, etc.

MATERIALS AND METHODS

Thus, the current state of scientific knowledge in the field of the molecular nature of migraine does not provide a complete understanding of the cause-and-effect relationship of its pathogenesis. Therefore, it seems reasonable to conclude that today the only adequate approach to studying the molecular mechanisms of migraine pathogenesis is the analysis of the available literature using modern software and the construction of signaling pathways of intermolecular interactions based on literature data with subsequent experimental verification of molecular -genetic and biochemical changes in patients with migraine.

Thesis submitted for defense

• 147 genes selected as a result of literature analysis are associated with migraine.

• Unique schemes of signaling pathways of intermolecular interactions described possible molecular mechanisms of disease pathogenesis.

Polymorphic variants of the genes CCKAR (rs1800857 genotypes CT+CC), CCKBR (rs1805000 genotypes CT+TT), MTHFR (rs1801133 genotypes CT+TT), NOS3 (rs2070744 genotype CC) and ACE (rs4646994 genotypes II+ID) have statistically significant associations with migraine.

• The identified complex genotypes confirm a significant contribution to the development of the disease of the C allele of the CCKAR gene (rs1800857), which increases the risk of development by 21 times.

• The results of experimental and bioinformatic studies support the dopamine

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theory of migraine pathogenesis.

RESULTS AND DISCUSSION

The allelic state of the genes CCK (rs1157184), CCK1R (rs1799723, rs1800908, rs1800857), CCK2R (rs1805002, rs1805000), DBH (rs1611115, rs2097629), MTHFR (rs1801133), MTR (rs1805087), BDNF (rs2049046, rs6265, rs11030107), CGRP (rs1553005) was assessed by the PCR-RFLP method using the enzymes Bsc4I, HinfI, PstI, Bst4CI, BstDEI, FauI, BstMAI, HaeIII, PspCI, TaqI, MnlI. Restriction products were separated in 2% agarose gel.

Reliable biomarkers of migraine, especially genetic markers, can predict predisposition to the disease and its severity. In the course of the work, a search for information on genetic markers associated with migraine was performed. The initial list of genes was compiled using the PathwayStudio9 ® program and the ResNet11 ® abstract database of Elsivier (USA). As a result of the analysis, all genes for which the program found a GeneticChange connection with migraine were selected. Then, articles from the list were analyzed in detail. Original articles with statistically significant results were taken into account, as well as reviews that included significant information (indication of how the gene is associated with migraine, the presence of a link to an article with the original study). Based on the obtained results, a table was constructed, including the following information: gene name, possible reasons for the connection with migraine, markers, detection method, sampling parameters, comments, link (PubMedID).

The analysis revealed 147 genes associated with migraine. For convenience, these genes are further classified by their involvement in various processes (vascular tone, neurotransmitter metabolism, neurotransmitter transport and reception, membrane potential, neurogenesis, inflammation, etc.) and the functioning of various systems (glutamatergic, serotonergic, dopaminergic, folate cycle, sex hormones, vessels, ion channels, immune system, extracellular matrix, pain sensitivity, etc.) involved in the pathogenesis of migraine.

At this stage of the work we have analyzed molecular and intercellular processes in the pathogenesis of 3 forms of familial hemiplegic migraine. The features of FHM are a high frequency of aura, neuronal hyperexcitability and RCD.

The CACNA1A gene encodes the main subunit of voltage-dependent neuronal calcium channels (Cav2.1, function - modulation of glutamate release) (Catterall, 1998). Mutations in the CACNA1A gene cause the development of FHM type I (FHM1) and are associated with various types of channelopathies: impaired ion channel conductivity,

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changes in its kinetics or structure (Cao et al., 2004; Hans et al., 1999; Kraus et al., 2000; Tottene et al., 2002), which leads to an increase in the current of calcium ions through voltage-dependent channels and the release of neurotransmitters. The ATP1A2 gene encodes the α2 subunit of glial and neuronal K+/Na+-ATPase, and mutations in this gene lead to the development of FHM type II (FHM2) (Maagdenberg et al., 2010). A decrease in the activity of K+/Na+-ATPase leads to a disruption in the reuptake of glutamate from the synaptic cleft by glial cells. The SCN1A gene, mutations in which lead to the development of FHM type III (FHM3), encodes the structure of the pore-forming α1-subunit of voltage-dependent sodium channels (Nav1.1). This type of ion channels is predominantly present in the body and proximal part of the dendrites of inhibitory interneurons (Yu et al., 2006). This specific location of Nav1.1 channels plays a key role in the development of dendritic hyperexcitability, an essential component of synaptic transmission.

Based on these data, hypothetical molecular signaling pathways were constructed to describe the causes and possible mechanisms for the development of aura and ICD in the case of SGM. The result is shown in Figure 1.

In the case of SGM1, an increase in intracellular calcium occurs, which leads to the fusion of vesicles with the membrane and the release of glutamate into the synaptic cleft. In SGM2, a decrease or loss of K+/Na+-ATPase activation activity leads to the accumulation of potassium in the intercellular space and sodium inside the cell. This disrupts the functioning of glutamate transporters and increases the concentration of glutamate in the synaptic cleft. A mutation in the SGM3 gene leads to a change in sodium transport across the membrane, which leads to an increase in intracellular calcium and the release of glutamate into the synaptic cleft. Thus, the key point in our scheme is the pathological increase in the concentration of glutamate in the synaptic cleft in all types of SHM. Further, in all types of SHM, the molecular processes proceed in the same way. Glutamate activates NMDA receptors on postsynaptic neurons. Activation of NMDA receptors leads to membrane depolarization by releasing potassium onto the cell surface from the intracellular space. Hyperdepolarization is the basis for the occurrence of RCD - spreading depolarization of brain cells. The aura preceding a migraine attack is a consequence of RCD.

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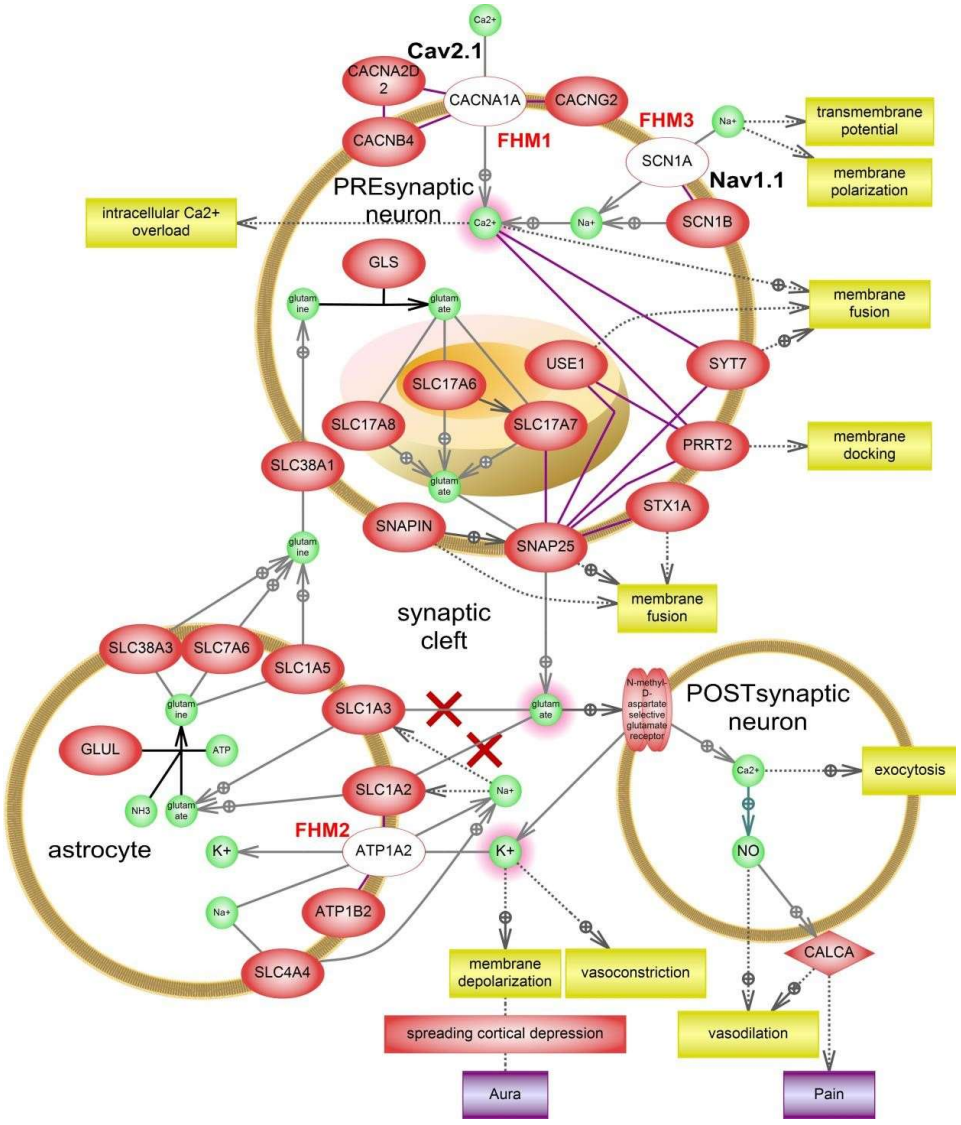

Figure 1. Signaling pathways leading to the development of cortical spreading depression => aura and vasodilation + pain. Whitened – proteins with loss of functionality. Highlighted in red – molecules with pathological increase in concentration.

Experiment Information

Run Name	1-60
Run Start	04.09.2024 11:18:57
Run Finish	04.09.2024 12:46:36
Operator	JUMAMURODOV
Notes	
Run On Software Version	Rotor-Gene Q Software 2.3.5.1
Run Signature	The Run Signature is valid.

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Gain Green	5,
Gain Yellow	5,
Gain Orange	5,
Gain Red	5,
Gain Blue	7,
Machine Serial No.	0121249

Scatter Analysis Information

Digital Filter	Light
Imported Analysis Settings	
No Template Control Threshold	% 15
Noise Slope Correction	No
Normalisation Method	Dynamic Tube Normalisation
Reaction Efficiency Threshold	Disabled
Start normalising from cycle	1

Potassium release also leads to vasoconstriction of nearby vessels. According to some authors, vasoconstriction precedes pain and vasodilation and occurs in parallel with RCD (Gunner et al., 2008; Viola et al., 2012). Then, in postsynaptic neurons, nitric oxide (NO) synthesis is induced, which in turn leads to the release of CGRP (CALCA), which, accordingly, leads to the development of vasodilation and pain. The glutamate-glutamine-glutamate cycle is shown on the left. Glutamate is synthesized in presynaptic neurons (GLS enzyme - glutaminase). Glutamate is removed from the synaptic cleft by astrocytes. In astrocytes, glutamate is converted to glutamine with the participation of ATP, NH3 and the GLUL enzyme (glutamate-ammonium ligase). Glutamine is then transported into the extracellular space and then captured by neurons, where it is converted back into glutamate. Thus, this study is the first to propose a model of signaling pathways for all forms of familial hemiplegic migraine. The novelty of this model lies in identifying a common point of intersection of pathological molecular processes - excess glutamate in the synaptic cleft, which then implements processes leading to the main symptoms of migraine. This model can be used as a starting point for creating signaling pathways for common migraine.

Sample No.	**Allele A**	**Allele G**	**Genotype**
1	22,55		AA
2	26,5	25,64	AG

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3	24,11		AA
4	22,45	21,66	AG
6	21,39		AA
7	23,18		AA
8	20,93		AA
9	21,98		AA
10	22,92	22,1	AG
11	22,11		AA
13	24,34		AA
15	23,19		AA
16	24,55	23,63	AG
17	23,87	23,08	AG
18		22,35	GG
19	23,35		AA
20	23,79		AA
21	25,45	24,78	AG
22	24,56	23,81	AG
23	23,84	23,2	AG
24		22,97	GG
25	24,44	23,79	AG
26	26,26	25,42	AG
27	25,16	24,57	AG
28		24,86	GG
29	26,27	25,65	AG
30	28,04	27,21	AG
31	26,53	25,89	AG
32	25,17		AA
33	22,64		AA
34	24,92		AA
35			
36	26,56	25,67	AG
37	27,33	26,7	AG
38	27,71	27,02	AG
39	27,42	26,88	AG
40	25,19		AA
41	25,62		AA
42	25,61	24,87	AG
43	29,78	29,1	AG
44	26,69	25,9	AG
45	26,71		AA
46	25,44	24,81	AG

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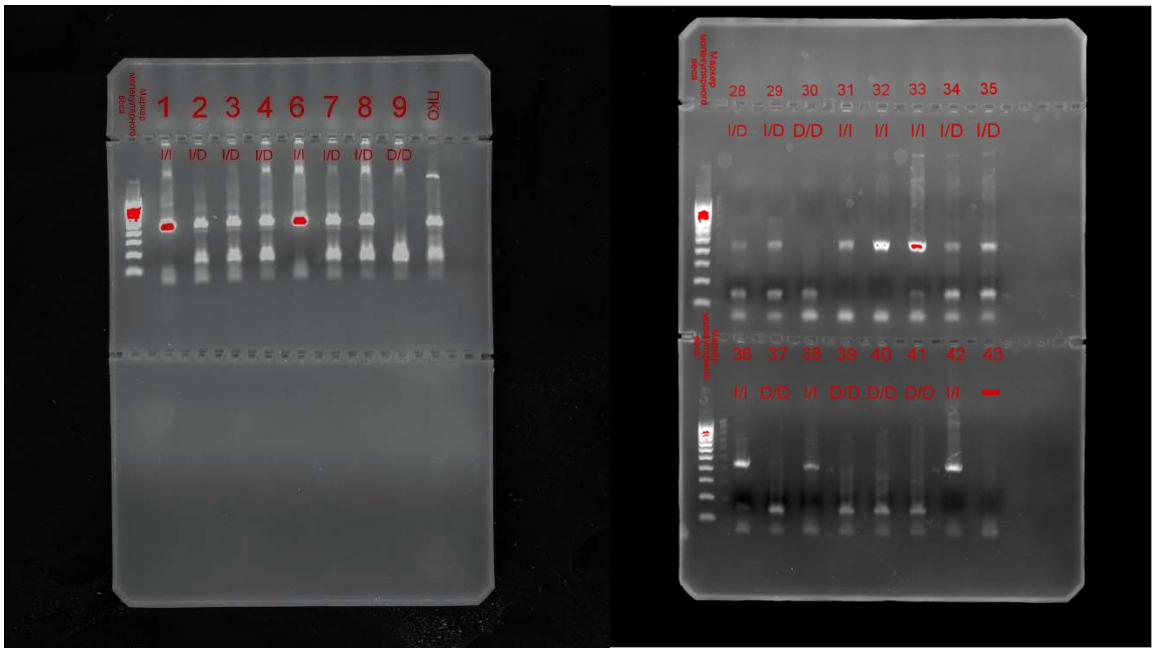
47	24,84		AA
48		24,01	GG
49	27,51	27,23	AG
50	26,43	26,15	AG
51	25,14	24,85	AG
52	25,28	24,91	AG
53	27,88	27,61	AG
54	22,93	22,82	AG
55	23,41		AA
56	24,42		AA
57	26,43	26,04	AG
58	31,64		AA
60	28,8	28,58	AG
Control genotype AA	24,17		AA
Control genotype AG	25,51	23,79	AG
Control genotype GG		21,77	GG

When constructing the signaling pathway diagrams of classical migraine, we relied on the existing signaling pathway diagrams of the SGM. The main theoretical premises were: the vascular theory of migraine, the role of spreading cortical depression at the initial stages of an attack, and the involvement of glutamate and dopamine in the pathogenesis of migraine.



Another criterion that also determined the strategy of the work was to place as

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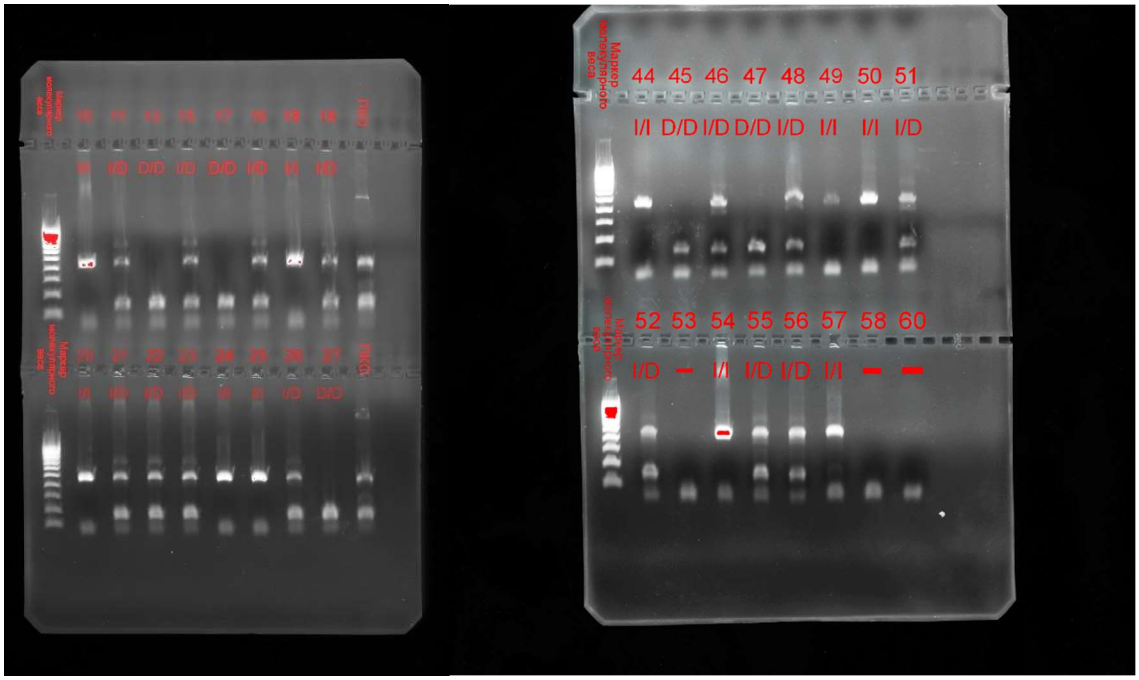
many molecules as possible from the created list of genes and proteins associated with migraine on the signaling pathway diagrams. A total of 8 signaling pathway diagrams were constructed: "Folate Cycle", "Dopamine Synthesis and Circulation", "Dopamine Effects in the Post-Synaptic Neuron", "Glutamate Release and Circulation", "Glutamate Effect in the Post-Synaptic Neuron", "Endothelial NO Synthase Activation in Vascular Endothelial Cells", "Processes Occurring in the Vascular Smooth Muscle Cell" and "Activation of CGRP Release (CALCA), Leading to Pain and Vasodilation". The first 7 were included in the abstract in a generalized form due to space limitations.



Recent studies (Eberhardt et al., 2014; Dux et al., 2015) have identified mechanisms that can simultaneously activate two processes associated with migraine: dilation of meningeal vessels (vasodilation) and pain. Nitric oxide (NO) and hydrogen sulfide (H2S) are synthesized in vascular endothelial cells and nerve endings. Endogenous H2S is produced primarily through the metabolism of sulfur-containing amino acids: starting with methionine and its conversion to homocysteine. In the body, H2S is formed with the participation of cystathionine-gamma-lyase (CTH) and cystathionine-beta-synthase (CBS). The CTH protein is localized mainly in vascular endothelial cells, and CBS is localized in neurons. Both enzymes use cysteine as the main substrate for H2S production (Huang et al., 2015). H2S also acts on the ATP-activated inward rectifier potassium channel of smooth muscle cells, which also promotes vasodilation.

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№	Full name	Year of birth	Date of capture of the BM	Diagnosis	DNA	Plasma	
1	Juraeva Xurshida	1982	06.03.2023	Migren	+	+	
2	Abdukadirova Dilfuza	1967	06.03.2023	Migren	+		sgustok
3	Nodirbekovna Xusnugul	1996	06.03.2023	Migren	+		sgustok
4	Akbarova Saida	1990	06.03.2023	Migren	+	+	
5							
6	Aslonova Gulxayo	1990	06.03.2023	Migren	+	+	
7	Abdugafforova Xilola	1997	06.03.2023	Migren	+	+	
8	Xolmatova Shoxida	1986	06.03.2023	Migren	+	+	
9	Ermatova Muazzam	1974	06.03.2023	Migren	+	+	
10	Abdullaeva O	1998	06.03.2023	Migren	+	+	
11	Sotvoldieva Maftuna	1993	06.03.2023	Migren	+	+	sgustok
12			yo‘q				
13	Abduraximova M	1983	13.05.2023	Migren	+		gemolis
14			yo‘q				
15	Abdullaeva Dilnoza	1992	13.05.2023	Migren	+	+	
16	Abdukadirov U T		13.05.2023	Migren	+	+	
17	Murodov Amir		13.05.2023	GBN	+	+	
18	Xafieva Ziyoda	1984	13.05.2023	Migren	+	+	
19	Mamatalieva Xurshida	1985	13.05.2023	Migren	+	+	
20	Eralieva Mashxura	1985	13.05.2023	Migren	+	+	
21	Mamadalieva Sh	1995	13.05.2023	Migren	+	+	
22	Samatova Gulnora	1981	13.05.2023	Migren	+	+	
23			13.05.2023	Migren	+	+	
24			13.05.2023	Migren	+	+	gemolis
25			13.05.2023	Migren	+	+	gemolis
26	Abdukodirov Ulugbek Taxirovich	1968		Migren	+	+	
27	Arevin Abramn Arminovna	1985		Migren	+	+	Gemolis
28	Kirgizova Lazokat	1984		Migren	+	+	
29	Munavvarova Mavluda	1988		Migren	+	+	
30	Gafurova Muxayyo	1991		Migren	+	+	
31	Dominova Umida	1991		Migren	+	+	
32	Mirzaeva Nilufar	1996		Migren	+	+	
33	Nizomova Nargiza	1980		Migren	+	+	
34	Ortikova Sadokat	1981		Migren	+	+	
35	Ismoilova Madina	1995		Migren	+	+	
36	Urinov Begzod	1991	19.01.2024	Migren	+	+	

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37	Oxunjonov Eldor	1991	19.01.2024	Migren	+	+	
38	Abduvoxidov Rasul		19.01.2024	Migren	+	+	
39	Chombarova Munira	1990	19.01.2024	Migren	+	+	
40	Xoshimova Muxlisa		19.01.2024	Migren	+	+	
41	Abdullaev Ibratullo		19.01.2024	Migren	+	+	
42	Toshboltaeva Gulola		19.01.2024	Migren	+	+	
43	Isroilova Madina		19.01.2024	Migren	+	+	
44	Xabibullaev Akmaljon		19.01.2024	Migren	+	+	
45	Tulanov Donier		19.01.2024	Migren	+	+	
46	Azimova Zilola	1987	19.01.2024	Migren	+	+	
47	Eminov Adxamjon	1998	19.01.2024	Migren	+	+	
48	Sarimsokava Zieda	1996	19.01.2024	Migren	+	+	
49	Abakulova Moxira	2001	19.01.2024	Migren	+	+	
50	Ukubjonova Rukiya	2001	19.02.2024	Migren	+	+	
51	Toxtasinova Farogat	1985	19.02.2024	Migren	+	+	
52	Abdulaxatova Feruza	1983	19.02.2024	Migren	+	+	
53	Tuxtasinova Farogat	1985	19.02.2024	Migren	+	+	
54	Sotvoldieva Xotira	1971	19.02.2024	Migren	+	+	
55	Ergasheva Dilrabo	1975	19.02.2024	Migren	+	+	
56	GafurovaGulnora	1982	19.02.2024	Migren	+	+	
57	Matkosimova Faragat	1990	19.02.2024	Migren	+	+	
58	MamadalievaUgiloy	1987	19.02.2024	Migren	+	-	gemolis
59	Izzatullaev Azizxon	2002	19..02.		empty proofreading		
60	Xotamjonov Farxod	2000		Migren	+	-	gemolis

CONCLUSION

1) For the first time, we constructed schemes of molecular signaling pathways describing hypothetical mechanisms of migraine pathogenesis based on a list of 147 genes functionally associated with migraine, which we created based on literature data. Fourteen genes (22 polymorphic sites) were selected for further molecular genetic analysis. 2) The frequencies of genotypes and alleles of substitutions in the ACE, BDNF, CCK, CCKAR, CCKBR, CGRP, DBH, MTDH, MTHFR, MTR, NOS1, NOS2, NOS3 and SNAP25 genes were determined in patients (n=146) suffering from migraine and the control group (n=363). Statistically significant associations with migraine were found for polymorphic variants of the genes CCKAR (rs1800857 TC+CC, p=9.2E-9), CCKBR (rs1805000 CT+TT, p=1.7E-9), MTHFR (rs1801133 CT+TT, p=0.001), NOS3 (rs2070744 genotype CC, p=0.026) and ACE (rs4646994 II+ID, p=0.030). The greatest

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contribution to the development of the disease is made by the CCKAR_rs1800857:C allele, which increases the risk of migraine by more than 9 times (RR=9.39). 3) 7 significant complex genotypes (OR>10) were identified, in which the migraine-associated allele CCKAR_rs1800857:C was presented. No new associations with migraine were identified. 4) The role of alleles of 5 genes included in 7 significant complex genotypes in changing molecular signaling pathways was assessed. Our data support the dopamine theory of migraine pathogenesis.

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