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Testing Genes Implicated in the Novel Case of Familial Hemiplegic Migraine

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Abstract Familial hemiplegic migraine (FHM) is a rare monogenetic form of migraine associated with aura and motor dysfunctions. Molecular mechanisms of FHM still remain unknown; however, genetic predisposition to the disease has been suggested. We report several cases of FHM diagnosed in three generations of ethnic Tatars, Russian Federation. Apart from clear family character, patients presented with typical features of FHM including aura and ataxia. In order to determine disease genetic markers in these cases, we sequenced several genes (*CACNA1A*, *ATP1A2*, and *SCN1A*) previously reported in FHM. Sequence analysis demonstrated absence of the previously identified mutations in genes studied. This is the first genetic study of FHM in the Tatar population. The lack of known FHM associated mutation in these patients suggests presence of yet unidentified mutations in some other genes. Further studies including full genome sequencing are needed to clarify the genetic background of FHM cases in the Tatar population.

Keywords Familial hemiplegic migraine (FHM) · *CACNA1A*, *ATP1A2*, *SCN1A* genes · Mutations

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1 Introduction

Migraine is the most common neurological disorder presented often (~30 % cases) with aura which is associated with sensory and motor disturbances. Familial hemiplegic migraine (FHM) is a rare monogenic type of migraine with aura for which several genes have been identified [1]. Each type of FHM is associated with mutations in representative gene. For example, mutations in *CACNA1A* are found in FHM1 [2]. Out of all these mutation, T666M was found in 40–50 % FHM1 cases [2, 3]. Also, mutations R1347Q and I1811L were found in several families with FHM1 [4]. More than 60 mutations in the gene *ATP1A2*, coding for α2 subunit of the glial and neuronal Na^+/K^+-ATPase, were found in FHM2 and sporadic migraine [2, 5–9]. It has been shown that, mutations in *SCN1A* gene, coding for α1 subunit of voltage-dependent Na+ channels, may cause FHM3. Currently, few mutations in these genes are identified [10–14].

In this study, the presence of known mutations in *CACNA1A*, *ATP1A2*, and *SCN1A* genes was investigated in three generations of FHM Tatar cases. Analysis revealed lack of mutations associated with FHM, suggesting novel mutations in cases studied. Further genetic analysis of the complete genome of Tatar FHM cases may reveal previously unknown mutations related to the disease pathogenesis.

2 Material and Methods

Blood samples collected from the four patients of the FHM family were used for DNA extraction (Lytech, Russia). The Institutional Review Board of the Kazan Federal University approved this study, and informed consent was obtained from each study subject according to

Table 1 Genes and tested amino acid changes (mutations) in our FHM patients

Gene	Codon	Amino acid change	SNP position (nt)	DNA variation
CACNA1A	192	R→Q	850	G→A
	666	T→M	2272	C→T
	714	V→A	2416	T→C
	1347	R→Q	4315	G→A
	1811	I→L	5706	A→C
ATP1A2	376	T→M	1231	C→T
	378	T→N	1237	C→A
	689	R→Q	2170	G→A
	717	N→K	2255	C→A/G
	731	M→T	2296	T→C
	999	D→H	2995	G→C
SCN1A	263	L→V	787	C→G
	1174	T→S	3521	C→G
	1489	Q→K	4450	C→A

the guidelines approved under this protocol (article 20, Federal Law "Protection of Health Right of Citizens of Russian Federation" N323–FZ, 11.21.2011). Informed consent for this publication was obtained from the living patients. DNA samples were analyzed for the presence of mutations in genes known associated with FHM, i.e., *CACNA1A*, *ATP1A2*, and *SCN1A*, using polymerase chain reaction (PCR). One microliter of DNA (25 ng) was mixed with 2 μL 10× PCR buffer, 2 μL 25 mM $MgCl_2$, 0.2 μL (100 pM) of each primer, 0.8 μL 25 mM dNTP, 13.3 μL LH_2O, and 0.5 μL (2.5 U) Taq DNA polymerase (Sileks, Russia). Amplification was performed as follows: (1) DNA denaturation at 95 °C, 5 min; (2) 40 cycles at 95 °C, 30 s; 56 °C (for *CACNA1A* exons 4, 16, 17, 25, and 36 and *ATP1A2* exons 9, 10, 15, 16, and 23) or 53 °C (for *SCN1A* exons 6, 17, and 23), 30 s; 72 °C, 25 s (for *CACNA1A*), 45 s (for *ATP1A2*), or 40 s (for *SCN1A*); (3) final extension at 72 °C, 5 min; (4) reaction termination at 4 °C. PCR products were sequenced using 3730 DNA Analyzer (Life technologies, Carlsbad, CA, USA). Analyzed mutations in *CACNA1A*, *ATP1A2*, and *SCN1A* genes are summarized in Table 1. Primers used for PCR amplification and sequencing analysis are summarized in Table 2.

3 Results and Discussion

The Tatar family involved in this study included 13 individuals, where four members were diagnosed with FHM at the regional neurological hospital. These patients include father (patient F; proband) and his brother (patient B), as well as the daughter (patient D) and son (patient S) of patient F. Severe clinical presentation of FHM was characteristic to proband and his brother. Both patients had headache lasting for several hours, accompanied by nausea and intolerance to light and sound. Migraine attacks appeared 2–3 times per year during the last 30 years. Notably, clinical symptoms of FHM were

Table 2 Primers used for PCR amplification and sequencing analysis

Primer	Sequence (5′→3′)	Reference
CAC-e4-F	aaaacccaccctctgttctc	[16]
CAC-e4-R	ttgtcagggtcggaaactca	[16]
CAC-e16-F	tccacagctgcatctccaag	[16]
CAC-e16-R	accctcccttgagcccct	[16]
CAC-e17-F	cagtggttgcttttcctgac	[16]
CAC-e17-R	ttgccagagaaacattctcc	[16]
CAC-e25-F	ctacccaacctgacctctgc	[16]
CAC-e25-R	acatgataaccctgacagtc	[16]
CAC-e36-F	ttcattccctcggtctctgc	[16]
CAC-e36-R	ctgactgaacctgtgagac	[16]
ATP-e9-F	gccacggtctagggtaaggt	[6]
ATP-e910-R	gcaagaggctttggagacac	[6]
ATP-e15-F	aggaggggctggtacaggt	[5]
ATP-e15-R	gaagacggccacacttgag	[5]
ATP-e16-F	ccaagacaagcatggagtga	[5]
ATP-e16-R	aagggacagggaacagaggt	[5]
ATP-e22-F	agaagaggctgttggaagaagaca	[9]
ATP-e22-R	gcaggaaccagtagtgggagtgga	[9]
SCN-e6-F	ttgcttctccactagcgttg	[11]
SCN-e6-R	ggatatccagcccctcaagt	
SCN-e17-F	cttcccctattcaatctctc	
SCN-e17-R	tcttccacatattgggcag	
SCN-e23-F	cagggatgttttttagagg	
SCN-e23-R	ggatagtgaatgacagagg	

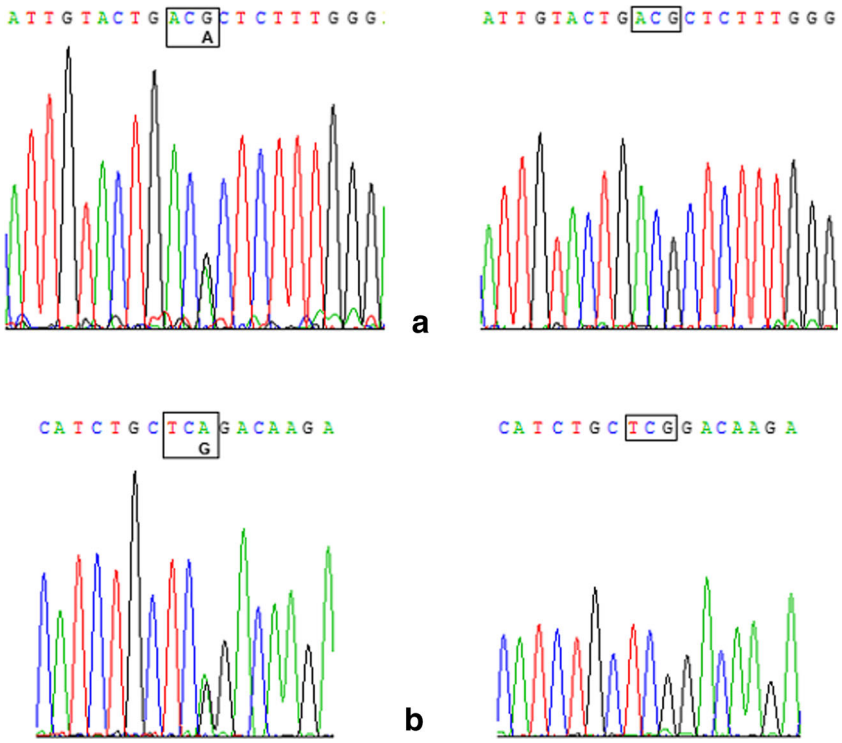

Fig. 1 **a** Electrophoregram of sequences of exon 16 of the gene *CACNA1A*. *Left*—with a mutation of G→A, *right*—control. **b** Electropherogram of sequences of exon 9 of the gene *ATP1A2*. *Left*—with a mutation of G→A, right—control

mild in patient S, which were limited to a single pulsation headache attack without clear aura.

Presence of mutations in *CACNA1A*, *ATP1A2*, and *SCN1A* genes, previously shown associated with FHM, was analyzed to establish the genetic predisposition to the disease. A total of 12 exons in three genes were analyzed using PCR. Analysis included four exons 4, 16, 17, 25, and 36 in the *CACNA1A* gene, encoding for the α1A subunit of voltage-gated Cav2.1 calcium channels. Missense mutations R192Q, T666M, V714A, R1347Q, and I1811L had been previously found in FHM [4, 15–17]. Additionally, the exons 9, 15, 16, and 22 in the *ATP1A2* gene were analyzed. The *ATP1A2* gene codes for the α2 subunit of Na^+/K^+-ATPase, where several mutations (T378N, R689Q, N717K, M731T, and D999H) were found in FHM cases [5–9, 18]. Finally, Tatar FHM cases studied were examined for the presence of mutations in the exons 6, 17, and 23 of the *SCN1A* gene, coding for the α1 subunit of voltage-gated sodium channel Nav1.1. Amino acid-altering mutations in these exons (L263V, T1174S, and Q1489K) were previously found in FHM cases [11–14]. Interestingly, Tatar FHM cases lack previously described mutations in these exons. However, there was a single nucleotide substitution identified in *CACNA1A* gene in patients F, B, and D at the third position of the codon 698 (ACG to ACA), which did not cause amino acid change. Additionally, silent mutation was identified in *ATP1A2* gene (codon 373; TCG→TCA) in patients F and S (Fig. 1). Mutations previously shown to be associated with FHM were absent in the Tatar cases. Similar to our results, absence of known mutations in FHM genes was reported by Christensen et al. and LaBianca et al. in FHM cases [19, 20].

4 Conclusions

We found lack of association between studied Tatar cases and known mutations in *CACNA1A*, *ATP1A2*, and *SCN1A* genes previously found in the FHM. We believe that FHM in studied Tatar cases is associated with a novel mutations, possibly, in genes not included in this study. We postulate that sequencing a complete genome of the Tatar FHM cases will help to identify new mutations, thus improving our understanding of the disease genetic predisposition. Molecular and genetic studies of FHM revealed fundamental role of the genetic mechanisms explaining many of clinical features of the migraine. Collected data will facilitate the identification of the novel genetics biomarkers of the FHM, which will improve diagnosis of the disease.

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Compliance with Ethical Standards The Institutional Review Board of the Kazan Federal University approved this study, and informed consent was obtained from each study subject according to the guidelines approved under this protocol (article 20, Federal Law "Protection of Health Right of Citizens of Russian Federation" N323–FZ, 11.21.2011). Informed consent for this publication was obtained from the living patients.

Conflict of Interest The authors declare that they have no conflict of interest.

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