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Case report

Identification of the first in Poland *CACNA1A* gene mutation in familial hemiplegic migraine. Case report

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ARTICLE INFO

Article history:
Received 8 August 2016
Accepted 9 January 2017
Available online 21 January 2017

Keywords:
Migraine
Familial hemiplegic migraine
Calcium channel
CACNA1A

ABSTRACT

Introduction: Migraine is a common neurological disorder characterized by a particular phenotype, complex pathophysiology and a heterogeneous genetic background. Among several heritable forms, familial hemiplegic migraine is the best described one. In the majority of cases it is caused by mutations in one of three different genes.

Case report: Clinical symptoms of a 47 year old proband (and independently described in his 20 year old son) as well as differential diagnosis are discussed in the presented report. The most characteristic were recurrent attacks of blurred vision, paresthesias and hemiparesis often accompanied by speech disturbances and followed by severe headache with vomiting. Advanced morphological and genetic procedures were required to exclude MELAS, CADASIL and Call-Fleming syndrome. Finally, the definite diagnosis was possible after the application of the whole exome sequencing technique. It confirmed, for the first time in the Polish population, a heterozygous T666M mutation (c.1997C>T; p.Thr666Met) in the *CACNA1A* gene in the proband, the proband's son and in several other family members.

Conclusion: The presented report provides clinical and genetic insight into familial hemiplegic migraine 1 resulting from a mutation in the *CACNA1A* gene.

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http://dx.doi.org/10.1016/j.pjnns.2017.01.005

1. Introduction

Migraine usually affects persons between the ages of 22 and 55 years and is considered to be one of the most common neurological disorders worldwide. It is estimated that about 17% of females and 8% of males in Europe suffer from migraine [1]. Familial hemiplegic migraine (FHM) was the first genetically confirmed heritable migraine described in the literature. Three subtypes of familial hemiplegic migraine have been identified. Familial hemiplegic migraine 1 (FHM1) is caused by mutations in the pore-forming $\alpha 1$ subunit of the P/Q type calcium channel (CACNA1A). FHM2 (ATP1A2) results from mutations in the $\alpha 2$ subunit of the Na^+/K^+-ATPase pump, while FHM3 (SCN1A) from mutations in the $\alpha 1$ subunit of the neuronal voltage-gated $Na_v1.1$ channel. Further genetic analyses provided evidence of other genes involved in migraine pathogenesis and explained neuronal hyperexcitability of the migraine brain [2].

Familial hemiplegic migraine is an autosomal dominant disorder which typically begins in the first or second decade of life [3]. Recurrent migraine attacks are usually preceded by aura. Attacks are characterized by unilateral motor weakness which lasts from minutes to days or even weeks and are accompanied or followed by headache. Rarely a decreased level of consciousness or fever are observed during the attacks. Detailed family history should reveal similar symptoms in close relatives. It is estimated that about 50% of cases are accompanied by progressive cerebellar syndrome. FHM should be always differentiated with epilepsy and postictal paresis and with numerous other syndromes such as cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), hereditary hemorrhagic teleangiectasia, hereditary amyloid angiopathy, familial cerebral cavernous malformation and benign familial infantile convulsions [3]. Population studies in Denmark estimated the prevalence of FHM as 0.01% with predominance in women. FHM begins in the first decade of life. According to one publication, mutations in three genes, *CACNA1A*, *ATP1A2* and *SCN1A* account for about 50–70% of familial cases and about 50% of cases are caused by mutations in the *CACNA1A* gene [3]. Studies in Denmark gave different results, only 14% of FHM families harbored mutations in the *CACNA1A* and *ATP1A2* genes [4].

2. Patients and methods

2.1. *Proband*

A 47 year old man (IV:12, Fig. 1) was for the first time admitted to our hospital in 2012. From 19 years of age he suffered from episodes of blurred vision, paresthesia and hemiparesis of left or right side sometimes accompanied by speech disturbances and followed by severe headache with vomiting. Till 2012 he was hospitalized several times in several neurological departments and many diagnoses were considered including mitochondrial encephalomyopathy, familial hemiplegic migraine and Call-Fleming syndrome.

At admission neurological examination revealed a high arched palate, horizontal nystagmus, mild dysarthria, discrete weakness and atrophy of forearm muscles, discreet left-sided ataxia, positive Romberg test and gait on expanded base. Routine laboratory tests were normal despite mild hypercholesterolemia (total cholesterol level 232 mg/dl, normal range 120–200 mg/dl), hypertrigliceridemia (382 mg/dl, normal range 50–150 mg/dl) and slightly reduced vitamin B_{12} level (178.8 pg/ml, normal range 191–663 pg/ml). Additionally in studies performed a few years earlier elevated blood lactate level and slightly increased cerebrospinal fluid protein level (47 mg/dl, normal range 15–45 mg/dl) were detected. Magnetic resonance imaging (MRI) showed on T2-weighted and FLAIR images numerous disseminated hyperintensive lesions in both hemispheres and moderate cortical and subcortical atrophy with marked cerebellum atrophy and slight enlargement of ventricular system (Fig. 2a–c). There was no progression comparing to the previous radiological studies. Additionally, magnetic resonance spectroscopy revealed unspecific decrease of N-acetylaspartate in the cingulate gyrus. Magnetic resonance angiography was normal. Initially Call–Fleming syndrome was suspected, however, the reversible cerebral

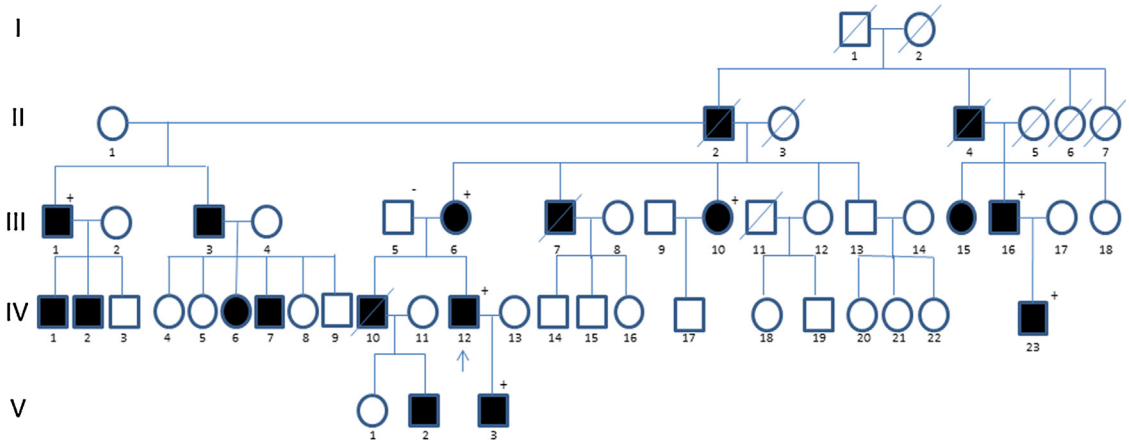

Fig. 1 – Pedigree of the family with T666M mutation in the *CACNA1A* gene: I–V indicate generations, "+" or "−" indicate the presence of a mutation or lack thereof, respectively. Proband (IV:12) marked by an arrow.

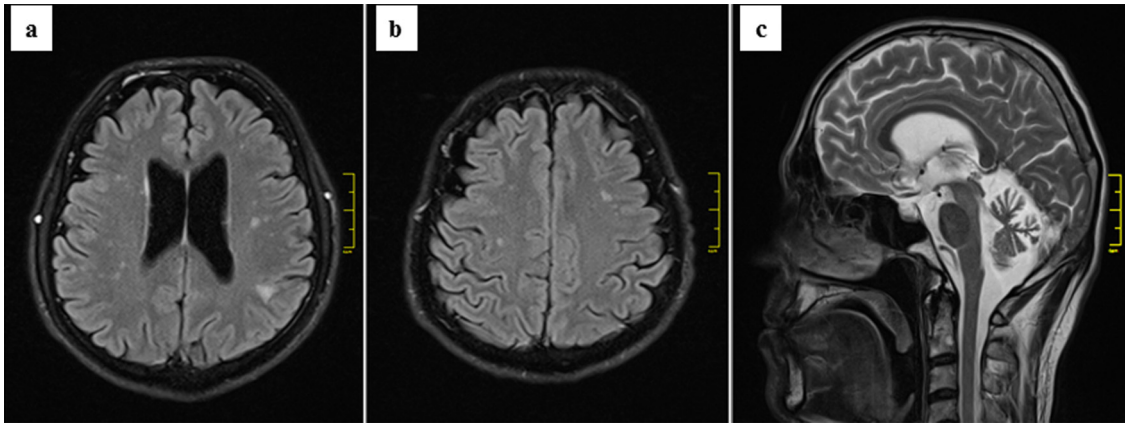

Fig. 2 – Proband's brain MRI: (a, b) diffused hyperintensive lesions in both hemispheres on FLAIR images; (c) cerebellum atrophy on T2-weighted image.

artery spasm was excluded in the test with intensive physical exercise. Ultrasound Doppler examination was normal apart from small atherosclerotic plaques in the bifurcation of common carotid arteries and in the interior carotid arteries. Echocardiography presented no abnormalities. Visual as well as brain stem auditory evoked potentials were normal. Both nerve conduction studies and electromyography (EMG) of biceps brachii and vastus lateralis muscles were normal. Electroencephalography (EEG) showed generalized epileptic discharges more pronounced in the left hemisphere. Only mild difficulties in learning and decreased direct memory were found on neuropsychological assessment. Skeletal muscle biopsy was performed twice, however, neither showed characteristic abnormalities and ragged-red fibers were not found.

Previous genetic studies excluded mutations in the *NOTCH3* gene, responsible for cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). In addition to the genetic studies detailed ultrastructural examination of vessels in muscle biopsy material was performed. It also excluded CADASIL but revealed numerous abnormal microvessels with pale, swollen endothelial cells and pericytes (Fig. 3). Edema of the former frequently considerably narrowed the vessel lumen. Affected cells showed clear cytoplasm, enlarged mitochondria, distended endoplasmic reticulum and abundant vacuolar structures.

2.2. *Proband's son*

The 20 year old man (V:3, Fig. 1) was hospitalized for the first time at the age of 8 years. He was born in 37 hbd, with hypotrophy (birth weight 1650 g) and he had 8 points on the Apgar scale. Nevertheless, later psychomotor development was normal. From the fifth year of age he suffered from recurrent paroxysmal episodes of intermittent aphasia, hemiparesis and paresthesia accompanied by headache and vomiting. Between the episodes neurological examination was normal. Blood tests showed an elevated lactate level. Brain magnetic resonance imaging showed on T2-weighted and FLAIR images disseminated hyperintensive lesions in both hemispheres. Both MRI angiography and spectroscopy excluded other specific abnormalities. At the age of 12 the occlusion of patent foramen ovale was performed. Electroencephalography examination showed generalized epileptic discharges both during neurological episodes and between them. Nerve conduction studies were normal and EMG showed myopathic changes in the biceps brachii muscle. To diagnose suspected mitochondrial encephalomyopathy a skeletal muscle biopsy was performed. Both histopathological and histochemical studies of the muscle sample presented no abnormalities. Ultrastructural examination of blood vessels in the skeletal muscle biopsy showed the same type of abnormalities as in the proband but of lesser severity (Fig. 4). Additionally spectrophotometric analysis of electron transport chain complexes did not detect any disturbances.

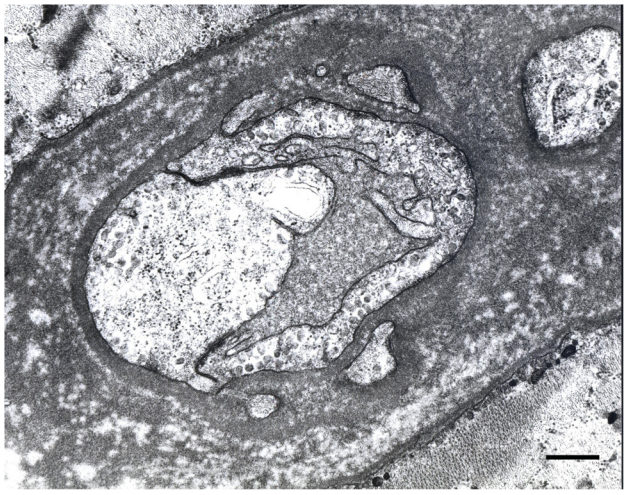

Fig. 3 – Proband's skeletal muscle. Capillary vessel with swollen endothelial cell considerably narrowing vessel lumen, and swollen pericyte processes. The affected cells show clear cytoplasm, enlarged mitochondria and distended endoplasmic reticulum. Bar 500 nm.

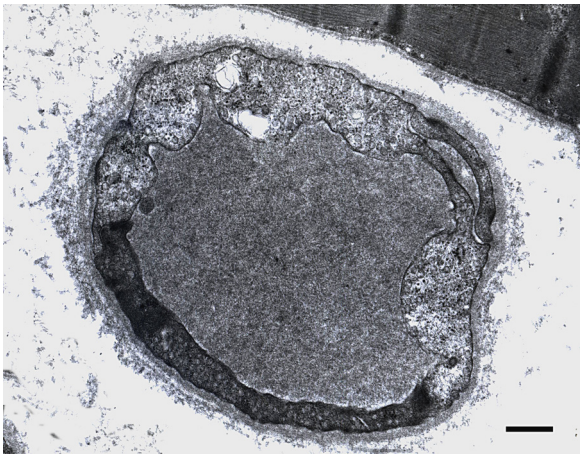

Fig. 4 – Proband's son's skeletal muscle. In capillary vessel visible moderately swollen endothelial cell with cytoplasm paler than nearby normal cell. Bar 500 nm.

2.3. *Proband's family*

Detailed family history disclosed that similar symptoms were recorded in 16 other family members, 12 men and 4 women. The presented family pedigree suggested autosomal dominant inheritance (Fig. 1).

2.4. *Molecular study*

Genetic studies toward mitochondrial encephalomyopathies were done in the proband and his son. Whole exome sequencing (WES) was performed as described previously [5].

3. Results and discussion

Molecular diagnosis toward mitochondrial encephalomyopathies in the proband revealed only the presence of a single mitochondrial DNA deletion with a low level of heteroplasmy in the muscle tissue. No mutations were detected in coding region of *POLG* and *C10orf2* genes. In the proband's son all the most common point mutations and mitochondrial DNA deletions were excluded by appropriate tests in blood. WES was performed in an arbitrarily chosen member of the family (III:16) to look for a candidate gene. The study revealed a known heterozygous T666M mutation in the *CACNA1A* gene (Fig. 5). The linkage of the T666M variant with the disease in the family was assessed by Sanger sequencing in 8 individuals, including the proband, his son and individual III:16 (Fig. 1). The heterozygous T666M mutation in the *CACNA1A* gene was detected in the proband (IV:12) and all affected relatives studied: the son (V:3), the proband's mother (III:6) and his four cousins (III:1, III:10, III:16, IV:23). The mutation was not detected in the healthy father of the proband (III:5). Till now blood samples from other family members are not available.

The presented study of a first to our knowledge Polish family with the heterozygous T666M mutation (c.1997C>T; p.Thr666Met) in the *CACNA1A* gene involved a comprehensive

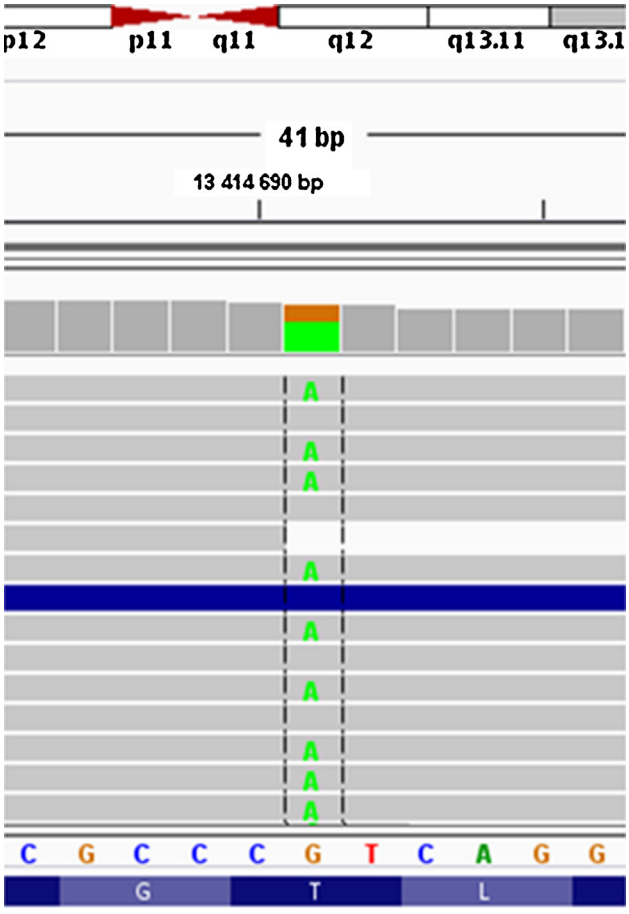

Fig. 5 – The T666M (c.1997C>T; p.Thr666Met) mutation in *CACNA1A* gene (RefSeq:NM_001127221.1) detected using whole-exome sequencing (WES). The analysis was performed using a Nextera Rapid Capture Exome kit (Illumina, San Diego, CA, USA) on the HiSeq 1500 according to the manufacturer's protocol.

and long lasting diagnostic work-up. The confirmed mutation was previously described in the literature, however, until recently such genetic studies were not available apart from strictly scientific purposes. Due to whole exome sequencing the definite diagnosis was made. The proband's migraine with transient hemiparesis as well as cerebellum atrophy and episodic ataxia were comparable to those previously described in the literature. In the meantime, several other hereditary disorders were excluded in our patient. Although generally mitochondrial disorders are maternally inherited, we considered the co-existence of multiple deletions or mitochondrial DNA depletion syndrome followed by the mutation in one of the nuclear genes responsible for imbalance of the respiratory chain. However, neither skeletal muscle biopsy nor further genetic analysis confirmed that. It is worth mentioning that an elevated lactate level is not specific and does not exclusively indicate a mitochondrial disorder. It may be connected with various conditions, for example with ischemic brain injury, epilepsy, encephalitis and with mitochondrial disorders. The most indicative for the final clinical diagnosis of FHM1 were episodes of paroxysmal and transient neurological deficits,

similar in a few family members. The symptoms of mitochondrial disorders are heterogeneous and highly variable even within one family with the same mutation. Additionally, symptoms are usually aggravated by infections, stressful situations and exercise. In the presented case the episodes were not correlated with any of the above conditions.

According to the literature data, recurrent and transient attacks of hemiparesis and paresthesias in several family members are the most characteristic for familial hemiplegic migraine. In single cases triggering coexistence with fever was reported, however, in all situations the patients recovered completely [6]. For many years patients were often suspected of having epilepsy rather than suffering from any genetic disorder and antiepileptic drugs were administered. Brain atrophy, especially in the cerebellum was reported in the literature and correlated with migraine-induced ischemic injury and not always with the disease duration [6]. In our case hyperintensive lesions were noted both in the proband's and his son's brain MRI, however, cerebellum atrophy was observed only in the proband's MRI (Fig. 2c). Chronic and progressive cerebellar ataxia was reported independently from migraine attacks. Among additional clinical features epileptic seizures and mental retardation were documented [7]. Several different mutations in the *CACNA1A* gene were also identified in episodic ataxia type 2 (EA-2) and spinocerebellar ataxia type 6 (SCA6) partially explaining the overlapping syndromes in some families [8,9]. However, the T666M mutation was almost exclusively associated with FHM1 with progressive cerebellar ataxia phenotype. The recurrence of cerebellar symptoms was observed in at least 20% of affected families [10,11]. Partial efficacy of acetazolamide in preventing paroxysmal attacks was also reported in FMH1 cases [10]. Extensive studies of the clinical spectrum of 117 patients with familial hemiplegic migraine revealed that symptoms usually started at a young age of about 11.7 years with a range from 1 to 51 years [12]. Moreover, the T666M mutation was more often associated with hemiplegic migraine, severe attacks with coma and nystagmus [12]. Taking all data into account in patients with the same mutation clinical variability was marked, which suggested the influence of other genetic or environmental factors [12]. Similar findings were obtained by other groups, for example from Korean patient studies [13]. They indicated even the absence of headache in some cases and generally strongly heterogeneous phenotypes and a meaningful role of neuroimaging studies. Abnormalities described on MRI are usually nonspecific, however, cerebellar atrophy as well as disturbances in cerebral blood flow during incidents were frequent [13].

Ultrastructural examination of vessels in the proband's and his son's muscle biopsies revealed pathological changes in microvessels resembling onkosis. Three of the six different types of voltage-gated calcium channels are known to be present in vessels: high-voltage activated L- and P/Q-type channels, and low-voltage activated T-type channel [14]. In the brain, P/Q-type calcium channels are mainly located in presynaptic terminals of neurons. However, there is growing evidence that they could be also present in the cerebral vasculature [15]. In familiar hemiplegic migraine with *CACNA1A* gene mutation altered channels open more easily than usual leading to increased cell influx of calcium ions and affecting endoplasmic reticulum and mitochondrial calcium concentrations [16]. Therefore ultrastructural abnormalities in cytoplasmic organelles and edema of vessel wall cells observed by us may be either a direct consequence of disturbed calcium influx or a manifestation of secondary damage due to increased susceptibility of vessels to glutaminergic transmission and/or cortical spreading depression.

Despite numerous studies, the definite pathogenesis of migraine is still not known. Among heritable forms, only in a few monogenic syndromes the direct genetic factors have been recognized [17]. Therefore, a wide collaboration is needed in order to identify different forms of migraine, its genetic background and pathogenesis.

4. Conclusions

Familial hemiplegic migraine is one of the monogenic autosomal dominant forms of migraine. Its comprehensive phenotype requires variable diagnostic procedures to exclude other conditions and establish a final diagnosis. Detailed family history and advanced genetic techniques are crucial tools both in diagnostic work-up and in better understanding of underlying pathophysiological mechanisms.

Conflict of interest

None declared.

Consent for publication

We obtained written informed consent from the proband and proband's son for publication of this case report and any accompanying images. A copy of the written consents is available for review by the corresponding author.

Authors' contributions

Study concept and design: BK, DDz, EP, AK. Acquisition of data: BK, DDz, AK. Family pedigree design: JT. Molecular analysis for mitochondrial disease design: KT. Sequencing and analysis *POLG* and *C10orf2* genes, mtDNA deletion screening: MK. Whole exome sequencing studies: JT, RP. Analysis and interpretation of data: BK, DDz, JT, DR, DP-A, RP, AK. Drafting of the manuscript: BK, DDz, EB. Critical revision of the manuscript for important intellectual content: EP, EB, AK. All authors read and approved the final manuscript.

Acknowledgements and financial support

Many physicians and biologists from different centers in Poland have contributed to the study: Dr. Malgorzata Kuzior-Plawiak, Dr. Marek Bodzioch and Dr. Rafal Rola should be mentioned among others. The diagnosis would not be possible without invaluable participation of the proband, who managed to stimulate joint efforts of professionals.

The study was supported by the Children's Memorial Health Institute project no. S136/13 and EU Structural Funds Project POIG.02.01.00-14-059/09. Mitochondrial DNA studies were supported by the Ministry of Science and Higher Education grant N N401 049238. Sequencing of *POLG* and *C10orf2* genes was supported by the EU OPI Program via the Foundation for Polish Science (VENTURES/2011-8/3).

Ethics

The study was approved by the Bioethical Committee at the Medical University of Warsaw (No KB/156/2008 and KB/17/R/2011) and by the Bioethical Committee at The Children's Memorial Health Institute (for project no. S136/13).

REFERENCES

[1] Stovner LJ, Hagen K. Prevalence, burden, and cost of headache disorders. Curr Opin Neurol 2006;19:281–5.
[2] Burstein R, Noseda R, Borsook D. Migraine: multiple processes, complex pathophysiology. J Neurosci 2015;35(17):6619–29.
[3] Kazemi H, Speckmann EJ, Gorji A. Familial hemiplegic migraine and spreading depression. Iran J Child Neurol 2014;8(3):6–11.
[4] Thomsen LL, Kirchmann M, Bjornsson A, Stefansson H, Jensen RM, Fasquel AC, et al. The genetic spectrum of a population-based sample of familial hemiplegic migraine. Brain 2007;130:346–56.
[5] Pronicka E, Piekutowska-Abramczuk D, Ciara E, Trubicka J, Rokicki D, Karkucińska-Więckowska A, et al. New perspective in diagnostics of mitochondrial disorders: two years' experience with whole-exome sequencing at a national paediatric centre. J Transl Med 2016;14:174. http://dx.doi.org/10.1186/s12967-016-0930-9
[6] Tashiro Y, Yamazaki T, Nagamine S, Mizuno Y, Yoshiki A, Okamoto K. Repeated encephalopathy and hemicerebral atrophy in a patient with familial hemiplegic migraine type 1. Intern Med 2014;53:2245–50.
[7] Carreño O, Corominas R, Serra SA, Sintas C, Fernández-Castillo N, Vila-Pueyo M, et al. Screening for CACNA1A and ATP1A2 genes in hemiplegic migraine: clinical, genetic and functional studies. Mol Genet Genomic Med 2013;1(4):206–22.
[8] Takahashi T, Igarashi S, Kimura T, Hozumi I, Kawachi I, Onodera O, et al. Japanese cases of familial hemiplegic migraine with cerebellar ataxia carrying a T666M mutation in the CACNA1A gene. J Neurol Neurosurg Psychiatry 2002;72(5):676–7.
[9] Ophoff RA, Terwindt GM, Vergouwe MN, van Eijk R, Oefner PJ, Hoffman SM, et al. Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca21 channel gene CACNL1A4. Cell 1996;87:543–52.
[10] Wada T, Kobayashi N, Takahashi Y, Aoki T, Watanabe T, Saitoh S. Wide clinical variability in a family with a CACNA1A T666M mutation: hemiplegic migraine, coma, and progressive ataxia. Pediatr Neurol 2002;26(1):47–50.
[11] Ducros A, Denier C, Joutel A, Vahedi K, Michel A, Darcel F, et al. Recurrence of the T666M calcium channel CACNA1A gene mutation in familial hemiplegic migraine with progressive cerebellar ataxia. Am J Hum Genet 1999;64(1):89–98.
[12] Ducros A, Denier C, Joutel A, Cecillon M, Lescoat C, Vahedi K, et al. The clinical spectrum of familial hemiplegic migraine associated with mutations in a neuronal calcium channel. N Engl J Med 2001;345(1):17–24.
[13] Choi KH, Kim JS, Lee SY, Ryu SW, Kim SS, Lee SH, et al. Familial hemiplegic migraine with prolonged coma and cerebellar atrophy: CACNA1A T666M mutation in a Korean family. J Korean Med Sci 2012;27(9):1124–7.
[14] Hansen PB, Jensen BL, Andreasen D, Friis UG, Skøtt O. Vascular smooth muscle cells express the alpha(1a) subunit of a P/Q-type voltage-dependent Ca(2+) channel, and it is functionally important in renal afferent arterioles. Circ Res 2000;87:896–902.
[15] Hansen PB. New role of P/Q-type voltage-gated calcium channels: from transmitter release to contraction of renal vasculature. J Cardiovasc Pharmacol 2015;65:406–11.
[16] Wheeler DG, Groth RD, Ma H, Barrett CF, Owen SF, Safa P, et al. Ca(V)1 and Ca(V)2 channels engage distinct modes of Ca(2+) signaling to control CREB-dependent gene expression. Cell 2012;149:1112–24.
[17] Schürks M. Genetics of migraine in the age of genome-wide association studies. J Headache Pain 2012;13:1–9.