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Original Article

Novel $KCNQ_4$ gene mutations contribute to susceptibility of genetic febrile seizures

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Abstract: Objective: To identify genes potentially accounting for pathogenesis of genetic febrile seizures. Methods: 31 febrile seizures families and 200 healthy patients were included in this study. A high throughput gene capture approach was used to identify candidate genes which were then validated using Sanger sequencing. Pedigree analysis was applied to evaluate phenotype and genotype. Sequencing PCR products was used to screen gene mutations in the control group. Results: We found three $KCNQ_4$ gene missense mutations c.1609C>T (P537S), c.1207G>A (A403T), and c.1177G>T (G393C) in exons 9, 10, and 13, respectively. Each patient only had one of these mutations among three of all 31 febrile seizures families. $KCNQ_4$ gene mutations were not found in the other 28 febrile seizures families and the healthy control group. We also found a missense SCN5A gene mutation c.2893C>T (R965C) in proband 1 family but not the other two febrile seizures families. Pedigree analysis indicated that some mutation-carriers in these members were not febrile seizures. However, the proband 1 and his elder brother carried both the $KCNQ_4$ and SCN5A gene mutations, and probands 2 and 3 carried $KCNQ_4$ gene mutation. Conclusion: Novel $KCNQ_4$ gene mutations and SCN5A gene mutation were not single causal factors for febrile seizures. They most likely interacted with other gene mutations to promote a susceptibility to febrile seizures. Our findings add to the literature that $KCNQ_4$ gene mutations are previously found to be only associated with hearing loss. Our finding provides a new clue to investigate the genetic etiology of febrile seizures.

Keywords: Genetic febrile seizures, $KCNQ_4$, SCN5A, gene mutation, pedigree analysis

Introduction

Febrile seizures have been defined as any seizure associated with >38°C (rectal or tympanic) fever without CNS infection in a child aged 6 months to 5 years. Febrile seizures occur in 2-5% of infants in Europe and North America and in 6-9% in Japan. It is the most common convulsive event in children younger than 5 years of age [1-3]. Most febrile seizures are self-limited with age, but people who have had febrile seizures have a higher risk of developing spontaneous afebrile seizures which defines epilepsy when they recur [4]. The cause of febrile seizures is multifactorial in nature. Several susceptibility genes have been identified, including (1) genes encoding ion channels, such as SCN1A, SCN1B, and SCN2A genes that encode neuronal voltage-gated sodium ion channels and GABRG2 genes that encode ligand-gated chloride ion channel of the GABAA receptor γ2 subunits [5-7]. (2) genes encoding proinflammatory and anti-inflammatory cytokines,such as interleukin 1α (IL-1α), IL-1 receptor antagonist (IL-1 Ra) IL-1β, IL-6, and tumor necrosis factorα (TNF-α) [8-11]. Mutations or polymorphisms in these genes are associated with development of febrile seizures. We hypothesized that other genes may also account for the pathogenesis of febrile seizures.

The KCNQ gene family encodes five Kv7 K^+ channel subunits (Kv7.1-Kv7.5), which are voltage gated potassium ion channels. Kv7 subunits are involved in reducing membrane excitability [12-14]. Kv7.2-Kv7.5 are expressed in the nervous system [15]. Studies have shown that Kv7.2 and Kv7.3 mutations are implicated in

Table 1. Primers for $KCNQ_4$ gene and SCN5A gene

Name	Sequence	Tm
$KCNQ_4$ E13-F	5'-TACTTGTCAGGTTCGTGTGTGG-3'	56.62
$KCNQ_4$ E13-R	5'-GAGGGGGCTCTTAGGTCTTCT-3	56.1
$KCNQ_4$ E10-F	5'-GCTAACTTGGCTCTCTCCC-3'	52.62
$KCNQ_4$ E10-R	5'-TGCTGCTAACCCCTATGAA-3'	50.95
$KCNQ_4$ E9-F	5'-GAACTGGGGGTAGAAAGGGA,-3'	58.1
$KCNQ_4$ E9-R	5'-AGGCAGGTCTGAGAGAGGATG-3'	58.2
SCN5A E-17-F	5'-TTAAAGGTCTCTGGGCCTCA -3'	60
SCN5A E-17-R	5'-TCATCTGTGTCTGACTCGGC -3'	60

Table 2. $KCNQ_4$ and SCN5A gene mutations in 31 febrile seizures families

Proband	Genes	Site	SNV	aa	Exon
1	$KCNQ_4$	chr1:41303362	c.1609C>T	p.Pro537Ser	13
2	$KCNQ_4$	chr1:41296832	c.1207G>A	p.Ala403Thr	10
3	$KCNQ_4$	chr1:41289815	c.1177G>T	p.Gly393Cys	9
1	SCN5A	Chr3:38622757	c.2893C>T	p.Arg965Cys	17

benign familial neonatal convulsions [16-20]. $KCNQ_4$ gene is located on human chromosome 1p34 and consists of 14 exons, encoding Kv7.4 with 695 amino acids [21]. $KCNQ_4$ is expressed mainly in the central auditory pathway, such as Cochlear nucleus, lateral lemniscus nucleus, and the hypothalamus [22]. $KCNQ_4$ gene mutations can result in human hereditary nonsyndromic progressive hearing loss [23, 24]. The association of $KCNQ_4$ gene with febrile seizures remains unknown.

In this study, we identified three $KCNQ_4$ and SCN5A gene mutations in febrile seizures patients using a high throughput screening approach. These mutations were not present in the healthy population and not single causal factors for febrile seizures clinical manifestation in three pedigrees. They may interact with other mutations to cause febrile seizures. Our findings suggest that $KCNQ_4$ mutations may contribute to susceptibility of febrile seizures, providing a novel clue to investigate the genetic background of epileptogenesis.

Materials and methods

Study subjects

Thirty-one febrile seizures families were included in the study in Guangdong General Hospital from January 2012 to November 2015. The inclusion criteria were based on the clinical guideline published by American Academy of Pediatrics in 2008 and clinical diagnosis. The included patients had a history of febrile convulsions before the age of 5. Exclusion criteria were: (1) patients had a history of afebrile seizures; (2) patients with progressive or degenerative nervous diseases, or inherited metabolic diseases; (3) patients with heart, liver, kidney, blood, and other essential organ disease; (4) patients with intracranial infection [25].

A total of 104 subjects, including 31 probands and their family members, were included in the study. 31 probands consisted of 19 males and 12 females, average age 4.73 ± 2.94, onset from 5 months to 5 years old. Among 31 probands, 29 cases were diagnosed as generalized seizures (28 cases in generalized tonic seizures and 1 case in atonic seizure), 1 case was focal seizures, and 1 case was mixed seizures. 200 healthy subjects were collected as the control group with male: female ratio 1.4:1, average age 11.27 ± 2.56. They did not have a family history of neurological disease. Detailed clinical data were collected and long-range video EEG and cranial MRI examination were performed on all subjects. Venous blood samples were collected from each subject into an anticoagulant EDTA-containing tube. All study subjects or their legal guardians signed informed consent. Our study was approved by the ethics committee of Guangdong General Hospital.

Extraction of DNA samples

DNA samples were extracted from blood samples using genomic DNA Extraction Kit (TIANamp genomic DNA kit, Shanghai, China) following the manufacturer's protocol and stored in -80°C until use. The concentration of DNA was determined using a NanoDrop 2000/2000c spectrophotometer (Thermo Fisher Scientific, life science reagents, USA). The quality of DNA was confirmed by the 260/280 OD value between 1.8 and 2.0, and by 1% agarose gel electrophoresis.

Capture of target genes

A total of 485 genes related to genetic epilepsy based on OMIM and HGMD databases were

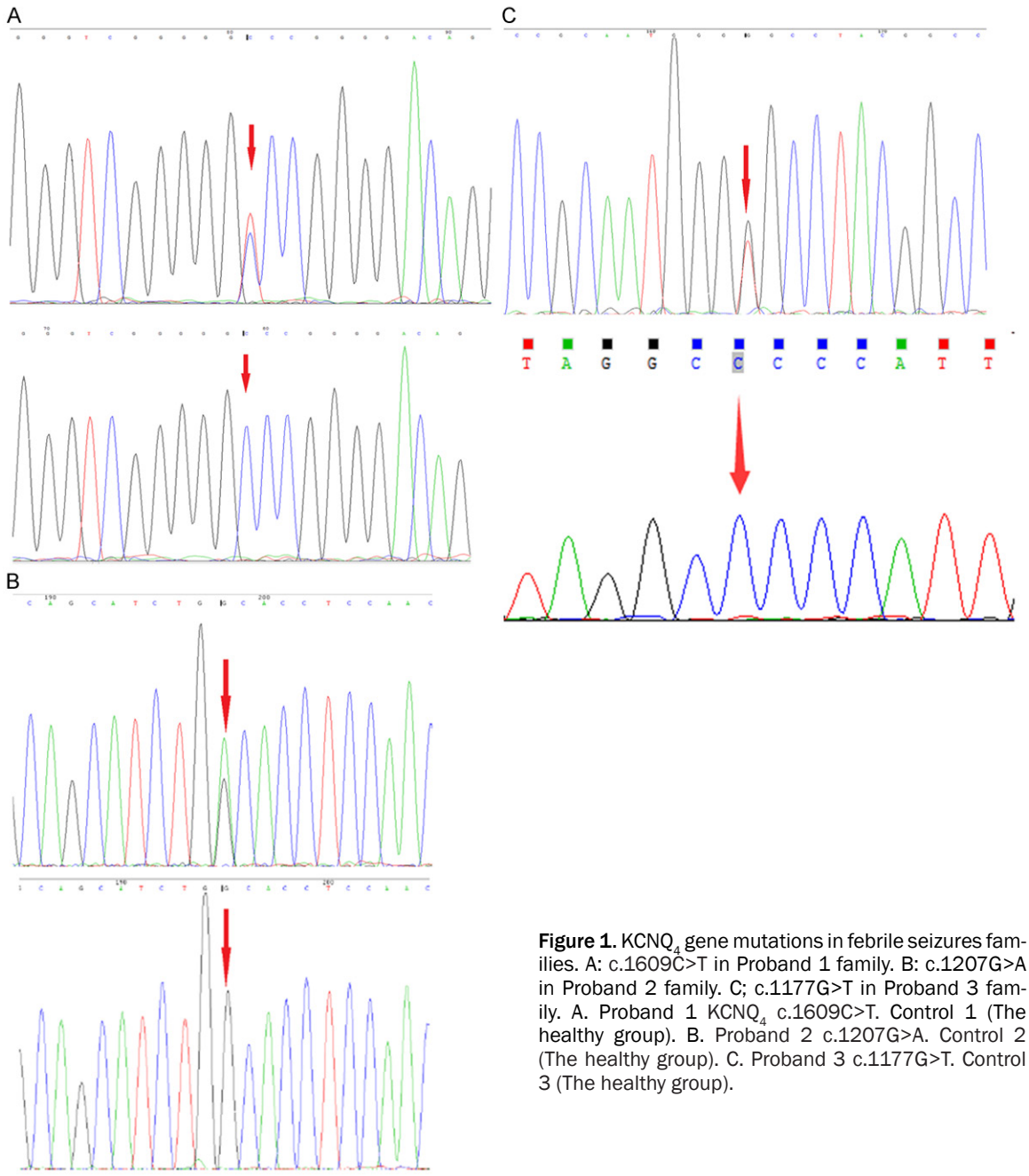

Figure 1. $KCNQ_4$ gene mutations in febrile seizures families. A: c.1609C>T in Proband 1 family. B: c.1207G>A in Proband 2 family. C; c.1177G>T in Proband 3 family. A. Proband 1 $KCNQ_4$ c.1609C>T. Control 1 (The healthy group). B. Proband 2 c.1207G>A. Control 2 (The healthy group). C. Proband 3 c.1177G>T. Control 3 (The healthy group).

selected. Probes for exons and the flanking sequences (±10 bp) were designed using the online tool SureDesign (Agilent, USA). The target gene capture kit was custom made by Agilent, USA, containing 60605 probes in a total of 1.961 Mbp. The target gene library was constructed from the DNA sample of febrile seizures patient using the Agilent SureSelect Target Enrichment System Kit (Agilent, USA) by following the manufacturer's instructions, including broken-down of DNA fragments using ultrasonic probe, library construction, hybridization of probes, amplification and purification of the library. Quality was controlled using DNA chips (Agilent Cat# 5067-1522), DNA 1000 reagent (Agilent Cat# 5067-1504), and an Agilent 2100 Bioanalyzer (Agilent). The library was sequenced using a NEXTSEQ 500 sequencer (Illumina, USA) by following the manufacturer's protocol. The raw sequencing data Fastq files were processed using RTA software (real-time analysis Illumina), Casava software v1.8.2 (Illumina), BWA, Genome analysis Toolkit (GATK). The sequencing quality was evaluated

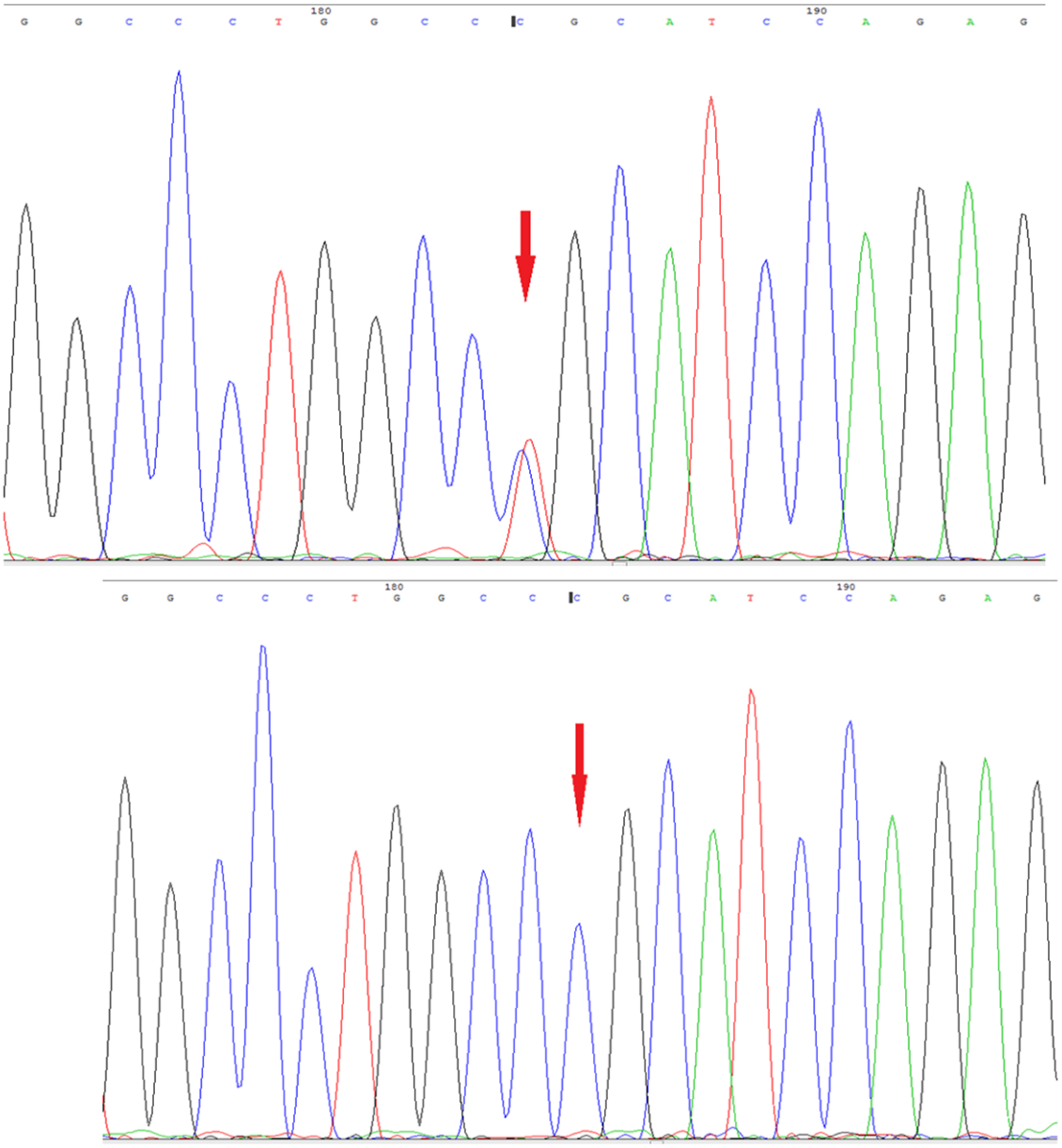

Figure 2. SCN5A gene mutation (c.2893C>T) in Proband 1 family. Proband 1 SCN5A c.2893C>T. Control 1 (The healthy group).

based on total reads number, the proportion of the reads that match the human genome sequence, the proportion of the reads that was located within the target gene sequence, average depth of sequencing, and the ratio of the fragments where sequencing depth greater than 20x, and uniformity analysis of coverage. The report on single nucleotide variants (SNV) was generated in VCF format and then annotated using PolyPhen2.2.2 and ANNOVAR software, HGMD, dbSNP, and 1000 Genome databases. The candidate variants were verified using the Sanger method.

Screening of $KCNQ_4$ and SCN5A gene mutations in population

$KCNQ_4$ and SCN5A gene mutations in the population were screened by sequencing PCR products. Primer pairs for $KCNQ_4$ and SCN5A genes were designed (**Table 1**) based on genome sequence (NC_000002 and NM_001099405, NCBI GenBank) and the $KCNQ_4$ and SCN5A gene mutations found in "2.3 Capture of target genes" section using Primer Premier V5.0. The primers were synthesized in the Guangzhou Gene Denovo Co., Ltd. PCR products of the DNA

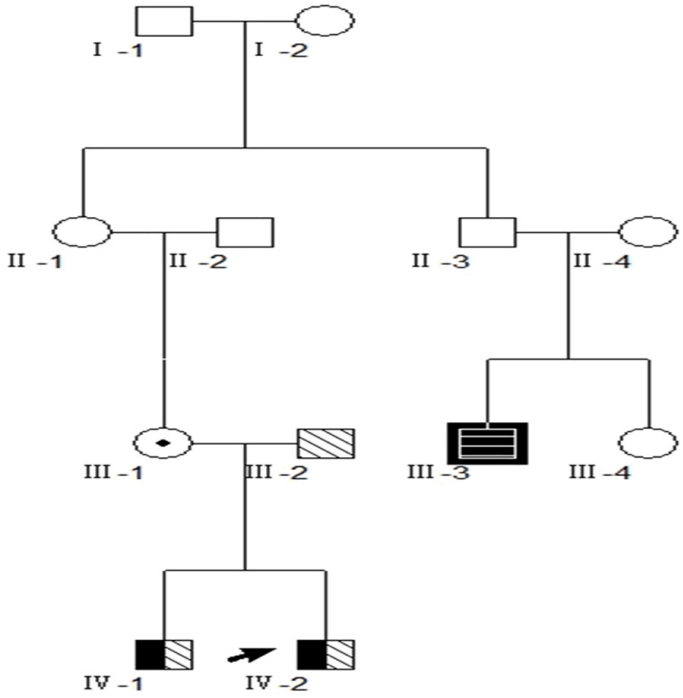

Figure 3. Pedigree of febrile seizures Proband 1 family with $KCNQ_4$ and SCN5A gene mutations. III-3 experienced two febrile convulsion at temperature 39°C before the age of three years old, who is now asymptomatic and studies in middle school. Gene mutation in III-3 is uncertain. III-2 father, IV-1 brother and III-2 proband have gene mutations in SCN5A. III-2 did not have history of FS. □ normal male; ○ normal female; ↗ proband ■ male patient with KCNQ4 gene mutaion; ●female patient with $KCNQ_4$ gene mutaion; ⊡ normal male with $KCNQ_4$ gene mutation; ⊙ normal female with $KCNQ_4$ gene mutaion ▬ male patient ▨ normal male with SCN5A gene mutaion ◪ male patient with $KCNQ_4$ and SCN5A gene mutaions.

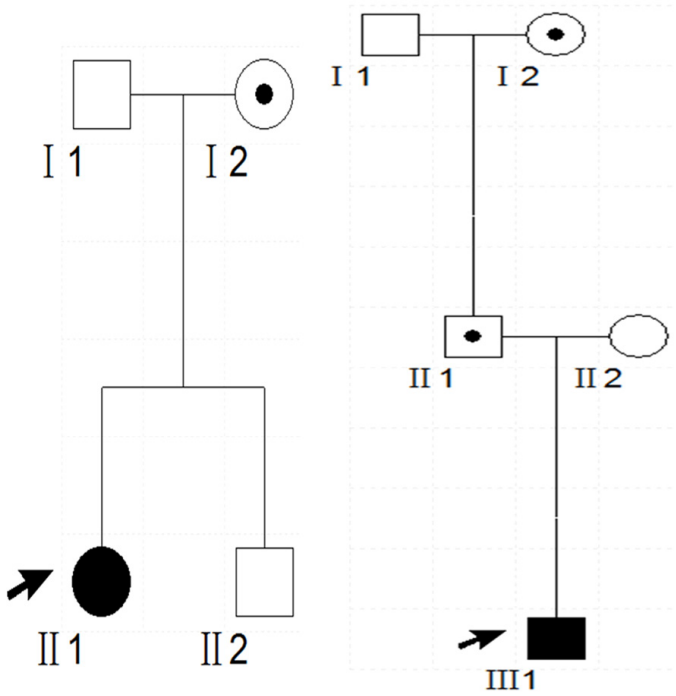

Figure 4. Pedigrees of febrile seizures families with $KCNQ_4$ gene mutations. A. Proband 2 family. B. Proband 3 family. □ normal male; ○ normal female; ↗, ■ febrile seizures male proband; ↗, ● febrile seizures female proband; ■ febrile seizures male patient with gene mutation; ⊡ normal male with gene mutation; ⊙ normal female with gene mutation.

samples from 31 febrile seizures families and 200 healthy subjects were sequenced and analyzed by alignment to sequence of chr1:41249684-41306124 (GRCH37, GenBank) and chr3:38591812-38674798 (GRCH37/hg19, GenBank).

Results

$KCNQ_4$ and SCN5A gene mutations in febrile seizures patients

We found three $KCNQ_4$ gene mutations c.1177G>T, c.1207G>A, and c.1609C>T in exons 9, 10, and 13, respectively, in febrile seizures patients through high-throughput target gene capture (**Table 2**; **Figure 1**). Each patient only had one of these mutations among three febrile seizures families (**Table 2**; **Figure 1**). These mutations were missense and may cause the changes p.Gly393Cys, p.Ala403Thr, and p.Pro537Ser, respectively, in the $KCNQ_4$ peptide (**Table 2**). We did not find any $KCNQ_4$ gene mutation in other 28 febrile seizures families.

We also found a missense SCN5A gene mutation c.2893C>T in exon 17, resulting in a p.Arg965Cys change, in proband 1 family but not in the other two families (**Figure 2**).

Pedigree analysis of $KCNQ_4$ and SCN5A gene mutation in three febrile seizures families

We performed pedigree analysis of $KCNQ_4$ and SCN5A gene mutations in three febrile seizures families. The results demonstrated that the proband 1's elder brother and mother carried $KCNQ_4$ c.1609C>T (**Figure 3**). No other member carried

Table 3. Clinical data of probands in three febrile seizures families

Probands	Sex	Age	Onset age	Attack	T[a]	Duration[b]	Total attack	Family history	Head MRI
1	M	2 y	1y9m	Generalized tonic/atonic seizure	1	1	>10	+ #	Normal
2	F	3 y	6m	Generalized tonic	1	1	7	-	Thin corpus callosum
3	M	4 y	1y8m	Generalized tonic	2	1	3	-	subependymal cysts

T, Highest body temperature during fever[a], 1<38.5°C, 2≥38.5°C; Duration[b], 1<15 min; 2≥15 min; Family history, "+" yes; "-" no; #, The brother of this patient had an onset of convulsion with fever 38°C at 2 years old.

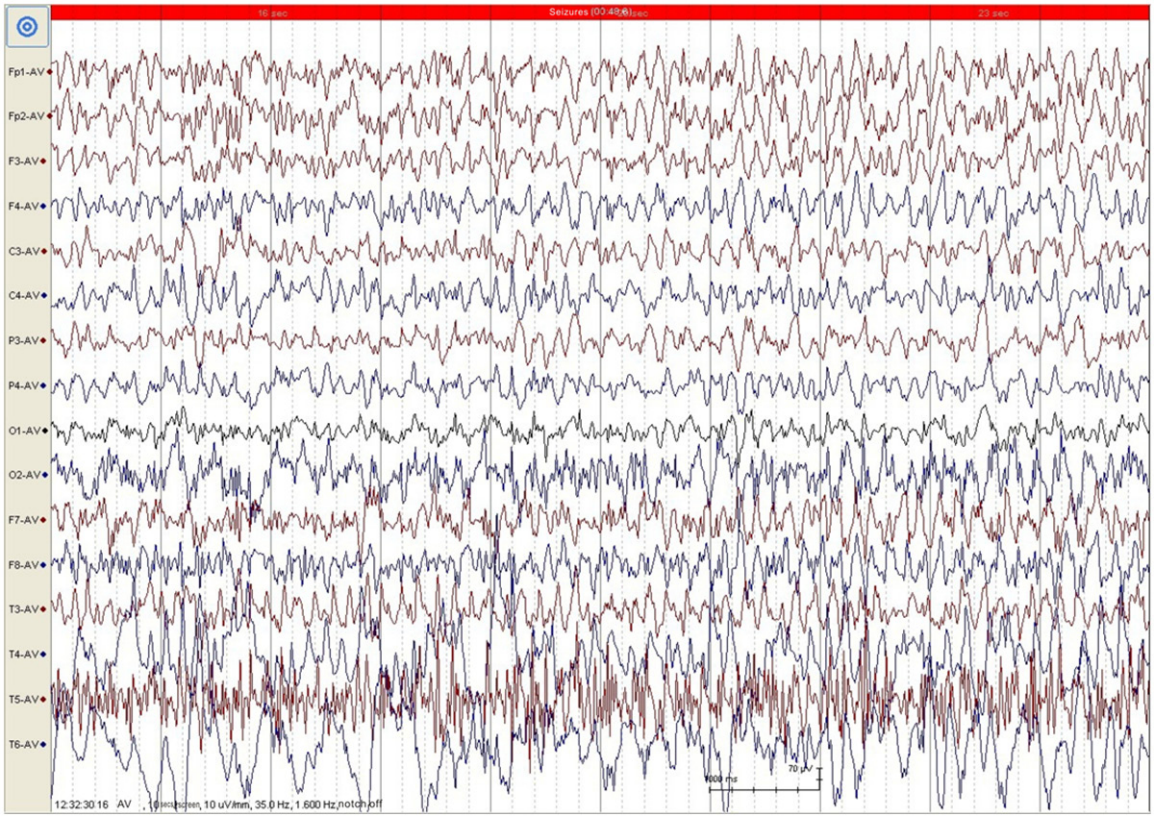

Figure 5. The EEG of proband 1 at 2 years old. The Video-EEG recorded multiple generalized seizures which arose from the central and temporal lobe and spread to all the brain area during monitoring time. There are continuously moderate to extreme high amplitude sharp waves, sharp and slow wave complex, spike and slow wave complex, and multiple spike and slow wave complex in all leads during ictal phase.

this $KCNQ_4$ gene mutation in this family. The proband 1 and his elder brother and father carried SCN5A c.2893C>T (**Figure 3**). No other member carried the SCN5A gene mutation in this family. Except for proband 1 and his brother, who were febrile seizures patients, no other member in this family had febrile seizures (**Figure 3**). This suggests that either $KCNQ_4$ c.1609C>T or SCN5A c.2893C>T mutations were not a single causal factor for febrile seizures. Combination of $KCNQ_4$ c.1609C>T and SCN5A c.2893C>T mutations may contribute to febrile seizures.

The proband 2's mother carried c.1207G>A, and the proband 3's father and paternal grandmother carried c.1177G>T (**Figure 4**). No other member carried any $KCNQ_4$ gene mutation in these two febrile seizures families (**Figure 4**). Clinical information on the febrile seizures patients with $KCNQ_4$ gene mutation was listed in **Table 3** and **Figures 5-8**. No member carried the SCN5A c.2893C>T gene mutation in these families. Except for the probands 2 and 3, no other member in these two families was febrile seizures patient. These results suggested that the $KCNQ_4$ c.1207G>A or c.1177G>T gene

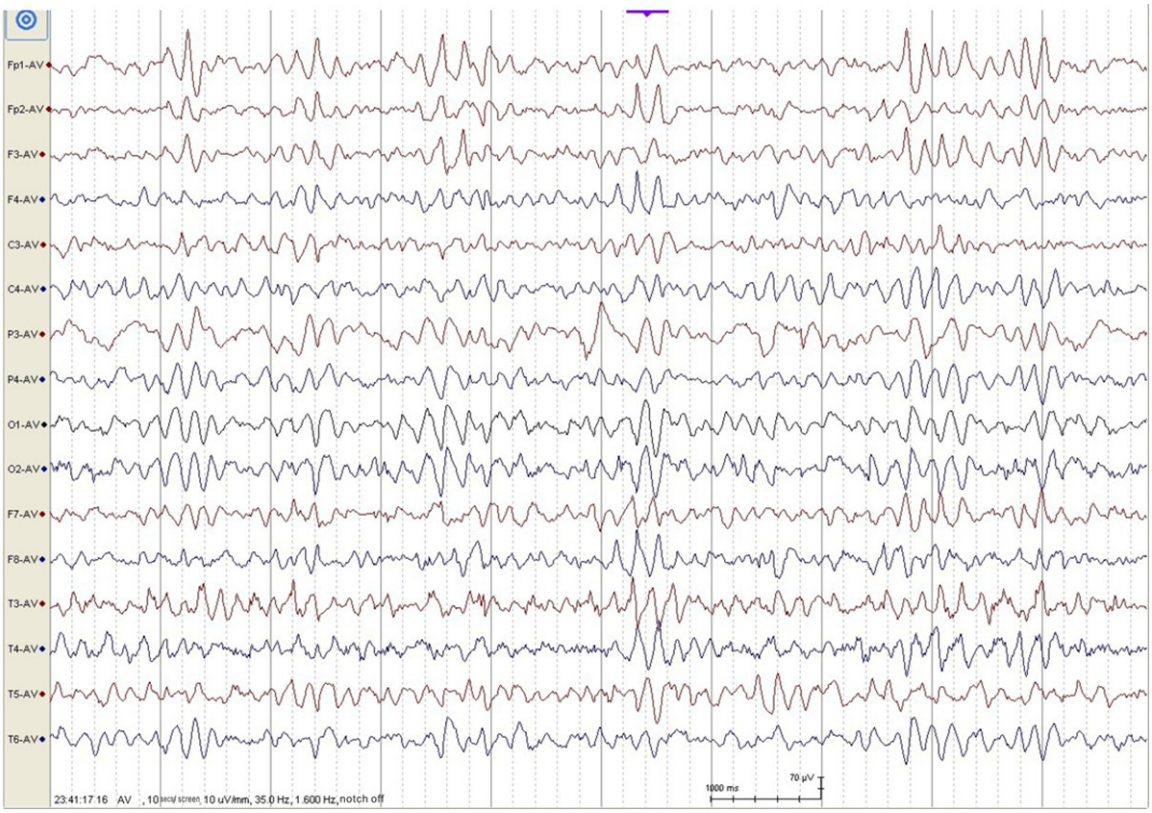

Figure 6. The EEG of proband 1's brother at the age of 3. There are some single and short-range theta activities mixed with sharp waves in the forehead, anterior temporal, and temporal regions during sleeping period.

mutation was not a single causal factor for febrile seizures, and may contribute to febrile seizures.

No $KCNQ_4$ and SCN5A gene mutation in the healthy control group

We examined whether each $KCNQ_4$ and SCN5A gene mutations was found in the healthy control group. The results showed that no $KCNQ_4$ and SCN5A gene mutation was found in the healthy control group of 200 patients.

Discussion

In the current study, we identified three $KCNQ_4$ gene mutations c.1177G>T, c.1207G>A, and c.1609C>T in exons 9, 10, and 13, respectively, in three febrile seizures families. Each patient only had one of these mutations among 3 febrile seizures families of all 31 febrile seizures families, and no $KCNQ_4$ gene mutations were found in the other 28 febrile seizures families or the healthy control group. We also found a known SCN5A gene mutation c.2893C>T in proband 1 family but not the other two families. Pedigree analysis indicated that these $KCNQ_4$ gene mutations and SCN5A gene mutation were not single causal factors for febrile seizures as some mutation-carriers in these members were not febrile seizures.

Previous studies showed that SCN1A and SCN2A gene mutations are associated with genetic epilepsy with febrile seizures (GEFS+) and epilepsy, and that SCN5A is associated with epilepsy [13, 26, 27]. A recent study showed that SCN5A mutation is related to the pathogenesis of epilepsy, long QT syndrome, and Jervell and Lange-Nielsen syndrome alone or alongside KCNQ1 and KCNH2 gene mutations [28]. In the current study, we found that the missense SCN5A gene mutation c.2893C>T in exon 17 results in a p.Arg965Cys change, in proband 1 family but not the other two families. The proband 1 and his elder brother carried both the $KCNQ_4$ gene mutation and SCN5A gene mutation, the probands 2 and 3 carried $KCNQ_4$ gene mutation, suggesting that they probably interacted with other gene mutations to promote susceptibility to febrile seizures.

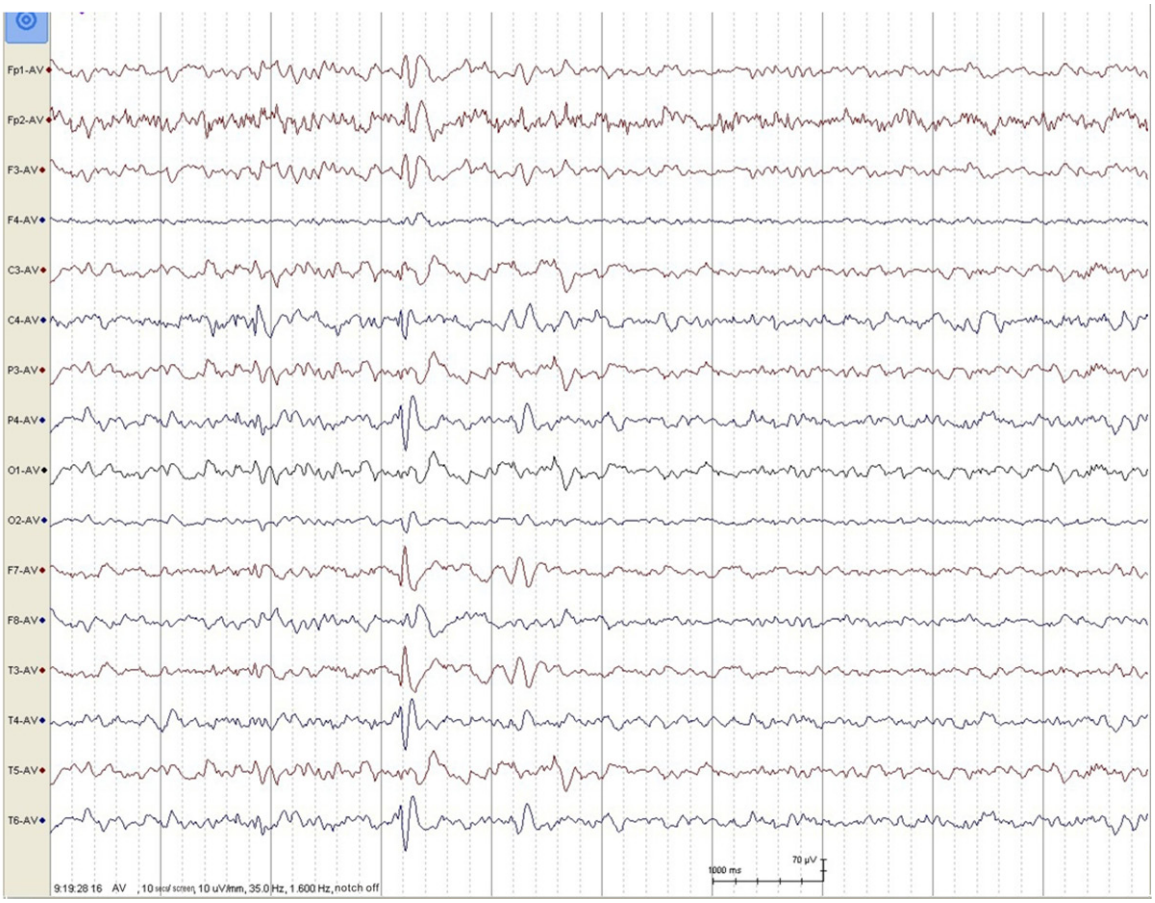

Figure 7. The EEG of proband 2 at 3 years old. There are many single asynchronous sharp waves, sharp and slow wave complex in anterior frontal lobe, anterior temporal, right parietal lobe, and bilateral posterior temporal area during sleeping period.

$KCNQ_4$ gene encodes Kv7.4 that has six transmembrane domains (S1-S6) with intracellular NH2 and COOH ends. Like other Kv7 subtypes, $KCNQ_4$ is characterized by a long COOH end, which determines its physical and functional interactions with other molecules and calcium regulatory proteins [29, 30] such as PIP2 (2-phosphate - inositol phosphate) and PKC (protein kinase C) [31, 32]. $KCNQ_4$ mutations (Gly-393Cys, p.Ala403Thr, and p.Pro537Ser) indentified in the current study are located in the COOH end. In the wild type which contains Gly, Ala, and Pro, Gly does not have a side chain, Ala has a hydrophobic side chain, and Pro has a conformational rigidity due to a specialized side chain. In the mutants which contains Cys, Thr, and Ser, Cys has a sulfhydryl group, and Thr and Ser have polar and uncharged side chain with hydroxyl. Therefore, these mutations may change the structure of the COOH end of the $KCNQ_4$ protein and affect the $KCNQ_4$ functions according to structure-activity correlation. In addition, the sulfhydryl and hydroxyl groups in the side chains of Cys, Thr, and Ser are subjected to modification, such as phosphorylation, thereby altering the signaling systems for $KCNQ_4$ functions. Moreover, Kv7.4 can form hetero-dimers with Kv7.3 in addition to form homotetramer in the regulation of the membrane potential [21, 33, 34]. Kv7.3 is widely expressed in the cerebral cortex, cerebellum, basal ganglia and, hippocampus [15]. We speculate that $KCNQ_4$ mutations identified in the current study may cause disorders in the structural and functional interactions between Kv7.4 and Kv7.3, and interact with other unknown factors to induce abnormal excitability of neurons and neural networks in the cerebral cortex, cerebellum, basal ganglia, hippocampus, leading to epilepsy. The underlying mechanism remains to be investigated further.

It has been well known that $KCNQ_4$ gene mutations cause human hereditary nonsyndromic progressive hearing loss [23, 24], such as one-base deletion in exon 1, c.211delC which gen-

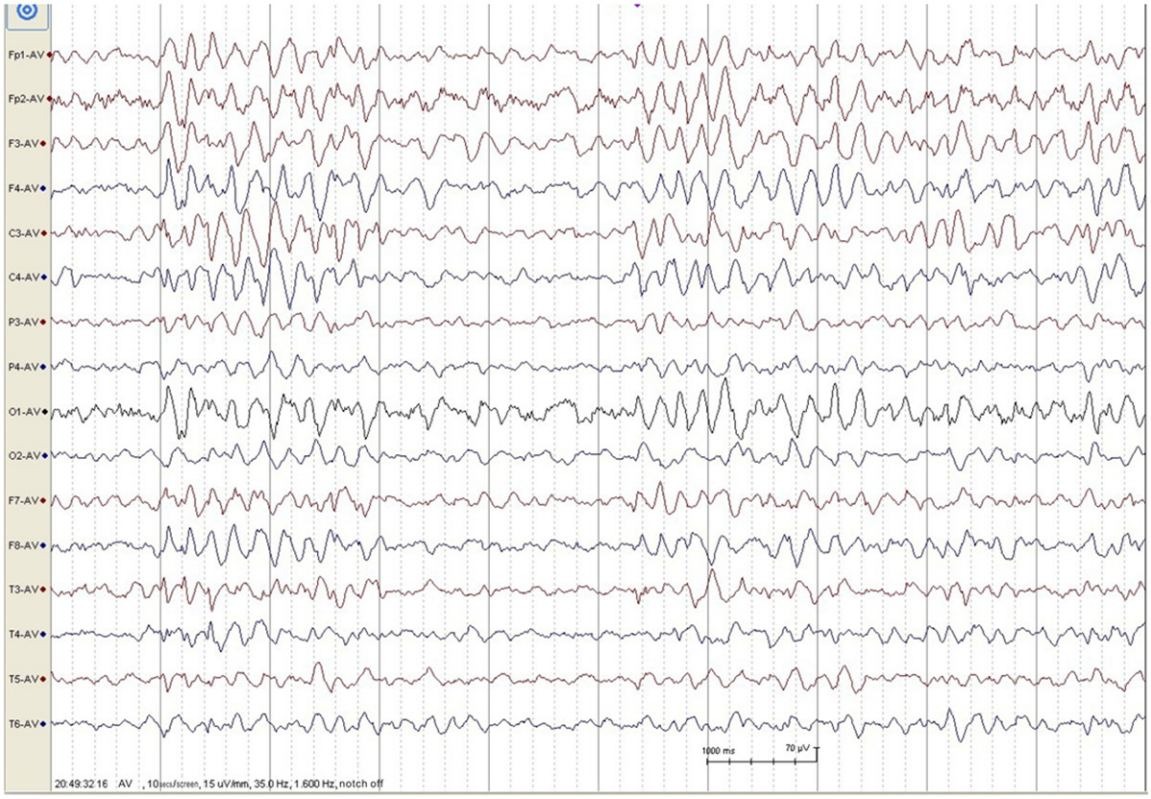

Figure 8. The EEG of proband 3 at the age of 4. There are some paroxysmal short to moderate range theta activities mixed with sharp waves, sharp and slow wave complex, spike and slow wave complex during sleeping period, which are obvious in central and frontal lobe.

erated a profoundly truncated protein without transmembrane domains (p.Q71fsX138) [35], and the changes of the copies of intron between exon 2 and exon 3 of $KCNQ_4$ [36]. Adding to the literature, we found $KCNQ_4$ gene mutations may contribute to febrile seizures in the current study, suggesting that $KCNQ_4$ is an essential gene in regulating nervous system and a patient predisposition for both hearing loss and febrile seizures. Even though some people with KCNQ4 mutation in the three family have no phenotype of febrile seizures, it might be due to variable penetrance. Syndromes expressed by individual members would be determined by other genes or environmental factors in febrile seizures [37]. How different $KCNQ_4$ gene mutations contribute to either hearing loss or febrile seizures is an important research goal and deserves further investigation.

In summary, we have identified three novel $KCNQ_4$ and SCN5A gene mutations which were not single causal factors for febrile seizures. They most likely interact with other gene mutations to promote susceptibility to febrile seizures. This adds to the literature that $KCNQ_4$ gene mutations are previously found to be only associated with hearing loss. Our finding provides a new clue to investigate the genetic etiology of febrile seizures.

Acknowledgements

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Disclosure of conflict of interest

None.

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References

[1] Camfield P and Camfield C. Febrile seizures and genetic epilepsy with febrile seizures plus (GEFS+). Epileptic Disord 2015; 17: 124-133.

[2] Practice parameter: the neurodiagnostic evaluation of the child with a first simple febrile seizure. American Academy of Pediatrics. Provisional Committee on Quality Improvement, Subcommittee on Febrile Seizures. Pediatrics 1996; 97: 769-772; discussion 773-765.

[3] Tsuboi T. Epidemiology of febrile and afebrile convulsions in children in Japan. Neurology 1984; 34: 175-181.

[4] Mastrangelo M, Midulla F and Moretti C. Actual insights into the clinical management of febrile seizures. Eur J Pediatr 2014; 173: 977-982.

[5] Mantegazza M, Gambardella A, Rusconi R, Schiavon E, Annesi F, Cassulini RR, Labate A, Carrideo S, Chifari R, Canevini MP, Canger R, Franceschetti S, Annesi G, Wanke E and Quattrone A. Identification of an Nav1.1 sodium channel (SCN1A) loss-of-function mutation associated with familial simple febrile seizures. Proc Natl Acad Sci U S A 2005; 102: 18177-18182.

[6] Fendri-Kriaa N, Kammoun F, Salem IH, Kifagi C, Mkaouar-Rebai E, Hsairi I, Rebai A, Triki C and Fakhfakh F. New mutation c.374C>T and a putative disease-associated haplotype within SCN1B gene in Tunisian families with febrile seizures. Eur J Neurol 2011; 18: 695-702.

[7] Xiumin W, Meichun X and Lizhong D. Association analysis of gamma2 subunit of gamma-aminobutyric acid (GABA) type A receptor and voltage-gated sodium channel type II alpha-polypeptide gene mutation in southern Chinese children with febrile seizures. J Child Neurol 2007; 22: 714-719.

[8] Soltani S, Zare-Shahabadi A, Shahrokhi A, Rezaei A, Zoghi S, Zamani GR, Mohammadi M, Ashrafi MR and Rezaei N. Association of Interleukin-1 Gene Cluster and Interleukin-1 Receptor Polymorphisms With Febrile Seizures. J Child Neurol 2016; 31: 673-677.

[9] Chou IC, Lin WD, Wang CH, Tsai CH, Li TC and Tsai FJ. Interleukin (IL)-1beta, IL-1 receptor antagonist, IL-6, IL-8, IL-10, and tumor necrosis factor alpha gene polymorphisms in patients with febrile seizures. J Clin Lab Anal 2010; 24: 154-159.

[10] Zare-Shahabadi A, Ashrafi MR, Shahrokhi A, Soltani S, Zoghi S, Soleimani F, Vameghi R, Badv RS and Rezaei N. Single nucleotide polymorphisms of TNF-Alpha gene in febrile seizures. J Neurol Sci 2015; 356: 153-156.

[11] Saghazadeh A, Mastrangelo M and Rezaei N. Genetic background of febrile seizures. Rev Neurosci 2014; 25: 129-161.

[12] Soldovieri MV, Miceli F and Taglialatela M. Driving with no brakes: molecular pathophysiology of Kv7 potassium channels. Physiology (Bethesda) 2011; 26: 365-376.

[13] Kullmann DM. Neurological channelopathies. Annu Rev Neurosci 2010; 33: 151-172.

[14] D'Adamo MC, Catacuzzeno L, Di Giovanni G, Franciolini F and Pessia M. K(+) channelepsy: progress in the neurobiology of potassium channels and epilepsy. Front Cell Neurosci 2013; 7: 134.

[15] Brown BS and Yu SP. Modulation and genetic identification of the M channel. Prog Biophys Mol Biol 2000; 73: 135-166.

[16] Maljevic S, Wuttke TV, Seebohm G and Lerche H. KV7 channelopathies. Pflugers Arch 2010; 460: 277-288.

[17] Maljevic S, Wuttke TV and Lerche H. Nervous system KV7 disorders: breakdown of a subthreshold brake. J Physiol 2008; 586: 1791-1801.

[18] Biervert C, Schroeder BC, Kubisch C, Berkovic SF, Propping P, Jentsch TJ and Steinlein OK. A potassium channel mutation in neonatal human epilepsy. Science 1998; 279: 403-406.

[19] Singh NA, Otto JF, Dahle EJ, Pappas C, Leslie JD, Vilaythong A, Noebels JL, White HS, Wilcox KS and Leppert MF. Mouse models of human KCNQ2 and KCNQ3 mutations for benign familial neonatal convulsions show seizures and neuronal plasticity without synaptic reorganization. J Physiol 2008; 586: 3405-3423.

[20] Miceli F, Striano P, Soldovieri MV, Fontana A, Nardello R, Robbiano A, Bellini G, Elia M, Zara F, Taglialatela M and Mangano S. A novel KCNQ3 mutation in familial epilepsy with focal seizures and intellectual disability. Epilepsia 2015; 56: e15-20.

[21] Kubisch C, Schroeder BC, Friedrich T, Lutjohann B, El-Amraoui A, Marlin S, Petit C and Jentsch TJ. KCNQ4, a novel potassium channel expressed in sensory outer hair cells, is mutated in dominant deafness. Cell 1999; 96: 437-446.

[22] Kharkovets T, Hardelin JP, Safieddine S, Schweizer M, El-Amraoui A, Petit C and Jentsch TJ. KCNQ4, a K+ channel mutated in a form of dominant deafness, is expressed in the inner ear and the central auditory pathway. Proc Natl Acad Sci U S A 2000; 97: 4333-4338.

[23] Naito T, Nishio SY, Iwasa Y, Yano T, Kumakawa K, Abe S, Ishikawa K, Kojima H, Namba A, Oshikawa C and Usami S. Comprehensive genetic screening of KCNQ4 in a large autosomal dominant nonsyndromic hearing loss cohort: genotype-phenotype correlations and a founder mutation. PLoS One 2013; 8: e63231.

[24] Kharkovets T, Dedek K, Maier H, Schweizer M, Khimich D, Nouvian R, Vardanyan V, Leuwer R, Moser T and Jentsch TJ. Mice with altered

KCNQ4 K+ channels implicate sensory outer hair cells in human progressive deafness. EMBO J 2006; 25: 642-652.
[25] Febrile seizures: clinical practice guideline for the long-term management of the child with simple febrile seizures. Pediatrics 2008; 121: 1281-1286.
[26] Aurlien D, Leren TP, Tauboll E and Gjerstad L. New SCN5A mutation in a SUDEP victim with idiopathic epilepsy. Seizure 2009; 18: 158-160.
[27] Mercimek-Mahmutoglu S, Patel J, Cordeiro D, Hewson S, Callen D, Donner EJ, Hahn CD, Kannu P, Kobayashi J, Minassian BA, Moharir M, Siriwardena K, Weiss SK, Weksberg R and Snead OC 3rd. Diagnostic yield of genetic testing in epileptic encephalopathy in childhood. Epilepsia 2015; 56: 707-716.
[28] Sanecka A, Biernacka EK, Szperl M, Sosna M, Mueller-Malesinska M, Kozicka U, Baranowski R, Kosiec A, Lazarczyk H, Skarzynski H, Hoffman P, Bieganowska K and Piotrowicz R. QTc prolongation in patients with hearing loss: Electrocardiographic and genetic study. Cardiol J 2016; 23: 34-41.
[29] Haitin Y and Attali B. The C-terminus of Kv7 channels: a multifunctional module. J Physiol 2008; 586: 1803-1810.
[30] Xu Q, Chang A, Tolia A and Minor DL, Jr. Structure of a Ca(2+)/CaM:Kv7.4 (KCNQ4) B-helix complex provides insight into M current modulation. J Mol Biol 2013; 425: 378-394.
[31] Hoshi N, Zhang JS, Omaki M, Takeuchi T, Yokoyama S, Wanaverbecq N, Langeberg LK, Yoneda Y, Scott JD, Brown DA and Higashida H. AKAP150 signaling complex promotes suppression of the M-current by muscarinic agonists. Nat Neurosci 2003; 6: 564-571.
[32] Li Y, Gamper N, Hilgemann DW and Shapiro MS. Regulation of Kv7 (KCNQ) K+ channel open probability by phosphatidylinositol 4,5-bisphosphate. J Neurosci 2005; 25: 9825-9835.
[33] Schwake M, Pusch M, Kharkovets T and Jentsch TJ. Surface expression and single channel properties of KCNQ2/KCNQ3, M-type K+ channels involved in epilepsy. J Biol Chem 2000; 275: 13343-13348.
[34] Bal M, Zhang J, Zaika O, Hernandez CC and Shapiro MS. Homomeric and heteromeric assembly of KCNQ (Kv7) K+ channels assayed by total internal reflection fluorescence/fluorescence resonance energy transfer and patch clamp analysis. J Biol Chem 2008; 283: 30668-30676.
[35] Kamada F, Kure S, Kudo T, Suzuki Y, Oshima T, Ichinohe A, Kojima K, Niihori T, Kanno J, Narumi Y, Narisawa A, Kato K, Aoki Y, Ikeda K, Kobayashi T and Matsubara Y. A novel KCNQ4 one-base deletion in a large pedigree with hearing loss: implication for the genotype-phenotype correlation. J Hum Genet 2006; 51: 455-460.
[36] Wang Q, Cao J, Li N, Yang Y, Yu L, Han D and Yang W. [KCNQ4 gene mutations affected a pedigree with autosomal dominant hereditary hearing loss]. Zhonghua Er Bi Yan Hou Ke Za Zhi 2002; 37: 343-347.
[37] Baulac S, Gourfinkel-An I, Nabbout R, Huberfeld G, Serratosa J, Leguern E and Baulac M. Fever, genes, and epilepsy. Lanc Neu 2004; 3: 421-430.