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BRIEF COMMUNICATION

G327E mutation in SCN9A gene causes idiopathic focal epilepsy with Rolandic spikes: a case report of twin sisters

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Abstract

The voltage-gated sodium channel NaV1.7, encoded by the gene *SCN9A*, is located in peripheral neurons and plays an important role in epileptogenesis. Previous studies have identified an increasing number of SCN9A mutations in patients with variable epilepsy phenotypes. Phenotypes of SCN9A mutations include febrile seizures (FS), genetic epilepsy with febrile seizures plus (GEFS+), and Dravet syndrome (DS), which pose challenges in clinical treatment. Here, we identified a heterozygous SCN9A mutation (c.980G > A chr2:167149868 p.G327E) from two twin sisters with Rolandic epilepsy by whole-exome sequencing. The patient became seizure free with a combination of levetiracetam and clonazepam. Identification of this mutation is also helpful for advancing our understanding of the role of SCN9A in epilepsy and provides deeper insights for *SCN9A* mutations associated with broad clinical spectrum of seizures.

Keywords SCN9A · Heterozygous mutation · Epilepsy · Rolandic spikes

Introduction

SCN9A gene encoding voltage-gated sodium channel NaV1.7 is preferentially expressed in dorsal root ganglion neurons and sympathetic ganglion neurons, and has an important role as "gatekeeper" within the peripheral pain-signaling pathway [1]. Thus, SCN9A mutations are mainly associated with pain disorders [2]. Recently, an increasing number of SCN9A mutations have been identified in patients of febrile seizures (FS) [3, 4], genetic epilepsy with febrile seizures plus (GEFS$^+$) [5, 6], or Dravet syndrome (DS) [3, 7]. In addition, SCN9A variants have also been suggested as a genetic modifier in SCN1A mutation-associated GEFS$^+$ and a possible susceptibility gene for DS [3, 7]. Recently, Yang et al. [6] reported one heterozygous SCN9A mutations (c.980G > A) associated with GEFS$^+$. Here, we would like to present a case of twin sisters diagnosed as Rolandic epilepsy with the same mutation.

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Material and methods

Targeted exon capture and sequencing

After the subject signed the informed consent for testing, 5 ml peripheral blood from the subject and 2 ml peripheral blood from each parent of the subject were collected. Then the blood samples were sent to Beijing Kangso Medical Inspection for whole-exome sequencing (WES). Qiagen FlexiGene DNA kit (Qiagen, Hilden, Germany) was used to extract genomic DNA from blood samples. To construct the DNA library, genomic

DNA sample was fragmented into 150~300-bp DNA fragments by ultrasonic processor. Adaptors to both ends of these DNA fragments were ligated and cohesive ends of the DNA fragments were trimmed. Then the DNA library was amplified and purified by polymerase chain reaction (PCR). The target DNA fragments from amplified DNA library were hybridized and captured by probes and then amplified through SureSelect Target Enrichment System (Agilgent, Santa Clara, California). Then the products were purified and quantified. Single-read sequencing was performed by NextSeq500 (Illumina, San Diego, California). Raw data were obtained in the format of Fastaq.

Sequence alignment and variant calling

Raw data can be transformed into identifiable base sequence with software CASAVA (1.8.2). Then Align analysis, SNP analysis, and DIP analysis were conducted to obtain information of mutation sites from targeted region. At last, protein damage analysis was conducted to qualitatively predict the probability of the results by SIFT, PolyPhen-2, MutationTaster, and MutationAssessor, and thus obtaining mutation sites which need further validation. The gene sequences of above mutation sites were obtained from GenBank (Fig. 1). The primers were designed by the website Primer Z (http://genepipe.ncgm.sinica.edu.tw/primerz/primerz4.do) and then synthesized. The mutation sites were amplified by PCR and then sequenced by the first-generation sequencing. The obtained sequences were aligned with the previous results, and false positive sites obtained by NGS (the next generation sequencing) were ruled out. Finally, multispecies alignments were performed using Blast (http://www.uniprot.org/blast/) to determine whether the affected amino acids were conserved and whether there was precedence for any of the variants in other species.

Sequence variant classification for the pathogenicity

The pathogenicity of sequence variant is classified to the following categories: (1) pathogenic, (2) likely pathogenic, (3) uncertain significance, (4) likely benign, and (5) benign. We

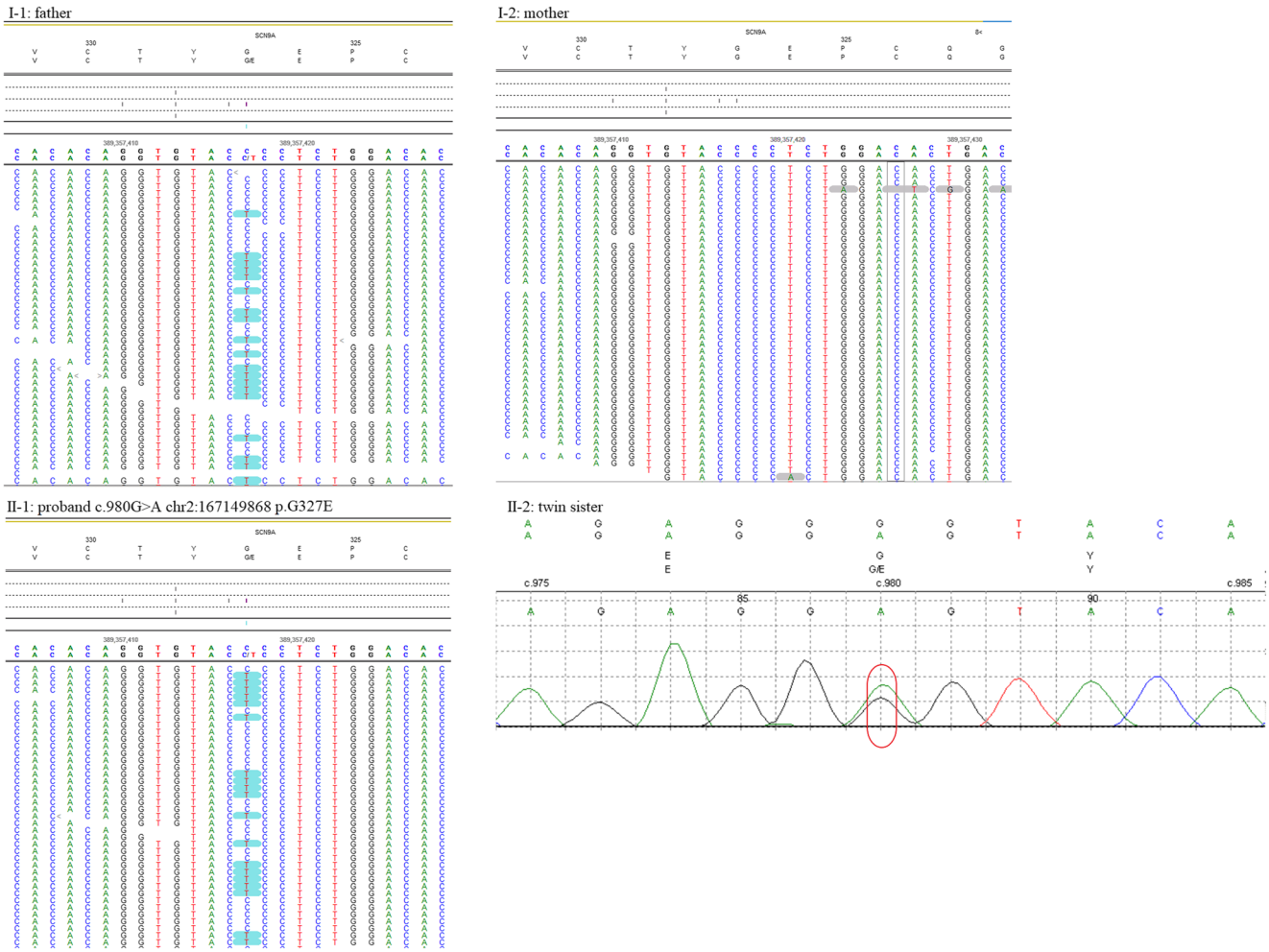

Fig. 1 Identification of a heterozygous mutation c.980G > A chr2:167149868 p.G327E from the first family. I-1: father, I-2: mother, II-1: proband, II-2: twin sister

followed the principle of standards and guidelines recommended by ACMG (American College of Medical Genetics) in recent publication [8].

Results

By using clinical whole-exome sequencing, a heterozygous SCN9A mutation (c.980G > A) was detected (Fig. 1), and the mutation were validated with Sanger sequencing. This variant (c.980G > A chr2:167149868 p.G327E) occurs in the population at a frequency of < 0.5% (0.008245% in the general population and 0.05172% in East Asians) in the ExAC database (http://exac.broadinstitute.org/variant/2-167149868-C-T). Multiple sequence alignment was performed using Blast (http://www.uniprot.org/blast/) and residue G327 is highly conserved across higher vertebrates. The variant (c.980G > A chr2:167149868 p.G327E) was predicted to damage the function of the protein by SIFT, PolyPhen-2, MutationTaster, and MutationAssessor. The pathogenicity of this variant is classified likely pathogenic followed the principle of standards and guidelines recommended by ACMG (American College of Medical Genetics) in recent publication [8].

Case report

The probands were two 10-year-old twin girls with normal delivery and development. The family history was unremarkable for genetic and neurological diseases. There was no teratogen exposure during the pregnancy. They presented their first seizure during sleep at 7 years of age. The seizure pattern was described as a nocturnal clonic seizure involving right upper extremity movements and oropharyngeal region, and it lasted for about 2 min. Six months later, they presented one generalized tonic-clonic seizures during sleep. Electroencephalogram monitoring showed remarkable interictal high-voltage spikes and spike-and-slow waves in the bilateral central-temporal regions. The discharges were aggravated by sleep, with spike-and-slow wave indexes ~50%–60%. Brain magnetic resonance imaging (MRI) and metabolic screening of amino acids and organic acid analyses were normal. A diagnosis of Rolandic epilepsy was made. Then, oxcarbazepine (OXC) was administered and their seizure was controlled. One year later, they presented three similar seizures during sleep. Electroencephalogram monitoring showed abundance of spike-and-slow waves (SW) in Rolandic areas during wake-up and sleep and the SW index was more than 80% during slow sleep. Then, they were given levetiracetam. After being seizure free for 3 months, the seizures recurred. Clonazepam was added, and the patients have been seizure free from that time.

Discussion

BECTS is the most common focal epilepsy syndrome in children. Previous studies reported that mutations in KCNQ2 and KCNQ3 encoding subunits of K+ channel (Kv7.2 and Kv7.3) have been identified as causes of BECTS [9], and mutations in GRIN2A, a subunit of the excitatory glutamate receptor N-methyl-D-aspartate (NMDA), play an important role in the pathogenesis of BECTS [10]. In addition, Wang et al. detected SCN3A mutation (c.1861 C > T, p.R621C) in patient of BECTS [11]. In this study, we detected a SCN9A mutation (c.980G > A) in two twin girls of BECTS. SCN9A encodes NaV1.7, which is mainly expressed in neurons of the dorsal root ganglia and has preliminarily been classified as a peripheral nervous system channel. SCN9A mutations have been identified in patients of various epilepsy phenotypes [3–7]. As *SCN9A* is also expressed in brain, we speculate that the G327E mutation also increases the central nervous system excitability and causes the phenotype of seizure disorders. There may be a similar genotype-phenotype correlation that more increased central nervous system excitability is associated with more severe seizure disorders (from FS to DS). On the other hand, most FS would remit spontaneously with age and might be missed by the pain disorders patients and their doctors. It is suggested that pain disorders patients with *SCN9A* mutations should be reinvestigated for their possible history of seizure disorders, which might help to further confirm the role of *SCN9A* in seizure disorders.

Interestingly, this missense mutation in the amino acid site c.980G > A (p.G327E) was recently reported as causative mutations of GEFS^{+} patients [6]. Is it due to the phenotypic heterogeneity or the other cause? In fact, clinical heterogeneity is common in genetic diseases. Even affected members in a GEFS+ pedigree with the same *SCN1A* mutation could present different phenotypes from FS to DS, a severe epileptic encephalopathy. Factors such as developmental variability, modifier genes, accumulation of somatic mutation in lifetime, and environmental insults may be involved to affect the pathogenesis and treatment effectiveness of epilepsy. To our knowledge, there is no mutation in *SCN9A* associated with seizure disorders also found in pain disorders patients previously. The underlying mechanism of such heterogeneity was still unclear [12].

One limitation in our study should be addressed. The family is too small to identify its pathogenicity for this variant (c.980G > A) of SCN9A gene, thus the functional effect of the mutation should be further studied to strengthen their assumption of causative mutation.

In conclusion, this report presents a SCN9A mutation G327A in two twin girls of BECTS. Our study will be helpful in the genetic diagnosis of epilepsy as well as in developing precision therapies for patients who carry SCN9A mutations. Identification of this mutation is also helpful for advancing our

understanding of the role of SCN9A in epilepsy and provides deeper insights for *SCN9A* mutations associated with broad clinical spectrum of seizures.

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Compliance with ethical standards

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

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