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Novel mutations in *SCN9A* occurring with fever-associated seizures or epilepsy

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ABSTRACT

Purpose: This study aimed to identify disease-causing gene mutations in individuals belonging to the Southern Chinese Han population diagnosed with fever-associated seizures or epilepsy (FASE).
Methods: Blood samples and clinical data were collected from 78 children with FASE. All subjects were screened for mutations using whole-exome sequencing, and mutations were validated using the Sanger sequencing method.
Results: Three novel*SCN9A* heterozygous missense mutations (I775M, R429C and A442T) were noted, which are associated with febrile seizures (FS), febrile seizures plus (FS^+) and genetic epilepsy with febrile seizures plus ($GEFS^+$), respectively. The R429C and A442T mutations are located in the large cytoplasmic loop between transmembrane topological domains, whereas I775M is located in the topological domain DIIS2. The I775M and R429C mutations have highly evolutionarily conserved residues and are predicted to affect the *SCN9A* protein function according to bioinformatics tools. These three mutations were not identified in 300 unrelated control subjects.
Conclusions: Mutations in the*SCN9A* gene may be linked with FASE.

1. Introduction

Fever-associated seizures or epilepsy (FASE), which is common in infants and children, is primarily characterised by the occurrence of an epileptic seizure accompanied with fever. FASE includes febrile seizures (FS), febrile seizures plus (FS^+), genetic epilepsy with febrile seizures plus ($GEFS^+$) and Dravet syndrome (DS), which is a severe form of epilepsy. Genetic factors, such as *SCN1A* and *GABRG2* mutations, have been demonstrated to play a key role in the pathogenesis of FASE [1–3]. However, the exact causative genetic changes are not defined in some patients with FASE. In this study, we performed whole-exome sequencing in children with FASE that belonged to the Han ethnicity and native to southern China. We aimed to identify the FASE causative genetic mutations in this subject population.

2. Results

In total, we recruited 78 patients diagnosed with a range of FASE; 53/78 (68.2%), 15/78 (18.7%), 7/78 (9.3%) and 3/78 (3.8%) were diagnosed with FS, FS^+, $GEFS^+$ and DS, respectively. At the time of study, the patients' age ranged from 2 to 9 years with an average age of 5 years and 6 months. Among the 78 patients with FASE, 45 causative genetic variants were detected. Nine *SCN9A* variants were identified (11.5% of patients) and showed the highest detection rates. Six patients harbouring the *SCN9A* mutations had other gene mutations (Table 1).

Three patients exclusively harboured the *SCN9A* mutation (probands 2, 3 and 4) and these patients were diagnosed with FS, FS^+ and $GEFS^+$, respectively. These three patients exhibited typical generalised tonic-clonic seizures with onset at 13, 15 and 48 months, respectively. Seizures occasionally occurred with a frequency of about four times per year, and were controlled with the administration of levetiracetam, valproate and lamotrigine, respectively, in these three patients. The three probands were born at term after an unremarkable pregnancy and showed normal psychomotor development. Further, results of physical examination, nervous system examination and magnetic resonance imaging of the brain revealed no abnormalities. Proband 4 had a family history of FASE and her uncle had FS during his childhood (Table 2).

The three *SCN9A* (NM_002977) (I775 M, R429C and A442 T)

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Table 1
Nine patients have *SCN9A* gene variants, six of them combined with other mutations.

No.	Gene name
Proband 1	*UBE3A*, *SCN9A*, *GPR98*
Proband 2	*SCN9A*
Proband 3	*SCN9A*
Proband 4	*SCN9A*
Proband 5	*CLCN2*, *SCN9A*, *SERPINI1*
Proband 6	*SCN9A*, *GPR98*, *TSC2*
Proband 7	*GLUD1*, *SCN9A*, *SHANK3*
Proband 8	*KCNA2*, *SCN9A*, *GABRA2*, *NIPA2*
Proband 9	*SCN9A*, *TSC2*, *PRRT2*

mutations identified in the probands were not detected in 300 unrelated control subjects; however, identical mutations were found in the family members. The mutations R429C and A442 T were located in the DI-DII linker, whereas the mutation I775 M was located in the topological domain DIIS2. I775 M and R429C are highly evolutionarily conserved. According to the bioinformatics tools PolyPhen-2 and PROVEAN, the mutations I775 M and R429C may affect the function of SCN9A protein (Table 3, Figs. 1, 2).

3. Discussion

SCN9A contains 27 exons on chromosome 2q24.3 and encodes the α-subunit of the voltage-gated sodium channel $Na_V1.7$. *SCN9A* is primarily *e*xpressed in the neurons of the dorsal root ganglia and was initially classified as a peripheral nervous system channel gene [4]. The expression of NaV1.7 in the brain, particularly in the embryonic hippocampus, was reported later, suggesting its role in the central nervous system [5].

In this study, we screened 78 patients with FASE using whole-exome sequencing. Nine variants of *SCN9A* were identified, six of which co-occurred with other mutations. Three patients exclusively harboured the *SCN9A* mutations (I775 M, R429C and A442 T). These mutations were heterozygous missense mutations and were confirmed by the Sanger sequencing method. Identical mutations were also detected in the unaffected fathers of all probands who showed no clinically recognisable phenotypes. Thus, we believe that these mutations may be related with incomplete hereditary dominance. The mutation c.1324 G > A/p.A442 T was also identified in an affected uncle of proband 4, who experienced FS during his childhood. However, these mutations were neither noted in 300 unrelated control subjects nor associated with polymorphic changes (reference database: 1000Genomes, dbSNP). Therefore, we believe that these mutations are pathogenic.

Previously, *SCN9A* has been reported to be associated with a group of heterogeneous phenotypes [6], including autosomal dominant primary erythermalgia, inherited erythromelalgia (IEM), paroxysmal extreme pain disorder, small-fibre neuropathy and congenital insensitivity to pain (CIP) [7–9]. An association between *SCN9A* and epilepsy was first suspected based on the linkage analysis that located an FS-related locus within the 2q23-24 region containing five sodium channel genes namely, *SCN1A*, *SCN2A*, *SCN3A*, *SCN6A* and *SCN9A* [10,11]. *SCN1A* is one of the most important causative genes for FASE [2], particularly for GEFS + and DS. Several *SCN9A* mutations have been identified in patients with FS-related epilepsies [11], and in a large family diagnosed with an autosomal dominant inherited disease GEFS + [6]. *SCN9A* is also considered as a genetic modifier that can aggravate slight *SCN1A* mutation-associated GEFS + and a susceptibility gene for DS [10,12,13]. In the current study, novel *SCN9A* mutations were identified in patients with FASE, supporting our hypothesis that *SCN9A* is a pathogenic gene for epilepsy. Based on the mechanisms underlying pain sensitivity, mutations in the SCN9A gene can cause sensitisation to pain

Table 2
Main clinical characteristics of three FASE children only with *SCN9A* gene mutation in the Southern Chinese Han population.

No.	Sex	Age at time of study (yrs)	Age at febrile seizure onset (yrs)	Age at afebrile seizure onset (yrs)	Clinical phenotype	Developmental status	Family history	Other comorbidities	Interictal EEG	MRI
Proband 2	G	6	1 +	No	FS	N	No	No	slow activity	N
Proband 3	B	5	1 +	6 +	FS +	N	No	No	Single spike and spike-slow waves	N
Proband 4	G	10	4	7	GEFS +	N	Yes	No	Sharp and spike-slow waves, Slow activity	N

B, boy; G, girl; N, normal.

Table 3
Three *SCN9A* gene variants in children with FASE in the Southern Chinese Han population.

No.	Varian type	Nucleotide changes	Amino acid changes	Father	Mother	Other family member	Highly evolutionary conservation	Topological domain	Score from PolyPhen-2	Prediction from PolyPhen-2
Proband 2	Missense	c.2325C > G	I775M	+	–	–	Yes	DIIS2	0.961	Probably damaging
Proband 3	Missense	c.1285C > T	R429C	+	–	–	Yes	DI-DII	0.981	Probably damaging
Proband 4	Missense	c.1324 G > A	A442 T	+	–	+	No	DI-DII	0.140	Benign

+, positive; -, negative.

in the neurons of the peripheral dorsal root ganglion. Genetic mutations in *SCN9A* cause changes in complement-related proteins or secondary messengers in the Nav1.7 channel, thereby resulting in decreased excitation threshold, increased repetitive discharges, altered brain sodium channel function and blocked connections between neurons, which may possibly trace the mechanism underlying epilepsy [14]. However, these studies cannot definitively attribute pathogenicity to the *SCN9A* variation, and further functional studies are required to exclude mutations in other genes. Further studies are also necessary as only a few reports on the role of *SCN9A* in seizure disorders are currently available; thus, its significance in these disorders remains controversial.

In conclusion, three novel heterozygous missense mutations in *SCN9A* were identified in patients with FASE by next-generation exome sequencing. This information may provide a novel perspective for understanding the pathology of FASE. Further research is necessary to confirm the role of *SCN9A* mutations in FASE and to reveal the effect of these mutations on protein function.

4. Materials and methods

4.1. Patients

We recruited 78 patients with FASE in strict accordance with the 2010 International Epilepsy Union classification criteria for the diagnosis of epilepsy and epilepsy syndrome. Patients were diagnosed on the basis of clinical history, electroencephalogram/ictal video electroencephalogram, polysomnography, neurological examination and cranial magnetic resonance imaging. A total of 300 healthy volunteers, native to the Southern Chinese Han population, without epilepsy and related diseases were selected as control subjects. Written informed consent was obtained from all subjects and healthy controls after explaining the nature and possible consequences of the study. The research programme was approved by the Ethics Committee for Human Research of Guangdong General Hospital, which conforms to the purpose of the Helsinki Declaration. Information was retrospectively obtained from medical records.

4.2. Gene sequencing and analysis

The experimental procedure in exome sequencing included ultrasonic fragmentation of DNA fragments, library construction, hybridisation capture, capture library amplification and purification and other standard procedures. The original data Fastq files generated by the sequencing platform were analysed using RTA software (real-time analysis, Illumina), CASAVA software v1.8.2 (Illumina), BWA and Genome Analysis Toolkit (GATK) bioinformatics software to evaluate the quality of sequencing. Subsequently, various bioinformatics platforms, including PolyPhen-2.2.2 software, ANNOVAR software, HGMD database, dbSNP database and 1000 Genome database, were used for annotating variations. Sanger sequencing was used to verify variation in candidate genes.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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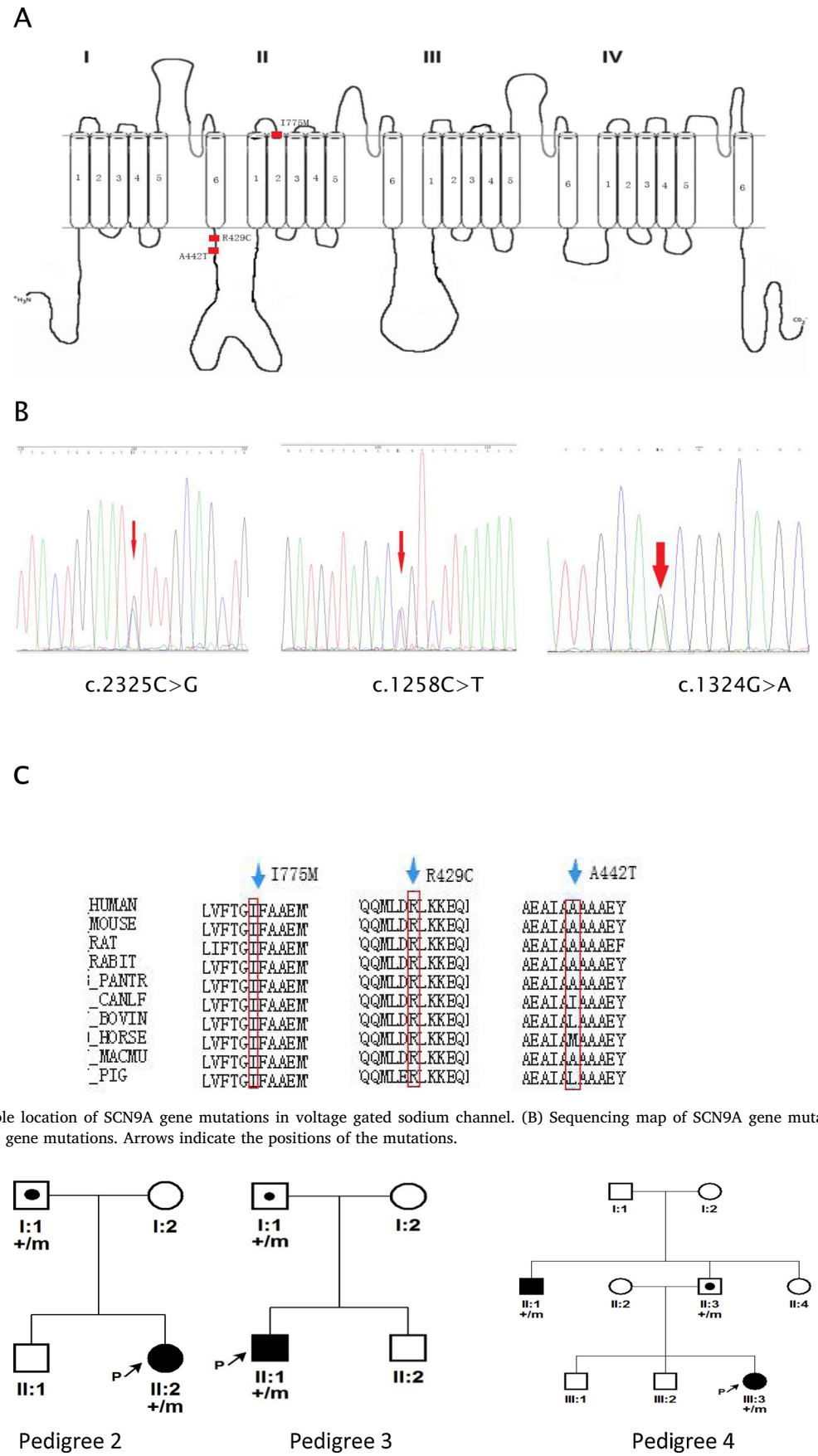

Fig. 1. (A) The probable location of SCN9A gene mutations in voltage gated sodium channel. (B) Sequencing map of SCN9A gene mutations. (C) Evolutionary conservation of SCN9A gene mutations. Arrows indicate the positions of the mutations.

Fig. 2. Pedigrees with mutation in the *SCN9A* gene. Filled symbols indicate affected individuals and clear symbols unaffected individuals; squares: males; circles: females; arrow represents the proband. Genotypes are indicated for each individual. "+" means wild type; "m" means mutant.

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