

Clinical and molecular analysis of epilepsy-related genes in patients with Dravet syndrome

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Abstract

Dravet syndrome is considered to be one of the most severe types of genetic epilepsy. Mutations in *SCN1A* gene have been found to be responsible for at least 80% of patients with Dravet syndrome, and 90% of these mutations arise de novo. The variable clinical phenotype is commonly observed among these patients with *SCN1A* mutations, suggesting that genetic modifiers may influence the phenotypic expression of Dravet syndrome. In the present study, we described the clinical, pathological, and molecular characteristics of 13 Han Chinese pedigrees clinically diagnosed with Dravet syndrome. By targeted-exome sequencing, bioinformatics analysis and Sanger sequencing verification, 11 variants were identified in *SCN1A* gene among 11 pedigrees including 7 missense mutations, 2 splice site mutations, and 2 frameshift mutations (9 novel variants and 2 reported mutations). Particularly, 2 of these Dravet syndrome patients with *SCN1A* variants also harbored *SCN9A*, *KCNQ2*, or *SLC6A8* variants. In addition, 2 subjects were failed to detect any pathogenic mutations in *SCN1A* and other epilepsy-related genes. These data suggested that *SCN1A* variants account for about 84.6% of Dravet syndrome in our cohort. This study expanded the mutational spectrum for the *SCN1A* gene, and also provided clinical and genetic evidence for the hypothesis that genetic modifiers may contribute to the variable manifestation of Dravet syndrome patients with *SCN1A* mutations. Thus, targeted-exome sequencing will make it possible to detect the interactions of epilepsy-related genes and reveal their modification on the severity of *SCN1A* mutation-related Dravet syndrome.

Abbreviations: CCDS1= cerebral creatine deficiency syndrome1, DS = Dravet syndrome, GEFS+ = generalized epilepsy with febrile seizures plus, MRS= magnetic resonance spectroscopy, Nav1.1 = type I voltage-gated sodium channel, SMEI = severe myoclonic epilepsy in infancy, SNV = single nucleotide variation.

Keywords: Dravet syndrome, genetic modifier, *SCN1A* mutation, targeted-exome sequencing

1. Introduction

Dravet syndrome (DS) is an infrequent progressive epileptic encephalopathy with an incidence between 1:20,000 and 1:60,000.^[1–3] It was firstly reported by Dravet in 1978, and initially called severe myoclonic epilepsy in infancy (SMEI). In 2001, the name was officially changed into DS by the International League Against Epilepsy. DS is one of the most severe types of genetic epilepsy characterized by febrile seizures and psychomotor developmental delay after seizures onset. Mutations in *SCN1A* gene, which encodes the voltage-gated sodium channel α -subunit, have been shown to be responsible for at least 80% of patients with DS, and 90% of these mutations arise de novo.^[4] Furthermore, DS rarely involves *PCDH19*, *STXBP1*, *GABRA1*, *GABARG2*, *SCN1B*, and *SCN2A* mutations.^[5–8] In addition, individuals with *SCN1A* mutations exhibit

widely different seizure severities ranges from intractable seizures of DS, through comparatively benign febrile seizure, to completely normal family members in some cases. It has been reported that other genetic susceptibility factors may exist and act as a partner with *SCN1A* mutations, leading to the various clinical phenotype of patients with DS.^[9,10] Genetic modifiers including *SCN2A*, *KCNQ2*, and *SCN8A* have been showed to influence the clinical presentation of human GEFS+ (generalized epilepsy with febrile seizures plus) associated *SCN1A* mutation by mouse models.^[11] Furthermore, Singh et al^[12] found that variants in *SCN9A* may act as a genetic modifier that exacerbated mild *SCN1A* mutation-associated GEFS+ and as a susceptibility gene for DS. However, more definitive clinical and genetic evidence is lacking. Here, the clinical and molecular characteristics of 13 DS cases were analyzed by targeted-exome sequencing to identify the novel variants in *SCN1A* gene among Han Chinese children and investigate the possibility of other epilepsy-related genes contributing to the variable manifestation of DS associated with *SCN1A* mutations.

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2. Materials and methods

2.1. Subjects

Peripheral blood samples from 13 DS pediatric probands hospitalized in the Department of Neurology, Children's Hospital of School of Medicine, Zhejiang University from January 2014 to September 2017, as well as their families, were collected. Furthermore, a comprehensive history and physical examination were performed on these subjects to fulfill the clinical registration forms including name, sex, date of birth, age at onset, inducing factor, clinical manifestations during onset, perinatal situation, previous history, family history, auxiliary

examination results, treatment, and prognosis. The study has been approved by Ethic Committee of Zhejiang University and written informed consents were obtained from all participants or their guardians (in the case of children).

2.2. Methods

2.2.1. Diagnostic criteria. According to Classification criteria of epilepsy and epilepsy syndrome (ILAE, 2010),^[13] the detailed diagnostic criteria for DS were as follows: onset age within 1 year with febrile seizures; normal mental and motor development before onset, but retrogression in the second year; longstanding generalized or lateral seizures or generalized tonic-clonic seizures, often induced by fever; multi-seizure types such as myoclonic seizures, partial seizures, and atypical absence seizures shown at 1 to 4-year-old, often presented as status epilepticus; onset characterized by heat sensitivity; mental and motor retardation after onset combined with ataxia and pyramidal signs; poor therapeutic efficacy of anti-epileptic drugs.

2.2.2. Mutational screening of epilepsy-related genes. Blood samples from subjects were collected (5 mL, EDTA anticoagulated), and FlexiGene DNA Kit (Qiagen, Germany) was used for whole genomic DNA extraction. Exon target capture and second-generation sequencing were used in combination to screen mutations in 308 epilepsy-related genes (Table S1, <http://links.lww.com/MD/C691>). Agilent SureDesign was employed to design target capture probes on target gene exons and flanking sequences (\pm 10 bp) (total 60,605 with 1.961 Mbp). Then,

Agilent SureSelect Target Enrichment System was combined with Illumina NextSeq 500 to perform high-throughput sequencing. Data analysis included fundamental assessment of original sequences and bioinformatics evaluation. After analysis by the RTA software (real-time analysis, Illumina San Diego, California), the CASAVA software v1.8.2 (Illumina), BWA and GATK, sequencing quality was assessed, and single nucleotide variation (SNV) reports were obtained. Furthermore, ANNOVAR, PolyPhen-2, SIFT, HGMD database, dbSNP database, and 1000 Genome database were used to conduct variation annotation and analysis to confirm candidate pathogenic mutations. To identify putative pathogenic mutation, variants associated with DS were evaluated using following criteria: recorded or reported as pathogenic mutation by HGMD and PubMed databases (reported mutation); minor allele frequency <0.01 in dbSNP and genotype frequency <0.005 in 1000 Genome databases; predicted as detrimental mutation by PolyPhen-2 and SIFT; alignment by National Center for Biotechnology Information (NCBI) BLAST Link showing highly conservation in evolution. Finally, the PCR-Sanger method was used to assess probands and their family members, as well as normal controls to confirm the pathogenic mutations.

3. Results

3.1. Mutational analysis of SCN1A gene in DS patients

As shown in Table 1, targeted-exome sequencing, bioinformatic analysis, and Sanger sequencing verification revealed that 11 out

Table 1
Molecular analysis of epilepsy-related genes in patients with Dravet syndrome.

| Family no. | Gene name | Mutation position (NC_000002.11) | Exon | Nucleotide change | Amino acid change | Hom/Het | Family genetic test |
|------------|---------------|----------------------------------|---------|-------------------|-------------------|---------|--|
| 1 | <i>SCN1A</i> | chr2-166856254 | Exon22 | c.4233_4234insAT | p.I1412fs | Het | Father: - Mother: - |
| | <i>SCN9A</i> | chr2-167137045 | Exon14 | c.2132T>C | p.L711S | Het | Father: + Mother: - |
| | <i>KCNQ2</i> | chr20-62045527 | Exon13 | c.1491G>C | P.G497D | Het | Father: + Mother: - |
| 2 | <i>SCN1A</i> | chr2-166854661 | Exon22 | c.4363G>A | p.E1455K | Het | Father: - Mother: - |
| | <i>SCN1A</i> | chr2-166896107 | Exon15 | c.2332-1G>C | Splice site | Het | Father: - Mother: - |
| 3 | <i>SLC6A8</i> | chrX-152959988 | Intron9 | c.1151-8C>T | Splice site | Hemi | Father: - Mother: + |
| | <i>SCN1A</i> | chr2-16894397 | Exon15 | c.2835C>G | p.F945L | Het | Father: - Mother: - |
| 5 | <i>SCN1A</i> | chr2-166856243 | Exon22 | c.4328A>T | p.D1443V | Het | Father: - Mother: - |
| 6 | <i>SCN1A</i> | chr2-166893002 | Exon18 | c.2985T>G | p.F995L | Het | Father: - Mother: - |
| 7 | <i>SCN1A</i> | chr2-166904281 | Exon9 | c.1029-3C>A | Splice site | Het | Father: - Mother: - |
| 8 | <i>SCN1A</i> | chr2-166894518 | Exon15 | c.2714C>A | p.A905D | Het | Father: - Mother: + Brother: + |
| 9 | <i>SCN1A</i> | chr2-166904189 | Exon8 | c.1118T>G | p.L373W | Het | Father: - Mother: - |
| 10 | <i>SCN1A</i> | chr2-166892909 | Exon16 | c.3078delG | p.V1026fs | Het | Father: - Mother: - |
| 11 | <i>SCN1A</i> | chr2-166852527 | Exon23 | c.4577C>T | p.P1526L | Het | Father: - Mother: - |

Screening conditions for the above putative pathogenic variants: recorded or reported as pathogenic mutation by HGMD and PubMed databases (known mutation); minor allele frequency <0.01 in dbSNP and genotype frequency <0.005 in 1000 Genome databases; predicted as detrimental mutation by PolyPhen-2.2.2 and SIFT; alignment by NCBI BLAST Link showing highly conservation in evolution; undetectable in local normal control individuals. The 2 reported mutations are underlined. + denotes mutant, - denotes wild type.

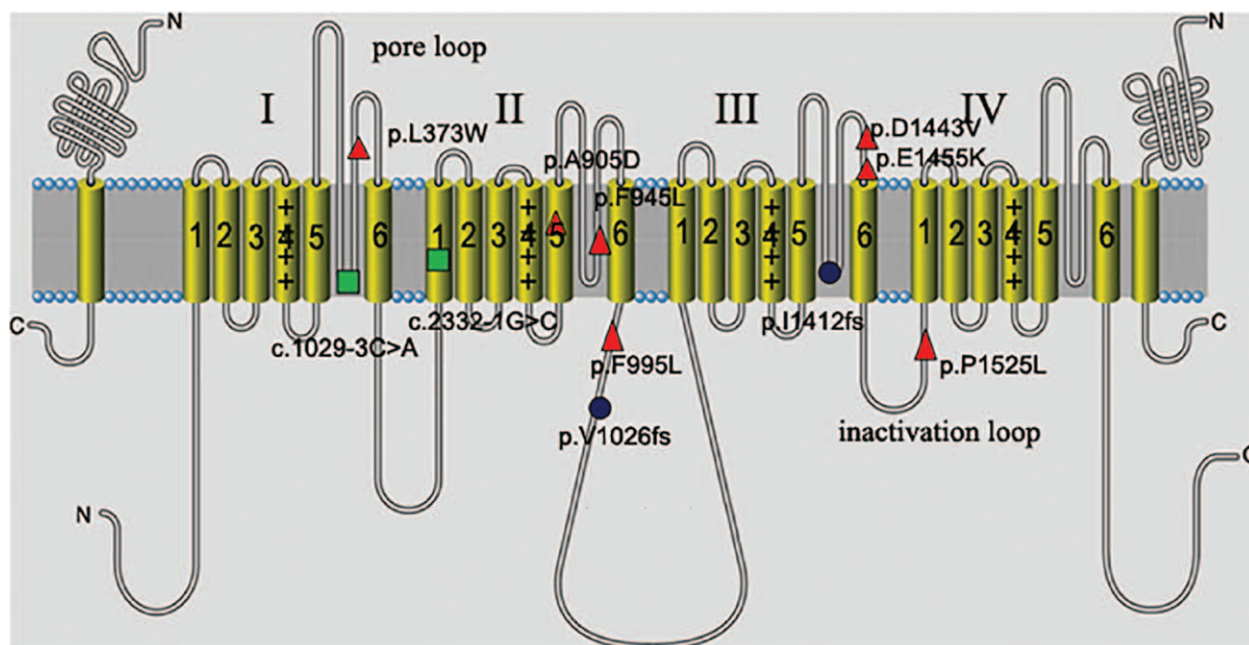


Figure 1. The schematic topology of the α -subunit of the sodium channel and the distribution of variants identified in our study. The α -subunit is composed of 4 homologous domains (DI–DIV) of 6 transmembrane segments each (S1–S6). S4 is the voltage zone. S5, S6, and the ring between them is the gate zone. Seven missense mutations were denoted by red triangles (p.L373W, p.A905D, p.F945L, p.F995L, p.D1443V, p.E1455K, and p.P1525L), 2 splice site mutations were indicated by green rectangles (c.2332-1G>C and c.1029-3C>A), and 2 frameshift mutations were in deep blue ellipses (p.I1412fs and p.V1026fs).

of 13 (84.6%) pediatric patients harbored *SCN1A* putative pathogenic variants in heterozygous state, including 7 missense mutations, 2 splice site mutations, and 2 frameshift mutations (9 novel variants and 2 reported mutations). Furthermore, the localization of these variants in the type I voltage-gated sodium channel (Nav1.1) alpha subunit was exhibited in Fig. 1. Among these missense variants, p.L373W, p.F945L, p.D1443V, and p.E1455K variants reside at the S5 to S6 pore loop regions,

p.P1525L variant is located at inactivation loop (connecting DIII and DIV), p.A905D variant occurs in S5 segment (DIII), and p.F995L variant resides at the loop between DII and DIII. The parental origin analyses for these variants by Sanger sequencings showed that 10 *SCN1A* variants in these DS pediatric patients were de novo, except the *SCN1A* c.2714C>A (p.A905D) variant. As shown in Fig. 2, the variant was inherited from his mother who displayed febrile seizures in Family 8, and the

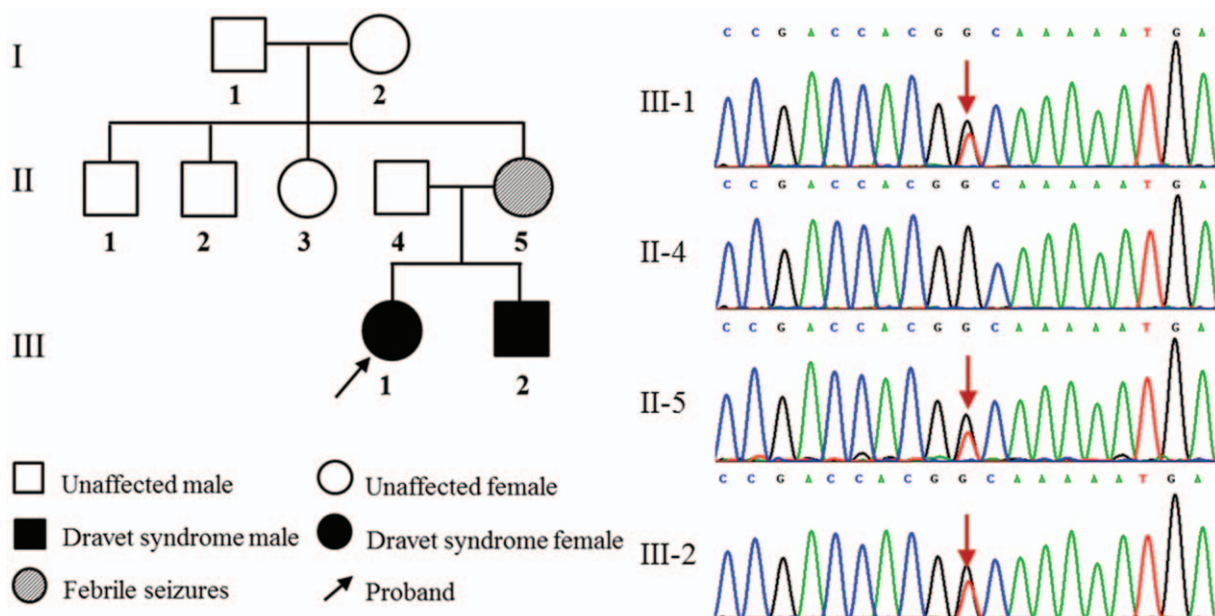


Figure 2. The *SCN1A* (c.2714C>A, p.A905D) variant was identified in Family 8. The variant was found in heterozygous state in mother with febrile seizures and 2 brothers with DS. The arrow denotes the position of the variant. DS=Dravet syndrome.

younger brother with DS phenotype also carried *SCN1A* c.2714C>A (p.A905D) variant. Additionally, 2 patients in our cohort were failed to detect any pathogenic mutations in 308 epilepsy-related genes.

3.2. Mutational analysis of other epilepsy-related genes in DS patients

In addition to the *SCN1A* variants mentioned above, putative pathogenic mutations in other epilepsy-related genes associated with DS were also evaluated (Fig. 3). In this cohort, 2 of these DS subjects with *SCN1A* variants were found to be in combination with the other epilepsy-related variants. Particularly, the pediatric patient from Family 1 carried *SCN9A* (c.2132T>C, p.L711S) and *KCNQ2* (c.1491G>C, P.G497D) variants along with *SCN1A* (c.4233_4234insAT, p.I1412Mfs37) variant. The variant p.L711S is located at the inactivation loop connecting DI and DII of voltage-gated sodium channel NaV1.7 which encoded by *SCN9A* and shared same overall structural motif as NaV1.1. The resulting P.G497D amino-acid change which occurred in the C-terminal of the potassium-channel subunit Kv7.2 (encoded by *KCNQ2*), was located in an evolutionarily conserved region. Furthermore, the proband (boy) in Family 3 with *SCN1A* c.2332-1G>C variant also harbored *SLC6A8* c.1151-8C>T variant, which may affect the process of precursor mRNA into mature mRNA and lead to the production of abnormal protein. In fact, neither of 2 variants was detected in the 100 unrelated population-matched controls by Sanger sequencing. The 2 missense mutations all localized at highly conserved amino acids of proteins and may cause potential structural and functional alterations. High probability of damaging effect by PolyPhen-2 and SIFT prediction of these variants further supported their pathogenic nature, and may contribute to the variable DS phenotype associated with *SCN1A* mutations.

3.3. Clinical and genetic characterization of *SCN1A* mutated patients with additional modifier variations

A genotype–phenotype correlation analysis was carried out among 2 *SCN1A* mutated patients carrying additional modifier variations (Table 2). In Family 1, the proband carried *SCN1A* variant along with *SCN9A* and *KCNQ2* variants has an unusually favorable cognitive and behavioral development. At admission to primary school, no obvious decline in cognitive level was observed. In Family 3, the proband with *SCN1A* and *SLC6A8* variants exhibited more serious of speech deficits, developmental delay, and intellectual deficiency. He is currently 5 years old, and has no speech and limited language comprehension. The interictal electroencephalogram (EEG) showed slow background activity and multifocal spike slow waves. The brain magnetic resonance imaging (MRI) suggested abnormal signal in ventricular trigone. Long echo time (288 ms) proton magnetic resonance spectroscopy (MRS) reveals diminished creatine signal which was acquired within frontal white matter. Furthermore, the patient's uncle from mother's side suffered seizures, mental, and motor retardation.

4. Discussion

Mutations in *SCN1A* gene have been found to be responsible for at least 80% of patients with Dravet syndrome, and 90% of these mutations arise de novo. In the present study, we detected 11 variants (including 10 de novo variants) in *SCN1A* gene among 11 pedigrees, accounting for about 84.6% (11/13) of DS in our cohort. Claes et al^[14] reported *SCN1A* gene mutation resulting in DS for the first time in 2001. Marini et al^[15] indicated that *SCN1A* mutation accounts for ~80% of all DS cases, while mutations in other genes only account for a small part. It has been reported that a total of 1727 *SCN1A* gene variants have been reported, including 1448 mutations associated with the DS

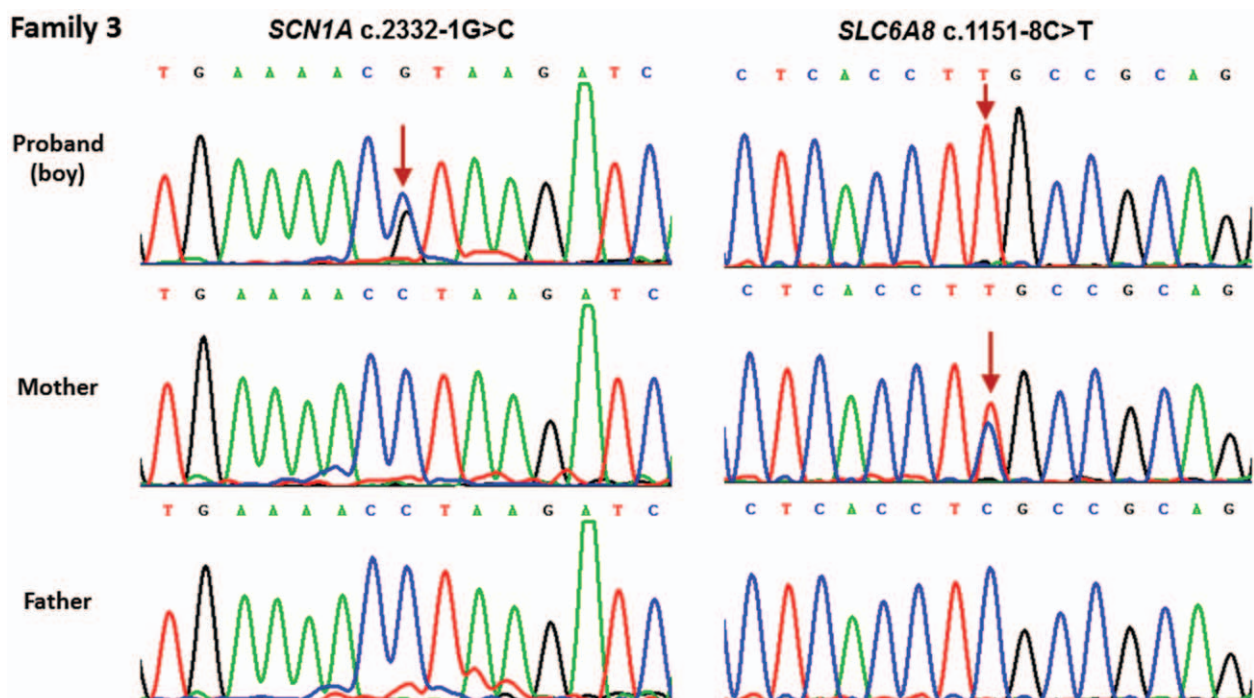


Figure 3. Genetic modifier was identified in Family 3 with *SCN1A* variant. The *SCN1A* c.2332-1G>C variant was de novo, and *SLC6A8* c.1151-8C>T variant was inherited from his mother in Family 3. The arrow denotes the position of the variant.

Table 2
Summary of clinical and molecular data for 11 DS patients with putative pathogenic variants on epilepsy-related genes.

| Family no. | Variants | Current age | Sex | Family history of seizure | Age at onset/ seizure type | Further seizure types | Neuroimaging (MR/MRS) | Current Seizure frequency | Cognition (WPPSI-IV) | Speech |
|------------|---|-------------|-----|---------------------------|----------------------------|-----------------------|---|---|---------------------------|------------|
| 1 | <i>SCN1A</i> c.4233_4234insAT <i>SCN9A</i> c.2132T>C <i>KCNQ2</i> c.1491G>C | 11y5m | F | No | 9m/GTCS | GTCS,MS,AAS,EMS | Normal (9m,9y) | Low frequency GTCS often induced by fever | Usual level (5y) | Normal |
| 2 | <i>SCN1A</i> c.4363G>A | 7y5m | M | No | 9m/GTCS | GTCS,PS | Normal (9m) | Low frequency GTCS and PS | Less than normal (5y) | Normal |
| 3 | <i>SCN1A</i> c.2332-1G>C SLC6A8c.1151-8C>T | 4y2m | M | Yes | 7m/GTCS | GTCS,PS,MS,AAS | Abnormal signal in ventricular trigone and diminished creatine signal (1y6m,3y9m) | GTCS and MS monthly | Mild mental deficits (4y) | No speech |
| 4 | <i>SCN1A</i> c.2835C>G | 4y3m | M | No | 9m/GTCS | GTCS,PS,MS | Normal (9m,2y) | GTCS and MS monthly | Borderline (4y) | Borderline |
| 5 | <i>SCN1A</i> c.4328A>T | 6y1m | F | No | 4m/PS | PS,GTCS,MS | Normal (5m,1y2m,3y) | PS and Low frequency GTCS | Borderline (5y) | Normal |
| 6 | <i>SCN1A</i> c.2985T>G | 6y1m | M | No | 7m/GTCS | GTCS,MS | Normal (7m,4y) | Low frequency GTCS often induced by fever | Less than normal (5y) | Normal |
| 7 | <i>SCN1A</i> c.1029-3C>A | 5y | M | No | 7m/GTCS | GTCS,MS,AAS? | Normal (7m) | GTCS and MS monthly | Less than normal (5y) | Normal |
| 8 | <i>SCN1A</i> c.2714C>A | 5y5m | F | Yes | 11m/GTCS | GTCS,MS,PS,AAS | Normal (11m,4y8m) | GTCS and AAS monthly | Borderline (5y) | Borderline |
| 9 | <i>SCN1A</i> c.1118T>G | 13y3m | F | No | 8m/GTCS | GTCS,MS,AAS? | Normal (8m,1y7m) | Low frequency GTCS often induced by fever | Borderline (5y) | Normal |
| 10 | <i>SCN1A</i> c.3078delG | 3y9m | M | No | 10m/PS | PS,GTCS,MS | Normal (10m) | GTCS and MS monthly | Unremarkable | Borderline |
| 11 | <i>SCN1A</i> c.4577C>T | 15y11m | M | No | 6m/GTCS | GTCS,PS,MS,AAS | Normal (4y,11y3m) | Low frequency GTCS often induced by fever | Borderline (5y) | Normal |

AAS = atypical absence seizure, EMS = eyelid myoclonia seizure, FS = febrile seizure, GTCS = generalized tonic-clonic seizure, MR = magnetic resonance, MRS = magnetic resonance spectroscopy, MS = myoclonic seizure, PS = partial seizure, WPPSI-IV = Wechsler Primary Preschool Scale for Children-Fourth Edition.

phenotype. Among these, 617 variants were missense mutations, and there was no hot spot mutation.^[4] In this study, *SCN1A* c.4233_4234insAT variant from Family 1 and *SCN1A* c.2985T>G variant from Family 6 has been previously reported^[16,17], while the remaining 9 variants were novel. These findings expanded the mutational spectrum for the *SCN1A* gene in Chinese Han population with DS.

A total of 11 *SCN1A* putative pathogenic variants were identified among 11 pedigrees including 2 frameshift mutations, 2 splice site mutations, and 7 missense mutations. *SCN1A* encodes the $\alpha 1$ subunit of the sodium channel, and is located on chromosome 2q24. The α -subunit includes 4 homologous regions with 6 transmembrane fragments (S1–S6) each. Among them, S4 is the voltage zone. S5, S6, and the ring between them is the gate zone. The *SCN1A* protein coding region, especially mutations in the gate zone can lead to permeability and electro-conductibility changes of the sodium channel that results in increased cell excitability and neuronal discharge with tiny stimulations, causing epilepsy. The c.4233_4234insAT variant in Family 1 and c.3078delG variant in family 10 caused an altered open reading frame, resulting in a completely different translation and dysfunction of Nav1.1. Two splice site mutations (c.2332-1G>C and c.1029-3C>A) which located at the acceptor-sites or recognition sites, leading to the abnormal processing of mRNA, were identified in Family 3 and Family 7, respectively. Amino acid synthesis and arrangement are greatly influenced by such mutations, and the function of the product is significantly changed, resulting in severe clinical manifestations. The remaining 7 missense mutations in *SCN1A* gene, including p.L373W, p.F945L, p.P1443V, and p.E1445K (S5–S6 pore loop regions),

p.A905D (S5), p.F995L (intracellular DII and DIII connecting ring), and p.L373W (intracellular DIII and DIV connecting ring). In our study, 5 missense mutations occurred in the gate zone (71.4%, 5/7), suggesting the *SCN1A* missense mutation associated with DS mostly located at important functional region. Our finding was consistent with previous studies by Zuberi et al^[18] and Meng et al.^[4]

SCN1A mutations in DS pediatric patients were mostly de novo, and their clinical phenotypes were greatly different.^[19] In our study, 10 of these *SCN1A* variants in DS patients were de novo, except the *SCN1A* c.2714C>A (p.A905D) variant. Sanger sequencing showed that this variant was inherited from his mother who exhibited febrile seizure before 6 years of age. In addition, his younger brother with DS phenotype also carried this variant. Several studies have reported that some DS subjects inherited *SCN1A* mutations from asymptomatic parents or mildly affected adult patients. Actually, somatic and germline *SCN1A* mosaicism has been reported in a small number of cases.^[20,21] Somatic mosaicism may account for variable expressivity of *SCN1A* mutations in SMEI families.

Genetic interaction between sodium channel mutations and variants in other channels have been described both in mouse models and affected patients.^[11] These results suggest that ion channel variants may contribute to the clinical variation observed in subjects with monogenic epilepsy. Particularly, interactions between genetic variations of *SCN1A* and *KCNQ2* observed in the mouse and mutations of *SCN1A* and *SCN9A* described in DS patients provide important models for potential genetic modifier effects of DS.^[12] In our cohort, *SCN1A*

mutations were found to be combined with other epilepsy-related mutations (*SCN9A* and *KCNQ2* variants in Family 1 and *SLC6A8* variant in Family 3) that may contribute to the variable phenotype of *SCN1A*-related DS. In Family 1, segregation analysis showed that the *SCN9A* p.L711S and *KCNQ2* p.G497D variations were inherited from the asymptomatic father without a family history of seizures. It has been reported that *SCN9A* variations themselves alone might be asymptomatic or cause infrequent febrile seizures due to incomplete penetrance and variable expressivity, but likely contribute to the modification of the severity of *SCN1A* related DS. There is indeed that a subset of DS patients commonly with mutations in *SCN1A* gene also harbor *SCN9A* mutations.^[22] In addition, *KCNQ2* mutation has been considered to be as a cause of seizures benign neonatal and epileptic encephalopathy early infantile.^[23] Previous study have demonstrated that *KCNQ2* mutation could exhibit increased susceptibility to induced seizures, and can dramatically influence the phenotype of mice carrying the *SCN1A* mutation.^[24] However, it is reassuring that the proband in Family 1 has undergone an unusually favorable cognitive and behavioral development in comparison with other DS subjects. Furthermore, mutations in *SLC6A8* has been reported to be associated with X-linked cerebral creatine deficiency syndrome1 (CCDS1), and speech difficulty is considered as a hallmark of CCDS.^[25] The defect of *SLC6A8* impaired the transport of creatine into brain and resulted in creatine transporter deficiency, thereby producing the phenotype of CCDS1. The *SLC6A8* c.1151–8C>T variant that identified in proband 3 has been reported to be pathogenic in X-linked recessive inherited model.^[26] Clinical evaluation showed that his speech deficits, developmental delay and intellectual deficiency are more serious than other DS patients, MRS reveals diminished creatine signal. Thus, these findings suggested that *SCN9A*, *KCNQ2*, and *SLC6A8* may act as modifiers influenced the phenotypic manifestation of the DS associated *SCN1A* mutations. Furthermore, these modifying alleles may preferentially found in DS patients with *SCN1A* mutations that are less deleterious when compared with complete heterozygous loss of function mutations. Additional functional data in mouse models to evaluate the interaction between the variants in genetic modifiers and *SCN1A* gene will be required to test if the modification is valid in certain DS subjects.

In conclusion, these data suggested that *SCN1A* putative pathogenic variants account for about 84.6% of DS in our cohort. The study expanded the mutational spectrum for the *SCN1A* gene, and also provided clinical and genetic evidence for the hypothesis that genetic modifiers may contribute to the variable manifestation of DS patients with *SCN1A* mutations. Thus, targeted-exome sequencing will make it possible to detect the interactions of epilepsy-related genes and reveal their modification on the severity of *SCN1A*-related DS. The hypothesis of genetic modifiers on *SCN1A*-related variable phenotype should require more evidences from clinical patients and mouse models. Additionally, the whole exome or genome screening would be helpful to explain the molecular pathogenesis of the 2 patients who failed to detect any pathogenic mutations in 308 epilepsy-related genes.

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Writing – review & editing: Zhefeng Yuan, Shanshan Mao, Feng Gao.

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