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De novo *SCN1A*, *SCN8A*, and *CLCN2* mutations in childhood absence epilepsy

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ABSTRACT

This study aimed to identify monogenic mutations from Chinese patients with childhood absence epilepsy (CAE) and summarize their characteristics. A total of 100 patients with CAE were recruited in Peking University First Hospital from 2005 to 2016 and underwent telephone and outpatient follow-up review. We used targeted disease-specific gene capture sequencing (involving 300 genes) to identify pathogenic variations for these patients. We identified three *de novo* epilepsy-related gene mutations, including missense mutations of *SCN1A* (c. 5399 T > A; p. Val1800Asp), *SCN8A* (c. 2371 G > T; p. Val791Phe), and *CLCN2* (c. 481 G > A; p. Gly161Ser), from three patients, separately. All recruited patients presented typical CAE features and good prognosis. To date, CAE has been considered a complex disease caused by multiple susceptibility genes. In this study, we observed that 3% of typical CAE patients had a *de novo* mutation of a known monogenic epilepsy-related gene. Our study suggests that a significant proportion of typical CAE cases may be monogenic forms of epilepsy. For genetic generalized epilepsies, such as CAE, further studies are needed to clarify the contributions of *de novo* or inherited rare monogenic coding, noncoding and copy number variants.

1. Introduction

Childhood absence epilepsy (CAE) is a major subtype of genetic generalized epilepsies (GGE). Previous studies classified the genes associated with CAE into two groups: ion channel and non-ion channel genes. The majority of the genes associated with CAE comprise ion channel genes, such as *CACAN1H, GABRA1, GABRB3* and *GABRG2* (Chen et al., 2003; Peloquin et al., 2006; Liang et al., 2006; Feng et al., 2017). Recently, many non-ion channel genes, such as *NIPA2,* were discovered to be related to CAE (Jiang et al., 2012). Since these known genes are identified in a minority of patients with CAE, we sought to further study the genetic basis of CAE. Although most with CAE have a resolution of seizures, some do not. They may develop other epileptic syndromes, such as juvenile absence epilepsy (JAE) and juvenile myoclonus epilepsy (JME), or have comorbidities, such as attention deficit and learning difficulties (Wirrell et al., 1996; Echenne et al., 2001). Thus, better understanding of the contribution of novel CAE-related genes should provide insights into better treatments.

2. Materials and methods

2.1. Patients

A total of 100 patients with CAE were recruited at the Peking University First Hospital from 2005 to 2016 and were followed up by telephone and outpatient follow-up visits. Those with high-quality DNA samples and complete clinical data were preferentially selected. All subjects were diagnosed with CAE by pediatric neurologists using the following criteria: (1) initial onset at 4–10 years of age; (2) absence seizures occurring multiple times per day; (3) electroencephalogram (EEG) during absence seizures showing bilateral, symmetric, and synchronous discharge of 3 Hz spike–waves with normal background; (4) normal neurological examination; (5) normal neuroradiological examinations. The Medical Ethics Committee of Peking University First Hospital approved this study. Informed consent was obtained from the parents of CAE patients. Clinical data (onset age, seizure frequency, development assessment, physical examination, EEG, neuroimaging, and treatment) were recorded by pediatric neurologists.

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Table 1
De novo missense mutations identified from Chinese patients with CAE.

Patient ID	Gene	Mutation	Inheritance	Protein	Polyphen2	MutationTaster
802	*SCN1A*	p. Val1800Asp	*de novo*	Nav1.1	Probably damaging	Disease causing
695	*SCN8A*	p. Val791Phe	*de novo*	Nav1.6	Probably damaging	Disease causing
931	*CLCN2*	p. Gly161Ser	*de novo*	CIC-2	Probably damaging	Disease causing

2.2. Targeted next-generation sequencing

Targeted next-generation sequencing (NGS) was performed on the genomic DNA of 100 CAE patients. On the basis of published literature and databases, 300 genes were selected as genes of interest (Table S1) (Zhang et al., 2015; Kong et al., 2015). The genes previously reported in the literature as being associated with CAE (the genes listed in the introduction) were included in this analysis. We designed a complete kit using the SureSelect Target Enrichment technique (Zhongguancun Huakang Gene Institute, Beijing, China). After library construction, we captured the coding regions, including the exon and exon–intron boundaries (1.285 Mbp), of the selected genes. Targeted NGS was subsequently performed on an Illumina GAIIx platform using paired-end sequencing of 110 bp to screen for mutations in the selected genes of 100 CAE patients and 50 controls. For these samples, clean paired-end reads were aligned on the human reference genome (hg19) and annotated. Insertion–deletions (indels) and single-nucleotide polymorphisms (SNPs) were excluded using the Genome Analysis Tool kit (GATK) and annotated by ANNOVAR. Sanger sequencing was performed on DNA samples of probands and their parents to determine the parental origin and verify the results of targeted next-generation sequencing.

2.3. Variation analysis

We used the following criteria to select possible pathogenic variations: (1) insertion/deletion variations; (2) termination codon variations; (3) splice site variations involving substitution at nucleotide +1/-1 of the intron; (4) missense variations predicted by Polyphen2 as probably/possibly damaging or benign. Variations meeting any one of the above criteria were regarded as candidate variations for further analysis (Zhang et al., 2015; Kong et al., 2015).

To assess evolutionary conservation, multiple sequence alignments of the affected amino acids in different species were performed using a sequence alignment program (ClustalW; The Biology Workbench, San Diego, CA, USA). The 1000 Genomes database (http://www.1000genomes.org/) and the Human Gene Mutation Database (http://www.hgmd.org/) were applied to identify SNPs or reported pathogenic mutations. After the assessment, on average, 10 possible pathogenic variations were identified in each patient (Table S2). To further prioritize variations, we selected heterozygous variations of genes with known autosomal or X-linked dominant disease-causing variants, homozygous or compound heterozygous variations of genes with autosomal recessive disease-causing variants and hemizygous variations of genes with X-linked recessive disease-causing variants as likely causative variations. Finally, we validated the likely causative variations by Sanger sequencing and determined the parental origin of the variations and clinical features of the patients (Zhang et al., 2015; Kong et al., 2015).

2.4. Protein structure modeling

The homology modeling server SWISS-MODEL was used to predict the tertiary structure of Nav1.1, Nav1.6, and ClC-2 proteins (Waterhouse et al., 2018; Bienert et al., 2017; Guex et al., 2009; Benkert et al., 2011; Bertoni et al., 2017).

3. Results

3.1. Entire cohort

One hundred Chinese Han CAE patients were recruited in trios from our pediatric neurology clinics at Peking University First Hospital. A total of 42 patients were male, and 58 were female. Mean onset age of the patients was 6.6 years (median 6.4 years; range 4.2–9.9 years). In total, 16 CAE patients presented a history of febrile seizures, 8 patients reported a family history of febrile seizures, and 13 patients claimed a family history of epilepsy. Absence status epilepticus was unobserved in the patients.

3.2. Mutation identification

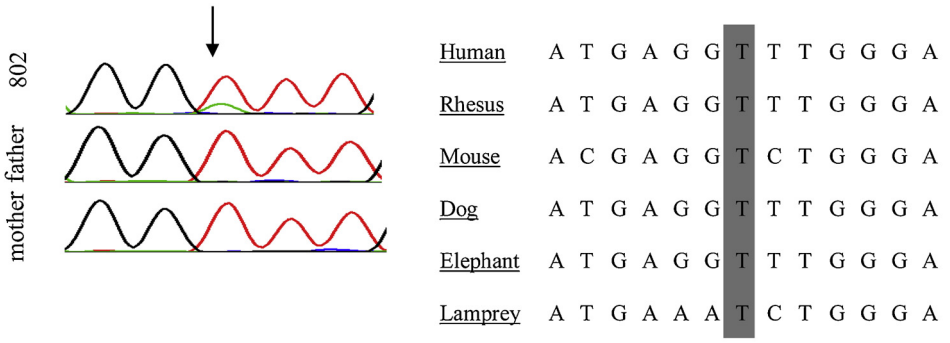
Using targeted next-generation sequencing for identification and Sanger sequencing for validation, *de novo* missense mutations, including *SCN1A* (c. 5399 T > A; p. Val1800Asp), *SCN8A* (c. 2371 G > T; p. Val791Phe), and *CLCN2* (c. 481 G > A; p. Gly161Ser), were identified from three independent cases among the 100 cases of clinically diagnosed typical CAE (positive rate of 3.0%). These mutations were not found in the 1000 Genomes database and 50 controls in this study. Table 1 shows details of the three mutations. All subjects but none of their parents were heterozygous. The mutations were all predicted as “probably damaging” in Polyphen2 and “disease-causing” in MutationTaster. The mutations were highly conserved according to homology comparison. Fig. 1 shows the sequencing data of each mutation.

The *SCN1A* mutation (Val1800Asp) in Patient 1 is located in the cytoplasmic C-terminus of Nav1.1 protein (amino acid 1786–2009). Val1800Asp. Glu1795Lys and Val1857Leu, which are associated with a milder phenotype, i.e., generalized epilepsy with febrile seizures plus 2 (GEFS + 2), are located nearby (Li et al., 2010; Nagao et al., 2005). However, several mutations related to early infantile epileptic encephalopathy (EIEE) and intractable childhood epilepsy with generalized tonic-clonic seizures (ICE-GTC) (e.g., Phe1808Leu and Trp1812Gly) are also found nearby (Fujiwara et al., 2003). We constructed a model of the Val1800Asp mutation through computational simulation and found no significant changes in protein structure (Fig. 2). Interestingly, our models of Glu1795Lys, Val1857Leu, Phe1808Leu and Trp1812Gly all suggest that they may also not cause significant changes in protein structure (Fig. 2).

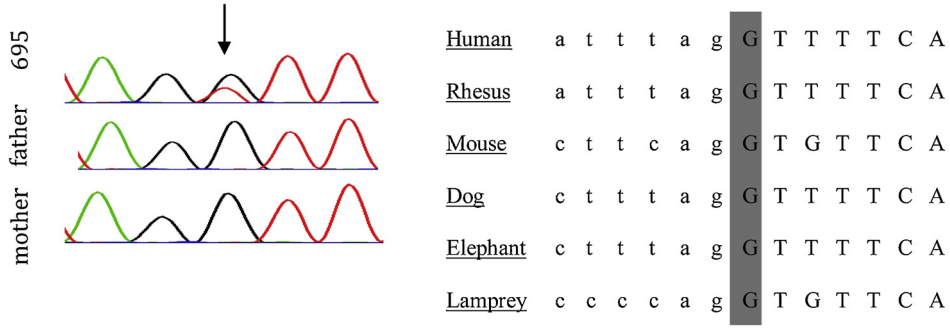
The *SCN8A* mutation Val791Phe in Patient 2 is located within the Nav1.6 segment S2 of domain II (amino acid 784–803). Reported mutations close to the site included Thr767Ile and Phe846Ser (Estacion et al., 2014; Ohba et al., 2014), which were all associated with EIEE. We created a model of Val791Phe through computational simulation and found a slight change in the loop region (Fig. 3). We also created models of Thr767Ile and Phe846Ser that predicted no significant changes in protein structure (Fig. 3).

For the Gly161Ser *CLCN2* mutation in Patient 3, we could not find nearby reported *CLCN2* mutations associated with CAE (Everett et al., 2007). Our structural model of Gly161Ser predicted an effect on the motif based on the selectivity filter, which might change the protein structure (Fig. 4).

Patient 802: *SCN1A* (c.5399T>A; p. Val1800Asp); the arrow showed the position of the mutation, and the red line stood for T, and the green line stood for A.



Patient 695: *SCN8A* (c.2371G>T; p. Val791Phe); the arrow showed the position of the mutation, and the black line stood for G, and the red line stood for T.



Patient 931: *CLCN2* (c.481G>A; p. Gly161Ser); the arrow showed the position of the mutation, and the black line stood for G, and the green line stood for A.

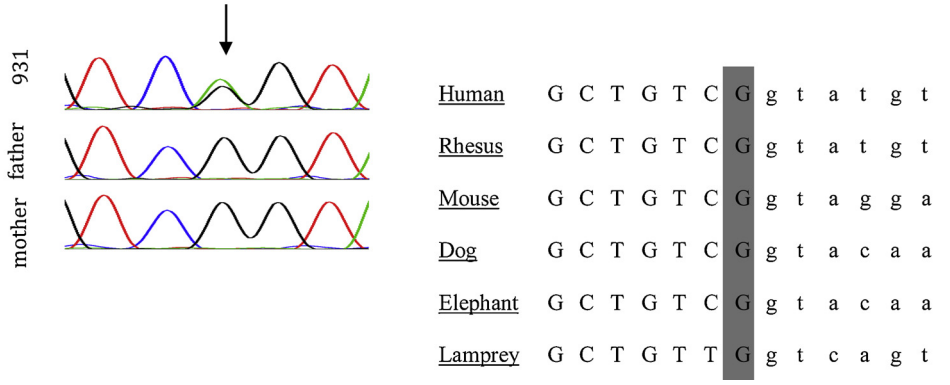


Fig. 1. Sanger sequencing and homology comparison of the three mutations identified from CAE patients.

3.3. Clinical findings of patients with a novel mutation

Patient 1 (ID 802) had the *de novo* p. Val1800Asp *SCN1A* mutation. Her family history was negative for epilepsy. Her initial epileptic seizures (absence seizures) occurred at 4.2 years of age and were occasionally accompanied by a mild tonic component at 4.5 years of age. The mild tonic component was occasionally associated with mild stiffness in both upper limbs during absence seizures, which occurred 10–20 times per day. She never experienced febrile seizures, and her seizures were insensitive to fever.

Patient 2 (ID 695) harbored the *de novo* p. Val791Phe *SCN8A* mutation. His family history was negative for epilepsy. His first absence seizure was noted at 7 years of age but then recurred 10–30 times per day.

Patient 3 (ID 931) had the *de novo* p. Gly161Ser *CLCN2* mutation. Her father suffered from febrile seizures during childhood, but none occurred after 5.5 years of age. Her first absence seizure occurred at 6.5 years of age. She only had typical absence seizures 5–10 times per day.

The EEG findings of the three patients showed bilateral, symmetric, and synchronous 3 Hz spike-and-wave discharges with normal background activity. All three had normal physical and neurological examinations and neuroimaging studies. Their seizures were all controlled well by valproic acid. Patient 1 became seizure-free after two weeks of treatment with valproic acid. At her last follow-up visit at 11 years of

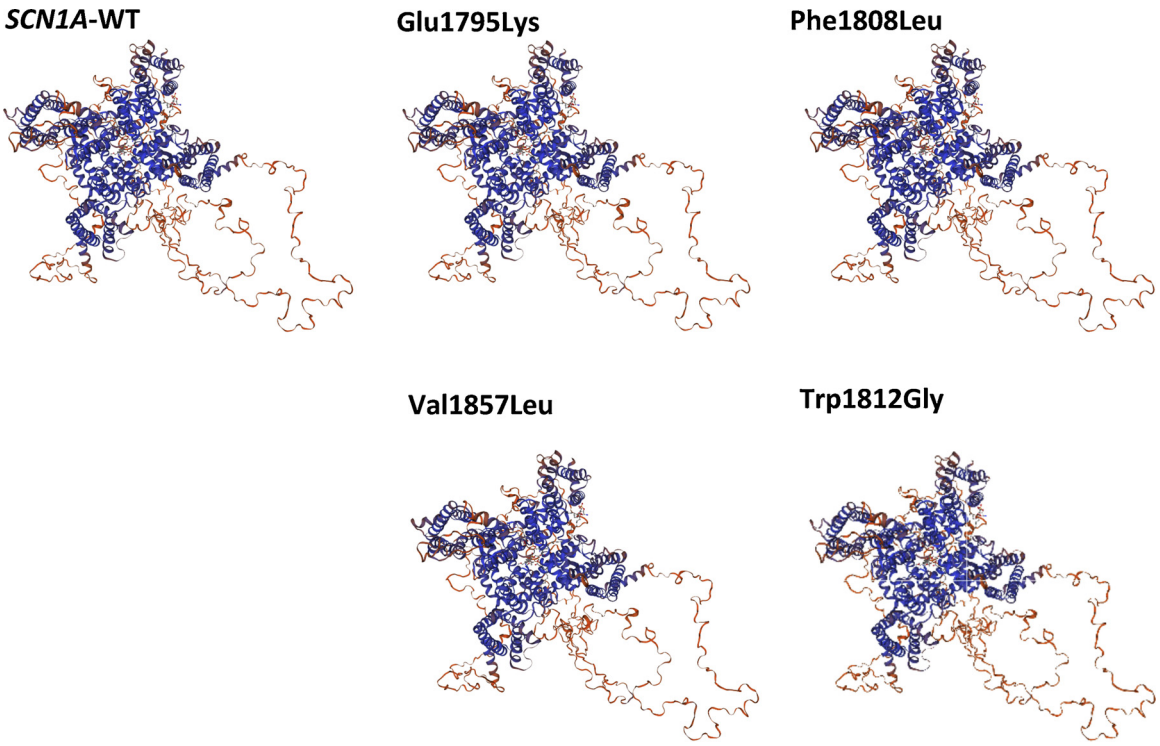


Fig. 2. Structure modeling of wild type and mutations of *SCN1A* with SWISS-MODEL. Compared with wild type, the mutations caused no significant changes in protein structure.

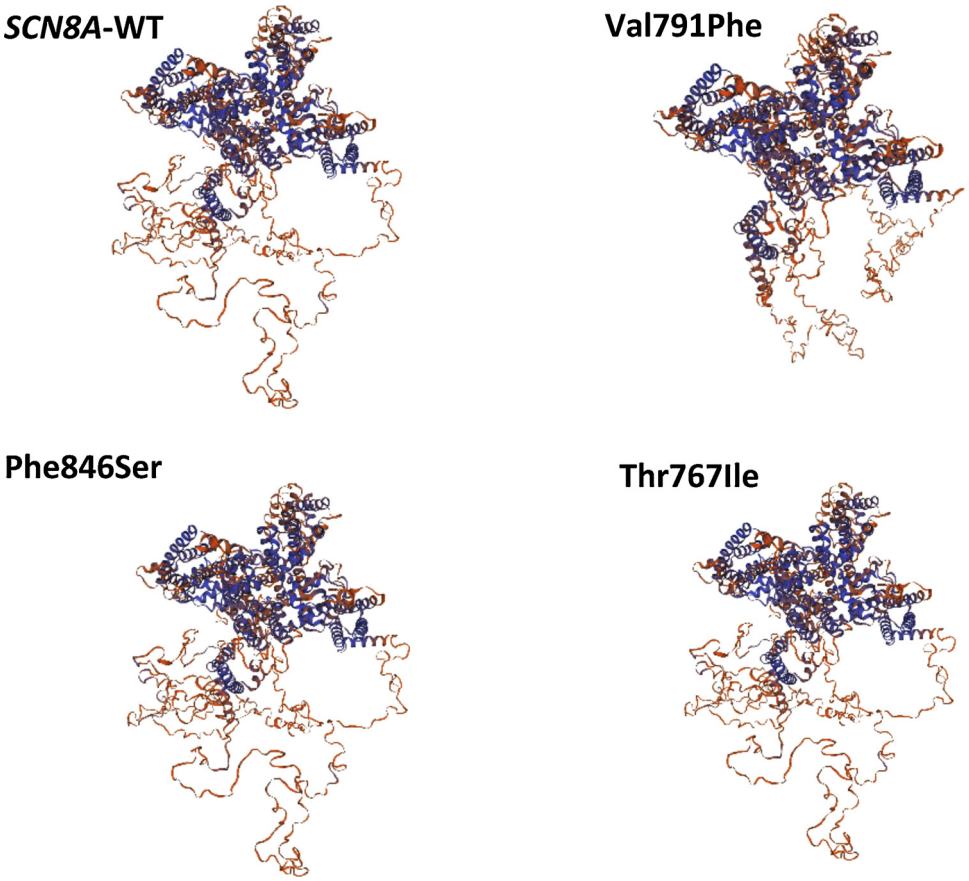


Fig. 3. Structure modeling of wild type and mutations of *SCN8A* with SWISS-MODEL. Compared with wild type, the mutation Val791Phe had a slight change in loop region, but other mutations caused no significant changes in protein structure.

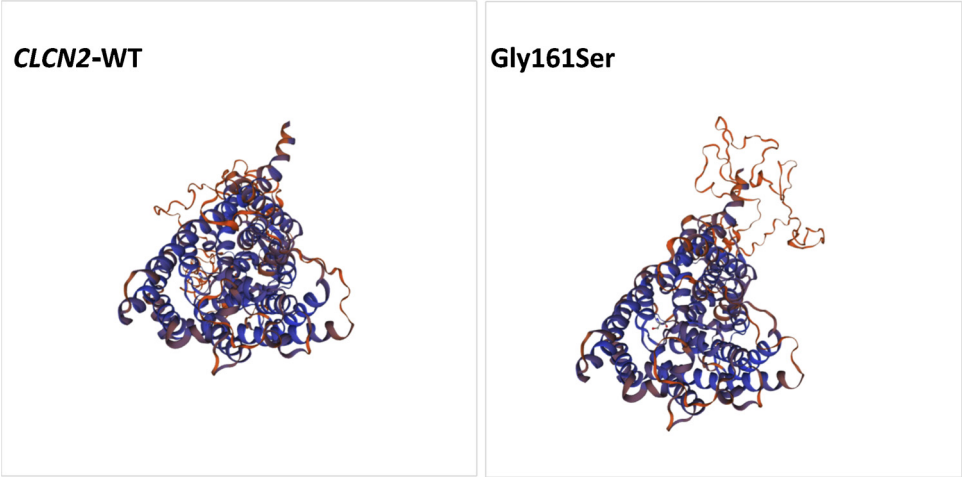


Fig. 4. Structure modeling of wild type and mutations of *CLCN2* with SWISS-MODEL. Compared with wild type, the mutation Gly161Ser affected the motif of selectivity filter part.

age, her psychomotor development was normal, and she had average academic grades. Patient 2 became seizure-free after two months of treatment. At his last follow-up visit at 12 years of age, his motor development was normal. His academic grades were below average, but he passed all his examinations. Patient 3 became seizure-free after two weeks of treatment. At her last follow-up visit at 15 years of age, her mental development was typical for her age, and she had average academic grades.

4. Discussion

4.1. Pathogenesis analysis of the three mutations

The *de novo* p. Val1800Asp *SCN1A*, p. Val791Phe *SCN8A* and p. Gly161Ser *CLCN2* mutations were identified in three independent cases in a cohort of 100 cases of clinically diagnosed with typical CAE. No mutations were found in the 1000 Genomes database or and 50 study controls. All mutations are highly conserved and are predicted to be "probably damaging" in Polyphen2 and "disease-causing" in MutationTaster. According to the guidelines for interpretation of sequence variants (The American College of Medical Genetics and Genomics), these mutations are classified as likely pathogenic (Richards et al., 2015).

4.2. CAE is a novel phenotype in SCN1A-related seizure disorders

CAE is a novel phenotype in the *SCN1A*-related spectrum of seizure disorders, which ranges from febrile seizures to Dravet syndrome and ICE-GTC. The reported phenotypes of *SCN1A*-related seizure disorders include GEFS+, ICE-GTC, Dravet syndrome, intractable infantile partial seizures, myoclonic–astatic epilepsy, simple febrile seizures, Lennox–Gastaut syndrome (LGS), infantile spasms, and vaccine-related seizures. Seizures reported with *SCN1A* mutations include tonic, clonic, tonic-clonic, myoclonic, and absence seizures. Our results indicate that *SCN1A* mutations may also induce absence epilepsy, which expands the spectrum of *SCN1A*-related seizure disorders. Patient 1's *de novo* p. Val1800Asp *SCN1A* mutation was associated with seizures insensitive but no myoclonic seizures, and she has no mental retardation. These features differ from those of reported cases with *SCN1A*-related seizure disorders. The *de novo SCN1A* missense mutation in Patient 1 may induce benign epileptic syndrome.

Nonsense and missense *SCN1A* variants in the voltage sensor or pore region usually cause more severe phenotypes (Zuberi et al., 2011; Suls et al., 2010; Yu et al., 2010). The Val1800Asp mutation in Patient 1 is located in the cytoplasmic C-terminus of Nav1.1 protein (amino acid 1786–2009), which is relatively unimportant as a voltage sensor or pore region. Mutations near p. Val1800Asp are associated with GEFS + 2, EIEE and ICE-GTC (Li et al., 2010; Nagao et al., 2005; Fujiwara et al., 2003). Thus, a weak relationship may exist between mutation location and disease severity. Since our computational models of these mutations found no significant changes in protein structure (Fig. 2), the mechanism of the Val1800Asp mutation inducing CAE remains unclear.

Our patient with *de novo* p. Val1800Asp *SCN1A* mutation presented typical absence seizures occasionally accompanied by mild tonic components. Since tonic seizures are associated with *SCN1A* mutations, and we will follow this subject to determine if these seizures increase over time.

We suggest that treatment of CAE patients with a *SCN1A* mutation with sodium channel blockers (SCBs) should be avoided because such treatment of patients with a loss-of-function mutation may further decrease sodium channel currents, which should reduce inhibitory neurotransmitters and increase seizures. Others suggest that SCBs treatment should be avoided if the loss or gain of function of a *SCN1A* mutation is unknown (Stafstrom, 2009; Catterall et al., 2008). We suggest that screening for *SCN1A* mutations be considered before treating CAE patients with SCBs. Patient 1's CAE and tonic episodes disappeared after treatment with valproic acid.

4.3. CAE is a novel phenotype in SCN8A-related epilepsies

SCN8A-related epilepsy with encephalopathy (Veeramah et al., 2012) is characterized by developmental delay and multiple seizure types, such as GTCS, absence, and infantile spasms. *SCN8A*-related epilepsy syndromes include LGS, West syndrome, and Dravet syndrome (Carvill et al., 2013; Allen et al., 2013). Importantly, CAE has not been reported to be associated with *SCN8A* mutations. Patient 2 had CAE with motor automatism, but the seizures were well controlled. In addition, his development was age appropriate, and he did not have status epilepticus or refractory seizures as typically observed (Larsen et al., 2015). His p. Val791Phe *SCN8A* mutation resides in the Nav1.6 segment S2 of domain II (amino acid 784–803), and nearby mutations include Thr767Ile and Phe846Ser (Estacion et al., 2014; Ohba et al., 2014), which are associated with EIEE. Our model of his Val791Phe mutation showed a slight change in the loop region, and our models of Thr767Ile and Phe846Ser showed no significant changes in protein structure (Fig. 3). These results imply that no definite relationship exists between disease severity and these structural changes. Therefore, further research is needed to determine how *SCN8A* mutations cause CAE.

4.4. CLCN2 is associated with CAE

We found a *de novo* p. Gly161Ser *CLCN2* mutation in Patient 3 with CAE. *CLCN2* encodes chloride channel 2 (CLC-2), which is a hyperpolarization-activated, inward rectifying chloride channel (Staley, 1994; Haug et al., 2009). ClC-2 leads to compensatory efflux of chloride, maintaining low intracellular chloride concentrations during chloride loading through repetitive activation of hyperpolarizing γ-aminobutyric acid (GABA) receptors. In previous studies, *CLCN2* mutations were found in patients with CAE (Everett et al., 2007), JAE, juvenile myoclonic epilepsy, or focal epilepsy (Haug et al., 2009; D'Agostino et al., 2004). Patient 3 had typical CAE. The sites of reported *CLCN2* mutations with CAE are distant from codon 161 (Everett et al., 2007). Our structural model of Gly161Ser predicted an effect on the selectivity filter motif (Fig. 4). The narrowest part of the pore in ion channels is regarded as a selectivity filter, and only selected ions can pass through the filter (Hanukoglu, 2017). If the structure of the selectivity filter motif is altered, ion selectivity will be changed. The change of ion selectivity may affect ClC-2 channel function, which should explain the pathogenesis of the *CLCN2* mutation.

4.5. CAE, monogenic, or complex disease?

CAE exhibits a complex hereditary basis. Our data suggest that monogenic inheritance should also be considered. Some calcium channel and GABA receptor genes are thought to induce CAE with monogenic inheritance (Chen et al., 2003; Peloquin et al., 2006; Liang et al., 2006; Annapurna and Daniel, 2011). In addition, *SLC2A1* mutations in glucose transporter type 1 (GLUT-1) have been identified in 10% of early-onset AE cases (onset age < 4 years) (Agostinelli et al., 2013a, 2013b; Muhle et al., 2013). Such monogenic mutations are thought to have strong pathogenicity in causing CAE. Minor mutations are thought to play modulating roles when no major mutation is present. In this study, the onset age of CAE patients was greater than 4 years, and all patients were diagnosed with typical CAE. No *SLC2A1* mutations were found in this study, and all the patients did not fit the criteria of GLUT-1 deficiency syndrome. Importantly, 3% of patients in this study had a pathogenic mutation of epilepsy-related genes (*SCN1A*, *SCN8A,* or *CLCN2*) that were considered to have an autosomal dominant mode. We suggest that the three mutations found in our patients cause CAE. Although CAE is presumed to be a disease with complex inheritance, we believe that the proportion of CAE caused by monogenic pathogenicity is likely underestimated. We suggest that further studies of single genes are needed in CAE patients as well as other GGE patients. We believe that some patients previously presumed to be affected by a complex disorder may actually have a monogenic disorder.

During our study period, targeted disease-specific gene capture sequencing is a reasonable and cost-effective method for gene screening. Usually, gene panels only include the reported genes associated with CAE. The limitation is that we cannot find novel causative genes from these gene panels. Therefore, to identify novel genes associated with CAE, we included some genes that have not been reported to be associated with CAE in our gene panels in this study. We eventually identified three novel genes related to CAE. However, our study still has limitations. We may miss causative genes associated with CAE due to the limited gene number in our panel. With advanced sequencing technology, such as whole exome sequencing (WES), becoming more feasible and cheaper, it should be more preferred for use for identifying novel genes.

5. Conclusion

In summary, we have discovered *de novo* p. Val1800Asp *SCN1A*, p. Val791Phe *SCN8A* and p. Gly161Ser *CLCN2* in three independent children with CAE. These data indicate that monogenic variations of major effects may play a role in other CAE patients. Therefore, monogenic mutation screening should be considered in CAE patients to clarify the etiology of the patient and the more comprehensive pathogenesis of CAE. We also suggest screening for monogenic mutations in CAE/GGE patients to further determine the contribution of monogenic causes and clarify clinical management.

Disclosure

None of the authors has any conflict of interest to disclose.

Acknowledgments

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.eplepsyres.2019.04.005.

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