



Genetic Diagnosis in Children with Epilepsy and Developmental Delay/Mental Retardation Using Targeted Gene Panel Analysis

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ABSTRACT

Purpose

Epileptic encephalopathies (EEs) are the severe form of childhood epilepsy and phenotypically heterogeneous disorders with different underlying genetic defects. EEs are always accompanied with developmental delay/mental retardation and behavior problems. Finding the genetic basis of epileptic encephalopathies with developmental delay/mental retardation can be valuable not only for diagnosis but also for guiding treatment and providing disease prognosis.

Methods and Results

A customized panel of 90 genes related to epileptic encephalopathies was utilized to screen for potential genetic variants via targeted next generation sequencing (NGS). A total of 78 children with epilepsy and developmental delay/mental retardation were analyzed with an average read depth of $265.3 \pm 68.3X$. Mutations were found in 23 (9 boys and 14 girls) probands, and the overall mutation identification rate was 29.5% (25.0% for males and 33.3% for females). Fifteen (65.2%) mutations involve ion channels, including *SCN1A*, *KCNT1*, *SCN2A*, *SCN8*, *KCNB1* and *KCNQ2*. The other (34.8%) mutations involve *CDKL5*, *ALG13*, *GFAP*, *SNAP25*, *STXBP1* and *SYNGAP1*. Sixteen (69.6%) of the identified mutations were confirmed to be *de novo* and one (4.3%) was found to be paternal mosaicism. Channelopathies were found to be the major cause of both early onset (≤ 6 months) and later onset of unclassified EE as well as febrile seizure cases. On the contrary, patients with infantile spasms or West syndrome were mainly caused by mutations in non-ion channel proteins, whereas patients of epilepsy with developmental delays and/or mental retardation were caused by mutations in both channel and non-channel genes.

Conclusion

NGS is a valuable and reliable diagnostic tool to detect the gene mutation in epileptic children with developmental delay/mental retardation. This cost-effective method shortens the course from seizure onset to genetic diagnosis. By understanding the gene mutations and genotype-phenotype correlation better, clinical practitioners could provide the optimal anticonvulsant and treatment.

Keywords

Epileptic encephalopathy (EE), Developmental delay (DD), Febrile seizure (FS), Infantile spasms (IS), West syndrome (WS), Targeted next generation sequencing (NGS), Mutation

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Introduction

Epilepsy is a common neurological disorder in children with broad phenotypic spectrum. Epileptic encephalopathies (EEs) are the most severe form of epilepsy. Heterogeneous clinical manifestations characterized by refractory seizures and developmental delay, which are often accompanied with various psychiatric comorbidities [1,2], age of onset, seizure types, interictal condition, electroencephalography (EEG) pattern and causes are variable in different epileptic encephalopathies. About one third of all EE cases are referred to as cryptogenic cases, where genetic factors are considered to play an important role [3-5]. More than 100 genes have been found to be involved in patients with epileptic encephalopathies [6]. Next generation sequencing (NGS) techniques, which allow massive parallel sequencing of as many genes as desired, are fast and cost-efficient diagnostic tools used to analyze the genetic cause of epileptic encephalopathies [6]. The genetic diagnostic yield can be increased from less than 10% to 28% in patients with epileptic encephalopathy using targeted NGS panels [7]. Not only the genes encoding membrane ion channel proteins, but also genes encoding proteins other than ion channels are now being identified as the cause of EE by NGS [8]. The present study is a retrospective cohort study for genetic testing in children with cryptogenic epilepsy and developmental delay/mental retardation (DD/MR) using NGS to identify the genetic characteristics and correlation with clinical features.

Materials and Methods

■ Participants

Seventy-eight Taiwanese children (36 boys and 42 girls) with epilepsy and DD/MR were collected with informed consent according to protocol (105142F) approved by the Internal Review Board of Far Eastern Memorial Hospital. Inclusion criteria were 70 children aged 7 months to 18 years 5 months (average 6 years 8 month \pm 4 years 6 months) and 8 long-term follow-up patients (aged 20 years 4 months to 26 years 8 months, average 23 years \pm 2 years 4 months) with cryptogenic epileptic encephalopathy. Children with family history of known neurological disorders, possible damage or infection of central nervous system (CNS), and known metabolic disturbance were excluded. Twenty three (13 males and 10 females) had early onset epileptic encephalopathy (EoEE)

with seizure onset before 6 month-old with or without suppression-burst on EEG. Ten (3 males and 7 females) had unclassified epileptic encephalopathy (EE) with seizure onset after 6-month-old. Twenty (6 males and 14 females) exhibited either infantile spasms (IS) or West syndrome (WS). Twenty one (11 males and 10 females) presented with epilepsy with developmental delay and/or mental retardation (E-DD/MR). Four (3 males and 1 females) had febrile seizure (FS) with developmental delay and/or mental retardation (FS-DD/MR). Genomic DNA (gDNA) were extracted from peripheral white blood cells and were evaluated both quantitatively and qualitatively by an Invitrogen™ Qubit™ 3.0 Fluorometer (ThermoFisher Scientific Inc., Waltham, MA, USA) and an Agilent 2200 TapeStation system (Agilent Technologies, Santa Clara, CA, USA) before being used for NGS analysis.

■ Library preparation and sequencing

A customized panel of 90 genes related to epileptic encephalopathy was created using SureDesign (Agilent Technologies, Santa Clara, CA, USA) and used as the HaloPlex probe capture library for the enrichment of DNA targets for mutational screening. For the generation of standard target-enriched libraries, the Agilent HaloPlex Target Enrichment System kit was used following the protocol for Illumina HiSeq paired-end sequencing library (Agilent Technologies, Santa Clara, CA, USA). The gDNA was digested in eight different restriction reactions (225 ng DNA/reaction), each containing two restriction enzymes. All restriction reactions were validated by using High Sensitivity D1000 ScreenTape in an Agilent 2200 TapeStation (Agilent Technologies). The collection of gDNA restriction fragments was hybridized to the HaloPlex probe capture library at 54°C for 3 hours for target enrichment. The circularized target DNA-HaloPlex probe hybrids were captured on streptavidin beads (HaloPlex Magnetic Beads, Agilent Technologies) and DNA ligase was then added to close nicks in the hybrids. Elution of captured DNA was carried out using NaOH, and 20 μ L of the eluted supernatant was used as template for PCR amplification. Twenty PCR cycles were performed to amplify target libraries and the PCR amplicons were purified using Agencourt AMPure XP beads (Beckman Coulter, Brea, CA, USA) for NGS. DNA sequencing was performed on an Illumina Miseq system using 150PE protocol (Illumina, Inc. San Diego, CA, USA).

■ Variants analysis

The sequencing data obtained from Illumina Miseq instrument were subjected to QC verification and the qualified read data went through genomic alignment against Ensembl human reference genome database using BWA software [9]. Variant calling of aligned sequences were created by Genome Analysis Toolkit (GATK) [10] and further analyzed using Illumina VariantStudio 3.0 software (Illumina, Inc.). Identified variants in probands and their parents were subjected to Sanger sequencing for further validation.

Results

A total of 78 Taiwanese patients with epileptic encephalopathies were collected and analyzed using targeted NGS. The average read depth on target sequences was $265.3 \pm 68.3X$ (ranged from 122-464X). As shown in **Figure 1**, mutations were found in 23 (9 boys and 14 girls) probands (Supplementary materials); the overall mutation identification rate via this designed panel was 29.5% (25.0% for males and 33.3% for females). Among them, 8 individuals were EoEE (identification rate: 8/23, 34.8%), 6 were IS/WS (6/20; 30.0%), 4 were E-DD/MR (4/21, 19.0%), 3 were EE (3/10, 30.0%), and 2 were FS-DD/MR (2/4, 50.0%). The details of patient clinical presentations and identified mutations are listed in **Table 1**. Fifteen (65.2%) mutations involve ion channels, including 5 (21.7%) in SCN1A, 4 (17.4%) in KCNT1, 2 (8.7%) each in SCN2A and SCN8A, and one (4.3%) of each for KCNB1 and KCNQ1. The

other 8 (34.8%) mutations include 3 (13.0%) in CDKL5 and one (4.3%) of each for ALG13, GFAP, SNAP25, STXBP1 and SYNGAP1 (**Figure 2**). Sixteen (69.6%) of the identified mutations were confirmed to de novo and one (4.3%) was found to be paternal mosaicism (**Figure 3**). Confirmation of paternity for de novo cases was performed by genotyping a short tandem repeat (STR) marker. Seven de novo mutations were identified in patients with EoEE (7/23, 30.4%), 5 for IS/WS (5/20, 25.0%), 1 (1/10, 10.0%) for EE, and 3 (3/21, 14.3%) for E-DD/MR. De novo mutation rate was 55.6% (5/9) for males and was 78.6% (11/14) for females. The others include 2 familial mutations (8.7%) and 5 (21.7%) were not determined due to the lack of parental samples. Seizure onset to diagnosis among 23 individuals with mutation identified by targeted NGS ranges from 1 month to 21 years and 8 months (average 6 years and 7 months \pm 5 years and 3 months). The frequency of all identified mutant alleles were relatively rare among all populations, including Asians, and the highest frequency reported was 8×10^{-6} for rs267607518 of the *GFAP* in the Exome Aggregation Consortium (ExAC) database. Twelve (12/23, 52.2%) of the 23 identified mutations have never been reported previously.

As shown in **Figure 2B**, channelopathy was the major cause of EoEE and EE. EoEE involves mainly KCNT1 followed by SCN8A, SCN1A and SCN2A as well as a non-channel protein CDKL5. EE involves SCN1A and KCNT1. In addition, two FS-DD/MR patients also carry mutations in the ion channel SCN1A. On the contrary, patients with IS/WS mainly

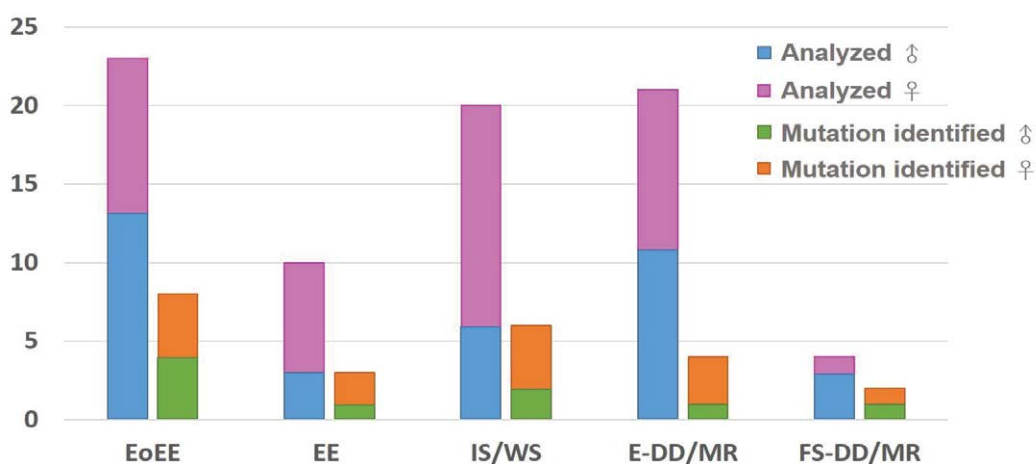


Figure 1: Summary of targeted NGS analyzed children with various epileptic encephalopathies. EoEE: Early-onset Epileptic Encephalopathy, EE: Unclassified Epileptic Encephalopathy, IS/WS: Infantile Spasms or West Syndrome, E-DD/MR: Epilepsy with Developmental Delays and/or Mental Retardation, FS-DD/MR: Febrile Seizure with Developmental Delays and/or Mental Retardation.

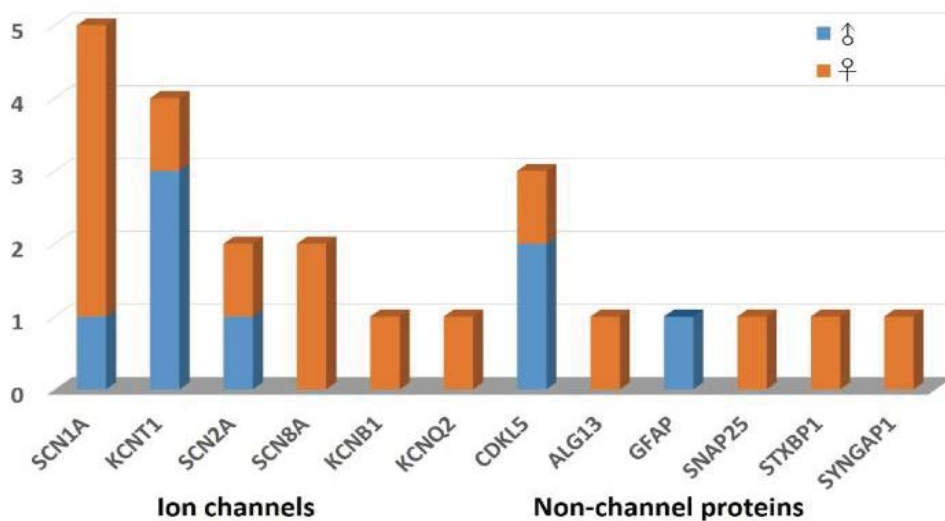
Table 1: Summary of clinical presentations and identified mutations.

| Gene (GRCh37 coordinate) | Sex | Age of Diagnosis | Seizures (Onset age) | Mutation | Parental Origin/ Novel(CS) | Diagnosis |
|-------------------------------------------------------------------------|-----|------------------|----------------------------|-------------------------------------------|----------------------------------------------------|------------------------------------|
| SCN1A (NM_001202435.1) (chr2:166,929,951) | F | 4y8m | FS, GS, PS (4m) | c.181delC p.L61Ffs*31 | <i>De novo</i> Novel | EoEE, MR |
| | | | | | | |
| | F | 2y5m | FS, GS, PS (4m) | c.482delT p.F161Sfs*11 | NA Novel | Complex FS, DD |
| (chr2:166,911,268) | | | | | | |
| | F | 23y2m | FS, GS, PS (1y) | c.1134_1136delAAT p.M379del | non-M Novel | EE, DD/MR |
| (chr2:166,904,171) | | | | | | |
| (chr2:166,903,480) | F | 8y | FS,GS,PS (1y) | c.1177C > T p.R393C | <i>De novo</i> rs398123588(P) | EE, DD/MR |
| | | | | | | |
| (chr2:166,852,549) | M | 9y | FS,GS,PS (1y1m) | c.4555C > A p.P1519T | Paternal rs796053021(U)*,# | GEFS+, DD, ADHD |
| | | | | | | |
| KCNT1 (NM_020822.2) (chr9:138,656,882) | M | 1y8m | SE (1m) | c.1041G > C p.E347D | <i>De novo</i> Novel*,# | EoEE, Expired |
| | | | | | | |
| (chr9:138,657,552) | F | 8y5m | GS, PS, TS (2m) | c.1283G > A p.R428Q | NA rs397515402(P) | EoEE, DD/MR |
| | | | | | | |
| (chr9:138,650,285) | M | 8y8m | GS,PS (1y) | c.785G > A p.R262Q | non-M SCV000589778.2(LP)*,# | EE, DD/MR |
| | | | | | | |
| (chr9:138,670,658) | M | 1y7m | GS,PS (2m) | c.2719A > G p.T907A | <i>De novo</i> (Paternal mosaicism) Novel*,# | EoEE, DD/MR |
| | | | | | | |
| SCN2A (NM_001040142.1) (chr2:166,170,505) | F | 11y | FS,IS GTCS, TS, MS (8m) | c.1270G > A p.V424M | <i>De novo</i> Novel*,# | WS, Rett-like syndrome, DD/MR [42] |
| | | | | | | |
| (chr2:166,165,272) | M | 25d | MS, TS, PS (3d) | c.573G > T p.W191C | <i>De novo</i> Novel*,# | EoEE, DD/MR |
| | | | | | | |
| SCN8A (NM_014191.3) (chr12:52,145,307) | F | 2y7m | GS (1m) | c.2300C > T p.T767I | <i>De novo</i> rs797045013(P) | EoEE, DD/MR |
| | | | | | | |
| (chr12:52,184,185) | F | 1y | PS,GS (4m) | c.4423G > A p.G1475R | <i>De novo</i> rs796053216(C)# | EoEE, DD |
| | | | | | | |
| KCNB1 (NM_004975.2) (chr20:47,991,468) | F | 7y4m | GS, PS (2y6m) | c.629C > T p.T210M | <i>De novo</i> Novel*,# | Epilepsy, MR |
| | | | | | | |
| KCNQ2 (NM_172107.2) (chr20:62,044,825) | F | 10y10m | GS,TS (7d) | c.1741C > T p.R581* | Maternal rs118192236(P) | Epilepsy, DD |
| | | | | | | |
| CDKL5 (NM_003159.2) (chrX:18,622,714) | M | 3y | IS, GS (2m) | c.1670C > G p.S557* | NA Novel | WS, MR |
| | | | | | | |
| (chrX:18,646,629) | F | 3y2m | MS, TS (2m) | c.2635_2636delCT p.L879Efs*30 | <i>De novo</i> rs61753251(P) | EoEE, DD/MR, Autism |
| | | | | | | |
| (chrX:18,616,605) | M | 9y1m | IS (3m) | c.849dupT p.D284* | <i>De novo</i> Novel | WS, DD/MR |
| | | | | | | |
| ALG13 (NM_001099922.2) (chrX:110,928,268) | F | 5y4m | IS (5m) | c.320A > G p.N107S | <i>De novo</i> rs398122394(P) | WS, DD/ MR |
| | | | | | | |
| GFAP (NM_001242376.1) (chr17:42,992,637; chr17:42,992,596) | M | 6y9m | PS (8m) | c.[218T > C; 259 G > T] p.[M73T; V87F] | <i>De novo</i> rs61060395(P); rs267607518(P) | Epilepsy, DD/MR |
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|------------------------------------------------------|---|-------|------------------------|------------------------|---------------------------------------------|-----------------|
| SNAP25 (NM_003081.3) (chr20:10,265,406) | F | 5y10m | IS,TS (7m) | c.149T > C p.L50S | <i>De novo</i> Novel* [#] | IS, DD/ MR |
| STXBP1 (NM_003165.3) (chr9:130,420,708) | F | 6y11m | IS, TS, GS, PS (4m) | c.224A > G p.Y75C | <i>De novo</i> rs796053352* [#] | WS, MR |
| SYNGAP1 (NM_006772.2) (chr6:33,408,615) | F | 11y | GS (1y3m) | c.1786C > A p.R596S | <i>De novo</i> Novel* [#] | Epilepsy, DD/MR |

DD: Developmental Delay; EE: Unclassified Epileptic Encephalopathy; EoEE: Early-onset Epileptic Encephalopathy; FS: Febrile Seizure; GEFS+: Generalized Epilepsy with Febrile Seizures Plus; GS: Generalized Seizure; GTCS: Generalized Tonic-Clonic Seizure; IS: Infantile Spasms; MR: Mental Retardation; MS: Myoclonic Seizure; PS: Partial Seizure; SE: Status Epilepticus; TS: Tonic Seizure; WS: West Syndrome; NA: Not Available; Non-M: Not Maternally Inherited; CS: Clinical Significance as listed in the ClinVar database; C: Conflicting Interpretations of Pathogenicity; LP: Likely Pathogenic; P: Pathogenic; U: Uncertain Significance; *: the variant is predicted to be damaging or deleterious by in silico analyses; #: the variant is located in a critical or evolutionarily conserved region of the protein.

(A)



(B)

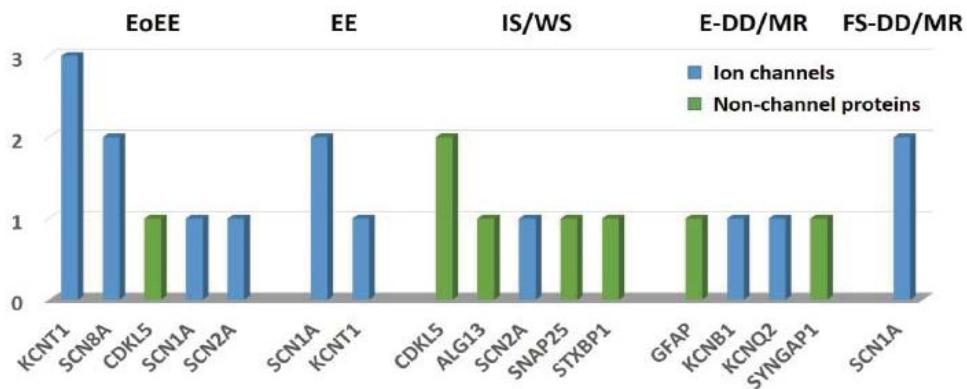


Figure 2: Summary of mutated genes identified by targeted NGS screening among children with various epileptic encephalopathies as grouped by (A) gene functions and (B) clinical presentations. EoEE: Early-onset Epileptic Encephalopathy, EE: Unclassified Epileptic Encephalopathy, IS/WS: Infantile Spasms or West Syndrome, E-DD/MR: Epilepsy with Developmental Delays and/or Mental Retardation, FS-DD/MR: Febrile Seizure with Developmental Delays and/or Mental Retardation.

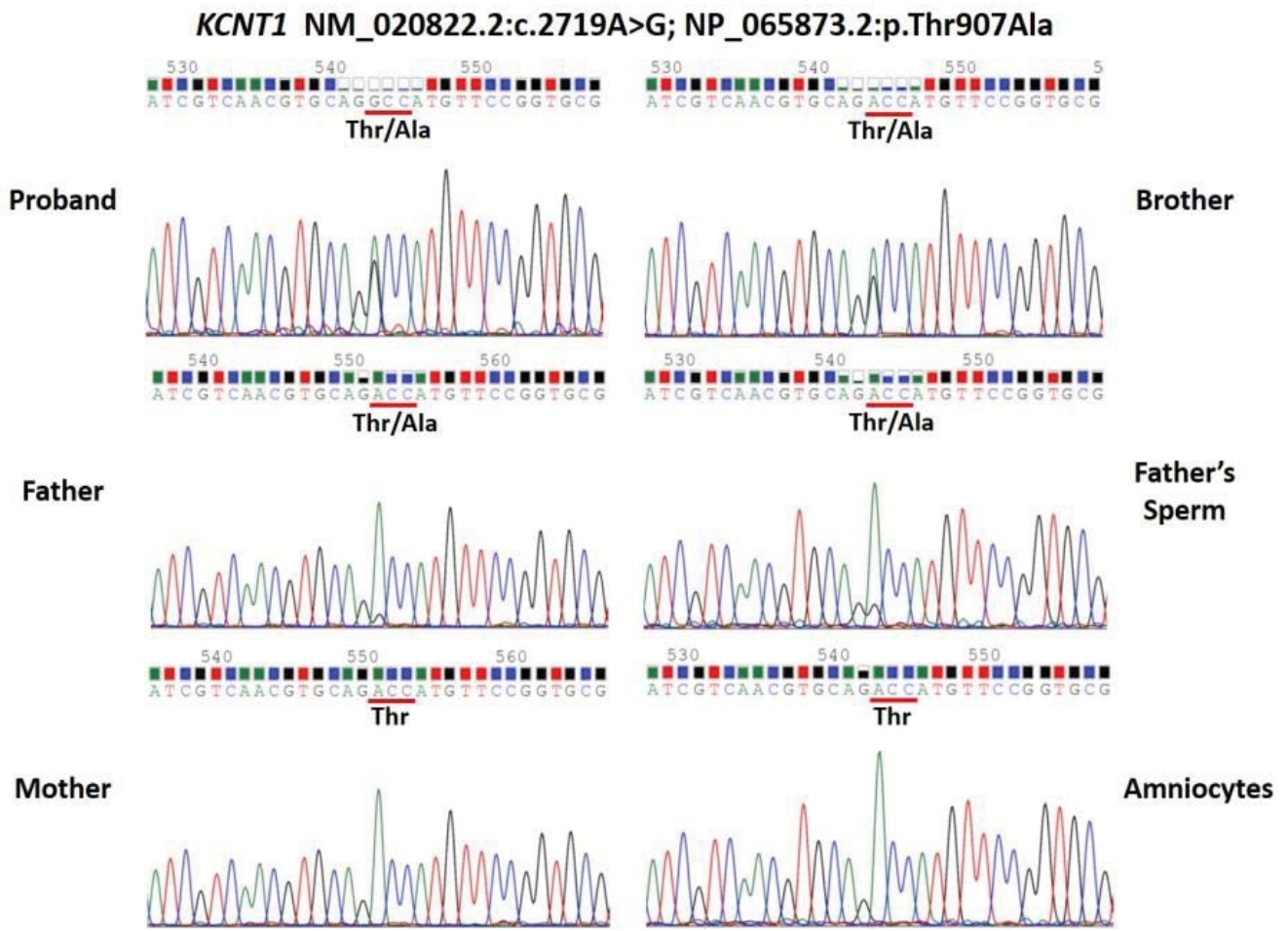


Figure 3: Sanger sequencing data of *KCNT1* (NM_020822.2:c.2719A>G; NP_065873.2:p.Thr907Ala) from peripheral blood of analyzed family members, paternal sperms and amniocytes of a new pregnancy.

resulted from mutations in non-ion channel proteins, including CDKL5, ALG13, SNAP25 and STXBP1 as well as one caused by SCN2A mutation. Patients with E-DD/MR were caused by mutations in both channel and non-channel proteins, including KCNB1 and KCNQ2, as well as GFAP, and SYNGAP1.

Discussion

It is often difficult to diagnose accurately for young children with seizures due to continuous evolving nature of age-dependent epileptic encephalopathies [11]. The phenotypic spectrum is broad for the same mutated gene and the phenotype may be similar among mutations in different genes. Therefore, a cost-effective genetic screening can help the practitioner make the diagnosis. A panel of 90 genes for targeted NGS screening was developed to facilitate the identification of underlying genetic causes in order to assist clinical diagnosis and prognosis as well as

to administer therapeutic choices. Targeted NGS analysis with an average read depth of ~265X, mutations were found in 23 (29.5%) out of 78 children with epileptic encephalopathies and developmental delay/mental retardation. The average read depth among these 23 individuals was $264.5 \pm 82.0X$ (ranged from 137-464X) as compared with that of $265.7 \pm 62.5X$ (ranged from 122-402X) among the other 55 individuals without identified mutation; the difference is not statistically significant ($p=0.944$). Furthermore, 4 (17.4%) of 23 individuals with identified mutation had an average read depth of less than 200X ($157.3 \pm 14.7X$, ranged from 137-170X). These results indicate that an average read depth above 250X is sufficient for this targeted panel to perform mutational screening in children with epileptic encephalopathies and DD/MR. Compared with two recent studies of about 18% of genetic diagnosis rate in patients with epilepsy [12,13], the higher mutation identification rate in our study would likely be due to the use of a

higher NGS read depth (250X *vs.* 30X), and a more focused patient cohort (mainly EoEE and IS/WS *vs.* clinically referred epilepsy patients).

The majority (65.2%) of the genetic causes for Taiwanese children with epilepsy and DD/MR are due to channelopathy as previously suggested for idiopathic epilepsies [14]. Most common involvement are Na⁺ channels, including SCN1A, SCN2A and SCN8A representing the subunit 1 (Nav1.1), 2 (Nav1.2) and 8 (Nav1.6), respectively, of the neuronal voltage-gated Na⁺ channel; followed by defects in K⁺ channels, including KCNT1, KCNB1 and KCNQ2 representing sodium-activated K⁺ channel subfamily T member 1 (Slc2.2), voltage-gated K⁺ channel subfamily B member 1 (Kv2.1) and subfamily Q member 2 (Kv7.2), respectively. Mutations in *SCN1A* have been reported to be associated with generalized epilepsy with febrile seizures plus (GEFS+) type 2 as the major culprit (~75%) for Dravet syndrome (DS) [15]. Among 5 patients identified to carry *SCN1A* mutations, two each were originally classified as EE and FS-DD/MR in addition to one as EoEE. One patient with EE was later confirmed to be DS. Three patients also exhibit MR which is commonly observed in DS due to developmental plateauing [16]. The significant features of our patients with *SCN1A* mutation is fever sensitive seizure. Mutations in *SCN2A* were reported to be associated with epileptic syndromes ranging from benign familial neonatal-infantile seizures [17] to severe epileptic encephalopathies, such as Ohtahara syndrome, malignant migrating focal seizures of infancy (MMFSI) [18,19] and late onset ataxia and autism spectrum disorders [20,21]. Two missense mutations were identified among a WS and an EoEE patient; the former exhibit Rett-like features and the latter is confirmed to be MMFSI. The Mutations in *SCN8A* have been reported for early infantile and unclassified EE, as well as in patients with Dravet-like presentation [22,23]. Similar to those of *SCN1A* in DS and *SCN2A* in MMFSI, the majority of *SCN8A* mutations in EE cases resulted from de novo mutations [13,16,24]. Six (66.7%) out of 9 analyzed patients with mutations in Na⁺ channels are de novo, which are consistent with observations in prior studies.

KCNT1, KCNB1 and KCNQ2 are members of large and diverse families of K⁺ channels, the disease causing mutations were found to be associated respectively with MMFSI [25] and with EoEE [26] *via* whole exome sequencing. KCNT1 is responsible for hyperpolarization

modulation [27] and KCNB1 is the main contributor to delayed rectifier K⁺ current that is critical for membrane repolarization following repetitive firing of action potentials [26]. Four missense mutations in *KCNT1* were identified in 3 EoEE and 1 EE patients. Two of them, including the only EE patient, were confirmed to be MMFSI. Importantly, the genetic diagnosis of *KCNT1* mutations helps the clinicians confirm the diagnosis of MMFSI. One missense mutation (R262Q) is localized to cytoplasmic end of the S4 transmembrane domain, the others are localized to cytoplasmic C-terminal domain, including E347D next to S6 transmembrane domain, R428Q in the first regulator of conductance of K⁺ (RCK) domain and T907A in the NAD⁺ binding domain. All identified mutations, except R428Q, are de novo. For *KCNB1*, a missense mutation (T210M) was localized to the extracellular linker between S1 and S2 segments of the transmembrane domain. *KCNQ2* encodes for Kv7.2 K⁺ channel subunits underlying the neuronal voltage-gated M-current (IKM) [28]. A maternally inherited nonsense mutation was identified in a familiar case of E-DD/MR.

The rest (34.8%) of the mutations were found in genes encoding non-ion channel proteins, including 3 in *CDKL5*, and one in each for *ALG13*, *GFAP*, *SNAP25*, *STXBP1* and *SYNGAP1*. *CDKL5* encodes for cyclin dependent kinase like Ser/Thr protein kinase and is one of the most frequently involved gene for X-linked IS, WS and atypical Rett syndrome predominantly in females [29,30]. A nonsense mutation (S557*) and a single nucleotide duplication leading to premature termination (D284*) of *CDKL5* were identified in two male IS patients. In addition, a recurrent two nucleotide deletion frameshift mutation (c.2635_2636delCT; p.L879Efs*30) was found in a female EoEE patient [31]. Only 25 mutations in *CDKL5* were previously reported in boys, as compared with 131 reported in females. The fact that two out of six of our analyzed male IS patients carry mutations warrants the necessity of mutational analysis of *CDKL5* in males with IS. Furthermore, two other analyzed female IS patients were found to carry de novo mutation in *ALG13* and *SNAP25* genes encoding a subunit of a bipartite UDP-N-acetylglucosaminyltransferase involving in N-linked glycosylation in endoplasmic reticulum and the synaptosome associated protein 25 for the regulation of neurotransmitter release in presynaptic plasma membranes, respectively.

Mutations in *STXBP1* have been reported to be associated with intellectual disability with or without epilepsy as well as EoEE mainly Ohtahara syndrome [32]. Some (~75%) patients with Ohtahara syndrome are known to develop WS between 2–6 months of age and could further evolve into Lennox-Gastaut syndrome (LGS) [33,34]. The gene product of *STXBP1*, syntaxin binding protein 1, is involved in modulating the release of synaptic vesicles via interaction with syntaxin A and soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) complex [35]. A de novo missense mutation was found in the *STXBP1* of a female Taiwanese WS patient. In addition, a de novo missense mutation in *SYNGAP1* was also identified in a female E-DD/MR patient. *SYNGAP1* encodes for synaptic Ras GTPase activating protein 1, which is highly enriched at excitatory synapses and is important for proper neuronal development and glutamate receptor trafficking [36]. It is previously reported to be associated with intellectual disability (ID), EE or autism spectrum disorders (ASD) [37, 38]. Thus, this supports the importance of genes involving trafficking of synaptic vesicles and its regulation in the pathogenesis of epileptic encephalopathies.

Double heterozygous missense mutations (c. [218T>C; 259 G>T]) in *GFAP* were identified in a boy with EoEE, who is confirmed to be diagnosed with Alexander disease (AD). Both mutations were found to be transmitted from the father, most likely due to de novo germline mutation [39]. *GFAP* is located on chromosome 17q21 and encodes glial fibrillary acidic protein which is the major intermediate filament proteins of mature astrocytes and is reported to be associated with a rare autosomal dominant neurological disorder AD [40]. Both missense mutations were localized to the 1st α -helical coil of the 4 coiled subdomain within the central rod domain of *GFAP* near known mutation hotspots (R79 and R239) [41].

In summary, overall mutation identification rate was 29.5% (23/78) among Taiwanese patients

using targeted gene panel for the investigation of underlying genetic causes of epilepsy with DD/MR. The diagnostic yield is highest in the EoEE subgroup. The majority of identified mutations involve genes encoding ion channels (65.2%) and more than two-thirds of identified mutations (69.6%) are de novo. Traditionally, the diagnosis of epileptic encephalopathies is based on clinical manifestations and EEG findings. Advances in high-throughput DNA sequencing technology have greatly facilitated detection of underlying genetic culprits of patient with various epileptic syndromes. Based on this study, targeted gene panels for epilepsy with DD/MR could facilitate the identification of mutation within a month from the onset of seizure. Thus, it would be a timely and cost-effective screening prior to considering whole exome sequencing as routine diagnostic tool. The use of targeted gene panels can shorten the course from seizure onset to genetic diagnosis. Current therapy for epileptic encephalopathies has mainly been focused on reducing the occurrence of seizure rather than influencing the underlying disease process. Based on limited genotype-phenotype correlations and available clinical evidences, some precision medicine in epilepsy has been proposed [42,43]. Improvements in comprehensive understanding of molecular pathogenesis underlying epileptic encephalopathies will pave the path to the development of effective precise therapeutic strategies for the diseases as early as possible.

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Conflicts of Interest

The authors declare no conflict of interest.

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