Genome sequencing of 320 Chinese children with epilepsy: a clinical and molecular study

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Abstract

To evaluate the diagnostic value of genome sequencing in children with epilepsy, and to provide genome sequencing-based insights into the molecular genetic mechanisms of epilepsy to help establish accurate diagnoses, design appropriate treatments, and assist in genetic counseling.

We performed genome sequencing on 320 Chinese children with epilepsy, and interpreted single nucleotide variants and copy number variants of all samples. The complete pedigree and clinical data of the probands were established and followed up. The clinical phenotypes, treatments, prognoses, and genotypes of the patients were analyzed.

Age at seizure onset ranged from 1 day to 17 years, with a median of 4.3 years. Pathogenic/likely pathogenic variants were found in 117 of the 320 children (36.6%), of whom 93 (29.1%) had single nucleotide variants, 22 (6.9%) had copy number variants, and 2 had both single nucleotide variants and copy number variants. Single nucleotide variants were most frequently found in SCN1A (10/95, 10.5%), which is associated with Dravet syndrome, followed by PRRT2 (8/95, 8.4%), which is associated with benign familial infantile epilepsy, and TSC2 (7/95, 7.4%), which is associated with tuberous sclerosis. Among the copy number variants, there were 3 with a length < 25Kb. The most common recurrent copy number variants were 17p13.3 deletions (5/24, 20.8%), 16p11.2 deletions (4/24, 16.7%), and 7q11.23

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duplications (2/24, 8.3%), which are associated with epilepsy, developmental retardation, and congenital abnormalities. Four particular 16p11.2 deletions and two 15q11.2 deletions were considered to be susceptibility factors contributing to neurodevelopmental disorders associated with epilepsy.

The diagnostic yield was 75.0% in patients with seizure onset during the first postnatal month, and gradually decreased in patients with seizure onset at a later age. Forty-two patients (13.1%) were found to be specifically treatable for the underlying genetic cause identified by genome sequencing. Three of them received corresponding targeted therapies and demonstrated favorable prognoses.

Genome sequencing provides complete genetic diagnosis, thus enabling individualized treatment and genetic counseling for the parents of the patients. Genome sequencing is expected to become the first choice of methods for genetic testing of patients with epilepsy.

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Introduction

Epilepsy is one of the most common diseases of the central nervous system. It most prominently appears in childhood and is characterized by recurrent seizures. According to the World Health Organization, epilepsy is present in about 50 million people worldwide. There are a multitude of causes that can lead to epilepsies including infections, genetic abnormalities, and structural, metabolic, or immune abnormalities without clear underlying genetic components. In about half of cases, however, the causes remain entirely unknown.¹

Among the genetic causes, a variety of sequence alterations can lead to epilepsies, such as single-nucleotide variants (SNVs), copy number variants (CNVs), small insertions or deletions (indels), and repeat expansions, each generating pathogenic alleles of one or multiple epilepsy genes. Many of these variants are associated not only with seizures but also other symptoms. Furthermore, different variants of the same gene can lead to distinct epileptic syndromes, and what appears as the same epileptic syndrome may be caused by variants of different genes.²
Since the identification of the first epilepsy gene in 1995, the number of genes associated with epilepsy and the types of genetic tests identifying variants have significantly increased. Most epilepsy patients have a good prognosis provided they are correctly treated, but the most appropriate treatment for any individual patient will depend on the underlying cause of the disease in that patient. Hence, genetic testing is very important to establish definitive diagnoses, accurate treatments, and genetic counseling.

There are several techniques available for genetic testing, such as next generation sequencing, capture-based targeted sequencing, exome sequencing (ES), and genome sequencing (GS). Among these, GS has several advantages. First, it can sequence the entire genome of an individual and identify not only SNVs but also CNVs and small fragment insertions and deletions simultaneously, the latter rendering it superior to chromosomal microarray analysis (CMA) that can only identify CNVs of larger sizes. Second, GS is better than capture-based targeted sequencing (which is limited to the identification of known genes) and ES, which misses potentially pathogenic non-coding and intronic SNVs (for instance a variation in an intronic part of SCN1A), non-coding repeat expansions (for instance in familial adult myoclonic epilepsy), SNVs in untranslated regions of mRNAs (unless specifically designed to identify them), non-coding RNAs, and small and large structural variants. Third, library preparation for GS is simpler and faster than for capture-based targeted sequencing or ES. Fourth, GS allows for a single test to capture almost all genomic variations in an unbiased manner due to even coverage throughout the genome as DNA amplification can be kept to a minimum with no capture step needed for enrichment.

Despite these numerous advantages, GS has not widely been applied to epilepsy patients (especially pediatric epilepsy patients) probably because of the higher costs associated with GS over other techniques. With the costs for GS gradually decreasing, however, GS should become an invaluable alternative to genetic testing by other methods even in clinical settings outside specific research projects.

Here, we performed GS on a cohort of 320 Chinese children with epilepsy and assess its
diagnostic yield and clinical utility.

Materials and methods

Participant recruitment and eligibility criteria

A cohort of 320 pediatric epilepsy patients was recruited from the Department of Neurology of Shenzhen Children’s Hospital (Shenzhen, China) during the period October 2016 to December 2017. All patients were evaluated clinically by pediatric neurologists and diagnosed based on the type of seizures, electroencephalographic findings, and brain imaging results. The criteria for inclusion were age <18 years and diagnosis of epilepsy based on International League Against Epilepsy criteria (2017)\textsuperscript{10} and suspicion for underlying genetic causes. Excluded were patients with seizures likely due to acquired brain injuries, including (but not limited to) head trauma, brain tumors, and central nervous system infections; patients with seizures likely due to cerebrovascular pathological changes; patients having undergone transplant surgery, stem cell therapy, or transfusion of allogeneic blood products within the past 6 months; and patients with hemolytic disease.

The study was approved by the Institutional Review Board on Bioethics and Biosafety of BGI and the ethics committee of the Shenzhen Children’s Hospital. Written informed consent was obtained from all parents or legal guardians of the patients according to the Declaration of Helsinki principles.

Genome sequencing and analysis

Genomic DNA was extracted from 2-4 ml of peripheral blood collected from patients and their parents (if available) with the QIAamp DNA Blood Mini Kit (Qiagen). Patient samples were subjected to GS by BGI-Shenzhen performing paired-end 100 sequencing using the BGISEQ-
500 platform as previously described. The patients were analyzed as singletons by GS while parental samples of patients with SNVs deemed pathogenic or likely pathogenic were subjected to Sanger sequencing to evaluate potential parental origin of the variants. Pedigrees were obtained through interviews, and clinical data of probands were followed up for 2 years.

All samples were analyzed for SNVs and CNVs. Deep sequencing data were aligned to the reference GRCh Build 37 (hg19) and variants were called using the Edico Dragen analysis pipeline. Edico Genome's Dragen Bio-IT Platform is based on the company's Dragen Bio-IT Processor, a bioinformatics application-specific integrated reference-based mapping, aligning, sorting, deduplication, and variant calling.

Variants were annotated using bcfanno (v1.4; https://github.com/shiquan/bcfanno) in the Frequency data (The Exome Aggregation Consortium, Genome Aggregation Database, 1000 Genomes, gene-disease database (ClinVar), Clinical Genomic Database, Online Mendelian Inheritance in Man database (OMIM), and Human Gene Mutation Database (HGMD)). The predictive programs SIFT (v5.2.2), Polyphen2 (v2.2.2), MutationTaster (NCBI 37/ Ensembl 69) and PROVEAN (v1.1.5) were used to access the pathogenic potential of the variants.

The procedure for SNVs filtering, classification, and interpretation is shown in Supplementary Fig. 1a. First, SNVs with allele frequencies above 5% were excluded. Second, the filtered SNVs were divided into functional and non-functional region variants, and the non-functional region variants were filtered and interpreted by the ClinVar and HGMD databases. Third, variants from the functional regions and filtered variants from the non-functional regions were then assigned to one of four categories (pathogenic, likely pathogenic, uncertain significance, negative) based on clinical significance according to the American College of Medical Genetics and Genomics (ACMG) Guidelines Revisions. Fourth, combined with phenotypes, we searched the variants through OMIM, HGMD, and PubMed databases, and the pathogenicity of a variant was then determined according to whether it belonged to a known epilepsy-associate gene or a known epilepsy phenotype in a syndrome caused by the gene.
Finally, Sanger sequencing was done for all patients with pathogenic, likely pathogenic sequence alterations, and uncertain significance favoring pathogenicity as well as for their parents for segregation analyses and for verification of the diagnostic value of the variants.

CNV calls were generated using the SpeedSeq SV pipeline (v.0.0.3a),\textsuperscript{27} which uses Lumpy (v.0.2.9)\textsuperscript{28} to process samples and SVtyper (v.0.0.2)\textsuperscript{27} to genotype variants. The pipeline outputs deletions, duplications, and inversions, and identifies breakpoints that cannot be assigned to any of these three types of genomic alterations. Low coverage data was analyzed based on a pipeline\textsuperscript{29} that calculates karyotype and ploidy based on the ratio of CNVs compared to reference genomes. Interpretation of CNVs was performed using in-house laboratory interpretation rules (Supplementary Fig. 1b), and re-evaluated using ACMG's latest CNV interpretation criteria\textsuperscript{26}. If a CNV involves a known epilepsy gene and the epilepsy phenotype was consistent with variants of that gene, we considered the CNV pathogenic. If a CNV did not involve a known epilepsy gene, we searched for what phenotypes associated with that CNV had ever been reported. If most of the reported phenotypes matched with the ones of our case and the CNV had been reported to be associated with epilepsy, then we considered this CNV to be pathogenic. Lastly, if a CNV did not involve a known epilepsy gene or was never reported to be associated with epilepsy, we considered this CNV to be of unknown clinical significance.

We divided pathogenic/likely pathogenic CNVs into three categories: recurrent CNVs with well-documented enrichment in epilepsy; CNVs that contained genes already implicated in epilepsy; and CNVs related to syndromes with epilepsy phenotypes reported in OMIM and literature. We also screened genome sequencing data for tandem repeat expansions using TRHist,\textsuperscript{31} which outputs information such as repeat units, length of tandem repeat expansion sequences, and surrounding flanking sequences. Then we interpreted the results regarding whether any of the tandem repeat expansions were known pathogenic variants.

The results were combined with the patient's phenotype and disease progression (as evaluated by pediatric neurologists with extensive clinical experience) to yield a final genetic diagnosis. The chromosomal locations of diagnostic SNVs and CNVs were plotted using the R package ‘Rideogram’.\textsuperscript{32}
Statistical analysis

A Chi-squared test of independence was performed using IBM SPSS v19 statistics software (SPSS 19.0; IBM Corp., Armonk, NY, USA) to evaluate the diagnostic yield across different patient groups. A $p$-value < 0.05 was considered statistically significant.

Data availability

All sequencing data was produced by China National GeneBank, Shenzhen, China. The data that support the findings of this study have been deposited in the CNSA (https://db.cngb.org/cnsa/) of CNGBdb with accession code CNP0000788.

Results

Demographics and clinical data

A total of 320 Chinese children were recruited and their demographic, clinical, and neuroimaging characteristics are shown in Table 1 and Supplementary Table 1. The ratio of male to female was 1.48:1, most likely due to the fact that epilepsy is more frequent in males. Age at seizure onset ranged from 1 day to 17 years, with a median of 4.3 years. As shown in Supplementary Fig. 2a, focal seizures (219/320, 68.4%) were the most common seizure type among the 320 patients, followed by infantile spasms (100/320, 31.3%), generalized tonic seizures (31/320, 9.7%), myoclonic seizures (26/320, 8.1%), absence seizures (10/320, 3.1%), generalized tonic-clonic seizures (4/320, 1.3%), and generalized clonic seizures (1/320, 0.3%).

In 30% of patients, there were two or more types of clinical seizures.

As shown in Supplementary Fig. 2b, many patients carried a diagnosis of an epileptic syndrome, such as West syndrome diagnosed in 68 of the 320 patients (21.3%); Ohtahara syndrome found
in 13 patients (4.1%); Dravet syndrome found in 11 patients (3.4%); Lennox-Gastaut syndrome found in 10 patients (3.1%); and benign familial infantile epilepsy found in 7 patients (2.2%). Furthermore, 245 of the 320 patients (76.6%) had comorbidities, such as global developmental delay in 219 (68.4%), and brain MRI detected potentially epileptogenic abnormalities in 170 (53.1%) of the patients, including brain malformations, brain dysplasia, and brain atrophy (Table 1).

We also screened for noncoding repeat expansions but found no known pathogenic repeat expansions variants in genes like \textit{CSTB}^{33,34}, \textit{SAMD12}^{35}, \textit{STARD7}^{36}, \textit{TNRC6A}^{37}, \textit{RAPGEF2}^{38}, \textit{MARCH6}^{39}, \textit{YEATS2}^{40}, and \textit{C9orf72}^{41}, or in any of the epilepsy genes identified in this research.

**Detection rates of SNV and CNV**

By simultaneously analyzing SNVs and CNVs in the 320 patients, pathogenic or likely pathogenic variants were found in 117 patients (36.6%). In 95 patients (29.7%), we detected a total of 107 pathogenic or likely pathogenic SNVs (Supplementary Table 1), and in 24 patients (7.5%), there were a total of 25 pathogenic or likely pathogenic CNVs (Supplementary Table 2. Note that this table lists 30 patients because it also includes 6 cases in which particular 15q11.2 and 16p11.2 deletion syndromes were considered susceptibility factors; see below). Concomitant pathogenic SNVs and CNVs were identified in 2 of the 117 patients (0.6%), including (in one of them) a compound heterozygosity with an SNV on one chromosome and a CNV on the corresponding part of the other.

A map of the chromosomal distribution of the SNVs and CNVs in the patient cohort is shown in Fig. 1. Interestingly, 31 of the 132 (23.5%) pathogenic or likely pathogenic variants, comprising 29 SNVs and 2 CNVs, were located on chromosome 16. Moreover, 17 of the pathogenic or likely pathogenic variants (12.9%) were located on chromosome 2, including 15 SNVs and 2 CNVs.

Of the 320 patients, 233 also underwent urine screening and tandem mass spectrometry of whole blood. Although no significant metabolic abnormalities were found in these samples, the genetic analysis revealed alterations in genes associated with metabolic diseases in 9 of
them (4% of the 233 patients).

**Comparison of clinical and genetic diagnoses**

Table 1 shows a comparison of the clinical findings in patients in which pathogenic/likely pathogenic variants were identified versus those in which no such variants were found. Two aspects are noteworthy. One concerned the age at onset of epileptic seizures. Interestingly, a genetic cause was overall more readily discoverable in patients whose seizure onset occurred at a younger age (average onset at 12.84 months versus 21.70 months in patients in which no genetic cause was found; \( p = 0.0002 \)). Furthermore, a division of the patients into different age cohorts showed that the detection rates of pathogenic/likely pathogenic variants steadily decreased as the age at seizure onset increased (Fig. 2).

The other aspect concerned the association of a genetic cause with the diagnosis of an epileptic syndrome. In fact, many of our patients were diagnosed with epileptic syndromes, and patients diagnosed with tuberous sclerosis syndrome, Dravet syndrome, and benign familial infantile epilepsy were more likely to show an underlying genetic cause than no such cause (\( p < 0.05 \)) (Tables 1 and 2). Patients with developmental and/or epileptic encephalopathies were also more likely to show an underlying genetic cause (\( p = 0.021 \)). The likelihood of association of a given SNV or CNV with the different phenotypic subgroups is shown in the heatmaps of Supplementary Fig. 3a and 3b.

A breakdown of the syndrome-associated genes in which we identified pathogenic/likely pathogenic variants is shown in Table 2. Of the 320 patients, 68 were clinically diagnosed as West syndrome, with 28 distinct variants detected among them (detection rate: 36.8%). Four SNVs were found in *PAFAH1B1*; two each in *UBA5* and *SUOX*, and one each in *DCX*, *DEPDC5*, *GNAO1*, *HCN1*, *KCN1H1*, *KCNQ2*, *PRKCG*, *TSC2*, *WDR45*, and *WWOX*, respectively. Furthermore, the following CNVs were found in cases of WS: five 17p13.3 deletions; one 9q31.1 duplication; one 7q11.22q11.23 deletion; one 7q11.22q11.23 duplication; one 16p13.3 deletion; and one 16q23.1 duplication that includes *WWOX*. Sixteen cases were clinically diagnosed as tuberous sclerosis complex, for which a total of 12 variants were
detected (detection rate: 75%) (7 variants in \textit{TSC2}, 4 variants in \textit{TSC1}, and one 16p13.3 deletion that included \textit{TSC2}). Eleven cases were clinically diagnosed as Dravet syndrome, 10 of which carrying a variant of \textit{SCN1A} and one a 2q24.3 deletion that included \textit{SCN1A} (detection rate: 100%). Thirteen cases were clinically diagnosed as Ohtahara syndrome, with 8 variants in 6 genes (detection rate: 61.5%) (2 variants each in \textit{ABAT}, \textit{CDKL5}, and \textit{GNAO1}, and 1 each in \textit{KCNT1}, \textit{STXBP1}, and \textit{WWOX}). Other epilepsy syndromes and the genes mutated for the corresponding syndromes are presented in Table 2.

**Single nucleotide variants**

The 107 pathogenic or likely pathogenic SNVs that we identified in 95 patients were assigned to a total of 52 different genes, among which the most frequently mutated one was \textit{SCN1A} (10/95, 10.5%), followed by \textit{PRRT2} (8/95, 8.4%), and \textit{TSC2} (7/95, 7.4%). As shown in Table 3, 4 patients (4.2%) were found with mutations in \textit{PAFAH1B1} and \textit{TSC1}, respectively; 3 patients (3.2%) with mutations in \textit{GNAO1}, \textit{STXBP1}, \textit{SCN8A}, and \textit{KCNQ2}; and 2 patients (2.1%) with mutations in \textit{ZEB2}, \textit{PCDH19}, \textit{CDKL5}, \textit{WWOX}, \textit{ALDH7A1}, \textit{DCX}, and \textit{SYNGAP1}.

As shown in Fig. 3, out of the total of 107 SNVs, 52 (48.6%) were missense variations, 29 (27.1%) frameshift variations, 15 (14.0%) nonsense variations, 8 (7.5%) splice variations, and one each (0.9%) in frame variation, synonymous variation affecting splicing, and intron variation. The parental origin of the variants was assessed by Sanger sequencing of the parents and is indicated (as far as it could be determined) in Supplementary Table 1. Based on OMIM and the literature, it is suggested that 68 variants (63.6%, 68/107) could be inherited in an autosomal dominant (AD) fashion, 27 (25.2%, 27/107) in an autosomal recessive (AR) fashion, and 12 (11.2%, 12/107) in an X-linked fashion (Fig. 3, Supplementary Table 3). Many of the SNVs were indeed of parental origin; however, 60 of the 107 SNVs (55.1%) were variants not present in either parent and hence were considered \textit{de novo}.

Further analysis revealed the following additional details. Of the 10 \textit{SCN1A} variants that we detected, one was inherited from the father, but the other nine appeared \textit{de novo} in the corresponding patients. Likewise, all four \textit{TSC1} and seven \textit{TSC2} variants appeared \textit{de novo}. 

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However, after verification by ddPCR, the father of the heterozygous TSC2 patient SZCH0052 was mosaic, suggesting that this patient inherited the variant from the father. Eight PRRT2 variants were of parental origin. Of these, five exhibited the same frameshift variation in a previously identified variation hotspot with incomplete penetrance [NM_001256442.1: c. 649dup (p. Arg217Profs8)], and one a different variant of codon 217.

One additional interesting case concerned patient SZCH0237 who, as mentioned above, is a compound heterozygote for variants of the BCKDK gene. Seizure onset in this patient was at 6 years of age and was combined with neurodevelopmental disorders and autism. The BCKDK gene is associated with a rare and treatable recessive disease called branched-chain ketoacid dehydrogenase kinase deficiency (OMIM #614923), described so far in only three consanguineous families with autism, epilepsy, and intellectual disability.

Copy number variants

Among 24 patients of our cohort, we identified 25 CNVs (1 patient, SZCH0370, harbored two distinct CNVs) (Supplementary Table 2). Their sizes ranged from 121bp to 30.47Mb and encompassed 18 deletions (72.0%) and 7 duplications (28.0%). According to our interpretation rules (Supplementary Fig. 1b), 19 CNVs (76.0%) were considered pathogenic, and 6 (24.0%) likely pathogenic. An additional four 16p11.2 deletions and two 15q11.2 BP1-BP2 deletions were considered susceptibility factors for the phenotypes due to reduced penetrance and variable expressivity of clinical features.

As shown in Fig. 1, the CNVs were distributed over 10 chromosomes. Many were associated with known syndromes, the most common being the chromosome 17p13.3 deletion syndrome, also known as Miller-Dieker lissencephaly syndrome (MIM#247200), that encompasses the deletion of PAFAH1B1, a known lissencephaly gene (OMIM#607432). In fact, we have identified 17 patients with lissencephaly, 5 of which harboring CNVs affecting PAFAH1B1 (Supplementary Tables 2 and 4), in addition to the four mentioned above with SNVs in PAFAH1B1 (Supplementary Tables 1 and 4). Patients with PAFAH1B1 gene variants in our cohort all presented with infantile spasms and had more severe alterations in the posterior brain
regions (posterior-to-anterior gradient), which was consistent with previous studies.\textsuperscript{44-46} The most common recurrent copy number variants were the 17p13.3 deletion (5/24, 20.8%), 16p11.2 deletions (4/24, 16.7%), and 7q11.23 duplications (2/24, 8.3%), all of which associated with epilepsy, developmental retardation, and congenital abnormalities.

The above results indicate that most of the chromosomal abnormalities identified in our patient cohort could be assigned to known syndromes in which seizures are at least one symptom. Apart from the 17p13.3 deletion syndrome, other CNVs may also cover epileptogenic genes, such as the 1.5-Mb deletion at 2q24.3 in patient SZCH0255, which includes several genes. Two of them (\textit{SCN1A}, and \textit{SCN9A}) are known epilepsy genes, and deletion of either is known to cause epilepsy.\textsuperscript{7,47}

Interestingly, among the 25 CNVs, the respective sizes of 3 CNVs were below 25kbp. The detection of small fragments of CNVs is a major advantage of GS because chromosomal microarrays can currently not detect CNVs below 25kbp\textsuperscript{48}. As shown in Supplementary Fig. 4, we verified one of these small deletions (the 1651bp deletion in chromosome 16p13.3 of patient SZCH0053) by PCR analysis that yielded, in addition to an expected wildtype band, a band of 237bp predicted to occur only in presence of the CNV. The deletion seemed to have appeared \textit{de novo} as the parents showed only the wildtype band. The analyses of additional CNVs revealed that the CNV fragment length correlated with a patient’s age at seizure onset (Supplementary Fig. 5).

**Effect on potential targeted therapies**

As mentioned above, seizures in many patients may be just one of the manifestations of an underlying neurogenetic disease. GS can help identify the pathogenic causes for the seizures and thereby help to design treatment modalities that specifically target these underlying causes. In our cohort, there were 42 patients (13.1%)—summarized in Table 4—diagnosed with a disorder whose conditions improved to varying degrees under targeted therapy or avoidance of specific drugs. Here we highlight two patients for whom specific treatments led to improvements in the seizure symptomatology.
An 8-month-old girl (SZCH0292) developed seizures on the 13th day after birth that were refractory to antiepileptic drugs. This patient was a compound heterozygote carrying two missense variants of the \textit{ALDH7A1} gene (OMIM\#107323), c.1547A>G (p. Tyr516Cys) (NM_001182.4) and c.965C>T (p. Ala322Val) (NM_001182.4). \textit{ALDH7A1} deficiency leads to pyridoxal phosphate deficiency associated with pyridoxin-responsive epilepsy that can be alleviated by high doses of pyridoxine (VitB6).\textsuperscript{49} With this treatment, the patient became seizure-free and had a good prognosis.

A 3-year-old boy (SZCH0283) had seizures at 3 months of age, combined with ataxia and mental retardation. We detected a heterozygous frameshift variant on \textit{SLC2A1} associated with deficiencies in the glucose transporter-1 (GLUT1) protein (OMIM\*138140, GLUT1 deficiency syndrome, infantile onset) [NM_006516.2: c.912del (p. Gln304Hisfs36)]. After treatment with a ketogenic diet, the boy was seizure-free and showed normal intellectual and motor development.

\textbf{Recurrence risk}

Among the 95 patients with pathogenic/likely pathogenic SNVs, 29 (30.5\%) patients inherited their variants from one or both parents. This result suggests that in these families, there is a risk of recurrence among other family members.

Nevertheless, among the 60 patients that harbored variants that appeared to have occurred \textit{de novo}, 3 families (5.0\%) had 2 children with the same birth defects. The most likely explanation for recurrence of “\textit{de novo}” mutations is parental mosaicism in germ cells that is missed or absent in DNA usually derived from peripheral blood leukocytes and that might be more prevalent than previously predicted.\textsuperscript{50} Indeed, in one case (SZCH0052), we found that the patient’s father was mosaic for the \textit{TSC2} variant detected in the child and so had likely transmitted it through the germline. Thus, GS can occasionally help to assess the risk for recurrence, even in cases where an original mutation seems to have arisen \textit{de novo}. 
Discussion

Despite the advantages of GS over other genetic methods, most large-scale genetic studies of pediatric epilepsy cases have been conducted using ES or capture-based targeted sequencing, probably because of cost considerations. Hence, the present GS study of 320 children with epilepsy remains one of few studies conducted on this scale thus underscoring its enhanced diagnostic yield and its feasibility in applying findings to clinical settings.

In our cohort, we found that 117 (36.6%) patients carried one (or in some cases more than one) pathogenic or likely pathogenic genetic variant. These variants included many of the possible genetic alterations such as SNVs in coding and non-coding sequences and copy number variations as well as small insertions and deletions, though no repeat expansions. Many of these alterations would be missed by ES or targeted capture methods. Nevertheless, a direct quantitative comparison of the diagnostic yields (provided by the different methods across different studies) is hampered by differences in patient cohorts, sequence coverage, and calling methods for what constitutes a pathogenic or likely pathogenic variant. Nevertheless, an extended comparison of GS with possible results of ES or capture-based targeted methods, as shown in Supplementary Fig. 6, would indicate that the increased diagnostic yield obtained by GS was due to the detection of CNVs and intronic SNVs. In fact, our study showed that the diagnostic yield of SNVs was 29.1% (6.9% for CNVs) thus comparing favorably with similar studies based on ES or targeted capture methods.

However, in the present study, four 16p11.2 deletions and two 15q11.2 BP1-BP2 deletions were considered to represent susceptibility loci for a variety of neurodevelopmental diseases, including seizures. Maya and coworkers showed that 15q11.2 BP1-BP2 deletions are common among affected and unaffected populations, with a calculated penetrance of 2.18% over the background risk. Rosenfeld and coworkers estimated that the risk of an abnormal phenotype ranged from 10.4% for 15q11.2 deletions to 46.8% for proximal 16p11.2 deletions. Due to the variable phenotypes and low penetrance, the pathogenicity of the deletions is low or not determined. Therefore, these CNVs may not be considered sufficient to be causal in epilepsy of the patients.
In the present study, we also found two special cases combining two types of variants. The first case was a 1-year-old girl (SZCH0677) presenting with seizures at 10 months of age and mental retardation and carrying a *SYNGAP1* [NM_001130066.1: c.1543C>T (p. Arg515Cys)] variant and a 1542kb deletion in 17q12 as part of the chromosome 17q12 deletion syndrome. Further analysis revealed that the *SYNGAP1* variant can result in autosomal dominant mental retardation-5 (OMIM#612621), which is characterized by moderate to severe intellectual disability with delayed psychomotor development. Most of these patients develop variable types of seizures.62 Furthermore, 17q12 deletion syndrome is also associated with mental retardation, seizures, and renal diseases. Hence, both the *SYNGAP1* variant and the 17q12 deletion may contribute to the patient’s phenotypes.

The second case was a 2-year-old boy (SZCH0268) who carried an SNV and an intragenic partial CNV. He presented with seizures in the first month of life, hypertonia, mental retardation, and corpus callosum dysplasia. GS revealed a splice variant (NM_001291997.1: c.453-1G>C) of the *WWOX* gene as well as a partial duplication of its exon 6-exon 8. Mutations in *WWOX* (MIM*605131) are associated with epileptic encephalopathy, early infantile epilepsy (OMIM#616211), and the alleles described thus far show autosomal recessive inheritance. A combined SNV and CNV compound heterozygosity in *WWOX* was also reported in an unrelated patient.63 Combined with clinical features, it is likely, therefore, that the SNV/CNV combination in our patient was the cause of the disease. In conclusion, a traditional assay designed to discover either SNVs or CNVs, but not both together, would easily miss detection of such particular genetic constellations.

Apart from the advantages that GS generally offers in detecting CNVs, it also is superior over CMA techniques, specifically in the discovery of small insertions.64 In fact, of all structural rearrangements that we discovered, three were insertions below 25kbp, the usual detection limit of CMA. Furthermore, GS can identify potentially pathogenic alterations in the UTRs of genes, as well as in intronic regions, such as in the highly conserved intronic region termed 20N of the epilepsy gene *SCN1A.*7 Lastly, it can detect alterations in the vast number of non-coding RNAs.
In this study, we found that the diagnostic yield of GS depended on a patient’s age at the first occurrence of a seizure, gradually decreasing with increasing age of seizure onset. This observation suggests that the earlier the onset, the more likely the cause was genetic.\textsuperscript{52} In fact, in patients with seizure onset within the first month of life, our detection rate (75\%) was similar to that found in a previous study indicating that 83\% of newborns with early-onset epilepsy had genetic etiologies,\textsuperscript{65} but exceeded that seen in other studies on this age group.\textsuperscript{66,67}

A particularly important contribution of genetic testing is to provide information about specific therapies targeted to an individual epilepsy patient. Thus, vitamin B6 therapy was successfully used to treat two patients with pyridoxine-dependent epilepsy, and one patient with GLUT1 deficiency syndrome was treated with a ketogenic diet. Before these two cases were identified molecularly, several therapeutic regimens had been used without real benefit to the patients; only the molecular findings made targeted and effective treatments possible as these treatments not only improved the seizure phenotype but also psychomotor development. Importantly, therefore, early detection and treatment initiation is critical for patients with early infantile epilepsy and is predictive for long-term cognitive and developmental outcomes.\textsuperscript{68,69} At present, treatment of epilepsy remains mainly empirical. Our findings of new genetic variants and potential therapeutic strategies (Table 4) will contribute to the establishment of personalized precision medicine and treatment stratification for individual patients. Genetic results can improve therapy by antiepileptic drugs selection and precise medication approaches. However, the change of clinical managements still relies on patient data or clinical studies. Further clinical studies need to be conducted to verify the potential therapies targeted on different cellular mechanisms or channel functions.

In summary, early GS testing can provide an accurate molecular diagnosis in a timely manner, predict possible phenotypes, initiate appropriate therapies and follow-up, predict prognosis, and assess the risk of recurrence, which are all extremely helpful for the clinical management of these patients.


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Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at Brain online.

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Figure legends

Figure 1. Distribution across chromosomes of associated diagnostic single nucleotide variants (SNVs) and diagnostic copy number variants (CNVs). Circles represent SNVs;
boxes represent CNVs; CNV number represents diagnostic CNV number in the region; SNV number represents diagnostic SNV number in a gene.

**Figure 2. Detection rate at different ages of seizure onset.** Detection rate: The ratio of the total number of positive samples in the age range of seizure onset.

**Figure 3. Variation type in 117 patients with epilepsy.**
Figure 1. Distribution across chromosomes of associated diagnostic SNVs and diagnostic CNVs/Circles represent SNVs; boxes represent CNVs; CNV number represents diagnostic CNV number in the region; SNV number represents diagnostic SNV number in a gene.

81x75mm (300 x 300 DPI)
Figure 2. Detection rate at different ages of seizure onset/Detection rate: The ratio of the total number of positive samples in the age range of seizure onset.
Figure 3. Variation type in 117 patients with epilepsy

43x32mm (600 x 600 DPI)
Table 1 Comparison of general clinical information in patients with pathogenic or likely pathogenic variants and patients without causative variants

| Variables                     | Category total (N=320) | Patients with pathogenic or likely pathogenic variants (N, %) | Patients without causative variants (N, %) | p-value  |
|-------------------------------|------------------------|---------------------------------------------------------------|-------------------------------------------|----------|
| Total                         | 320                    | 117 (36.6)                                                    | 203 (63.4)                                |          |
| Age at seizure onset          |                        |                                                              |                                           |          |
| Neonatal (0–1 mo)             | 28                     | 21 (71.9)                                                     | 7 (3.4)                                   | 0.0002   |
| Infancy (1 mo–1 y)            | 179                    | 65 (55.6)                                                    | 114 (56.2)                                |          |
| Toddler (1–3 y)               | 69                     | 22 (31.9)                                                     | 47 (23.2)                                 |          |
| Early childhood (3–6 y)       | 33                     | 8 (24.2)                                                      | 25 (7.8)                                  |          |
| Middle childhood (6–12 y)     | 10                     | 1 (10.0)                                                      | 9 (4.4)                                   |          |
| Adolescent (12–18 y)          | 1                      | 0 (0.0)                                                       | 1 (0.5)                                   |          |
| Sex                           |                        |                                                              |                                           |          |
| Male                          | 191                    | 74 (38.6)                                                     | 117 (57.6)                                | 0.346    |
| Female                        | 129                    | 43 (33.3)                                                     | 86 (42.4)                                 |          |
| DEE                           | 209                    | 86 (41.5)                                                     | 123 (60.0)                                | 0.021    |
| Epilepsy classifications      |                        |                                                              |                                           |          |
| Generalized epilepsy          | 101                    | 28 (27.8)                                                     | 73 (71.8)                                 |          |
| Focal epilepsy                | 152                    | 60 (39.5)                                                     | 92 (59.9)                                 |          |
| Generalized & focal epilepsy  | 67                     | 29 (43.3)                                                     | 38 (57.1)                                 | 0.203    |
| Epilepsy syndromes            |                        |                                                              |                                           |          |
| WS                            | 68                     | 25 (36.6)                                                     | 43 (61.2)                                 | 0.969    |
| CSWS                          | 21                     | 6 (28.6)                                                      | 15 (71.4)                                 | 0.491    |
| TSC                           | 16                     | 12 (75.0)                                                     | 4 (25.0)                                  | 0.002    |
| OS                            | 13                     | 8 (61.5)                                                      | 5 (38.5)                                  | 0.076    |
| DS                            | 11                     | 11 (100.0)                                                    | 0 (0.0)                                   | 1.14E-5  |
| LGS                           | 10                     | 5 (50.0)                                                      | 5 (50.0)                                  | 0.506    |
| FS+                           | 10                     | 1 (10)                                                        | 9 (90)                                    | 0.099    |
| BFIE                          | 7                      | 6 (85.7)                                                      | 1 (14.3)                                  | 0.011    |
| Other phenotypes              |                        |                                                              |                                           |          |
| Developmental delay           | 219                    | 100 (46.0)                                                    | 119 (58.6)                                | 6.4E-7   |
| Dystonia                      | 62                     | 31 (50.0)                                                     | 31 (50.0)                                 | 0.019    |
| Microcephaly                  | 33                     | 14 (42.4)                                                     | 19 (57.6)                                 | 0.577    |
| Premature                     | 30                     | 13 (43.3)                                                     | 17 (56.7)                                 | 0.419    |
| MGS                           | 20                     | 10 (50.0)                                                     | 10 (50.0)                                 | 0.197    |
| CHD                           | 13                     | 6 (46.2)                                                      | 7 (53.8)                                  | 0.463    |
| ASD                           | 13                     | 7 (53.8)                                                      | 6 (46.2)                                  | 0.240    |
| SGA                           | 11                     | 4 (36.4)                                                      | 7 (63.6)                                  | 0.989    |
| Lissencephaly                 | 17                     | 11 (64.7)                                                     | 6 (57.1)                                  | 0.019    |
| Abnormal limbs                | 15                     | 10 (66.7)                                                     | 5 (33.3)                                  | 0.024    |
| Brain MRI                     |                        |                                                              |                                           |          |
| With findings                 | 170                    | 77 (45.3)                                                     | 93 (54.7)                                 | 0.007    |
| No findings                   | 122                    | 36 (30.0)                                                     | 86 (60.0)                                 |          |
| Not determined                | 28                     | 4 (14.3)                                                      | 24 (85.7)                                 |          |

ASD, autism spectrum disorder; BFIE, benign familial infantile epilepsy; CHD, congenital heart disease; CSWS, epileptic encephalopathy with continuous spike-and-wave during sleep; DEE, developmental and/or epileptic encephalopathies; DS, Dravet syndrome; FS+, febrile seizures plus; MGS, malformations of the genitourinary system; LGS, Lennox-Gastaut syndrome; mo, month; OS, Ohtahara syndrome; SGA, small for gestational age infant; TSC, tuberous sclerosis; WS, West syndrome; y, year.

a % among the 117 cases; b % among the 203 cases.
| Clinically diagnosed syndrome | Diagnosis yield | Associated genes mutated |
|-------------------------------|----------------|--------------------------|
| WS                            | 36.8% (25/68)  | PAFAH1B1 (4), UBA5 (2), SUOX (2), DCX (1), DEPDC5 (1), GNAO1 (1), HCNI (1), KCNN4 (1), KCNN5 (1), PRKCG (1), TSC2 (1), WDR45 (1), WWOX (1), 17p13.3 del (5), 9q31.11 dup (1), 7q11.22q11.23 del (1), 7q11.22q11.23 dup (1), 16p13.3 del (1), 16q23.11 dup (1) # |
| TSC                           | 75.0% (12/16)  | TSC2 (7), TSC1 (4), 16p13.3 del incl TSC2 (1) |
| DS                            | 100.0% (11/11) | SCN1A (10), 2q24.3 del incl SCN1A (1) |
| OS                            | 61.5% (8/13)   | ABAT (2), CKB (2), GNAOI (2), KCN1 (1), STXBP1 (1), WWOX (1) |
| CSWS                          | 28.6% (6/21)   | TSC1 (2), FGFI2 (1), GRIN2A (1), 7q11.22q21.3 del (1), 15q11.22q13.1 del (1) |
| BFIE                          | 85.7% (6/7)    | PRRT2 (6) |
| LGS                           | 50.0% (5/10)   | ALOH7A1 (2), SCN8A (1), STXBP1 (1) 7q11.22q21.3 del (1), 22q11.33 del (1) |
| MEI                           | 50.0% (2/4)    | GRIN2B (1), KCN2 (1) |
| FS+                           | 10.0% (1/10)   | SETBP1 (1) |
| PS                            | 25.0% (1/4)    | 1q43p44 del (1) |
| CECT                          | 25.0% (1/4)    | KIF4A (1) |
| EIMFS                         | 33.3% (1/3)    | KCNQ2 (1) |
| MAE                           | 100.0% (1/1)   | ITPR1 (1) |
| EME                           | 100.0% (1/1)   | EIF1A2 (1) |
| CAE                           | 0.0% (0/3)     | — |

BFIE, benign familial infantile epilepsy; CECT, childhood epilepsy with centrotemporal spikes; CSWS, epileptic encephalopathy with continuous spike-and-wave during sleep; del, deletion; DS, Dravet syndrome; dup, duplication; EIMFS, epilepsy of infancy with migrating focal seizures; EME, early myoclonic encephalopathy; FS+, febrile seizures plus incl, including; LGs, Lennox-Gastaut syndrome; MAE, epilepsy with myoclonic-atonic seizures; MEI, myoclonic epilepsy in infancy; OS, Ohtahara syndrome; PS, Panayiotopoulos syndrome; TSC, tuberous sclerosis; WS, West syndrome.

# 16q23.1 dup was an intragenic variant in WWOX.
Table 3. Pathogenic/likely pathogenic genes identified in 320 patients with seizures

| Number of patients per gene | Gene/Syndromes                                                                 |
|-----------------------------|-------------------------------------------------------------------------------|
| 1                           | AARS, ABAT, ALG11, ARK, BCKDK, COG4, CHD2, COL4A1, CYP27A1, DARS2, DEPDC5,  |
|                             | EEFA2, FGF12, GRIN2A, GRIN2B, HCN1, ITPR1, KAT6A, KCNA2, KCNH1, KCNMA1,      |
|                             | KCNT1, KIF4A, MECP2, PRKCG, RTTN, SCN3A, SETBP1, SHANK5, SLC2A1, SMCA1, SUOX, |
|                             | TAF1, TARS2, UBAS, WDR45                                                      |
| 2                           | ZEB2, WWOX, SYNGAP1, PCDH19, DCX, CDKL5, ALDH1A                                |
| 3                           | STXBP1, SCN8A, KCNQ2, GNAO1                                                   |
| 4                           | TSCI, PAFAH1B1                                                                |
| 7                           | TSC2                                                                         |
| 8                           | PRRT2                                                                        |
| 10                          | SCN1A                                                                        |
| Gene   | Sample number | Recommended drugs                                                                 | Aggravating drugs                                      |
|--------|---------------|-----------------------------------------------------------------------------------|--------------------------------------------------------|
| SCN1A  | 10            | Valproate, benzodiazepines, cannabinoids, stiripentol, ketogenic diet, fenfluramine | Sodium channel blockers, e.g. carbamazepine, oxcarbazepine, lamotrigine, and phenytoin |
| SCN8A  | 3             | Sodium channel blockers, e.g. carbamazepine, oxcarbazepine, lacosamide, lamotrigine, rufinamide, oxcarbazepine, and phenytoin | NA                                                     |
| KCNT1  | 1             | Quinidine                                                                         | NA                                                     |
| TSC1   | 4             | Everolimus, vigabatrin                                                            | NA                                                     |
| TSC2   | 7             | Everolimus, vigabatrin                                                            | NA                                                     |
| PCDH19 | 2             | Bromide, clobazam                                                                | Carbamazepine, oxcarbazepine, rufinamide               |
| ALDH7A | 2             | Pyridoxine (vitamin B6), folinic acid                                             | NA                                                     |
| 1      |               |                                                                                    | Lacosamide and phenytoin                              |
| HCN1   | 1             | NA                                                                                | Ethosuximide                                          |
| KCNMA1 | 1             | Vaproate and/or lamotrigine, valproate and/or levetiracetam                       | NA                                                     |
| PRRT2  | 8             | Carbamazepine                                                                     | NA                                                     |
| SLC2A1 | 1             | Ketogenic diet                                                                    | NA                                                     |
| CDKL5  | 2             | Ketogenic diet                                                                    | NA                                                     |