BRAIN
ORIGINAL ARTICLE

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

Dongfang Zou,[1,†] Lin Wang,[2,†] Jianxiang Liao,[1,†] Hongdou Xiao,[2,†] Jing Duan,[1] Tongda Zhang,[2] Jianbiao Li,[2] Zhenzhen Yin,[2] Jing Zhou,[2] Haisheng Yan,[2] Yushan Huang,[2] Nianji Zhan,[2] Ying Yang,[2] Jingyu Ye,[2] Fang Chen,[2] Shida Zhu,[2] Feiqiu Wen[3] and Jian Guo[2]

†These authors contributed equally to this work.

The aim of this study is 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 counselling.

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 analysed.

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 two 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 three with a length < 25 kilobases. 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 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 favourable prognoses.

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

1   Department of Neurology, Shenzhen Children's Hospital, Shenzhen, China
2   BGI-Shenzhen, Shenzhen 518083, China
3   Department of Hematology and Oncology, Shenzhen Children's Hospital, Shenzhen, China

Correspondence to: Jian Guo
BGI-Shenzhen, Beishan Industry Zone
Shenzhen 518083, Guangdong, China
E-mail: guojian@genomics.cn

Correspondence may also be addressed to: Feiqiu Wen
Shenzhen Children's Hospital
No. 7019 Yitian Road, Shenzhen 518038
Guangdong, China
E-mail: fwen62@126.com

Introduction

Epilepsy is one of the most common diseases of the CNS. 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,[3] the number of genes associated with epilepsy[4] and the types of genetic test identifying variants have significantly increased. Most patients with epilepsy 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 counselling.

There are several techniques available for genetic testing, such as next generation sequencing, capture-based targeted sequencing, exome sequencing (exome sequencing) and genome sequencing (genome sequencing).[5] Among these, genome sequencing 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 last rendering it superior to chromosomal microarray analysis (CMA) that can only identify CNVs of larger sizes.[6] Second, genome sequencing is better than capture-based targeted sequencing (which is limited to the identification of known genes) and exome sequencing, which misses potentially pathogenic non-coding and intronic SNVs (for instance a variation in an intronic part of *SCN1A*),[7] non-coding repeat expansions (for instance in familial adult myoclonic epilepsy),[8] 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 genome sequencing is simpler and faster than for capture-based

targeted sequencing or exome sequencing. Fourth, genome sequencing 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.[9]

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

Here, we performed genome sequencing 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 paediatric patients with epilepsy 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 paediatric neurologists and diagnosed based on the type of seizure, 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)[10] and suspicion of underlying genetic causes. Excluded were patients with seizures probably due to acquired brain injuries, including (but not limited to): head trauma, brain tumours and CNS infections; patients with seizures probably 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 haemolytic 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 genome sequencing by BGI-Shenzhen performing paired-end 100 sequencing using the BGISEQ-500 platform as previously described.[11,12] The patients were analysed as singletons by genome sequencing while parental samples of patients with SNVs deemed pathogenic or probably 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 analysed 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.[13] 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 (v.1.4; https://github.com/shiquan/bcfanno; accessed 10 November 2021) in the Frequency data (The Exome Aggregation Consortium,[14] Genome Aggregation Database,[15] 1000 Genomes[16]) gene-disease database (ClinVar),[17] Clinical Genomic Database,[18] Online Mendelian Inheritance in Man database (OMIM),[19] and Human Gene Mutation Database (HGMD).[20] The predictive programs SIFT (v.5.2.2),[21] PolyPhen-2 (v.2.2.2),[22] MutationTaster (NCBI 37/Ensembl 69)[23] and PROVEAN (v.1.1.5)[24] 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.[25,26] 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 favouring 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),[27] which uses Lumpy (v.0.2.9)[28] to process samples and SVtyper (v.0.0.2)[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 alteration. Low coverage data were analysed based on a pipeline[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.[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. Last, 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[30]: 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,[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 paediatric 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'.[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 were 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/; accessed 10 November 2021) 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 seizure.

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 seven 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 non-coding repeat expansions but found no known pathogenic repeat expansion variants in genes like CSTB,[33,34] SAMD12,[35] STARD7,[36] TNRC6A,[37] RAPGEF2,[38] MARCH6,[39] YEATS2[40] and C9orf72,[41] or in any of the epilepsy genes identified in this research.

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, %[a])	Patients without causative variants (n, %[b])	P-value
Total	320	117 (36.6)	203 (63.4)	
Age at seizure onset				
Neonatal (0–1 month)	28	21 (17.9)	7 (3.4)	0.0002
Infancy (1 month–1 year)	179	65 (55.6)	114 (56.2)	
Toddler (1–3 years)	69	22 (18.8)	47 (23.2)	
Early childhood (3–6 years)	33	8 (6.8)	25 (12.3)	
Middle childhood (6–12 years)	10	1 (0.9)	9 (4.4)	
Adolescent (12–18 years)	1	0 (0.0)	1 (0.5)	
Sex				
Male	191	74 (63.2)	117 (57.6)	0.346
Female	129	43 (36.8)	86 (42.4)	
DEE	209	86 (73.5)	123 (60.6)	0.021
Epilepsy classifications				
Generalized epilepsy	101	28 (23.9)	73 (36.0)	0.033
Focal epilepsy	152	60 (51.3)	92 (45.3)	0.353
Generalized and focal epilepsy	67	29 (24.8)	38 (18.7)	0.203
Epilepsy syndromes				
West syndrome	68	25 (21.4)	43 (21.2)	0.969
CSWS	21	6 (5.1)	15 (7.4)	0.491
Tuberous sclerosis	16	12 (10.3)	4 (2.0)	0.002
Ohtahara syndrome	13	8 (6.8)	5 (2.5)	0.076
Dravet syndrome	11	11 (9.4)	0 (0.0)	1.14×10^{-5}
Lennox–Gastaut syndrome	10	5 (4.3)	5 (2.5)	0.506
Febrile seizures plus	10	1 (0.9)	9 (4.4)	0.099
Benign familial infantile epilepsy	7	6 (5.1)	1 (0.5)	0.011
Other phenotypes				
Developmental delay	219	100 (85.5)	119 (58.6)	6.4×10^{-7}
Dystonia	62	31 (26.5)	31 (15.3)	0.019
Microcephaly	33	14 (12.0)	19 (9.4)	0.577
Premature	30	13 (11.1)	17 (8.4)	0.419
MGS	20	10 (8.5)	10 (4.9)	0.197
Congenital heart disease	13	6 (5.1)	7 (3.4)	0.463
Autism spectrum disorder	13	7 (6.0)	6 (3.0)	0.240
SGA	11	4 (3.4)	7 (3.4)	0.989
Lissencephaly	17	11 (9.4)	6 (3.0)	0.019
Abnormal limbs	15	10 (8.5)	5 (2.5)	0.024
Brain MRI				
With findings	170	77 (65.8)	93 (45.8)	0.007
No findings	122	36 (30.8)	86 (42.4)	
Not determined	28	4 (3.4)	24 (11.8)	

CSWS = epileptic encephalopathy with continuous spike-and-wave during sleep; DEE = developmental and/or epileptic encephalopathies; MGS = malformations of the genitourinary system; SGA = small for gestational age infant.
[a]Per cent among the 117 cases.
[b]Per cent among the 203 cases.

Detection rates of single-nucleotide and copy number variants

By simultaneously analysing 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 six 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 two 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 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 nine 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
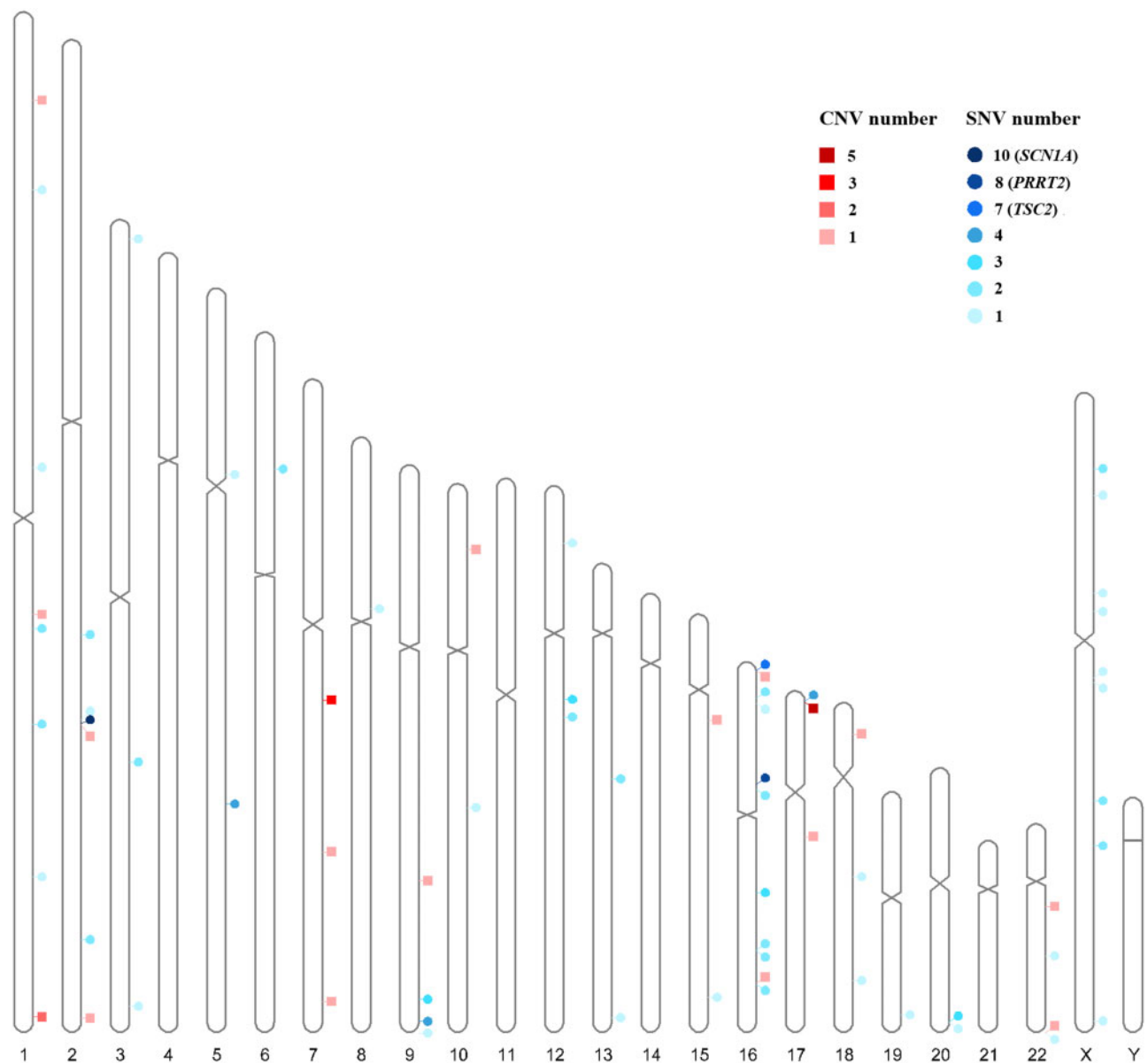
Figure 1 Distribution across chromosomes of associated diagnostic SNVs and diagnostic CNVs. Circles represent SNVs; squares represent CNVs; CNV number represents diagnostic CNV number in the region; SNV number represents diagnostic SNV number in a gene.

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 heat maps of Supplementary Fig. 3A and B.

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*, *KCNH1*, *KCNQ2*, *PRKCG*, *TSC2*, *WDR45* and *WWOX*, respectively. Furthermore, the following CNVs were found in cases of West syndrome: 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%) (seven variants in *TSC2*, four variants in *TSC1* and one 16p13.3 deletion that included *TSC2*). Eleven cases were clinically diagnosed as Dravet syndrome, 10 of which carrying a variant of *SCN1A* and
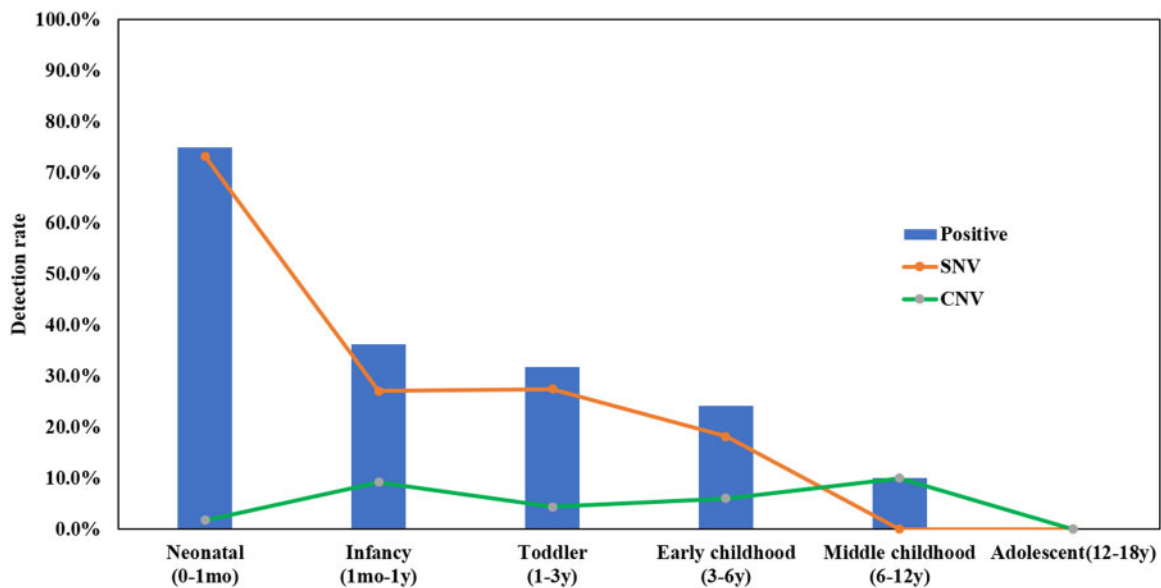
Figure 2 Detection rates at different ages of seizure onset. Detection rate: The ratio of the total number of positive samples in the age range of seizure onset. mo = month(s); y = years.

Table 2 Mutated genes associated with clinically diagnosed syndromes

Clinically diagnosed syndrome	Diagnosis yield	Associated genes mutated
West syndrome	36.8% (25/68)	*PAFAH1B1* (4), *UBA5* (2), *SUOX* (2), *DCX* (1), *DEPDC5* (1), *GNAO1* (1), *HCN1* (1), *KCNH1* (1), *KCNQ2* (1), *PRKCG* (1), *TSC2* (1), *WDR45* (1), *WWOX* (1), 17p13.3 del (5), 9q31.1 dup (1), 7q11.22q11.23 del (1), 7q11.22q11.23 dup (1), 16p13.3 del (1), 16q23.1 dup (1)[a]
Tuberous sclerosis	75.0% (12/16)	*TSC2* (7), *TSC1* (4), 16p13.3 del including *TSC2* (1)
Dravet syndrome	100.0% (11/11)	*SCN1A* (10), 2q24.3 del including *SCN1A* (1)
Ohtahara syndrome	61.5% (8/13)	*ABAT* (2), *CDKL5* (2), *GNAO1* (2), *KCNT1* (1), *STXBP1* (1), *WWOX* (1)
Epileptic encephalopathy with continuous spike-and-wave during sleep	28.6% (6/21)	*TSC1* (2), *FGF12* (1), *GRIN2A* (1); 7q11.21q21.3 dup (1), 15q11.2q13.1 del (1)
Benign familial infantile epilepsy	85.7% (6/7)	*PRRT2* (6)
Lennox–Gastaut syndrome	50.0% (5/10)	*ALDH7A1* (2), *SCN8A* (1), *STXBP1* (1) 7q11.22q11.23 dup (1), 22q13.33 del (1)
Myotonic epilepsy in infancy	50.0% (2/4)	*GRIN2B* (1), *KCNA2* (1)
Febrile seizures plus	10.0% (1/10)	*SETBP1* (1)
Panayiotopoulos syndrome	25.0% (1/4)	1q43q44 del (1)
Childhood epilepsy with centrotemporal spikes	25.0% (1/4)	*KIF4A* (1)
Epilepsy of infancy with migrating focal seizures	33.3% (1/3)	*KCNQ2* (1)
Epliespy with myoclonic-atonc seizures	100.0% (1/1)	*ITPR1* (1)
Early myoclonic encephalopathy	100.0% (1/1)	*EEF1A2* (1)
Childhood absence epilepsy	0.0% (0/3)	-

del = deletion; dup = duplication.
[a]16q23.1 dup was an intragenic variant in *WWOX*.

one a 2q24.3 deletion that included *SCN1A* (detection rate: 100%). Thirteen cases were clinically diagnosed as Ohtahara syndrome, with eight variants in six genes (detection rate: 61.5%) (two variants each in *ABAT*, *CDKL5* and *GNAO1* and one each in *KCNT1*, *STXBP1* and *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 *SCN1A* (10/95, 10.5%), followed by *PRRT2* (8/95, 8.4%) and *TSC2* (7/95, 7.4%). As shown in Table 3, four patients (4.2%) were found with mutations in

PAFAH1B1 and *TSC1*, respectively; three patients (3.2%) with mutations in *GNAO1*, *STXBP1*, *SCN8A* and *KCNQ2*; and two patients (2.1%) with mutations in *ZEB2*, *PCDH19*, *CDKL5*, *WWOX*, *ALDH7A1*, *DCX* and *SYNGAP1*.

As shown in Fig. 3, 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 fashion, 27 (25.2%, 27/107) in an autosomal recessive fashion and 12 (11.2%, 12/107) in an X-linked fashion (Fig. 3, Supplementary Table 3). Many of the

Table 3 Pathogenic/likely pathogenic genes identified in 320 patients with seizures

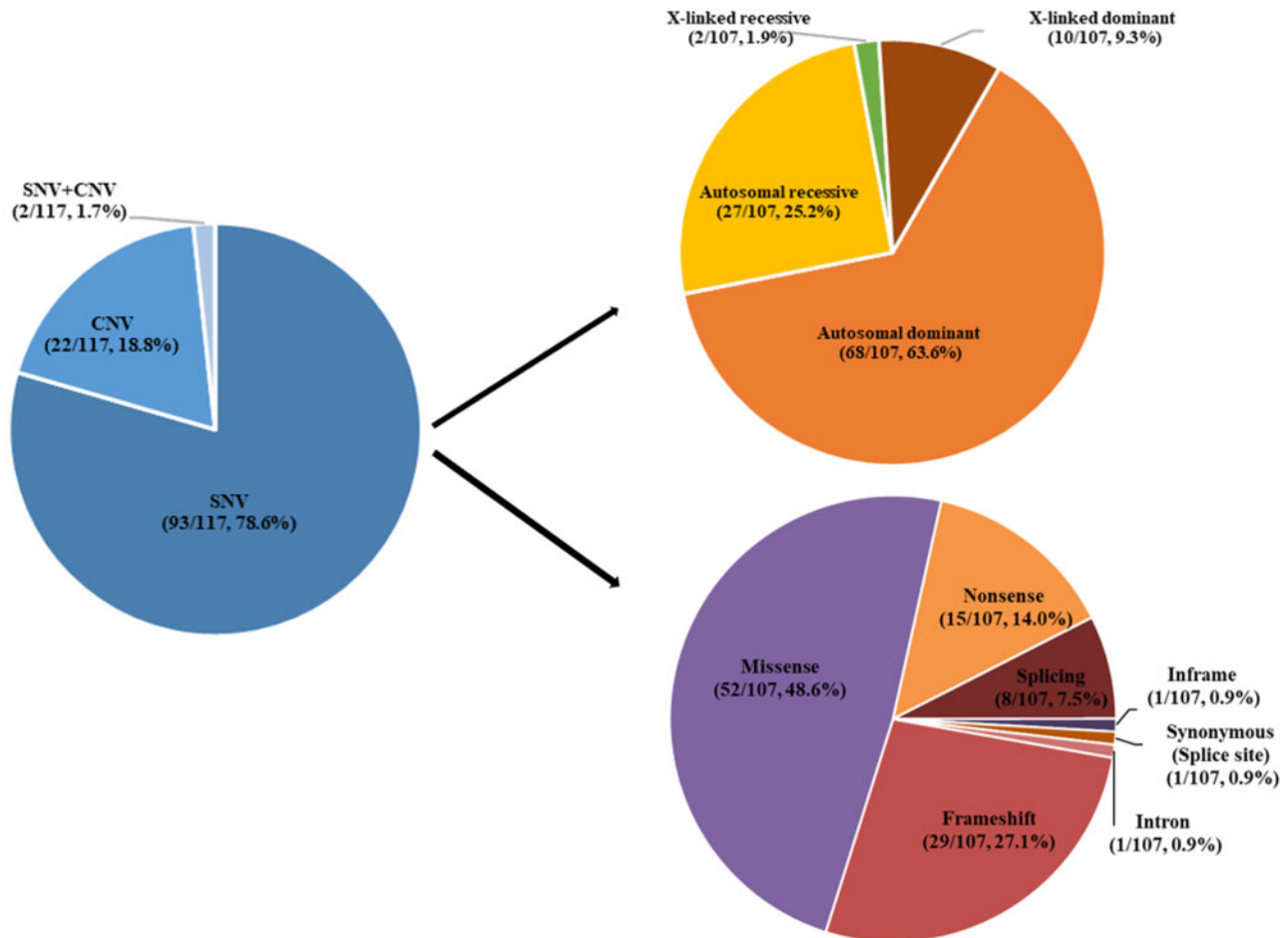| Number of patients per gene	Gene/syndromes
1	AARS, ABAT, ALG11, ARX, BCKDK, COG4, CHD2, COL4A1, CYP27A1, DARS2, DEPDC5, EEF1A2, FGF12, GRIN2A, GRIN2B, HCN1, ITPR1, KAT6A, KCNA2, KCNH1, KCNMA1, KCNT1, KIF4A, MECP2, PRKCG, RTTN, SCN3A, SETBP1, SHANK3, SLC2A1, SMC1A, SUOX, TAF1, TARS2, UBA5, WDR45
2	ZEB2, WWOX, SYNGAP1, PCDH19, DCX, CDKL5, ALDH7A1
3	STXBP1, SCN8A, KCNQ2, GNAO1
4	TSC1, PAFAH1B1
7	TSC2
8	PRRT2
10	SCN1A

Figure 3 Variation type in 117 patients with epilepsy.

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 *de novo*.

Further analysis revealed the following additional details. Of the 10 *SCN1A* variants that we detected, one was inherited from the father, but the other nine appeared *de novo* in the corresponding patients. Likewise, all four *TSC1* and seven *TSC2* variants appeared *de novo*. However, after verification by droplet digitnal PCR (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)],[42] 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.[43]

Copy number variants

Among 24 patients of our cohort, we identified 25 CNVs (1 patient, Patient SZCH0370, harboured two distinct CNVs) (Supplementary Table 2). Their sizes ranged from 121 base pairs (bp) to 30.47 megabase

Table 4 Gene variants associated with potential targeted therapies

Gene	Sample number	Recommended drugs	Aggravating drugs
SCN1A	10	Valproate,[70] benzodiazepines,[70] cannabinoids,[71] stiripentol,[72] ketogenic diet,[73] fenfluramine[74]	Sodium channel blockers, e.g. carbamazepine, oxcarbazepine, lamotrigine, and phenytoin[70,75]
SCN8A	3	Sodium channel blockers, e.g. carbamazepine, oxcarbazepine, lacosamide, lamotrigine, rufinamide, oxcarbazepine, and phenytoin[76,77]	NA
KCNT1	1	Quinidine[78]	NA
TSC1	4	Everolimus,[79,80] vigabatrin[81]	NA
TSC2	7	Everolimus,[79] vigabatrin[81]	NA
PCDH19	2	Bromide, clobazam[82]	Carbamazepine, oxcarbazepine, rufinamide[82]
ALDH7A1	2	Pyridoxine (vitamin B6),[83] folinic acid[84]	NA
HCN1	1	NA	Lacosamide and phenytoin[85]
KCNMA1	1	Vaproate and/or lamotrigine,[86] valproate and/or levetiracetam[87,88]	Ethosuximide[87]
PRRT2	8	Carbamazepine[89]	NA
SLC2A1	1	Ketogenic diet[90,91]	NA
CDKL5	2	Ketogenic diet[92]	NA

pairs (Mbp) and encompassed 18 deletions (72.0%) and seven duplications (28.0%). According to our interpretation rules (Supplementary Fig. 1B), 19 CNVs (76.0%) were considered pathogenic and six (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), which encompasses the deletion of *PAFAH1B1*, a known lissencephaly gene (OMIM #607432). In fact, we have identified 17 patients with lissencephaly, five of which harbouring 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.[44–46]

The most common recurrent CNVs 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.

These 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 (*SCN1A* and *SCN9A*) are known epilepsy genes, and deletion of either is known to cause epilepsy.[7,47]

Interestingly, among the 25 CNVs, the respective sizes of three CNVs were below 25 kilobase pairs (kbp). The detection of small fragments of CNVs is a major advantage of genome sequencing because chromosomal microarrays can currently not detect CNVs below 25 kbp.[48] As shown in Supplementary Fig. 4, we verified one of these small deletions (the 1651-bp deletion in chromosome 16p13.3 of Patient SZCH0053) by PCR analysis that yielded, in addition to an expected wild-type band, a band of 237 bp predicted to occur only in presence of the CNV. The deletion seemed to have appeared *de novo* as the parents showed only the wild-type 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 before, seizures in many patients may be just one of the manifestations of an underlying neurogenetic disease. Genome sequencing 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 female (Patient SZCH0292) developed seizures on the 13th day after birth that were refractory to anti-epileptic drugs. This patient was a compound heterozygote carrying two missense variants of the *ALDH7A1* gene (OMIM #107323), c.1547A > G (p.Tyr516Cys) (NM_001182.4) and c.965C > T (p.Ala322Val) (NM_001182.4). *ALDH7A1* deficiency leads to pyridoxal phosphate deficiency associated with pyridoxin-responsive epilepsy that can be alleviated by high doses of pyridoxine (vitamin B6).[49] With this treatment, the patient became seizure-free and had a good prognosis.

A 3-year-old male (Patient SZCH0283) had seizures at 3 months of age, combined with ataxia and mental retardation. We detected a heterozygous frameshift variant on *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 male was seizure-free and showed normal intellectual and motor development.

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 harboured variants that appeared to have occurred *de novo*, three families (5.0%) had two children with the same birth defects. The most likely explanation for recurrence of *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.[50] Indeed, in one case (Patient SZCH0052), we found that the patient's father was mosaic for the TSC2 variant detected in the child and so had probably transmitted it through the germline. Thus, genome sequencing can occasionally help to assess the risk for recurrence, even in cases where an original mutation seems to have arisen de novo.

Discussion

Despite the advantages of genome sequencing over other genetic methods, most large-scale genetic studies of paediatric epilepsy cases have been conducted using exome sequencing or capture-based targeted sequencing,[51–54] probably because of cost considerations. Hence, the present genome sequencing 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 CNVs as well as small indels, although no repeat expansions. Many of these alterations would be missed by exome sequencing 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 genome sequencing with possible results of exome sequencing or capture-based targeted methods, as shown in Supplementary Fig. 6, would indicate that the increased diagnostic yield obtained by genome sequencing 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 favourably with similar studies based on exome sequencing or targeted capture methods.[55–59]

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[60] 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[61] estimated that the risk of an abnormal phenotype ranged from 10.4% for 15q11.2 deletions to 46.8% for proximal 16p11.2 deletions. Because of 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 variant. The first case was a 1-year-old female (Patient 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 1542 kb 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 seizure.[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 male (Patient 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. Genome sequencing 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 genome sequencing generally offers in detecting CNVs, it also is superior over CMA techniques, specifically in the discovery of small indels.[64] In fact, of all structural rearrangements that we discovered, three were indels below 25 kbp, the usual detection limit of CMA. Furthermore, genome sequencing 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] Last, it can detect alterations in the vast number of non-coding RNAs.

In this study, we found that the diagnostic yield of genome sequencing 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.[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 aetiologies,[65] but exceeded that seen in other studies on this age group.[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.[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 anti-epileptic 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 genome sequencing 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.

Acknowledgements

Funding

Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

References

1. Neligan A, Hauser WA, Sander JW. The epidemiology of the epilepsies. *Handb Clin Neurol.* 2012;107:113–133.
2. Weber YG, Biskup S, Helbig KL, Von Spiczak S, Lerche H. The role of genetic testing in epilepsy diagnosis and management. *Expert Rev Mol Diagn.* 2017;17(8):739–750.
3. Steinlein OK, Mulley JC, Propping P, et al. A missense mutation in the neuronal nicotinic acetylcholine receptor alpha 4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy. *Nat Genet.* 1995;11(2):201–203.
4. Wang J, Lin ZJ, Liu L, et al. Epilepsy-associated genes. *Seizure.* 2017;44:11–20.
5. Dunn P, Albury CL, Maksemous N, et al. Next generation sequencing methods for diagnosis of epilepsy syndromes. *Front Genet.* 2018;9:20.
6. Stavropoulos DJ, Merico D, Jobling R, et al. Whole-genome sequencing expands diagnostic utility and improves clinical management in paediatric medicine. *NPJ Genomic Medicine.* 2016;1(1):1–9.
7. Carvill GL, Engel KL, Ramamurthy A, et al.; EuroEPINOMICS Rare Epilepsy Syndrome, Myoclonic-Astatic Epilepsy, and Dravet Working Group. Aberrant inclusion of a poison exon causes Dravet syndrome and related SCN1A-associated genetic epilepsies. *Am J Hum Genet.* 2018;103(6):1022–1029.
8. Corbett MA, Kroes T, Veneziano L, et al. Intronic ATTTC repeat expansions in STARD7 in familial adult myoclonic epilepsy linked to chromosome 2. *Nat Commun.* 2019;10(1):4920.
9. Meienberg J, Zerjavic K, Keller I, et al. New insights into the performance of human whole-exome capture platforms. *Nucleic Acids Res.* 2015;43(11):e76.
10. Fisher RS, Cross JH, French JA, et al. Operational classification of seizure types by the International League Against Epilepsy: Position Paper of the ILAE Commission for Classification and Terminology. *Epilepsia.* 2017;58(4):522–530.
11. Huang J, Liang X, Xuan Y, et al. A reference human genome dataset of the BGISEQ-500 sequencer. *Gigascience.* 2017;6(5):1–9.
12. Xu Y, Lin Z, Tang C, et al. A new massively parallel nanoball sequencing platform for whole exome research. *BMC Bioinform.* 2019;20(1):153.
13. Goyal A, Kwon HJ, Lee K et al. Ultra-fast next generation human genome sequencing data processing using DRAGENTM bio-IT processor for precision medicine. *Open Journal of Genetics.* 2017; 7(1): 9–19.
14. Lek M, Karczewski KJ, Minikel EV, et al.; Exome Aggregation Consortium. Analysis of protein-coding genetic variation in 60,706 humans. *Nature.* 2016;536(7616):285–291.
15. Karczewski KJ, Francioli LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature.* 2020;581(7809):434–443.
16. Auton A, Brooks LD, Durbin RM, et al.; Genomes Project C. A global reference for human genetic variation. *Nature.* 2015; 526(7571):68–74.
17. Landrum MJ, Lee JM, Benson M, et al. ClinVar: public archive of interpretations of clinically relevant variants. *Nucleic Acids Res.* 2016;44(D1):D862–8.
18. Solomon BD, Nguyen AD, Bear KA, Wolfsberg TG. Clinical genomic database. *Proc Natl Acad Sci U S A.* 2013;110(24):9851–9855.
19. Hamosh A, Scott AF, Amberger JS, Bocchini CA, McKusick VA. Online Mendelian Inheritance in Man (OMIM), a knowledgebase of human genes and genetic disorders. *Nucleic Acids Res.* 2005; 33(Database issue):D514–D517.
20. Stenson PD, Ball EV, Mort M, et al. Human gene mutation database (HGMD®): 2003 update. *Hum Mutat.* 2003;21(6):577–581.
21. Ng PC, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. *Nucleic Acids Res.* 2003;31(13):3812–3814.
22. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Meth.* 2010; 7(4):248–249.
23. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: Mutation prediction for the deep-sequencing age. *Nat Meth.* 2014;11(4):361–362.
24. Choi Y, Sims GE, Murphy S, Miller JR, Chan AP. Predicting the functional effect of amino acid substitutions and indels. *PLoS ONE.* 2012;7(10):e46688.
25. Richards S, Aziz N, Bale S, et al.; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17(5):405–424.
26. Riggs ER, Andersen EF, Cherry AM, et al. Technical standards for the interpretation and reporting of constitutional copy-number variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen). *Genet Med.* 2020;22(2):245–257.
27. Chiang C, Layer RM, Faust GG, et al. SpeedSeq: Ultra-fast personal genome analysis and interpretation. *Nat Methods.* 2015; 12(10):966–968.
28. Layer RM, Chiang C, Quinlan AR, Hall IM. LUMPY: A probabilistic framework for structural variant discovery. *Genome Biol.* 2014; 15(6):R84.
29. Dong Z, Zhang J, Hu P, et al. Low-pass whole-genome sequencing in clinical cytogenetics: A validated approach. *Genet Med.* 2016;18(9):940–948.
30. Coppola A, Cellini E, Stamberger H, et al.; EpiCNV Consortium. Diagnostic implications of genetic copy number variation in epilepsy plus. *Epilepsia.* 2019;60(4):689–706.
31. Doi K, Monjo T, Hoang PH, et al. Rapid detection of expanded short tandem repeats in personal genomics using hybrid sequencing. *Bioinformatics.* 2014;30(6):815–822.
32. Hao Z, Lv D, Ge Y, et al. RIdeogram: Drawing SVG graphics to visualize and map genome-wide data on the idiograms. *PeerJ Computer Sci.* 2020;6:e251.

33. Borel C, Migliavacca E, Letourneau A, et al. Tandem repeat sequence variation as causative *cis*-eQTLs for protein-coding gene expression variation: The case of CSTB. *Hum Mutat*. 2012;33(8):1302–1309.

34. Bosak M, Sułek A, Łukasik M, et al. Genetic testing and the phenotype of Polish patients with Unverricht-Lundborg disease (EPM1) - A cohort study. *Epilepsy Behav*. 2020;112:107439.

35. Ishiura H, Doi K, Mitsui J, et al. Expansions of intronic TTTCA and TTTTA repeats in benign adult familial myoclonic epilepsy. *Nat Genet*. 2018;50(4):581–590.

36. Corbett MA, Kroes T, Veneziano L, et al. Intronic ATTTC repeat expansions in STARD7 in familial adult myoclonic epilepsy linked to chromosome 2. *Nat Commun*. 2019;10(1):4920.

37. Terasaki A, Nakamura M, Urata Y, et al. DNA analysis of benign adult familial myoclonic epilepsy reveals associations between the pathogenic TTTCA repeat insertion in SAMD12 and the nonpathogenic TTTTA repeat expansion in TNRC6A. *J Hum Genet*. 2021;66(4):419–429.

38. Lei X, Liu Q, Lu Q, et al. TTTCA repeat expansion causes familial cortical myoclonic tremor with epilepsy. *Eur J Neurol*. 2019;26(3):513–518.

39. Florian RT, Kraft F, Leitão E, et al.; FAME consortium. Unstable TTTTA/TTTCA expansions in MARCH6 are associated with Familial Adult Myoclonic Epilepsy type 3. *Nat Commun*. 2019;10(1):4919-

40. Yeetong P, Pongpanich M, Srichomthong C, et al. TTTCA repeat insertions in an intron of YEATS2 in benign adult familial myoclonic epilepsy type 4. *Brain*. 2019;142(11):3360–3366.

41. van den Ameele J, Jedlickova I, Pristoupilova A, et al. Teenage-onset progressive myoclonic epilepsy due to a familial C9orf72 repeat expansion. *Neurology*. 2018;90(8):e658–e63.

42. Meneret A, Grabli D, Depienne C, et al. PRRT2 mutations: A major cause of paroxysmal kinesigenic dyskinesia in the European population. *Neurology*. 2012;79(2):170–174.

43. Novarino G, El-Fishawy P, Kayserili H, et al. Mutations in BCKD-kinase lead to a potentially treatable form of autism with epilepsy. *Science*. 2012;338(6105):394–397.

44. Pilz DT, Matsumoto N, Minnerath S, et al. LIS1 and XLIS (DCX) mutations cause most classical lissencephaly, but different patterns of malformation. *Hum Mol Genet*. 1998;7(13):2029–2037.

45. Parrini E, Conti V, Dobyns WB, Guerrini R. Genetic basis of brain malformations. *Mol Syndromol*. 2016;7(4):220–233.

46. Mills PB, Struys E, Jakobs C, et al. Mutations in antiquitin in individuals with pyridoxine-dependent seizures. *Nat Med*. 2006;12(3):307–309.

47. Singh NA, Pappas C, Dahle EJ, et al. A role of SCN9A in human epilepsies, as a cause of febrile seizures and as a potential modifier of Dravet syndrome. *PLoS Genet*. 2009;5(9):e1000649.

48. Haraksingh RR, Abyzov A, Urban AE. Comprehensive performance comparison of high-resolution array platforms for genome-wide Copy Number Variation (CNV) analysis in humans. *BMC Genomics*. 2017;18(1):14-

49. Wilson MP, Plecko B, Mills PB, Clayton PT. Disorders affecting vitamin B6 metabolism. *J Inherit Metab Dis*. 2019;42(4):629–646.

50. Campbell IM, Yuan B, Robberecht C, et al. Parental somatic mosaicism is underrecognized and influences recurrence risk of genomic disorders. *Am J Hum Genet*. 2014;95(2):173–182.

51. Costain G, Cordeiro D, Matviychuk D, Mercimek-Andrews S. Clinical application of targeted next-generation sequencing panels and whole exome sequencing in childhood epilepsy. *Neuroscience*. 2019;418:291–310.

52. Demos M, Guella I, DeGuzman C, et al. Diagnostic yield and treatment impact of targeted exome sequencing in early-onset epilepsy. *Front Neurol*. 2019;10:434.

53. Epi25 Collaborative. Electronic address: s.berkovic@unimelb.edu.au; Epi25 Collaborative. Ultra-rare genetic variation in the epilepsies: a whole-exome sequencing study of 17,606 individuals. *Am J Hum Genet*. 2019;105:267–282.

54. Truty R, Patil N, Sankar R, et al. Possible precision medicine implications from genetic testing using combined detection of sequence and intragenic copy number variants in a large cohort with childhood epilepsy. *Epilepsia Open*. 2019;4(3):397–408.

55. Butler KM, da Silva C, Alexander JJ, Hegde M, Escayg A. Diagnostic yield from 339 epilepsy patients screened on a clinical gene panel. *Pediatr Neurol*. 2017;77:61–66.

56. Helbig KL, Hagman KDF, Shinde DN, et al. Diagnostic exome sequencing provides a molecular diagnosis for a significant proportion of patients with epilepsy. *Genet Med*. 2016;18(9):898–905.

57. Liang JS, Wang JS, Lin LJ, et al. Genetic diagnosis in children with epilepsy and developmental delay/mental retardation using targeted gene panel analysis. *Neuropsychiatry*. 2018;8(5):1577–1585.

58. Tumienė B, Maver A, Writzl K, et al. Diagnostic exome sequencing of syndromic epilepsy patients in clinical practice. *Clin Genet*. 2018;93(5):1057–1062.

59. Yang L, Kong Y, Dong X, et al. Clinical and genetic spectrum of a large cohort of children with epilepsy in China. *Genet Med*. 2019;21(3):564–571.

60. Maya I, Perlman S, Shohat M, et al. Should We Report 15q11.2 BP1-BP2 deletions and duplications in the prenatal setting? *J Clin Med*. 2020;9(8):2602.

61. Rosenfeld JA, Coe BP, Eichler EE, Cuckle H, Shaffer LG. Estimates of penetrance for recurrent pathogenic copy-number variations. *Genet Med*. 2013;15(6):478–481.

62. Berryer MH, Hamdan FF, Klitten LL, et al. Mutations in SYNGAP1 cause intellectual disability, autism, and a specific form of epilepsy by inducing haploinsufficiency. *Hum Mutat*. 2013;34(2):385–394.

63. Mignot C, Lambert L, Pasquier L, et al. WWOX-related encephalopathies: Delineation of the phenotypical spectrum and emerging genotype-phenotype correlation. *J Med Genet*. 2015;52(1):61–70.

64. Zhou B, Ho SS, Zhang X, et al. Whole-genome sequencing analysis of CNV using low-coverage and paired-end strategies is efficient and outperforms array-based CNV analysis. *J Med Genet*. 2018;55(11):735–743.

65. Shellhaas RA, Wusthoff CJ, Tsuchida TN, et al.; Neonatal Seizure Registry. Profile of neonatal epilepsies: characteristics of a prospective US cohort. *Neurology*. 2017;89(9):893–899.

66. Jang SS, Kim SY, Kim H, et al. Diagnostic yield of epilepsy panel testing in patients with seizure onset within the first year of Life. *Front Neurol*. 2019;10:988.

67. Olson HE, Kelly M, LaCoursiere CM, et al. Genetics and genotype-phenotype correlations in early onset epileptic encephalopathy with burst suppression. *Ann Neurol*. 2017;81(3):419–429.

68. Mizrahi EM, Clancy RR. Neonatal seizures: Early-onset seizure syndromes and their consequences for development. *Ment Retard Dev Disabil Res Rev*. 2000;6(4):229–241.

69. Tadic BV, Kravljanac R, Sretenovic V, Vukomanovic V. Long-term outcome in children with neonatal seizures: A tertiary center experience in cohort of 168 patients. *Epilepsy Behav*. 2018;84:107–113.

70. Wirrell EC, Laux L, Donner E, et al. Optimizing the diagnosis and management of Dravet syndrome: Recommendations from a North American consensus panel. *Pediatr Neurol*. 2017;68:18–34.e3.

71. Devinsky O, Cross JH, Laux L, et al.; Cannabidiol in Dravet Syndrome Study Group. Trial of cannabidiol for drug-resistant seizures in the Dravet syndrome. *N Engl J Med*. 2017;376(21):2011–2020.

72. Chiron C, Marchand M, Tran A, et al. Stiripentol in severe myoclonic epilepsy in infancy: A randomised placebo-controlled syndrome-dedicated trial. *Lancet*. 2000;356(9242):1638–1642.

73. Ko A, Jung DE, Kim SH, et al. The efficacy of ketogenic diet for specific genetic mutation in developmental and epileptic encephalopathy. *Front Neurol*. 2018;9:530.

74. Bialer M, Johannessen SI, Koepp MJ, et al. Progress report on new antiepileptic drugs: A summary of the Fourteenth Eilat Conference on new antiepileptic drugs and devices (EILAT XIV). I. Drugs in preclinical and early clinical development. *Epilepsia*. 2018;59(10):1811–1841.

75. Shi X-Y, Tomonoh Y, Wang W-Z, et al.; Epilepsy Genetic Study Group, Japan. Efficacy of antiepileptic drugs for the treatment of Dravet syndrome with different genotypes. *Brain Dev*. 2016; 38(1):40–46.

76. Boerma RS, Braun KP, van de Broek MP, et al. Remarkable phenytoin sensitivity in 4 children with SCN8A-related epilepsy: A molecular neuropharmacological approach. *Neurotherapeutics*. 2016; 13(1):192–197.

77. Møller RS, Johannesen KM. Precision medicine: SCN8A encephalopathy treated with sodium channel blockers. *Neurotherapeutics*. 2016;13(1):190–191.

78. Bearden D, Strong A, Ehnot J, et al. Targeted treatment of migrating partial seizures of infancy with quinidine. *Ann Neurol*. 2014;76(3):457–461.

79. Franz DN, Lawson JA, Yapici Z, et al. Everolimus for treatment-refractory seizures in TSC: Extension of a randomized controlled trial. *Neurol Clin Pract*. 2018;8(5):412–420.

80. French JA, Lawson JA, Yapici Z, et al. Adjunctive everolimus therapy for treatment-resistant focal-onset seizures associated with tuberous sclerosis (EXIST-3): A phase 3, randomised, double-blind, placebo-controlled study. *Lancet*. 2016;388(10056):2153–2163.

81. Schubert-Bast S, Rosenow F, Klein KM, et al. The role of mTOR inhibitors in preventing epileptogenesis in patients with TSC: Current evidence and future perspectives. *Epilepsy Behav*. 2019; 91:94–98.

82. Lotte J, Bast T, Borusiak P, et al. Effectiveness of antiepileptic therapy in patients with PCDH19 mutations. *Seizure*. 2016;35:106–110.

83. van Karnebeek CD, Tiebout SA, Niermeijer J, et al. Pyridoxine-dependent epilepsy: An expanding clinical spectrum. *Pediatr Neurol*. 2016;59:6–12.

84. Campistol J, Plecko B. Treatable newborn and infant seizures due to inborn errors of metabolism. *Epileptic Disord*. 2015;17(3):229–242.

85. Marini C, Porro A, Rastetter A, et al. HCN1 mutation spectrum: From neonatal epileptic encephalopathy to benign generalized epilepsy and beyond. *Brain*. 2018;141(11):3160–3178.

86. Du W, Bautista JF, Yang H, et al. Calcium-sensitive potassium channelopathy in human epilepsy and paroxysmal movement disorder. *Nat Genet*. 2005;37(7):733–738.

87. Li X, Poschmann S, Chen Q, et al. De novo BK channel variant causes epilepsy by affecting voltage gating but not Ca 2+ sensitivity. *Eur J Hum Genet*. 2018;26(2):220–229.

88. Tabarki B, AlMajhad N, AlHashem A, Shaheen R, Alkuraya FS. Homozygous KCNMA1 mutation as a cause of cerebellar atrophy, developmental delay and seizures. *Hum Genet*. 2016; 135(11):1295–1298.

89. Huang X-J, Wang T, Wang J-L, et al. Paroxysmal kinesigenic dyskinesia: Clinical and genetic analyses of 110 patients. *Neurology*. 2015;85(18):1546–1553.

90. Klepper J, Diefenbach S, Kohlschütter A, Voit T. Effects of the ketogenic diet in the glucose transporter 1 deficiency syndrome. *Prostaglandins Leukot Essent Fatty Acids*. 2004;70(3):321–327.

91. Klepper J, Scheffer H, Leiendecker B, et al. Seizure control and acceptance of the ketogenic diet in GLUT1 deficiency syndrome: A 2-to 5-year follow-up of 15 children enrolled prospectively. *Neuropediatrics*. 2005;36(5):302–308.

92. Lim Z, Wong K, Olson HE, et al. Use of the ketogenic diet to manage refractory epilepsy in CDKL 5 disorder: Experience of > 100 patients. *Epilepsia*. 2017;58(8):1415–1422.