BRAIN
LETTER TO THE EDITOR

Clinical and genetic spectrum of 355 Chinese children with epilepsy: a trio-sequencing-based study

Jing Duan,[1,†] Yuanzhen Ye,[1,†] Dezhi Cao,[1] Dongfang Zou,[1] Xinguo Lu,[1] Li Chen,[1] Jialun Wen,[1] Huafang Zou,[1] Jian Gao,[2] Bingying Li,[2] Zhanqi Hu[1] and Jianxiang Liao[1]

[†]These authors contributed equally to this work.

1 Department of Neurology, Shenzhen Children's Hospital, Shenzhen, China
2 Aegicare (Shenzhen) Technology Co., Ltd., Shenzhen 518060, China

Correspondence to: Professor Jianxiang Liao
Department of Neurology, Shenzhen Children's Hospital
7019 Yitian Road, Futian District, Shenzhen
Guangdong Province 518038, China
E-mail: liaojianxiang@vip.sina.com

Correspondence may also be addressed to: Dr Zhanqi Hu
Department of Neurology, Shenzhen Children's Hospital
7019 Yitian Road, Futian District, Shenzhen
Guangdong Province 518038, China
E-mail: huzhanqi1983@aliyun.com

We read with great interest the cohort study of children with epilepsy that was recently published by Zou *et al.*[1] The authors recruited 320 paediatric epilepsy patients between October 2016 and December 2017 and performed genome sequencing on probands. They analysed genome sequencing data with comprehensive pedigree and clinical data and concluded that genome sequencing should be the first choice for genetic testing in epilepsy patients. We agree that the application of genome sequencing in children with epilepsy will lead to accurate interpretation of genetic testing and thus benefit therapeutic decision-making and precision medicine. However, there are several methods and strategies to applying next-generation sequencing for clinical diagnostics, which vary in the type of sequencing regions and cost. According to the American College of Medical Genetics and Genomics guidelines, trio-based genetic analysis of the proband and both biological parents is important in determining if a variant is inherited or if it is *de novo* and thus affects variant classification and identification.[2] Hence, our group retrospectively reviewed and analysed paediatric epilepsy patients who underwent diagnostic trio-based clinical genetic testing at Shenzhen Children's Hospital between September 2019 and June 2020. A total of 355 cases were included; 168 of the patients and their parents underwent whole-exome sequencing (WES), 48 patients and their parents underwent whole genome sequencing (WGS) and 139 patients underwent WGS while their parents underwent WES. We systematically reviewed detailed clinical records of patients in all groups. We evaluated the clinical characteristics that were associated with a positive genetic diagnosis and assessed the potential impact of the genetic diagnosis on management strategy.

Zou's group[1] performed genome sequencing on 320 Chinese children with epilepsy and uncovered pathogenic/likely pathogenic variants in 117 of the 320 children (36.6%). A similar diagnostic rate was observed in our analysis, with 117 of the 355 patients (32.96%) showing causative results (79 with single nucleotide variations or insertion deletions, 32 with copy number variations and five with mitochondrial mutations; Fig. 1A). We identified 89 causative single nucleotide variations or insertion deletion variants in 79 patients (Supplementary Table 1). The variants were most frequently found in *PRRT2* (10/88, 11.36%), which is associated with benign familial infantile epilepsy, followed by *SCN1A* (7/88, 7.95%), which is associated with Dravet syndrome and *TSC2* (5/88, 5.68%), which is associated with tuberous sclerosis. Interestingly, five patients were identified with multilocus disease-causing genomic variations, which may lead to multiple genetic diagnoses (Fig. 1B and Supplementary Tables 1 and 2). All patients with two molecular diagnoses showed two pathogenic variants that cause autosomal dominant disease, and three of them showed two *de novo* mutations in autosomal dominant disease genes. A 6-month-old boy (Case GT110) had a *de novo* missense mutation in *SYNGAP1* and a 16p11.2 recurrent microdeletion (524.61 Kb) inherited from his mother. Another 4-month-old boy (Case GT130) had a *de novo* missense mutation in *PACS1* and a recurrent frameshift mutation in *PRRT2* inherited from his father, who experienced seizures as a

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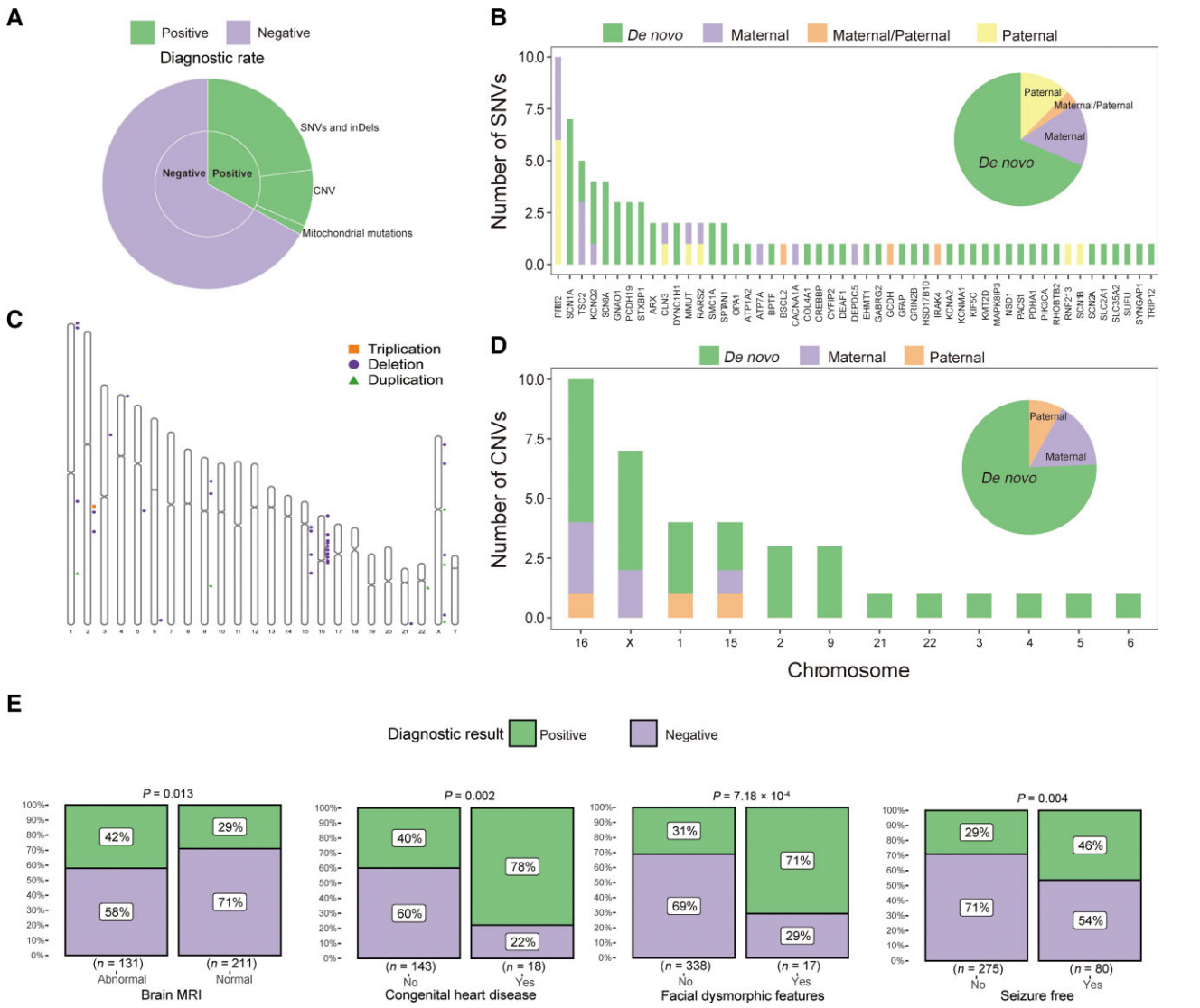

Figure 1 The genomic landscape of Chinese children with epilepsy. (**A**) Variation type in 335 patients with epilepsy. (**B**) Candidate single nucleotide variations found in 81 epilepsy patients. (**C**) Distribution of associated diagnostic copy number variations across chromosomes; circles represent deletion copy number variations, boxes represent triplicate copy number variations, triangles represent duplicate copy number variations. (**D**) Candidate copy number variations found in 32 epilepsy patients. (**E**) Comparison of patients with pathogenic or likely pathogenic variants and patients without causative variants.

child. The percentage of positive cases with *de novo* mutations in our group was slightly higher compared with that of Zou's group[1] (67.82% and 60%). Trio-based genetic testing has been proved to be particularly successful in identifying *de novo* variations[3,4] and diagnosing with a high pathogenic variant rate.[5] Our data support that the trio-based approach should be treated as a good option in light of the known clinical and genetic heterogeneity seen in epilepsy, especially when considering multiple genetic diagnoses.

We also identified 37 causative copy number variants in 32 patients (Supplementary Table 2) that ranged from 258 bp to 97.79 Mb and encompassed 30 deletions (81.1%), six duplications (16.2%) and one triplication (2.7%; Fig. 1C). As shown in Fig. 1D, the numbers of copy number variants varied on each chromosome, and the proportion of (28/37, 75.7%) of *de novo* copy number variants was similar to that of *de novo* single nucleotide variations. The most frequent copy number variant was 16p11.2 recurrent deletions, which were found in seven patients. Interestingly, *PRRT2*, the most common gene harbouring single nucleotide variations or insertion deletion variants, is contained in the 16p11.2 recurrent deletion region, which indicates that mutations involving this gene were found in 17 patients, accounting for 14% of all positive cases (Supplementary Tables 1 and 2). Combined with the results from Zou's group that reported 10 cases with a mutation in *PRRT2*, these findings suggest that aberrations in *PRRT2* may be one of the most common causes of monogenic epilepsies in Chinese children. Additionally, although mitochondrial mutations were not mentioned in Zou's study, we performed mitochondrial analysis in patients who underwent both WES and mitochondrial gene testing, as well as patients who underwent WGS with an enhanced WGS pipeline. The results identified four mitochondrial mutations in five patients including m.3243A>G (two patients), m.8993T>G (one patient), m.4810-15538del (one patient) and m.621_15950del (one patient) (Supplementary Table 2) In addition, m.827A>G, which may cause hearing loss after aminoglycoside treatment, was found in

Table 1 Impact of genetic testing

Affected gene	Sample number	Phenotype (OMIM)	Changes of management and diagnostic workup
With recommended drugs			
PRRT2	17	Seizures, benign familial infantile, 2	Recommended carbamazepin or oxcarbazepine
SCN8A	4	Developmental and epileptic encephalopathy 13	Recommended sodium channel blockers, e.g. carbamazepin, oxcarbazepine, lacosamide, lamotrigine and phenytoin
KCNQ2	4	Developmental and epileptic encephalopathy 7	Recommended carbamazepine, phenytoin
PCDH19	3	Developmental and epileptic encephalopathy 9	Recommended clobazam, bromide
CACNA1A	1	Developmental and epileptic encephalopathy 42	Recommended lamotrigine
SCN2A	1	Developmental and epileptic encephalopathy 11	Recommended carbamazepine, phenytoin
DEPDC5	1	Epilepsy, familial focal, with variable foci 1	Recommended oxcarbazepine, lacosamide. It may also help guide the selection of candidates for presurgical evaluation
SLC35A2	1	Congenital disorder of glycosylation, type IIm	Recommended ketogenic diet
To avoid aggravating drugs			
SCN1A	7	Dravet syndrome	Stiripentol, valproate, clobazam, ketogenic diet, and cannabidiol are recommended; avoid carbamazepine/lamotrigine
Special follow-up content			
COL4A1	1	Brain small vessel disease with or without ocular anomalies	MRI and MRA was used for dynamic follow-up.
RNF213	1	Moyamoya disease 2	Aspirin was added to the therapy to prevent thrombosis; MRI and MRA was used for dynamic follow-up.
Treated with precision therapy			
TSC2	5	Tuberous sclerosis	Vigabatrin for infantile spasms, everolimus while needed
HSD17B10	1	HSD10 mitochondrial disease	Cocktail therapy for mitochondrial disease; did not recommend surgical treatment.
IRAK4	1	Immunodeficiency 67	Gamma globulin was used regularly to prevent infection
SLC2A1	1	GLUT1 deficiency syndrome 1, infantile onset, severe	Ketogenic diet
Ion channel or synapse related disease, which did not recommend surgical treatment			
SMC1A	2	Developmental and epileptic encephalopathy 85, with or without midline brain defects	Periodic pharma co-resistant cluster seizures, focal onset, severe development delay; surgical treatment was not recommended.
SPTAN1	2	Developmental and epileptic encephalopathy 5	Pharmaco-resistant seizures, severe development delay; surgical treatment was not recommended.
RARS2	1	Pontocerebellar hypoplasia, type 6	No developmental milestones were attained; brain MRI revealed progressive atrophy of the cerebellum, pons, cerebral cortex, and white matter; surgical treatment was not recommended.
TRIP12	1	Mental retardation, autosomal dominant 49	Clinical synopsis was wide and epilepsy was only one of the symptoms; behavioural psychiatric manifestations were also seen; MRI was normal; surgical treatment was not recommended.
ARX	1	Developmental and epileptic encephalopathy 1	Lissencephaly was seen in the brain MRI; surgical treatment was not recommended
KCNA2	1	Developmental and epileptic encephalopathy 32	Ion channel disease; did not recommend surgical treatment.
CACNA1A	1	Developmental and epileptic encephalopathy 42	Ion channel disease; did not recommend surgical treatment.
GABRG2	1	Developmental and epileptic encephalopathy 74	Ion channel disease; did not recommend surgical treatment.
SPTAN1	1	Developmental and epileptic encephalopathy 5	No developmental milestones were attained; brain MRI showed widespread brain atrophy; did not recommend surgical treatment.

six patients. This finding is a reminder that aminoglycoside should be avoided for infectious disease treatment in these patients.

Zou *et al.*[1] reported that the age at onset of epileptic seizures and diagnosis of an epileptic syndrome associated with positive genetic diagnosis. Similarly, these two factors were significantly correlated with the detection of disease-causing variants in our group based on the chi-squared test (Supplementary Table 3). Three other factors also showed an association with positive genetic diagnosis in our study, including positive brain MRI, congenital heart disease and facial dysmorphic features (Fig. 1D and Supplementary Table 2). The positive hit-rates in patients with facial dysmorphia and congenital heart disease were particularly high (71% and 78%, respectively). We identified 12 disease-causing variants in 17 patients with facial dysmorphic features and 14 disease-causing variants in 18 patients with congenital heart disease. In terms of brain MRI, the positive rate in patients who were positive was slightly higher than that in patients with normal results (42% and 29%, respectively). We also divided patients according to birth weight or dystonia into abnormal groups and normal groups. There was no significant difference in the positive rates between these groups.

Genetic testing has become an essential part of clinical practice for epilepsy. It helps in establishing an aetiological diagnosis, providing prognostic information, precisely guiding therapy indicated for the patient and avoiding drugs that may worsen the seizures.[6,7] This is supported by our study, in which patients with a disease-causing mutation were more commonly seizure-free than those with no disease-causing mutation (46% and 29%, respectively). In our cohort, 32 patients chose a more suitable drug after their genetic diagnosis was confirmed (Table 1). Adjustment of treatment was frequently observed in patients with mutations involving *PRRT2* (17 cases), which is associated with benign familial infantile epilepsy. With oxcarbazepine treatment, these patients became seizure-free and had a good prognosis. Seven patients were told to avoid specific drugs because of loss of *SCN1A* function. Two patients were followed up with special follow-up content. Bilateral anterior and middle cerebral arteries were narrowed, and collateral circulation was observed in brain MRI of one of these two patients with *RNF213* pathogenic mutation. Evidence has suggested that susceptibility to Moyamoya disease 2 (MYMY2) may be conferred by variations in the *RNF213* gene (OMIM: 613768) on chromosome 17q25. Based on the condition of this patient and the positive testing result, aspirin was added to the therapy, and MRI and magnetic resonance angiography (MRA) were conducted for dynamic follow-up. Nine patients were treated with precision therapy after diagnosis, such as everolimus for patients with tuberous sclerosis with *TSC1/2* mutation. In addition, 11 patients were diagnosed with ion channel- or synapse-related disease, which helped to prevent further invasive investigations and surgical treatment was not recommended. Thirty-three patients went to an antenatal reproductive centre for genetic counselling to have another child after the genetic diagnosis was confirmed. These results confirmed that, in addition to clarifying the ecological diagnosis and treatment, genetic counselling and social support could also be useful for many families.

In conclusion, with the fast-approaching personal genomics era and advances in high-throughput sequencing, WES as well as WGS are now commonly used as diagnostic tools in the clinical setting.[7–10] Our results provided evidence that nearly 70% of positive patients carried de novo mutations and five patients showed mitochondrial diseases features. In addition, our data indicated that patients with multilocus disease–causing genomic variations are not rare among children with epilepsy, which challenges clinic diagnosis and genetic counselling. Incorporation of next generation sequencing into clinical practice for epilepsy patients continues to expand the list of variants, posing particular challenges for clinical decision-making for carriers of pathogenic variants regarding personalized drug therapy and genetic counselling. Our strategy that combines multiple sequencing technologies with a trio approach (proband, mother and proband) not only benefits patients with epilepsy features but will also accelerate the interpretation of pathogenic variants for precision medicine. Moreover, our research supports the essential role of genetic testing in the clinical practice of epilepsy.

Data availability

All data are available from the corresponding author upon reasonable request, with the exception of primary patient sequencing data that cannot be made available due to consent regulations.

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Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary material

Supplementary material is available at *Brain* online.

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