We read with great interest the cohort study of children with epilepsy that was recently published by Zou et al. 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. 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 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, 5.8%), 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
child. The percentage of positive cases with de novo mutations in our group was slightly higher compared with that of Zou’s group1 (67.82% and 60%). Trio-based genetic testing has been proved to be particularly successful in identifying de novo variations3,4 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...
six patients. This finding is a reminder that aminoglycoside should be avoided for infectious disease treatment in these patients.

Zou et al.\textsuperscript{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 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.

### Table 1  Impact of genetic testing

| Affected gene | Sample number | Phenotype (OMIM) | Changes of management and diagnostic workup |
|---------------|---------------|------------------|--------------------------------------------|
| PPRRT2        | 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, lamotriginie 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 IIa | Recommended ketogenic diet |

**To avoid aggravating drugs**

| SCN1A         | 7             | Dravet syndrome | Stiripentol, valproate, clobazam, ketogenic diet, and cannabidiol are recommended; avoid carbamazepine/lamotrigrine |

**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. |
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. 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. 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 multic locus disease-causing genomic variations are not rare among children with epilepsy, which challenges the 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.

References

1. Zou D, Wang L, Liao J, et al. Genome sequencing of 320 Chinese children with epilepsy: a clinical and molecular study. Brain. 2021;144:3623–3634.
2. Deignan JL, Chao E, Gannon JL, et al. Points to consider when assessing relationships (or suspecting misattributed relationships) during family-based clinical genomic testing: a statement of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2020;22(8):1285–1287.
3. Myers CT, Mefford HC. Genetic investigations of the epileptic encephalopathies of infancy: Recent advances. Prog Brain Res. 2016;226:35–60.
4. McTague A, Howell KB, Cross JH, Kurian MA, Scheffer IE. The genetic landscape of the epileptic encephalopathies of infancy and childhood. Lancet Neurol. 2016;15(3):304–316.
5. Helbig KL, Farwell Hagman KD, 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.
6. Dyment DA, Prasad AN, Boycott KM, et al. Implementation of epilepsy multigene panel testing in Ontario, Canada. Can J Neurol Sci. 2020;47(1):61–68.
7. Wright CF, FitzPatrick DR, Firth HV. Paediatric genomics: diagnosing rare disease in children. Nat Rev Genet. 2018;19(5):325.
8. Posey JE. Genome sequencing and implications for rare disorders. Orphanet J Rare Dis. 2019;14(1):153.
9. Foo JN, Liu JJ, Tan EK. Whole-genome and whole-exome sequencing in neurological diseases. Nat Rev Neurol. 2012;8(9):508–517.
10. Marshall CR, Bick D, Belmont JW, et al. The medical genome initiative: moving whole-genome sequencing for rare disease diagnosis to the clinic. Genome Med. 2020;12(1):48.