

Early diagnosis improving the outcome of an infant with epileptic encephalopathy with cytoplasmic FMRP interacting protein 2 mutation

Case report and literature review

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Abstract

Rationale: Early infantile epileptic encephalopathy (EIEE) 65 was recently shown to be caused by the cytoplasmic FMRP interacting protein 2 (*CYFIP2*) mutation. To date, only 5 cases have been reported in two articles, and all the outcomes in all cases were poor.

Patient concerns: In this study, we reported an 8-month-old girl with a 1 month-long history of seizures and developmental delay. Over 1 month later, she developed epileptic spasms in clusters with hypersarrhythmia on electroencephalography.

Diagnosis: The patient was diagnosed with EIEE 65 and trio-based whole-exome sequencing revealed a causative de novo *CYFIP2* mutation c.260G >T (p.Arg87Leu).

Interventions: The proband was successively treated with multiple antiepileptic drugs, including levetiracetam, phenobarbital, VitB6, topiramate, methylprednisolone, prednisone, valproic acid and vigabatrin.

Outcomes: After resistance to multiple anti-epileptic drugs over 2 months of treatment, she finally achieved seizure-free several days after vigabatrin administration and her developmental delay steadily improved.

Lessons: Our case confirmed that *CYFIP2* was the pathogenic gene of EIEE 65. We also first demonstrated vigabatrin might be effective for control of seizures and helpful for the improved outcomes of these patients.

Abbreviations: *CYFIP2* = cytoplasmic FMRP interacting protein 2, EE = epileptic encephalopathy, EEG = electroencephalography, EIEE = early infantile epileptic encephalopathy, GTCSS = generalized tonic-clonic seizures, MRI = magnetic resonance imaging, SD = standard deviation, WAVE = WASP-family verprolin-homologous protein, WES = whole-exome sequencing.

Keywords: *CYFIP2*, epileptic encephalopathy, hypersarrhythmia, vigabatrin, whole-exome sequencing

1. Introduction

Epileptic encephalopathy (EE) was formally defined as epileptic activity that contributes to severe and progressive cognitive and behavioral impairment above and beyond what might be expected from the underlying pathology alone. Most EE cases

are affected by onset in the early infantile year of life (EIEE),^[1,2] and nearly all patients experience a devastating process with a high risk of comorbidities and poor prognosis. In addition to the cerebral structural abnormalities found in a minority of these diseases, accumulating evidence indicates a major genetic

Editor: N/A.

Informed written consent was obtained from the patient's parents for publication of this case report and accompanying images.

This study was supported in part by a grant from the Children's Hospital of Chongqing Medical University for Special Project on Difficult and Rare Diseases (grant no. HJYN2013-4).

The authors have no conflicts of interest to disclose.

Supplemental Digital Content is available for this article.

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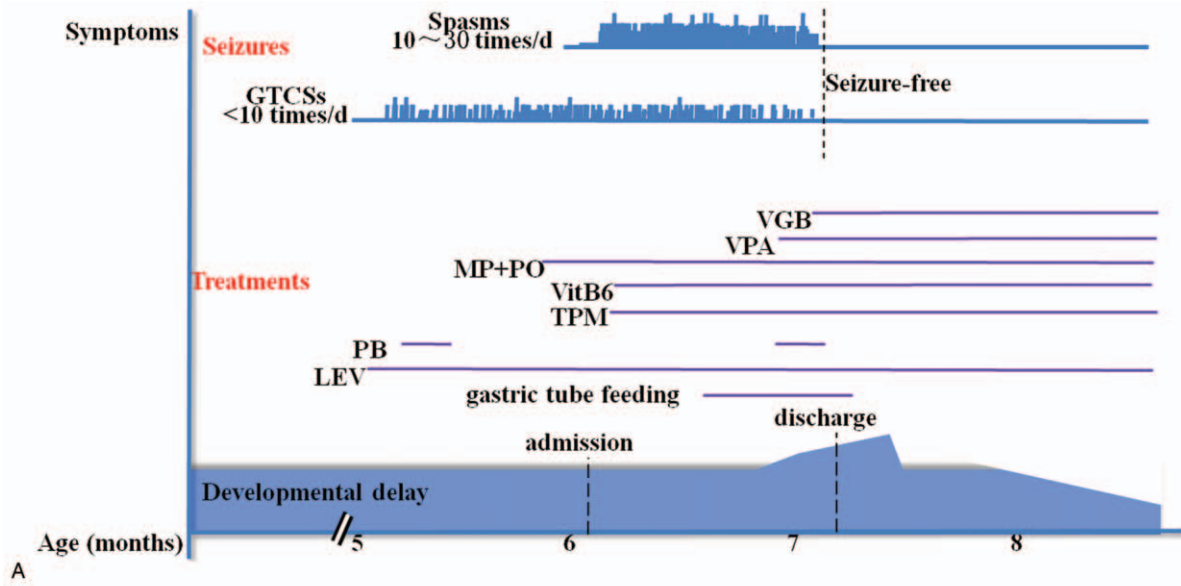
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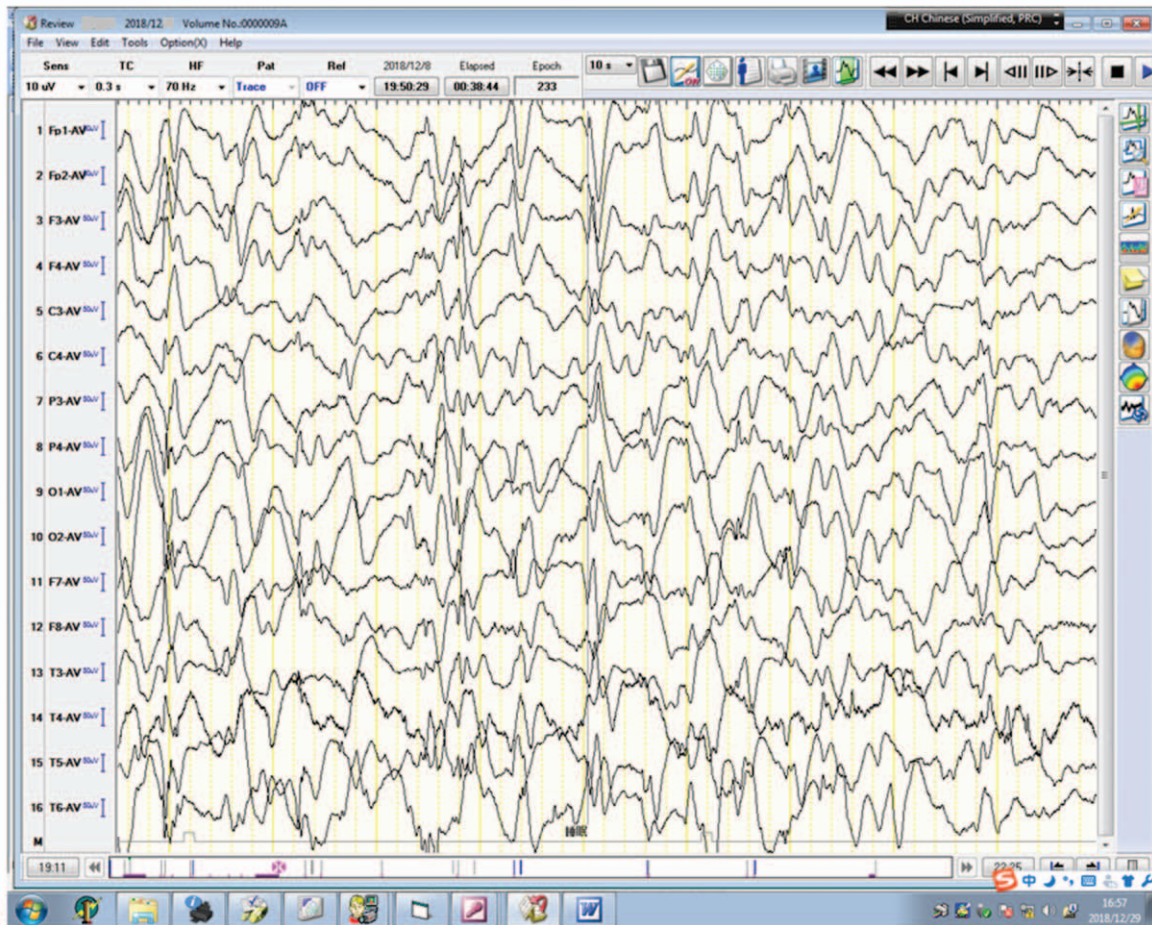
How to cite this article: Zhong M, Liao S, Li T, Wu P, Wang Y, Wu F, Li X, Hong S, Yan L, Jiang L. Early diagnosis improving the outcome of an infant with epileptic encephalopathy with cytoplasmic FMRP interacting protein 2 mutation. *Medicine* 2019;98:44(e17749).

Received: 27 April 2019 / Received in final form: 26 September 2019 / Accepted: 1 October 2019

<http://dx.doi.org/10.1097/MD.00000000000017749>



A

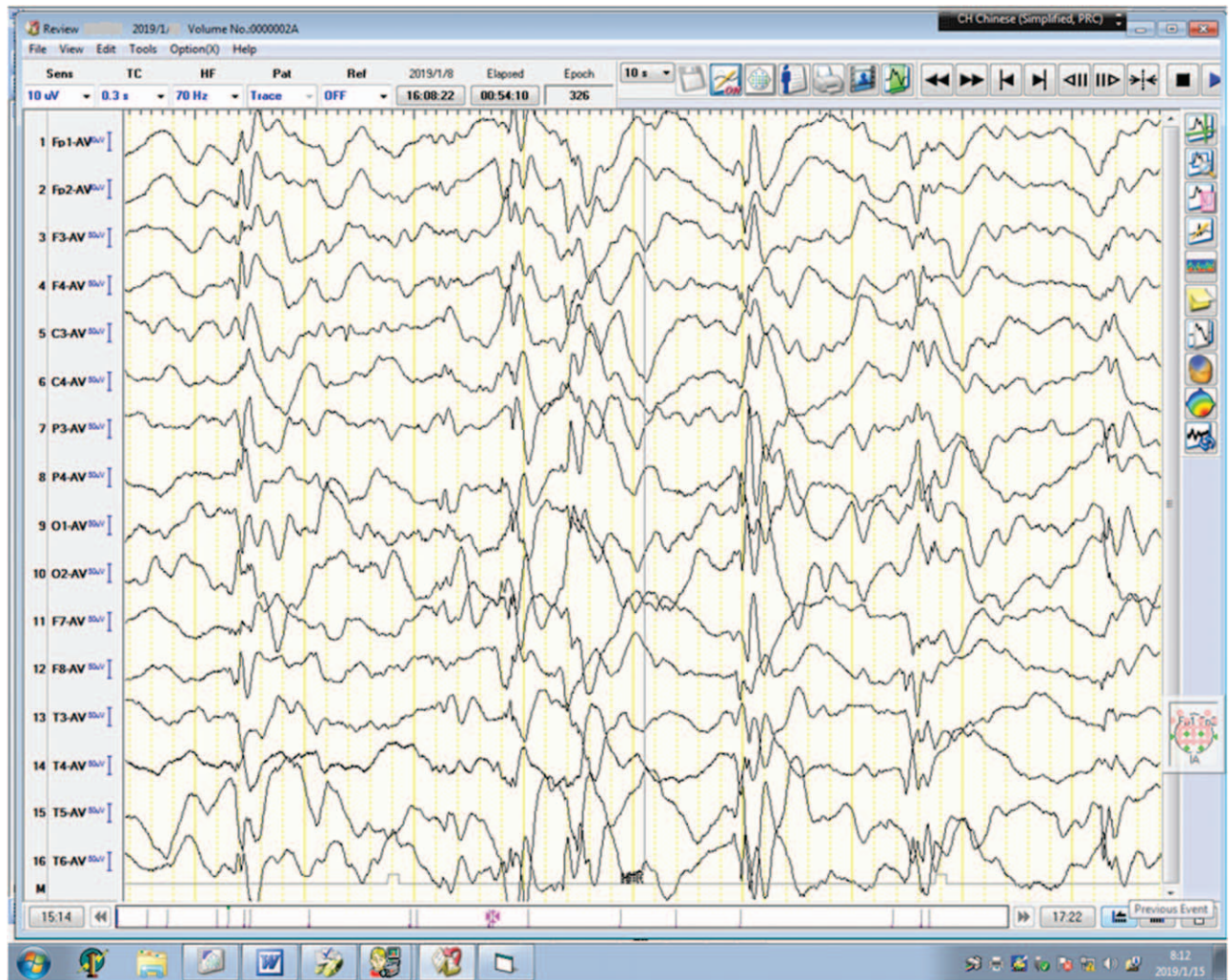


B

Figure 1. Summary of the clinical course of the patient (A). Seizures occurred approximately 10 times/d during the first month after the seizure onset. One and a half months later, the patient’s condition deteriorated with epileptic spasms occurring in clusters accompanied by hypsarrhythmia on EEG (B). Several days after adding vigabatrin, the patient became seizure-free and her condition steadily improved, but still with hypsarrhythmia 1 mo later (C). EEG = electroencephalography.

component in the etiology of EIEEs. In recent years, numerous causative genes (>80) have been identified in epilepsy patients^[3] providing greater insight into the pathogenesis of EIEEs. To date, about 70 single-gene mutations associated with EIEEs have been

submitted to the Online Mendelian Inheritance in Man database (www.omim.org). Several months ago, 4 cases of EIEE were reported to have cytoplasmic FMRP interacting protein 2 (*CYFIP2*) gene mutations that were designated EIEE 65 (OMIM: 618008).^[4]



C

Figure 1. (Continued).

Here, we present the case of a baby girl with global intellectual disability and refractory epilepsy. Whole-exome sequencing (WES) of the proband identified a *de novo* missense mutation of the *CYFIP2* gene. We also compared our findings with those of 5 previously reported cases.

2. Methods

2.1. Ethical approval

Ethical approval for this study was obtained from the Institutional Review Board, Children's Hospital of Chongqing Medical University (2018-64). Informed consent was obtained from the patient's parents.

2.2. Patient information

Here, we report the case of a baby girl born to non-consanguineous Chinese parents at 40 weeks and 2 days of gestation (G1P1). Her mother had a cold during the first 6 months of pregnancy. The girl was born by normal vaginal

delivery with a birth weight of 3010 g, body length of 50.5 cm and head circumference of 33 cm. The child was unable to support her head and roll over until she was 5 months of age.

The child was admitted to a local hospital for rehabilitation because of developmental delay; however, generalized tonic-clonic seizures (GTCSs) were identified by the clinicians and levetiracetam (35 mg/kg.d) was administered. Phenobarbital was added but stopped 2 weeks later. The seizures did not subside over the following month and the patient was transferred to our neurologic ward at the age of 6 months and 8 days. On admission, she had 10 GTCSs/d (Video 1, <http://links.lww.com/MD/D314>), was still unable to support her head and responded poorly to voices and light. No characteristic dysmorphic features were observed except for a small forehead. According to the World Health Organization Anthro guidelines, the patient's anthropometric Z-scores were as follows: body weight 7 kg (Z-score, -2.17 standard deviations [SD]), body length 65 cm (Z-score, -2.75 SD), and head circumference 45 cm (Z-score, 0.02 SD).

During hospitalization, we added topiramate (6.5 mg/kg.d) and vitamin B6 to the patient's treatment regimen, but her

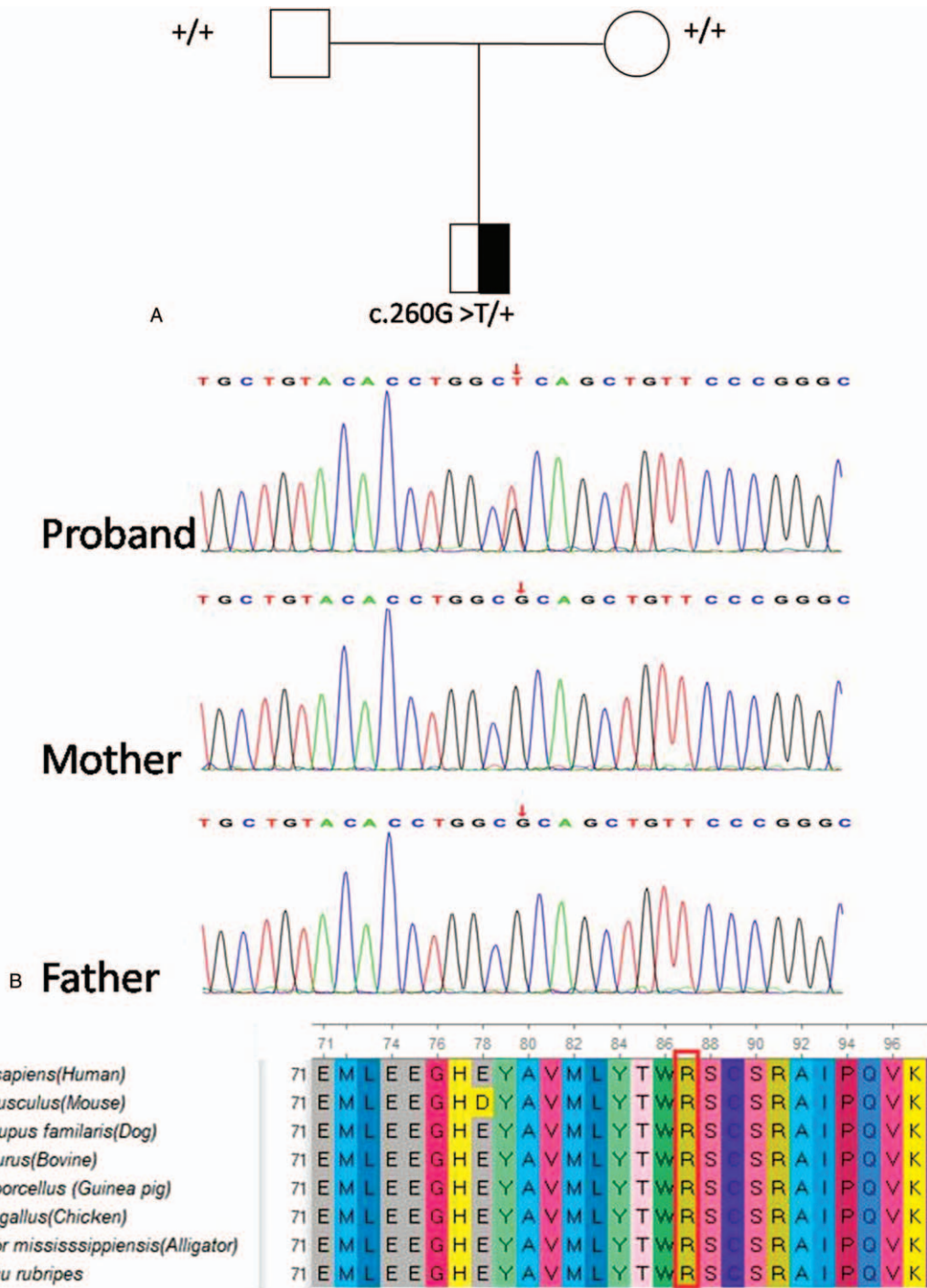


Figure 2. Whole-exome sequencing showed a de novo heterozygous mutation c.260G >T in the *CYFIP2* gene (A and B). The *CYFIP2* missense mutation (red arrow) is located in a highly conserved amino acid sequence among representative species (C). *CYFIP2*=cytoplasmic FMRP interacting protein 2.

seizures deteriorated and 10 days after hospitalization she developed epileptic spasms (Video 2, <http://links.lww.com/MD/D315>) in clusters with hypsarrhythmia on electroencephalography (EEG). The patient was diagnosed as West syndrome, and methylprednisolone (15 mg/kg.d × 3 days) was administered by intravenous injection followed by oral prednisone (20 mg/kg.d) and valproic acid (30 mg/kg.d), but without improvement. Seizures occurred over 30 times a day and gastric tube feeding was started because of feeding problems. Finally,

vigabatrin (50 mg/kg.d) was started at the request of the parents. After 2 to 3 days, the patient had no seizures and she was able to suck milk dispensing with gastric tube feeding. The patient was seizure-free and discharged 33 days after admission. During the 1-month follow-up, the seizures were totally controlled, and her psychomotor development improved (Fig. 1A).

The magnetic resonance imaging scans showed increased extra-axial spaces. Interictal EEG showed preliminary spike- and/

Table 1
Clinical characteristics of confirmed patients with CYFIP2 mutations from the literatures and our report.

Basic features	Nakashima et al (2018)					Peng et al (2018)	
	Case 1	Case 2	Case 3	Case 4	Case 5	Case 5	Our case
Ethnic origin	Japanese	Japanese	Israeli	Malaysian	Chinese	Chinese	Chinese
GA, wk	40	30	38	38	41	41	40 ^{±2}
BW, g	3748, +1.9SD	1938, -2.7SD	3564, +1.4SD	2670, -0.7 SD	3400, -0.92 SD	3400, -0.92 SD	3010, -0.71 SD
Length, cm	51.8, +1.3SD	41.0, -3.5SD	NA	49, -1 SD	NA	NA	50.5, +0.7 SD
HC, cm	34.5, +0.8 SD	31.5, -1.0SD	34.5, +1.1 SD	32, -3 SD	NA	NA	33, -1.15 SD
Sex/age	Male/5 yr	Male/11 yr	Male/3 yr	Male/5 yr	Female/5 yr	Female/5 yr	Female/6 mo
CYFIP2 variant	c.260G>T, De novo (p. Arg87Leu)	c.259C>T, De novo (p. Arg87Cys)	c.260G>C, De novo (p. Arg87Pro)	c.259C>T, De novo (p. Arg87Cys)	c.259C>T, De novo (p. Arg87Cys)	c.259C>T, De novo (p. Arg87Cys)	c.260G>T, De novo (p. Arg87Leu)
Phenotype	West syndrome (3)	Ohtahara syndrome (3)	Ohtahara syndrome (5)	West syndrome (6)	West syndrome (3 mo)	West syndrome (3 mo)	Infantile spasm (5), EIEE
Diagnosis, mo	generalized convulsions (<1), epileptic spasms in clusters (3), Tonic	generalized convulsions (<1), epileptic spasms (6), tonic (NA), myoclonus (NA),	complex partial seizures (1.5), epileptic spasms and myoclonus (5),	GTCs (5)	focal to bilateral tonic-clonic (1), epileptic spasms in clusters (3)	focal to bilateral tonic-clonic (1), epileptic spasms in clusters (3)	GTCs (5), epileptic spasms nonclusters, partial seizures, tonic (6)
PMD*	+/+	+/+	+/+	+/+	+/+	+/+	+/+
Dysmorphology	AE and SLG	AE and SLG	- and PC	Tented mouth	-	-	-
Microcephaly	-	+	+ and PC	+	+	+	+
Hypotonia	-	+	+	+	Hypertonia	Hypertonia	+
Status at report	Bed-ridden	Bed-ridden	Bed-ridden	Bed-ridden	Bed-ridden	Bed-ridden	Profound delay
EEG, mo Initial; follow-up	HH; Slow spike-and-wave (23)	SB; HH (5) and multifocal epileptic (8 years)	SB; HH (5)	SB and HH; N.A.	Spike and spike-slow waves (3); HH, SB (5)	Spike and spike-slow waves (3); HH, SB (5)	Spike- and/or spark-slow wave (5); HH (7 and 8)
Brain MRI	Diffuse cerebral atrophy (may be caused by ACTH)	Mild cerebral and cerebellar atrophy, a split CC	PC, a dysmorphic CC, mild expanded SS, B00, VE	Normal	Increased extra-axial spaces	Increased extra-axial spaces	Increased extra-axial spaces
Treatments	PP, ACTH, VPA, potassium bromide partially effective; refractory	Multiple AEDs (no details), ACTH, corpus callosotomy; refractory	Vigabatrin, VPA, topiramate; partially effective	Sodium valproate; seizure-free since age 3 yr	LEV, CBZ, TPM, ACTH, VGB, VPA; partially effective	LEV, CBZ, TPM, ACTH, VGB, VPA; partially effective	Levetiracetam, VIB6, topiramate, MP, VPA, vigabatrin; seizure-free until today

ACTH = adrenocorticotropic hormone, AE = arched eyebrows, B00 = bilateral operculum opening, BW = birth weight, CC = corpus callosum, GA = gestational age, GTCs = generalized tonic-clonic seizures, HH = hyposarhythmia, MP = methylprednisolone, PC = plagiocephaly, PMD = psychomotor development, PP = pyridoxal phosphate, SB = suppression-burst, SLG = swelling of lip and gingiva, SS = subarachnoid spaces, VE = ventricular enlargement, VPA = valproic acid.
 * Before/after seizure onset.

or spark-slow waves across the whole brain, and this later developed to hypsarrhythmia (Fig. 1B and C). Blood/urinary metabolic screening results were normal.

2.3. Molecular genetic analysis

Trio-based WES and copy number variation detection was performed on the proband and her parents by Chigene Biotech (Beijing, China).

No pathogenic copy number variants were found. WES revealed a de novo heterozygous mutation c.260G>T, p.Arg87Leu (NM_001037333) in Exon 4 of the *CYFIP2* gene; this was confirmed by Sanger sequencing (Fig. 2A and B). Her parents were clinically healthy and did not carry this *CYFIP2* mutation. The child's diagnosis was revised as EIEE 65 at the age of 8 months.

The amino acid residue encoded by the *CYFIP2* gene was evolutionarily conserved among representative species predicted using the GERP++ (Fig. 2C), phyloP and phastCons tools. The missense mutation was predicted to be a deleterious variant by multiple in silico tools including Polyphen2_HDIV, Polyphen2_HVAR, MutationTaster, M-CAP and REVEL.

2.4. Literature review

Five cases of *CYFIP2* mutations were identified by searching the PubMed database (<https://www.ncbi.nlm.nih.gov/pubmed/>).^[4,5] All the patients were born to non-consanguineous parents and had no similar familial history. They developed early-onset epilepsy and global intellectual disability. Their clinical features are summarized in Table 1.

3. Discussion

CYFIP1 and *CYFIP2*, which are members of the highly conserved *CYFIP* gene family, are components of the WASP-family verprolin-homologous protein (WAVE) regulatory complex, which is involved in actin dynamics. WAVE family proteins play central roles in actin remodeling, axon elongation, dendritic spine morphogenesis, and synaptic plasticity.^[4,6]

In a *Drosophila* model, mutants with only 1 *CYFIP* family homolog (dCYFIP) have shorter synapse terminals and a higher number of buds than their wild-type counterparts, which is indicative of impaired synapse growth. These abnormalities suggest that dCYFIP may regulate endocytosis and/or vesicle recycling by inhibiting F-actin assembly.^[6,7]

In recent years, accumulating evidence suggests that *CYFIP* is closely associated with human cognition and/or behavior. Ten de novo *CYFIP2* variants (6 intronic, 1 synonymous, and 3 missense) have been reported in patients with autism and developmental disorders.^[8,9] Moreover, novel *CYFIP2* variants have recently been identified in 5 patients with severe early-onset epileptic encephalopathies by 2 different research groups^[4,5] and these pathogenic mutations have been functionally verified.^[4] These *CYFIP2* mutations were designated EIEE 65 (OMIM database). Interestingly, the only 2 heterozygous *CYFIP2* mutations found in these 5 cases affect the same amino acid residue (p.Arg87Cys, p.Arg87Pro, and p.Arg87Leu).^[4,5] Bioinformatics analysis and other research indicates that these *CYFIP2* variants cause gain-of-function of the WAVE signaling pathway, resulting in dysregulation of the neurological function in the brain. These mutations are thought to contribute to the

pathogenesis of this form of EE, although further studies are needed to fully elucidate the biological functions of the wild-type and mutant *CYFIP2*. In addition to the *CYFIP2* mutation, the patient in Case 5 also had a de novo lysine methyltransferase 2D (*KMT2D*) mutation. Mutations in the *KMT2D* gene are reported mainly in association with Kabuki syndrome; thus, it is possible that the refractory epilepsy and developmental delay observed in this patient were associated with the *KMT2D* mutation.^[5]

All 5 of the cases of early-onset EE reported with *CYFIP2* mutations were primarily diagnosed as Ohtahara syndrome (n = 2) or West syndrome (n = 3). Furthermore, all of the patients had profound developmental delay and refractory epilepsy, with recurrent GCTSs and/or focal seizures and epileptic spasms. Patients 1, 2, 3, and 5 had hypsarrhythmia and/or suppression-burst pattern on EEG, and responded poorly to multiple anti-epileptic drugs. Patient 4 had been seizure-free with valproic acid treatment for nearly 3 years and had only frequent generalized epileptiform activities on EEG during the whole course of the disease.

In our patient, psychomotor developmental delay was observed as early as 2 months of age. No improvement was observed after rehabilitation, and the delay was exacerbated after the onset of epilepsy. Aged 5 months, she developed GCTSs with spike- and/or spark-slow wave on EEG. The frequency of the seizures was not reduced by levetiracetam, topiramate, and vitamin B6 treatment and approximately 1 month later, her condition evolved to West syndrome. The patient responded poorly to treatment with corticosteroids and valproic acid. Fortunately, her seizures were almost completely controlled by vigabatrin, and her psychomotor response also improved. The patient did not have any seizures during the 1-month follow-up and developmental delay steadily improved, although hypsarrhythmia was still present on her EEG (Fig. 1C). Here, we have reported the case of the earliest genetic diagnosis (8 months) of EIEE 65, with the patient responding well to treatment. The therapeutic effects and prognosis of early diagnosis and intervention in such cases will be clarified by long-term follow-up.

In conclusion, we have reported a case of EIEE 65 with a de novo heterozygous mutation of the *CYFIP2* gene, resulting in the (p.Arg87Leu) variant, which may be a pathogenic hot spot in *CYFIP2* associated with EIEE 65. Based on the case presented here and the previously reported cases, this newly identified form of EIEE appears to be refractory to multiple treatments. Thus, the results of this study expand the clinical spectrum of *CYFIP2* mutations and provide the first evidence indicating that vigabatrin is effective for controlling seizures and improving outcome in this patient population.

Acknowledgments

We thank the patient and her family members who participated in this study. We also thank Prof. Fei Yin and Nan Pang (Xiangya Hospital, Central South University, Changsha, China) provided more details of the case 5.

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