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 hypsarrhythmia 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, 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, hypsarrhythmia, 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...
component in the etiology of EIEEs. In recent years, numerous causative genes (>80) have been identified in epilepsy patients\(^1\) 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).\(^4\)

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.
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 3010g, 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 (35mg/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 7kg (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 (6.5mg/kg.d) and vitamin B6 to the patient’s treatment regimen, but her
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) 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).

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.
| Basic features | Case 1 | Case 2 | Case 3 | Case 4 | Case 5 | Our case |
|----------------|--------|--------|--------|--------|--------|----------|
| Ethnic origin  | Japanese | Japanese | Israeli | Malaysian | Chinese | Chinese |
| GA, wk         | 40      | 30     | 38     | 38     | 38     | 41       |
| BW, g          | 3748, +1.9SD | 1938, −2.7SD | 3564, +1.4SD | 2670, −0.7 SD | 3400, −0.92 SD | 3010, −0.71 SD |
| Length, cm     | 51.8, +1.3SD | 41.0, −3.6SD | NA     | 49, −1.3SD | NA     | 50.5, +0.7 SD |
| HC, cm         | 34.5, +0.8SD | 31.8, −1.0SD | 34.5, +1.1 SD | 32, −3 SD | 33, −1.15 SD |
| Sex/age        | Male5 yr | Male11 yr | Male3 yr | Male6 yr | Female5 yr | Female6 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) |
| Phenotype      | Generalized convulsions | Focal seizures (3) | Complex partial seizures | GTCSs (5) | Focal to bilateral tonic-clonic seizures (1), epileptic spasms in clusters (3) |
| Diagnosis, mo  | West syndrome (3) | Ohtahara syndrome (3) | Ohtahara syndrome (5) | West syndrome (6) | West syndrome (3 mo) |
| Type of seizures (onset mo) | (<1), epileptic spasms in clusters (9), Tonic | Focal seizures (3), epilepsy spasms (6), tonic (NA), myoclonus (NA), myoclonus (9), | Complex partial seizures (1.5), epileptic spasms (6), tonic (NA), myoclonus (9), | GTCSs, focal tonic-clonic seizures, partial seizures, tonic-clonic seizures |
| PMD*           | +/+     | +/+     | +/+     | +/+     | +/+     |
| Dysmorphology  | AE and SLG | AE and SLG | AE and SLG | Tented mouth | AE and SLG |
| Hypotonia       | −       | + and PC | +        | +        | +        |
| Status at report | Bed-ridden | Bed-ridden | Bed-ridden | Bed-ridden | Bed-ridden |
| 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. | SB and HH (5) |
| 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, B0C, VE | Normal | Increased extra-axial spaces |
| Treatments     | PP, ACTH, VPA, potassium bromide | 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 |

ACTH = adrenocorticotropic hormone, AE = arched eyebrows, BOO = bilateral operculum opening, BW = birth weight, CC = corpus callosum, GA = gestational age, GTCSs = generalized tonic-clonic seizures, HH = hypsarrhythmia, MP = methylprednisolone, PC = plagiocphaly, 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 variant numbers 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.[5,7]

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 to be closely associated with autism and developmental delay in patients with EIEE[5] with recurrent GCTSs and 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 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.

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References
[1] Scheffer IE, Berkovic S, Capovilla G, et al. ILAE classification of the epilepsies: position paper of the ILAE commission for classification and terminology. Epilepsia 2017;58:512–21.
[2] Alsahli S, Al-Twaijri W, Al Mutairi F. Confirming the pathogenicity of NECAP1 in early onset epileptic encephalopathy. Epilepsia Open 2018;3:524–7.
[3] Myers KA, Johnstone DL, Dyment DA. Epilepsy genetics: current knowledge, applications, and future directions. Clin Genet 2019;95:95–111.
[4] Nakashima M, Kato M, Aoto K, et al. De novo hotspot variants in CYFIP2 cause early-onset epileptic encephalopathy. Ann Neurol 2018;83:794–806.
[5] Peng J, Wang Y, He F, et al. Novel West syndrome candidate genes in a Chinese cohort. CNS Neurosci Ther 2018;24:1196–206.
[6] Abekhoukh S, Bardoni B. CYFIP family proteins between autism and intellectual disability: links with Fragile X syndrome. Front Cell Neurosci 2014;8:81.
[7] Zhao L, Wang D, Wang Q, et al. Drosophila cyfp regulates synaptic development and endocytosis by suppressing filamentous actin assembly. PLoS Genet 2013;9:e1003450.
[8] CYuen RK, Merico D, Bookman M, et al. Whole genome sequencing resource identifies 18 new candidate genes for autism spectrum disorder. Nat Neurosci 2017;20:602–11.
[9] Deciphering Developmental Disorders Study. Prevalence and architecture of de novo mutations in developmental disorders. Nature 2017;542:433–8.