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ORIGINAL ARTICLE

Clinical and genetic analysis of infants with pontocerebellar hypoplasia type 6 caused by *RARS2* variations

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Abstract

Objective: Defects in *RARS2* cause cerebellopontine hypoplasia type 6 (pontocerebellar hypoplasia type 6, PCH6, OMIM: #611523), a rare autosomal recessive inherited mitochondrial disease. Here, we report two male patients and their respective family histories.

Methods: We describe the clinical presentation and magnetic resonance imaging (MRI) findings of these patients. Whole-exome sequencing was used to identify the genetic mutations.

Results: One patient showed hypoglycemia, high lactic acid levels (fluctuating from 6.7 to 14.1 mmol/L), and frequent seizures after birth, with progressive atrophy of the cerebrum, cerebellum, and pons. The other patient presented with early infantile developmental and epileptic encephalopathies (EIDEEs) with an initial developmental delay followed by infantile epileptic spasm syndrome (IESS) at 5 months old, with no imaging changes. Whole-exome sequencing identified compound heterozygous *RARS2* variants c.25A>G (p.I9V) with c.1261C>T (p.Q421*) and c.1A>G (p.M1V) with c.122A>G (p.D41G) in these two patients. Of these loci, c.1261C>T and c.122A>G have not been previously reported.

Significance: Our findings have expanded the *RARS2* gene variant spectrum and present EIDEEs and IESS as phenotypes which deepened the association between PCH6 and *RARS2*.

Plain Language Summary: Defects in RARS2 cause cerebellopontine hypoplasia type 6, a rare autosomal recessive inherited mitochondrial disease. Two patients with RARS2 variants were reported in this article. One patient showed hypoglycemia, high lactic acid levels, and frequent seizures after birth, with progressive atrophy of the cerebrum, cerebellum, and Page 3 of 21 Epilepsia OpenFor Review Only pons. The other patient presented with an initial developmental delay followed by refractory epilepsy at 5 months old, with no imaging changes. Our findings deepened the association between PCH6 and RARS2.

KEYWORDS
EIDEEs, IESS, pontocerebellar hypoplasia type 6, *RARS2*

1 | INTRODUCTION

Barth described pontocerebellar hypoplasia (PCH) as a heterogeneous group of neurodegenerative disorders with severe hypoplasia or atrophy of the cerebellum and pons.[1] Several subtypes have been reported. Pontocerebellar hypoplasia type 6 (PCH6) is an autosomal recessive form of mitochondrial encephalopathy characterized by intractable epilepsy, microcephaly, feeding difficulties, lactic acidosis, and developmental delays and presents with cerebral hypoplasia and progressive cerebellar and pontine atrophy on with magnetic resonance imaging (MRI). PCH6 is caused by a homozygous or compound heterozygous mutations in *RARS2*, which encodes the mitochondrial arginyl-tRNA synthetase. To date, more than 30 PCH6 patients with *RARS2* variants have been reported.

Here, we report two Chinese patients diagnosed with PCH6 and review their medical records, including clinical presentation, MRI and electroencephalography (EEG) features, and follow-up information. Using trio-whole exome sequencing (WES), we identified two compound heterozygous *RARS2* variants (c.25A>G and c.1261C>T) and (c.1A>G and c.122A>G). Our findings broaden our knowledge of the PCH6 and *RARS2* genes.

Key Points

- Two novel variants broaden the spectrum of RARS2 mutations.
- Pontocerebellar Hypoplasia Type 6 can present IESS with no imaging changes.
- One typical PCH6 patient was reported.
- RARS2 needed to be related to early infantile developmental and epileptic encephalopathies (EIDEEs).

2 | METHODS

2.1 | Patients

Two unrelated consanguineous patients were diagnosed with PCH6 based on clinical manifestations, MRI, EEG, and *RARS2* mutation analyses. Informed consent was obtained from the parents of the patients, and ethical statements were obtained from the Ethics Committee of the Third Affiliated Hospital of Zhengzhou University (2021-062-01). All the procedures were performed in accordance with the ethical standards of the Declaration of Helsinki.

2.2 | Genetic analysis

Whole exome sequencing was performed after informed consent was obtained from the patients and their parents. Peripheral blood was collected from both patients and their parents. DNA was extracted using a blood genome extraction kit (Kang Wei Century), and the DNA fragments were enriched after quality control and library establishment to construct a whole exome library. High-throughput sequencing was performed using the Illumina NovaSeq 6000 series sequencer. The sequencing process was completed by Beijing Chigene Translational Medical Research Centre Co., Ltd. Raw sequencing reads were processed using FASTP (https://github.com/OpenGene/fastp) for adapter removal and low-quality read filtering. High-quality sequencing data were generated on the Ensemble GRCh37/hg19 reference genome using Burrows-Wheeler Aligner (BWA, https://github.com/lh3/bwa). GATK (http://www.broadinstitute.org/gatk/) was used for base quality score recalibration, single nucleotide polymorphisms (SNPs), and indel calling. A professional bioinformatics analysis pipeline was used to determine the pathogenicity of the variations, and regular population distribution frequency queries (dbSNP, 1000 Genomes, EXAC, ESP, etc.) were performed for the variants using variant annotation software. A series of bioinformatics tools, such as PROVEAN, SIFT, Polyphen2, MutationTaster, Reval, and CADD, were used to predict the possible deleterious effects of variations. Variants were classified according to the American College of Medical Genetics (ACMG) guidelines. MEGA7.0 software was used to analyze the conservation of amino acid variation sites, and PyMOL software was used to model protein structure and analyze the effect of the variation on spatial protein structure.

3 | RESULTS

3.1 | Case 1

The child was delivered naturally at 41 weeks and was the first pregnancy of a nonconsanguineous couple. His Apgar score was 9 points at 1 min (deducted based on skin color) and 10 points at 5 min. His birth weight was low (2400 g; −3 SD ~ −2 SD), but his birth length and head circumference were normal.

The patient was admitted to the neonatal department of our hospital because he was hypoglycemic and small for his gestational age after birth. After admission, he was found to have a high lactic acid level, fluctuating from 6.7 to 14.1 mmol/L. On the third day of hospitalization, the patient developed frequent seizures (mainly focal clonic seizures) and status epilepticus. Midazolam was continuously pumped and phenobarbital was intermittently administered; however, his seizures remained frequent. During this period, cocktail therapy (Vitamin B2, Vitamin B1, l-carnitine, coenzyme Q10, vitamin C, Vitamin E) was administered to actively treat the primary disease, and suspended red blood cells were infused to correct anemia. At 14 days of age, the patient was successively treated with a ketogenic diet, levetiracetam, and topiramate. The treatments were ineffective. He did not have status epilepticus; however, focal clonic seizures were still observed 1–2 times a day which showed on ambulatory EEG. Blood tandem mass spectrometry results suggested that his alanine, methionine, and proline levels were increased.

MRI revealed a small cerebellum 20 days after birth (Figure 1A). When the patient was 7.5 months old, MRI revealed that the white matter in the bilateral cerebral hemispheres had decreased and aggregated, the corpus callosum and brain stem were thin, the cerebrum and cerebellum were atrophied (Figure 1B), and the electroencephalogram (EEG) of the patient was abnormal, with a lack of sleep spindles, a larger number of low-voltage pattern bursts, and multiple isolated or clustered epileptic spasms, partly followed by tonic seizures (Figure 1C,D), and severe developmental delays.

Genetic testing was performed, and compound heterozygous mutations of the RARS2 gene were identified from the paternal c.25A>G (p.I9V) and maternal c.1261C>T (p.Q421*) alleles (Figure 2A).

He still had frequent seizures and severe developmental delay at 7.5 months old. Physical examination showed microcephaly (36 cm; <−3 SD), dystrophia (Weight: 6.5 kg; −3 SD ~ −2 SD), a large auricle, small jaw, high palatal arch, visible neck reverse tension posture, and increased muscle tension of the limbs. He did not interact with the outside world and showed no obvious response to the stimuli. Griffiths neurodevelopmental assessment showed that his development corresponds to 1 month of age. The patient was followed up for 8 months after discharge. He continued treatment with levetiracetam and topiramate, received an irregular ketogenic diet and cocktail therapy, and experienced frequent seizures and poor weight gain. The patient generally exhibited delayed cognitive and motor development.

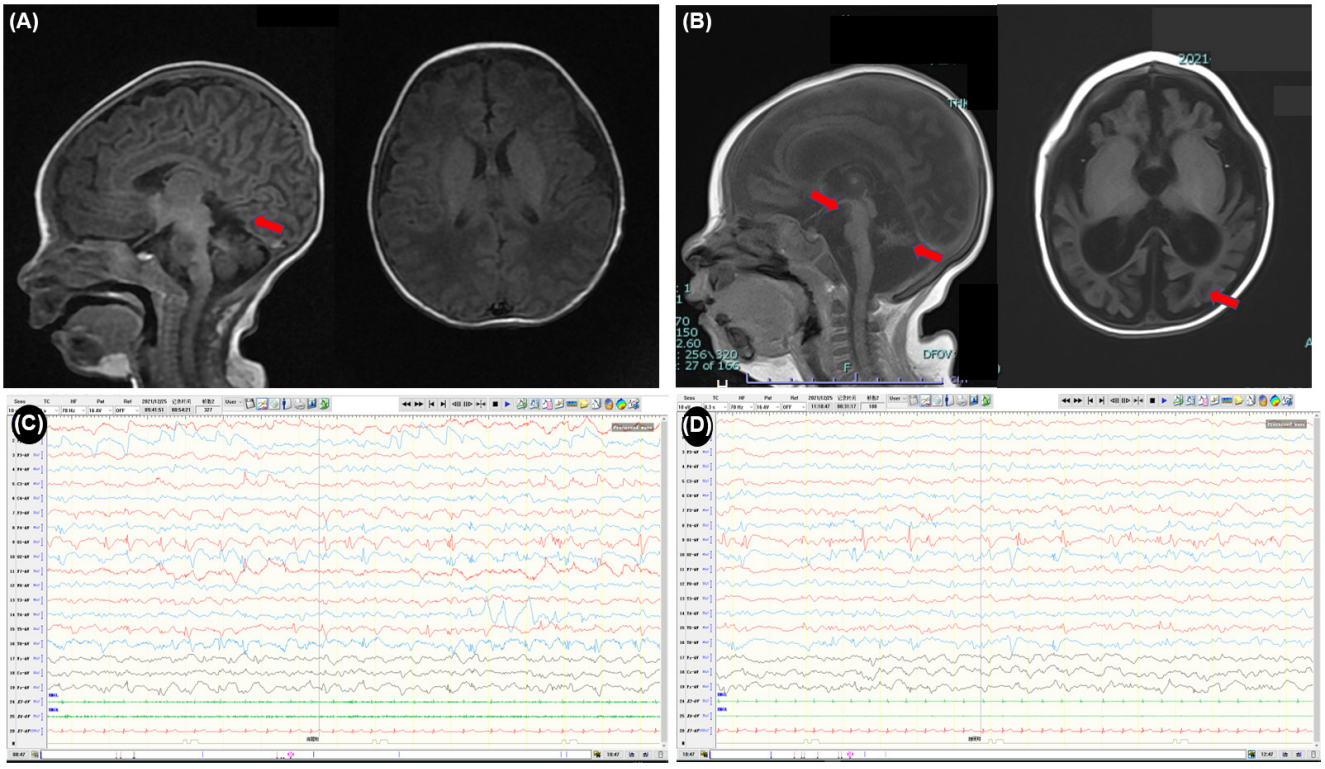

FIGURE 1 Cranial MRI (Sagittal T1-weighted and coronal T2-weighted imaging) and EEG features of Patient 1. (A) MRI showing small cerebellar volume at the age of 20 days (red arrow). (B) MRI showing bilateral cerebellar atrophy and thin brainstem, deepened cerebral sulci in the cerebral hemispheres bilaterally, bilateral cerebral atrophy, reduced and tangled cerebral white matter in the cerebral hemispheres bilaterally, thin corpus callosum, dilated ventricles, and a small amount of subdural fluid in the right frontal region at the age of 7.5 months (red arrow). (C and D) EEG showing a lack of sleep spindles, a larger number of low-voltage pattern bursts, and multiple isolated or clustered epileptic spasms, partly followed by tonic seizures at the age of 7.5 months.

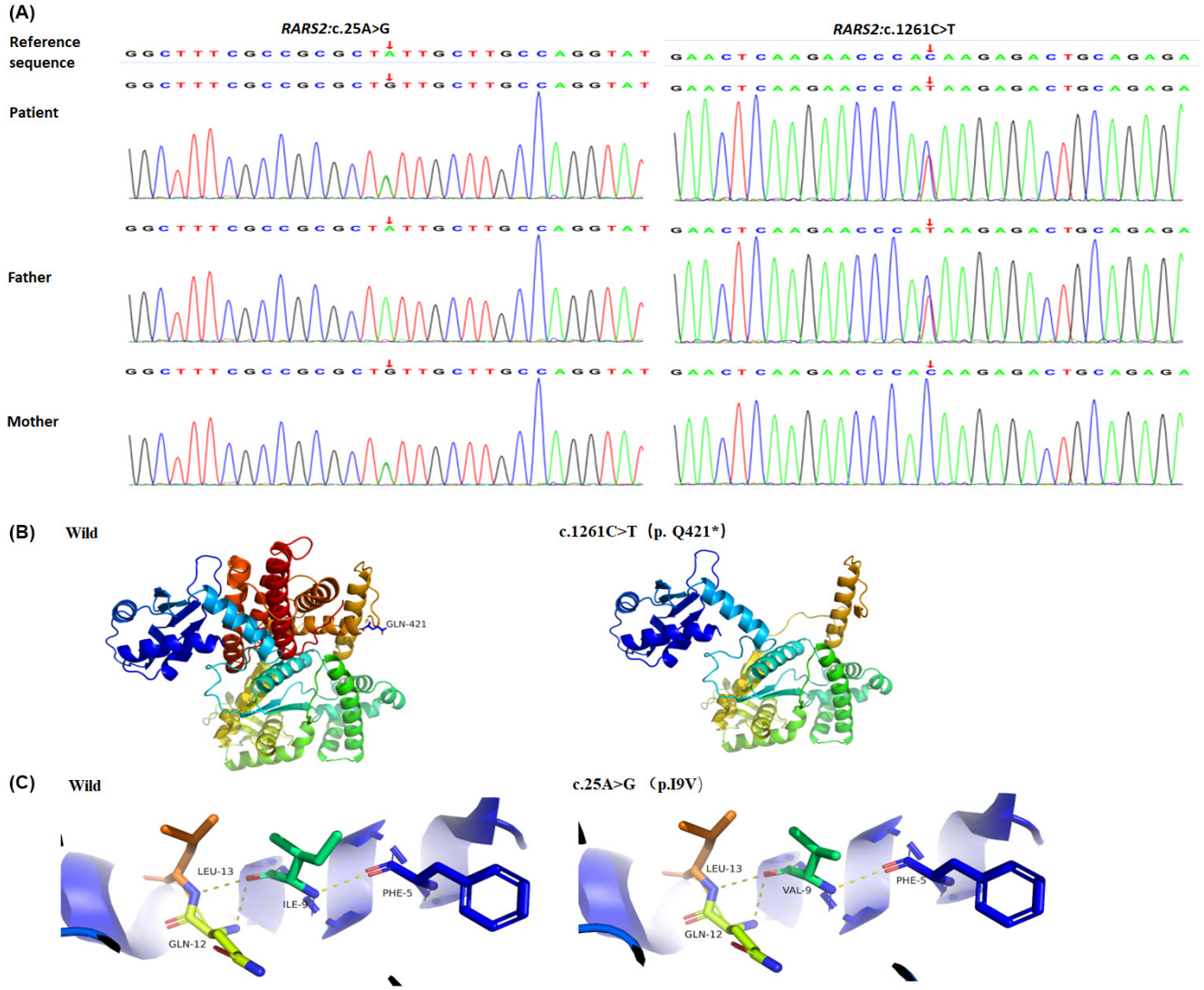

FIGURE 2 RARS2 variation of Patient 1 and three-dimensional protein structure. (A) Genetic analysis showed RARS2 variations including the paternal c.25A>G (P.I9V) and maternal c.1261C>T (p.Q421*) mutations. (B) The nonsense mutation, c.1261C>T, resulted in a shortened protein of 158 amino acids. (C) Mutation c.25A>G resulted in a change in the amino acid side chain, which may have an effect on the protein structure and thus the function.

3.2 | Case 2

Patient 2 was a second pregnancy in a nonconsanguineous parent with full-term spontaneous delivery. There was no remarkable family history.

At the age of 5 months, he appeared to have intermittent seizures that initially manifested as bilateral upper limb tremors, which lasted for several minutes and gradually evolved from a nodding hug-like action into a series of attacks, 4–5 times/series, 3–4 series/day. He was admitted to our hospital with infantile epileptic spasm syndrome (IESS) and developmental delay. The patient received intravenous vitamin B6 injections, sodium valproate oral solution (initial dose 20 mg/kg/day, maintenance dose 30 mg/kg/day; total 4 months), topiramate tablets (initial dose 1.5 mg/kg/d, maintenance dose 8 mg/kg/day; total 10 months), and one course of steroid pulse therapy (5 months old, methylprednisolone 20 mg/kg/day for 5 days). Although oral prednisone was administered for 3 months, he still experienced epileptic spasms. After vigabatrin (starting dose 15 mg/kg/d, maintenance dose 100 mg/kg/d) was administered, the seizures were controlled; however, the EEG remained abnormal.

Tandem mass spectrometry and lactate tests revealed no abnormalities. MRI revealed a wide subarachnoid space in the frontotemporal region bilaterally at the age of 5 months, and a left temporal pole cyst at the age of 20 months (Figure 3A,B). EEG revealed a high degree of arrhythmia when the spasms appeared. After treatment at the age of 15 months, EEG showed a large number

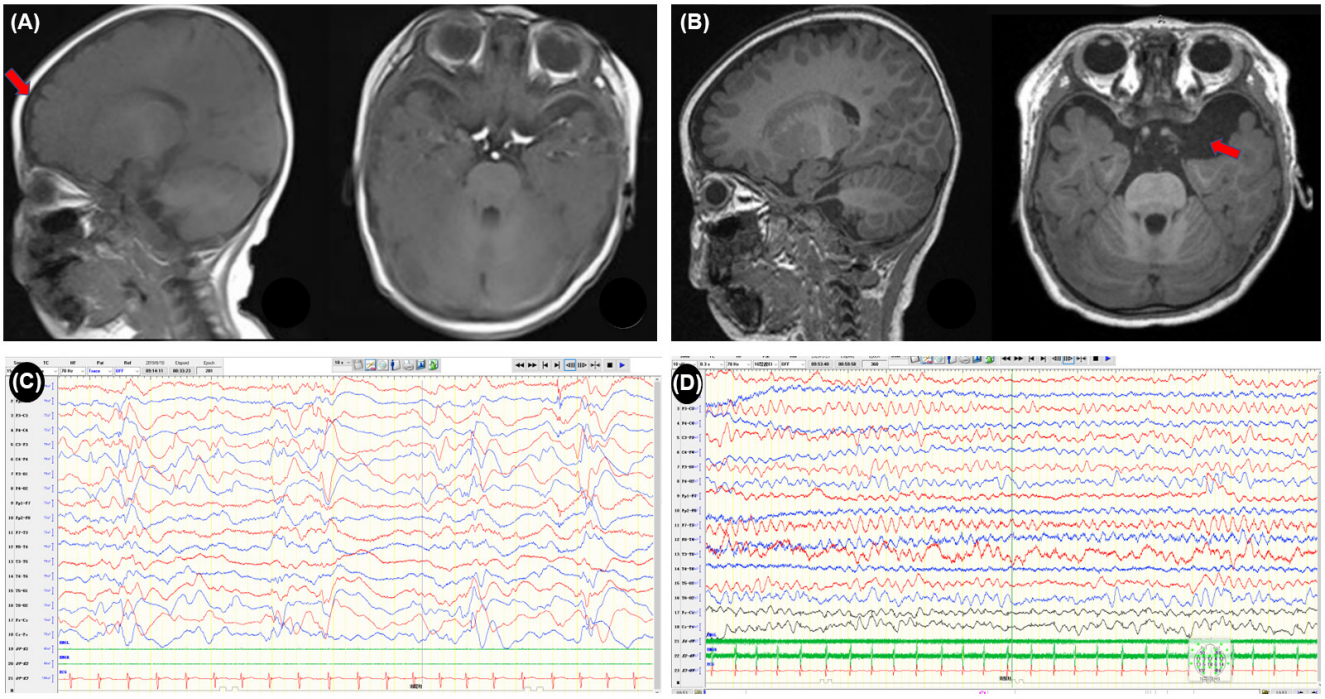

FIGURE 3 Cranial MRI (Sagittal T1-weighted and coronal T2-weighted imaging) and EEG features of Patient 2. (A) MRI showing wide subarachnoid space in the frontotemporal region bilaterally at the age of 5 months (red arrow). (B) MRI showing left temporal pole cyst at the age of 20 months (red arrow). (C) EEG displayed highly dysrhythmic patterns at 5 months old. (D) EEG showed a large number of multifocal slow and spike-and-slow waves issued in each conductor during sleep, with the left posterior head dominant.

of multifocal slow and spike-and-slow waves issued by each conductor during sleep, with the left posterior head being dominant (Figure 3C,D). The patient had inherited c.1A>G (p.M1V) *RARS2* variations from his father and c.122A>G (p.D41G) from his mother (Figure 4A). Based on clinical features and genetic testing results, the patient was diagnosed with PCH6.

The patient is now 2 years old and has no seizures, but still has a psychomotor delay. He can roll over, but cannot sit alone and can only pronounce "mama" and "baba." Griffiths neurodevelopmental assessment showed that his development corresponds to 5 months of age.

3.3 | *RARS2* gene mutation

Whole-exome sequencing confirmed that Patient 1 harbored paternal c.25A>G (p.I9V) and maternal c.1261C>T (p.Q421*) mutations in *RARS2*, which were both predicted to be pathogenic mutations. The c.1261C>T (p.Q421*) mutation was inherited from his father, leading to premature termination of translation. The variant pathogenicity was evaluated according to the medical genetics criteria and guidelines of the American Academy of Genetics and Genomics (ACMG). This variation has not been reported in the dbSNP, EXAC, or 1000 Genomes databases. The mutation was highly conserved and predicted to be a pathogenic mutation using several software packages. The missense mutation c.25A>G (p.I9V) was inherited from the mother, similarly highly conserved, located in constrained coding regions, and had been previously reported in a child with severe neonatal onset of epileptic encephalopathy, with a clinical presentation similar to that of this case.[2] Further mapping using PyMOL predicted that both mutations might affect protein function (Figure 2B,C).

Patient 2 had compound heterozygous variants c.1A>G (p.M1V) and c.122A>G (p.D41G) in the *RARS2* gene, which were inherited from his father and mother, respectively. The compound heterozygous variants were confirmed by Sanger sequencing. Two mutations were not present in the dbSNP, EXAC, or 1000 Genomes databases and had a low MAF frequency (<0.0005). SIFT, Polyphen2, and Mutation Taster analyses showed that the mutation could lead to structural changes in the protein, and conservation analysis suggested that the variant was highly conserved across multiple species (Figure 4B). Variant c.1A>G, classified as pathogenic, was reported in one patient who rapidly progressed to death and showed cardiomyopathy, hydrops fetalis, and severe multisystem respiratory chain deficiency.[2] The c.122A>G mutation has not yet been reported and is classified as a variant of unknown significance (VUS).

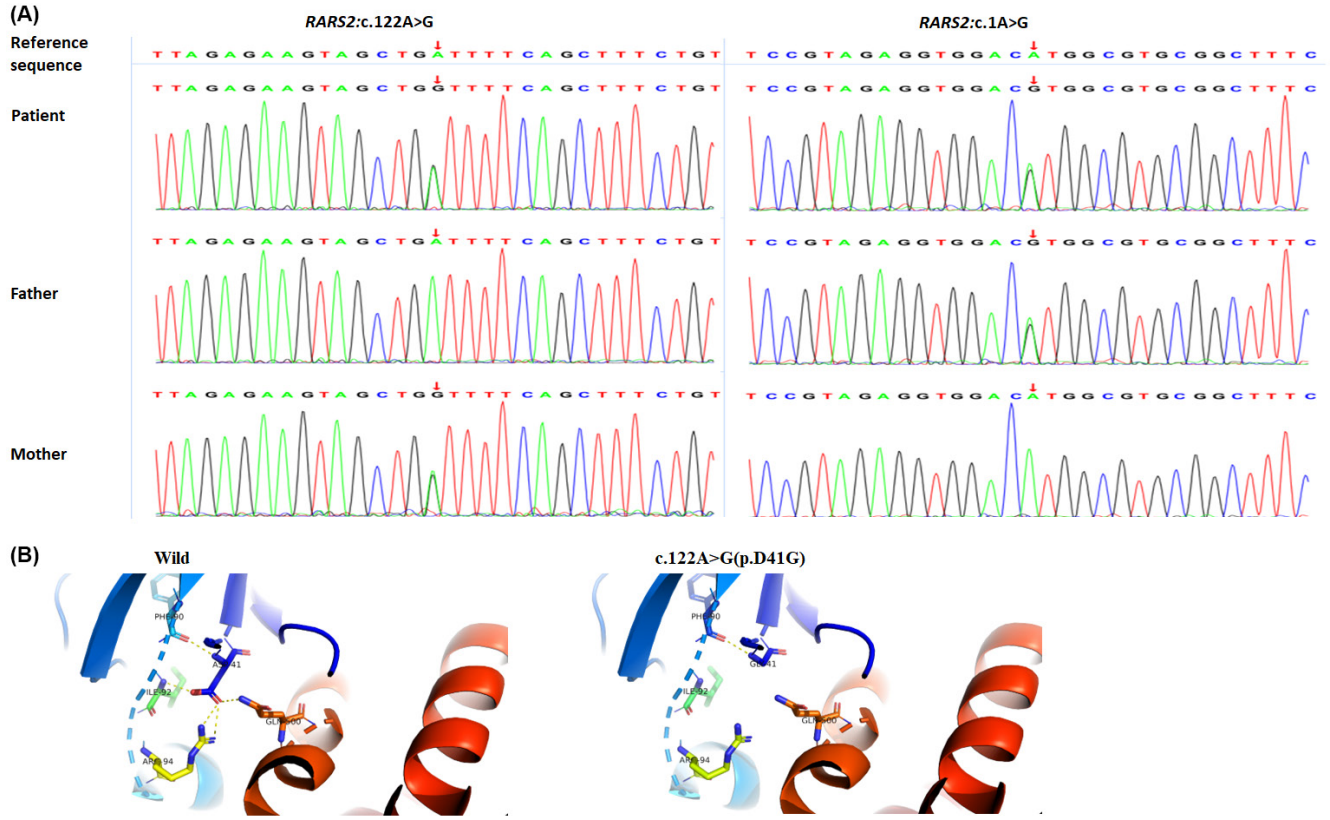

FIGURE 4 Heterozygous variants of *RARS2* gene in Patient 2 and three-dimensional protein structure. (A) Genetic analysis detected c.1A>G (p.M1V) inherited from his father and c.122A>G (p.D41G) inherited from his mother. (B) Mutation c.122A>G (p.D41G) formed hydrogen bonds with PHE at position 90 and no hydrogen bonding interaction with position 92, 94, or 500 and thus affect protein function.

4 | DISCUSSION

The *RARS2* gene comprises 20 exons and encodes a mitochondrial aminoacyl transfer-RNA synthetase that mediates mitochondrial protein synthesis by binding amino acid arginine to its cognate tRNA. It plays a vital role in maintaining cellular integrity.[3] *RARS2* gene variations have been reported in PCH6, which is a classification type of PCH syndrome characterized by severe early onset encephalopathy, lactic acidosis, abnormal respiratory chain, backward psychomotor function, and progressive pontocerebellar atrophy.[3] Some children show progressive encephalopathy with edema, hypsarrhythmia, and optic atrophy (PEHO) syndrome.[4] Typical MRI findings include cerebellar hypoplasia with progressive cerebellar atrophy and pons and white matter atrophy.[5] Since it was first reported by Edvardson et al.[6] in 2007, around 30 cases have been reported,[7] few of which were from China.[8,9]

Patients with *RARS2* mutations are more likely to develop symptoms within 3 months of birth. Initial symptoms include seizures, hypotonia, difficulty feeding, dyspnea, and hypoglycemia. Seizure types include focal clonic, generalized tonic–clonic, myoclonic, and epileptic spasms, many of which are refractory to treatment. Edema, progressive encephalopathy, and optic atrophy have also been reported in PCH6 patients. Depending on the number and activity of the remaining aminoacylases, the clinical symptoms vary and can progress rapidly to death after onset. Patient 1 suffered from the typical clinical manifestations of PCH6, including hypoglycemia, multiple increased lactic acid levels, microcephaly, intractable epilepsy, and cognitive and motor delay after birth. A sibship with infantile epileptic spasm syndrome and *RARS2* mutations was first reported by Ngoh A firstly[3]; Patient 2 presented with a similar clinical performance, including epileptic spasms at 5 months old.

In addition to typical brain atrophy, some patients were regular at the time of onset and exhibited gradually progressive brain atrophy. Nishri et al.[10] reported a patient with PCH6 without pontocerebellar hypoplasia or diffuse brain atrophy, which was more prominent in the frontal regions with cerebellar atrophy. Two siblings with typical clinical symptoms without the characteristic neuroradiological abnormalities of PCH6 (such as vermis, cerebellar hypoplasia, and progressive pontocerebellar atrophy) have been reported. Therefore, as the nomenclature of PCH6 is misleading,[11] we propose to

replace it with *RARS2* mutations. In this study, Patient 1 had typical MRI brain atrophy, whereas Patient 2 had a normal brain MRI scan. Based on his clinical manifestations and genetic results, we diagnosed him with PCH6. Our report broadens the phenotypic spectrum of *RARS2* mutations; however, this patient requires regular MRI checks.

The reported variants of *RARS2* include missense, frameshift, and splicing-site mutations. Compound heterozygous variants c.25A>G and c.1261C>T in *RARS2* were detected in Patient 1, both of which were pathogenic. c.25A>G was reported in a patient who presented with severe acidosis, intractable epilepsy, and microcephaly after birth.[12] They developed severe mental delay and progressive cerebellar and cerebral atrophy, similar to Patient 1 in this study. The nonsense variant c.1261C>T, resulting in the protein being shortened by 158 amino acids and seriously affecting its structure, was considered pathogenic. Molecular investigations of *RARS2* revealed c.1A>G and c.122A>G mutations in Patient 2. Variant c.1A>G has been reported in PCH6 patients with cardiomyopathy, hydrops fetalis, and severe multisystem respiratory chain deficiency, who died at 1 day and 14 days of age.[2] This mutation was predicted to abolish the initiator methionine; therefore, it was identified as a disease-causing variant. Here, we report a missense mutation, c.122A>G, for the first time. After analysis, we considered that it might affect protein structure, thereby affecting protein function.

Although most reported cases with *RARS2* gene mutations are classified as PCH6, the clinical phenotype of *RARS2* gene mutations is more extensive than that of PCH6. Therefore, EIDEEs were used to describe the *RARS2* mutation.[10] Some authors even consider that the diagnosis of PCH6 cannot be used to generalize all phenotypes of *RARS2* mutations, and suggest that "early onset mitochondrial encephalopathy" or "*RARS2* mutations" should be used instead.[11,13] In two recent domestic studies, Xu et al.[8] reported early myoclonic encephalopathy, suggesting that the *RARS2* gene be included in the detection of early myoclonic encephalopathy. Jiang et al. suggested that PCH6, a *RARS2* mutation, was no longer suitable for clinical diagnosis.[9] Namavar et al. have shown that pontocerebellar hypoplasia type 1 and elevated cerebrospinal fluid lactate levels may be caused by *RARS2* mutations.[14] The phenotype of Patient 2 was different from that of the typical PCH6 phenotype. Additional cases should be reported to deepen our understanding of PCH6- and *RARS2*-related diseases.

Here, we reported two patients diagnosed with clinically distinct PCH6 from unrelated families; one patient presented with IESS and did not undergo cranial MRI. These two novel variants broadened the spectrum of *RARS2* mutations; however, further functional studies are required. In conclusion, our findings expand the *RARS2* gene variant spectrum and deepen our understanding of PCH6 and *RARS2*. Genetic testing is recommended for patients with refractory epilepsy and developmental delays.

AUTHOR CONTRIBUTIONS

Shichao Zhao participated in drafting the manuscript and in collecting, analyzing, and interpreting the data. Ruofei Lian, Liang Jin, Mengchun Li, Lijun Wang, and Qiliang Guo participated in collecting and interpreting the data. Tianming Jia, Falin Xu, and Kaixian Du participated in the experimental design and confirmed the authenticity of the raw data. Yan Dong made substantial contributions to the conception, designed of this study, revised the manuscript, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

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

DATA AVAILABILITY STATEMENT

The original contributions presented in this study are included in the article; further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethical standards of the Third Affiliated Hospital of Zhengzhou University's Institutional Research Committee (Zhengzhou, China; no. 2021-062-01). Written informed consent from the participants' legal guardian/next of kin was waived by the committee. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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REFERENCES

1. Barth PG. Pontocerebellar hypoplasias. An overview of a group of inherited neurodegenerative disorders with fetal onset. Brain Dev. 1993;15:411–22. https://doi.org/10.1016/0387-7604(93)90080-r
2. Lax NZ, Alston CL, Schon K, Park SM, Krishnakumar D, He L, et al. Neuropathologic characterization of pontocerebellar hypoplasia type 6 associated with cardiomyopathy and hydrops Fetalis and severe multisystem respiratory chain deficiency due to novel RARS2 mutations. J Neuropathol Exp Neurol. 2015;74:688–703. https://doi.org/10.1097/nen.0000000000000209
3. Ngoh A, Bras J, Guerreiro R, Meyer E, McTague A, Dawson E, et al. RARS2 mutations in a sibship with infantile spasms. Epilepsia. 2016;57:e97–e102. https://doi.org/10.1111/epi.13358
4. Rankin J, Brown R, Dobyns WB, Harington J, Patel J, Quinn M, et al. Pontocerebellar hypoplasia type 6: a British case with PEHO-like features. Am J Med Genet A. 2010;152A:2079–84. https://doi.org/10.1002/ajmg.a.33531
5. Rudnik-Schoneborn S, Barth PG, Zerres K. Pontocerebellar hypoplasia. Am J Med Genet C Semin Med Genet. 2014;166C:173–83. https://doi.org/10.1002/ajmg.c.31403
6. Edvardson S, Shaag A, Kolesnikova O, Gomori JM, Tarassov I, Einbinder T, et al. Deleterious mutation in the mitochondrial arginyl-transfer RNA synthetase gene is associated with pontocerebellar hypoplasia. Am J Hum Genet. 2007;81:857–62. https://doi.org/10.1086/521227
7. Nevanlinna V, Konovalova S, Ceulemans B, Muona M, Laari A, Hilander T, et al. A patient with pontocerebellar hypoplasia type 6: novel RARS2 mutations, comparison to previously published patients and clinical distinction from PEHO syndrome. Eur J Med Genet. 2020;63:103766. https://doi.org/10.1016/j.ejmg.2019.103766
8. Xu Y, Wu BB, Wang HJ, Zhou SZ, Cheng GQ, Zhou YF. A term neonate with early myoclonic encephalopathy caused by RARS2 gene variants: a case report. Transl Pediatr. 2020;9:707–12. https://doi.org/10.21037/tp-20-110
9. Jiang HF, Deng J, Fang F, Li H, Wang XH, Dai LF. Early onset epileptic encephalopathy caused by mitochondrial arginyl-tRNA synthetase gene deficiency: report of two cases and literature review. Zhonghua Er Ke Za Zhi. 2020;58:893–9. https://doi.org/10.3760/cma.j.cn112140-20200716-00729
10. Nishri D, Goldberg-Stern H, Noyman I, Blumkin L, Kivity S, Saitsu H, et al. RARS2 mutations cause early onset epileptic encephalopathy without ponto-cerebellar hypoplasia. Eur J Paediatr Neurol. 2016;20:412–7. https://doi.org/10.1016/j.ejpn.2016.02.012
11. van Dijk T, van Ruissen F, Jaeger B, Rodenburg RJ, Tamminga S, van Maarle M, et al. RARS2 mutations: is pontocerebellar hypoplasia type 6 a mitochondrial encephalopathy? JIMD Rep. 2017;33:87–92. https://doi.org/10.1007/8904_2016_584
12. Cassandrini D, Cilio MR, Bianchi M, Doimo M, Balestri M, Tessa A, et al. Pontocerebellar hypoplasia type 6 caused by mutations in RARS2: definition of the clinical spectrum and molecular findings in five patients. J Inherit Metab Dis. 2013;36:43–53. https://doi.org/10.1007/s10545-012-9487-9
13. Lühl S, Bode H, Schlötzer W, Bartsakoulia M, Horvath R, Abicht A, et al. Novel homozygous RARS2 mutation in two siblings without pontocerebellar hypoplasia – further expansion of the phenotypic spectrum. Orphanet J Rare Dis. 2016;11:140. https://doi.org/10.1186/s13023-016-0525-9
14. Namavar Y, Barth PG, Kasher PR, van Ruissen F, Brockmann K, Bernert G, et al. Clinical, neuroradiological and genetic findings in pontocerebellar hypoplasia. Brain. 2011;134:143–56. https://doi.org/10.1093/brain/awq287

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