Two Chinese siblings of combined oxidative phosphorylation deficiency 14 caused by compound heterozygous variants in FARS2

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Abstract

Background: As a rare mitochondrial disease, combined oxidative phosphorylation deficiency 14 (COXPD14) is caused by biallelic variants in the phenylalanyl-tRNA synthetase 2, mitochondrial gene (FARS2) with clinical features of developmental delay, an elevated lactate level, early-onset encephalopathy, liver failure, and hypotonia. The objectives of this study were to analyze the clinical and molecular features of two Chinese siblings affected with COXPD14, and to review relevant literature.

Methods: Mutation screening was performed by whole exome sequencing (WES) in combination with Sanger sequencing validation to identify the disease-causing variants of the two patients.

Results: The two siblings presented with severe clinical features and both progressed aggressively and failed to survive after treatment abandonment. We identified two compound heterozygous FARS2 variants c.925G>A p.Gly309Ser and c.943G>C p.Gly315Arg in this proband, which were inherited from the unaffected father and mother, respectively. In addition, Sanger sequencing confirmed that the elder affected sister carried the same compound heterozygous variants. The c.925G>A p.Gly309Ser variant is known and commonly reported in COXPD14 patients, while c.943G>C p.Gly315Arg is a novel one. Neither of the variants was found in 100 Chinese healthy controls. Both variants were classified as “deleterious” and were located in the highly conserved regions of the protein. The above results suggested that the two variants were likely causative in this COXPD14-affected pedigree.

Conclusions: Our study expands the mutation spectrum of FARS2 and highlights the importance of genetic testing in the diagnosis of diseases with a wide variety of phenotypes, especially in the differential diagnosis of diseases.

Keywords: Combined oxidative phosphorylation deficiency 14, FARS2, Compound heterozygous variants, Whole exome sequencing

Introduction

As the consequences of defects in nuclear DNA (nDNA) or mitochondrial DNA (mtDNA), mitochondrial diseases are a highly heterogeneous group of inherited metabolic disorders characterized by a broad phenotypic spectrum, differential disease course, varying age of onset, and diverse consequences[1, 2]. They are due to impairments in mitochondrial respiratory chain oxidative phosphorylation (OXPHOS) function, which in turn have effects on multiple systems of the human body [3, 4]. The estimated prevalence of adult mitochondrial diseases given rise to...
causative variants of mitochondrial and nuclear genomes is 1 per 4300 individuals [5].

Combined oxidative phosphorylation deficiency (COXPD) is a severe disorder belonging to mitochondrial diseases with an autosomal recessive inheritance pattern. To date, COXPD has been divided into 51 types (COXPD1–COXPD51) based on different disease-causing genes in Online Mendelian Inheritance in Man (OMIM). Among these, combined oxidative phosphorylation deficiency 14 (COXPD14, MIM: 614946) is caused by biallelic variants in phenylalanyl-tRNA synthetase 2, mitochondrial (FARS2) (MIM: 611592). The clinical features encompass developmental delay, an elevated lactate level, early-onset epileptic encephalopathy, microcephaly, thin corpus callosum, brain atrophy, liver disease, and axial hypotonia [6, 7].

FARS2 is a nuclear gene that maps to chromosome 6p25.1 and spans over 510 kb with seven exons, six of which are coding [6, 8]. The encoded protein mitochondrial phenylalanyl-tRNA synthetase (mtPheRS) is composed of 451 amino acids and could transfer phenylalanine to its cognate mitochondrial tRNA, which is essential for the translation of mitochondrial DNA-encoded proteins [9]. The four major domains of mtPheRS consist of an N-terminal domain (residues 37–83), a catalytic aminoacylation domain (residues 84–325), a linker domain (residues 326–358), and a C-terminal anticodon-binding domain (residues 359–451) [6, 10]. Review of the literature demonstrates that homozygous or compound heterozygous FARS2 pathogenic variants are responsible for three distinct clinical phenotypes, including early-onset epileptic encephalopathy, spastic paraplegia, and the latest report of juvenile-onset refractory epilepsy [11].

In this study, we described the clinical presentations of two Chinese siblings affected by COXPD14 and found two compound heterozygous variants in FARS2 by utilizing whole exome sequencing (WES) combined with Sanger sequencing validation, which further expand the molecular and phenotypic spectrum of COXPD14 caused by genetic defects in FARS2. Additionally, we retrospectively reviewed and summarized the clinical and molecular data of the reported patients with FARS2 variants.

Materials and methods

Patients

The male proband (Patient II: 2, Fig. 1) and his families were recruited and examined in Zibo Maternal and Child Health Hospital. This study was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University and Zibo Maternal and Child Health Hospital. Peripheral blood samples were collected from the proband, his elder sister and parents, and 100 healthy controls of Chinese Han origin after informed consent was obtained.

WES

Following genomic DNA extraction, qualified DNA sample was randomly sheared to generate 180–280 bp DNA fragments, which were selected for the preparation of DNA libraries. The currently identified 6259 genetic phenotypes by OMIM were detected, and a total of 1839 genes associated with clinical phenotypes of the proband were focused. The libraries were hybridized with biotin-labeled probes in liquid phase; then the streptavidin magnetic beads were used to bind with biotin-containing target fragments for the capture of the exons of these genes. The paired-end reads of 150 bp sequencing was performed on an Illumina NextSeq 500 sequencer (Illumina, San Diego, CA, USA) after enrichment and quality inspection of the libraries.

Sequencing data analysis

Low-quality reads and raw reads with adaptor were removed. The Burrows-Wheeler Aligner (BWA) software was used to align the clean reads to the human reference genome (hg19). Subsequently, the alignment results were sorted using SAMtools and duplicated reads were marked with the Picard software for the statistical analyses of sequencing depth and coverage. On the completion of these, single nucleotide polymorphisms (SNPs) and insertions and deletions (InDels) variation sites were detected and annotated. Filter SNPs and InDels with minimum allele frequency (MAF)>0.02. Pathogenicity assessment of the nonsynonymous variants was performed by in silico analysis.
Sanger sequencing validation
The likely pathogenic variants detected in this proband were confirmed among all available family members and 100 Chinese healthy subjects by Sanger sequencing. Primers involving the mutation sites were designed by Primer Premier version 5.0 software. The primer sequences for PCR amplification were as follows: forward 5′-GAGGCGATCCGGAATATGG-3′ and reverse 5′-CCTTGATCGATCCTTGACAGCC-3′. Sequencing primer sequence was as follows: reverse 5′-CCTGTGATCCCTTGACAGCC-3′. After Polymerase Chain Reaction (PCR) and agarose gel electrophoresis, the gel-recovered PCR products were analyzed on an ABI 3730 analyzer (Applied Biosystem). The sequencing data were aligned to the reference sequence on the National Center Biotechnology Information (NCBI) website for the determination of the mutation sites.

Results
Clinical manifestations
The male proband (Patient II: 2, Fig. 1) was born to healthy and nonconsanguineous Chinese parents. No family history of genetic disorders was found in both maternal and paternal families. He was born at a gestational age of 37 weeks by cesarean section with a birth weight of 3500 g and head circumference of 33 cm. He postnatally displayed poor mental response and was initially admitted to the hospital for shortness of breath, foaming at the mouth accompanied by moaning without obvious inducement for 1 h after birth. No improvement was observed after airway clearing stimulation. Physical examination revealed clear consciousness, poor mental reaction, less ruddy skin, shortness of breath, dry and moist rales, thick breath sounds in both lungs, no pathologic murmurs in the valve areas, thick skin with many folds, popliteal angle< 90°, hypotonia of the limbs, and diminished neonatal reflexes, such as sucking, swallowing, and hugging.

Blood routine showed significantly elevated white blood cells. Biochemical results indicated that the levels of total protein, albumin and blood glucose were decreased, while the levels of total bilirubin, indirect bilirubin, lactate dehydrogenase, creatine kinase, and α-hydroxybutyrate dehydrogenase were increased. Blood gas analysis revealed that the lactate level was 3.2 mmol/L (normal range: 0.5–1.6 mmol/L). Chest X-ray showed increased bilateral lung texture in the lower lung fields. Cranial color ultrasound revealed abnormal right subependymal echo and subependymal bleeding was considered. Cranio cerebrovascular magnetic resonance imaging (MRI) indicated that large patchy of slightly long T1 and slightly long T2 signal shadows was seen in the brain parenchyma of bilateral cerebral hemispheres with unclear boundary. The cortex became thin. There were fissure-like long T1 and long T2 signal shadows in the bilateral external capsule with clear boundary. Patchy and striped DWI high signal shadows were observed in the white matter around the posterior horn of bilateral lateral ventricles. The bilateral ventricles were slightly enlarged, cerebral sulci, and fissure were widened and deepened, subarachnoid spaces were widened in frontal, parietal and temporal regions, and midline structure was in the middle (Fig. 2A-D).

The proband was diagnosed with neonatal pneumonia, neonatal encephalopathy, intracranial hemorrhage, neonatal hypoglycemia, and neonatal hyperbilirubinemia and was given symptomatic treatment and supportive care during hospitalization. He underwent WES for gene mutation screening to determine the clinical diagnosis. Four days after admission, his parents refused further treatment and asked to be discharged. Unfortunately, the proband died at 37 days of age.

His 32-day-old elder sister (Patient II: 1, Fig. 1), born at full term by cesarean section with a history of intrauterine asphyxia, was hospitalized for no weight gain for more than 1 month. She was given mixed feeding with feeding amount of 30 mL/2 h accompanied by nonprojectile vomiting. Physical examination showed pale skin, flat anterior fontanelle, normal auscultation of heart and lungs, soft abdomen and hypotonia of the limbs.

Laboratory examinations showed lower value of hemoglobin and increased levels of creatine kinase isoenzyme MB, γ-glutamyl transpeptidase, total bile acid, lactate dehydrogenase, α-hydroxybutyrate dehydroge nase, blood ammonia, aspartate aminotransferase, and alanine aminotransferase, supporting the diagnosis of liver dysfunction. Cranio cerebrovascular MRI showed unclear boundary between cortex and the medulla of the bilateral cerebral hemispheres; there was large patchy of long T1 and long T2 signal shadows in the bilateral frontal, parietal, and occipital lobes. In addition, the ventricles were enlarged, cerebral sulci and fissure were widened, and midline structure was in the middle (Fig. 2E–G). After severe hypoxic-ischemic encephalopathy and liver dysfunction were made at initial diagnosis, the patient was recommended to complete various examinations and be hospitalized. However, the parents refused to continue treatment, and the patient died at 34 days.

Molecular genetic analyses
In our study, one previously reported missense variant c.925G>A p.Gly309Ser [12] (Fig. 3A) and one novel missense variant c.943G>C p.Gly315Arg (Fig. 3E) in the FARS2 gene (NM_006567.5) were detected in this proband by WES. Sanger sequencing revealed that the unaffected father (I: 1, Fig. 1) was a heterozygous carrier...
for p.Gly309Ser (Fig. 3B), while the asymptomatic mother (I: 2, Fig. 1) carried the heterozygous p.Gly315Arg variant (Fig. 3F). In addition, genetic analysis confirmed that his elder sister carried identical compound heterozygous variants of FARS2 (Fig. 3C, G). Neither of the variants was found in 100 healthy unrelated controls of Chinese Han origin and has been reported in the gnomAD database. American College of Medical Genetics and Genomics (ACMG) guidelines [13] indicated that p.Gly309Ser was “likely pathogenic” and p.Gly315Arg was “uncertain” with evidence of PM2_Supporting + PM3_Strong + PP3_Moderate and PM2_Supporting + PP3_Moderate, respectively. In addition, p.Gly309Ser and p.Gly315Arg were classified as “Likely pathogenic” and “Uncertain significance,” respectively, using InterVar software based on ACMG guidelines [14]. In silico prediction revealed that the two FARS2 variants were deleterious (Table 1). Based on these findings, the identified compound heterozygous FARS2 variants were considered to be causative for disease phenotypes of this proband and his female sibling. They both were finally diagnosed with COXPD14 based on the clinical and laboratory findings as well as molecular genetic data.

**Sequence conservation analysis of mtPheRS protein**

The mtPheRS protein sequences of various species were obtained from the NCBI website. Sequence alignment of the mtPheRS protein sequences from these species was done by using the DNAMAN software, which revealed that Gly309 and Gly315 are both highly conserved residues (Fig. 4).

**Discussion**

In the present study, we reported two siblings with autosomal recessive COXPD14 and identified two damaging compound heterozygous FARS2 variants c.925G>A p.Gly309Ser and c.943G>C p.Gly315Arg by WES. The p.Gly309Ser variant has been reported previously, while p.Gly315Arg is a novel one. The patients who presented with hypoxic-ischemic encephalopathy, hypotonia of the limbs, and abnormal cranioencephalic MRI findings were severely affected. In addition, a high lactate level was
Fig. 3  Partial DNA sequence chromatograms of FARS2. The red arrows represent the location of the variants c.925G>A and c.943G>C. A Heterozygous variant c.925G>A identified in this proband. B Heterozygous variant c.925G>A identified in the father. C Heterozygous variant c.925G>A identified in the elder sister. D Normal DNA sequence of the mother. E Heterozygous variant c.943G>C identified in this proband. F Heterozygous variant c.943G>C identified in the mother. G Heterozygous variant c.943G>C identified in the elder sister. H Normal DNA sequence of the father.

Table 1  Pathogenicity analysis of the two FARS2 variants c.925G>A and c.943G>C

| Gene   | Nucleotide change | Amino acid change | Status          | Prediction tools          |
|--------|-------------------|-------------------|-----------------|---------------------------|
|        |                   |                   |                 | REVEL         | PolyPhen-2 | MutationTaster | SIFT | ClinPred    | PROVEAN       |
| FARS2  | c.925G>A          | p.G309S           | Known (0.885)   | Probably damaging (1)    | Disease causing (1) | 0.99848169 Deleterious (−5.027) |
| FARS2  | c.943G>C          | p.G315R           | Novel (0.921)   | Probably damaging (1)    | Disease causing (1) | 0.99980956 Deleterious (−7.680) |

Fig. 4  Protein sequence conservation analysis among different species. The red rectangles represent Gly309 and Gly315.
observed in this proband and the sister suffered from feeding difficulties, developmental delay, and abnormal liver function. However, both of them died more than 30 days after birth before they experienced seizures. Our study highlights that genetic testing is of great significance in the diagnosis of diseases with a wide variety of phenotypes, especially in the differential diagnosis of diseases, and can be served as a gold standard for diseases that cannot be made definitive diagnosis clinically.

COXPD14 is an unusual autosomal recessive disorder caused by defects in FARS2 and is characterized by early-onset encephalopathy with or without epilepsy, developmental delay, high levels of lactate, and short or long lifetimes [15]. The first FARS2 variant p.Tyr144Cys was reported by Shamseldin et al. in 2012, who described a 2-year-old female with clinical presentations of seizures, muscle weakness, developmental delay, and an increased lactate level [16]. Since then, a growing number of FARS2 deleterious variants have been identified in three different disorders: COXPD14 with early-onset encephalopathy with or without epilepsy, COXPD14 with juvenile-onset epilepsy, and spastic paraplegia type 77 (SPG77, MIM: 617046) [9]. The disease type of the patient should be determined based on the main clinical findings combined with age at onset because patients can develop identical symptoms at different ages and phenotypes among these diseases overlap, such as increased lactate level, developmental delay, and seizures.

The clinical and genetic features of our patients and the previously reported cases with FARS2 variants affected by early-onset encephalopathy, juvenile-onset epilepsy, and spastic paraplegia were summarized in Tables 2, 3, and 4, respectively, after literature review. Approximately 44 subjects with disease-causing variants in FARS2 have been reported, including 27 cases of early-onset encephalopathy with or without epilepsy, 3 cases of juvenile-onset epilepsy, and 14 cases of spastic paraplegia.

In our patients and the reported cases in the literature with early-onset encephalopathy, although the majority of patients died within 2 years of age, we found that some could survive beyond the age of 2, with the oldest surviving at age 16. Barcia et al. have described three early-onset patients with or without epileptic seizures, all of whom had longer lifespans and they were still alive at the time of the study, which highlighted that not all patients with early-onset form experience seizures or have poor outcomes [15]. To date, COXPD14 with juvenile-onset epilepsy was found in only three individuals. The first case exhibited developmental delay and died of likely pneumonia and urinary tract infections at the age of 15 years [17], whereas another patient reported by Chen et al. in 2109 had normal development but failed to survive due to pulmonary infection at age 20 years [9]. For patients suffered from spastic paraplegia, they showed the lowest disease severity and had a good prognosis in comparison with the other two disorders, with many being able to survive into adulthood. Interestingly, all cases with spastic paraplegia in the literature were alive at the time of report.

A total of 32 different FARS2 variants were discovered so far, including 23 missense variants, 1 three-base-pair deletion, 1 eight-base-pair duplication, 1 nonsense variant, and 6 microdeletions. The microdeletions were associated with three FARS2-related disorders and all were combined with missense variants to form compound heterozygous states. Similarly, patients with deletion, duplication, and nonsense variants also had a heterozygous missense variant. Both of the mutation sites identified in this study were evolutionarily conserved and located in the catalytic domain, which resulted in severe phenotypes of the two siblings. Nevertheless, the causal correlation between the protein domains affected by the mutation sites and disease phenotypes or severity remains unclear. Patients suffering from COXPD14 with early-onset encephalopathy had variants in the catalytic domain, linker region, and anticodon-binding domain, while COXPD14 with juvenile-onset epilepsy and SPG77 were associated with variants in the catalytic and anticodon-binding domains.

Of all the variants, only three have been presented in homozygous states. Both homozygous p.Gly309Ser and p.Tyr144Cys could give rise to early-onset epileptic encephalopathy, while patients with homozygous p.Asp142Tyr manifested spastic paraplegia. The FARS2 variant p.Gly309Ser was revealed as a Korean founder pathogenic variant and p.Tyr144Cys was a founder variant in Arabs despite the fact that it was initially identified in a Saudi female patient. All Arab patients presented by Almannai et al. carried this variant, 11 out of the 12 subjects were homozygotes and only one patient were compound heterozygous for p.Tyr144Cys and p.Val177Asp [6]. Different variants in the same locus may be responsible for distinct phenotypes. For example, the p.Arg419His variant is linked to early-onset encephalopathy without seizures [15], whereas p.Arg419Cys was only reported in cases with spastic paraplegia [6, 8]. The same variant could be compound heterozygous with other different variants. The variant p.Val197Met could be in combination with 1 exon 2 microdeletion or one p.Phe402Ser variant, respectively. In addition, for our patients and the four Korean patients described by Cho et al.[12], because they all harbored identical variant p.Gly309Ser, both presented with similar clinical features, such as abnormal brain MRI, elevated lactate level, hypotonia, developmental delay, and liver dysfunction. However, disease severity between the two groups could not be compared.
Table 2 The clinical and genetic features of our patients and the previously reported cases with FARS2 mutations affected by early-onset encephalopathy

| References          | Subject | Ethnicity | Consanguinity | Gender | Seizures and age of onset | Brain MRI                                                                 | Other clinical phenotypes                                      | Death of age | FARS2 variants                          |
|---------------------|---------|-----------|---------------|--------|---------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------|--------------|-----------------------------------------|
| This study          | 1       | Chinese   | No            | M      | No                        | Long T1 and long T2 signal shadows in the brain parenchyma of bilateral cerebral hemispheres, cortical thinning, long T1 and long T2 signal shadows in the bilateral external capsule, DWI high signal shadows in the white matter around the posterior horn of bilateral lateral ventricles, enlargement of the ventricles, widened and deepened cerebral sulci and fissure, widened subarachnoid spaces in frontal, parietal, and temporal regions | Hypoxic-ischemic encephalopathy, hypotonia of the limbs, a high lactate level | 37 days      | pG309S/p.G315R (het)                    |
|                     | 2       | Chinese   | No            | F      | No                        | Unclear corticomedullary demarcation of the bilateral cerebral hemispheres, long T1 and long T2 signal shadows in the bilateral frontal, parietal and occipital lobes, enlargement of the ventricles, widened cerebral sulci and fissure | Hypoxic-ischemic encephalopathy, hypotonia of the limbs, feeding difficulties, developmental delay and abnormal liver function | 34 days      | pG309S/p.G315R (het)                    |
| Shamseldin et al. [16] | 3       | Saudi     | Yes           | F      | Seizures, myoclonus, NA   | Similar to MRI findings of Leigh syndrome                                  | Muscle weakness, developmental delay, lactic acidosis           | 22 months    | pY144C (hom)                           |
| Elo et al. [18]     | 4       | Finnish   | No            | F      | Myoclonic jerks, 2 days   | Severe central and cortical atrophy with slight bilateral signal increase in the putamina | Elevated lactate, microcephaly, narrowed, and atrophic gyri      | 8 months     | pI329T/p.D391V (het)                    |
|                     | 5       | Finnish   | No            | F      | Seizures, 4 days          | NA                                                                         | Elevated lactate                                               | 21 months    | pI329T/p.D391V (het)                    |
| References          | Subject | Ethnicity | Consanguinity | Gender | Seizures and age of onset | Brain MRI | Other clinical phenotypes | Death of age | FARS2 variants                      |
|---------------------|---------|-----------|---------------|--------|---------------------------|-----------|--------------------------|-------------|------------------------------------|
| Almalki et al. [10] | 6       | White British | No           | M      | Infantile spams, 6 months | Symmetrical subcortical white matter lesions with thinning of the anterior and genu of the corpus callosum | Developmental delay, small, round, anteriorly rotated ears, and a broad nasal root | Alive at 30 months of age | p.D325Y/an 88 kb microdeletion (het) |
| Cho et al. [12]     | 7       | Korean    | No            | M      | Generalized tonic-clonic seizures, 3 months | A diffusely atrophic brain at 3 months; Progression of atrophic changes and myelination delay at 6 months | Hypotonia, delayed motor development, spastic four extremities, and increased deep tendon reflexes | Alive at 3 years of age | p.G309S (hom)                        |
|                     | 8       | Korean    | No            | F      | Myoclonic movement starting from the right hand and being generalized to the entire body, 4 months | A thin corpus callosum and generalized brain atrophy | NA | Alive at 17 months of age | p.G309S (hom)                        |
|                     | 9       | Korean    | No            | M      | Infantile spasms, 4 months | Mild brain atrophy | Abnormal liver function, an elevated lactate level | 8 months | p.G309S (hom)                        |
|                     | 10      | Korean    | No            | F      | Generalized tonic-clonic seizures, 3 months | Mild brain atrophy | Abnormal liver function, an elevated lactate level | 4 months | p.G309S (hom)                        |
| Raviglione et al. [7]| 11      | Romanian | No            | M      | Infantile spasms, 3 months | Microcephaly, enlargement of frontal subarachnoid spaces, and lateral ventricles due to a reduction in volume of the cerebral white matter, slight hyper-intensity of hemispheric white matter on T2-weighted images, thin corpus callosum, thinning of the cortical rim | Psychomotor delay, microcephaly, widely spaced eyes, large ears, bilateral divergent strabismus with visual impairment, and bilateral horizontal nystagmus, axial hypotonia and mild distal hypertonia | Alive at 3 years of age | p.R386G/a 134 kb microdeletion (het) |
| Almannai et al. [6] | 12      | Arab      | Yes           | F      | Seizures, NA | Brain atrophy, thin corpus callosum | Developmental delay, microcephaly, liver disease, elevated lactate | 23 months | p.Y144C (hom)                        |
| References | Subject | Ethnicity | Consanguinity | Gender | Seizures and age of onset | Brain MRI | Other clinical phenotypes | Death of age | FARS2 variants |
|------------|---------|-----------|---------------|--------|---------------------------|-----------|---------------------------|-------------|----------------|
| 13         | Arab    | Yes       | F             | Seizures, NA | Brain atrophy              | Developmental delay, microcephaly, liver disease, elevated lactate | 3 months | p.Y144C (hom) |
| 14         | Arab    | Yes       | M             | Seizures, 1 month | Brain atrophy, thin corpus callosum | Developmental delay, microcephaly, liver disease, elevated lactate | Alive at 2 years of age | p.Y144C (hom) |
| 15         | Arab    | NA        | F             | Seizures, 2 months | NA | Developmental delay, elevated lactate | NA | p.Y144C (hom) |
| 16         | Arab    | No        | F             | Seizures, 1 month | Brain atrophy, thin corpus callosum | Developmental delay, microcephaly, liver disease, elevated lactate | Alive at 1 year of age | p.Y144C (hom) |
| 17         | Arab    | Yes       | M             | Seizures, 1 month | Thin corpus callosum | Developmental delay, microcephaly, liver disease, elevated lactate | 3 months | p.Y144C (hom) |
| 18         | Arab    | No        | F             | Seizures, 1 month | Brain atrophy, thin corpus callosum | Developmental delay, microcephaly, liver disease, elevated lactate | Alive at 13 months of age | p.Y144C (hom) |
| 19         | Arab    | Yes       | F             | Seizures, 5 months | Brain atrophy, thin corpus callosum | Developmental delay, microcephaly, liver disease, elevated lactate | 2 years | p.Y144C (hom) |
| 20         | Arab    | Yes       | F             | Seizures, 1 month | Brain atrophy, thin corpus callosum | Developmental delay, microcephaly, liver disease, elevated lactate | Alive at 4.5 months of age | p.Y144C (hom) |
| 21         | Arab    | Yes       | F             | Seizures, 20 days | Brain atrophy, thin corpus callosum | Developmental delay, microcephaly, liver disease, elevated lactate | 4 months | p.Y144C (hom) |
| 22         | Arab    | Yes       | F             | Seizures, 25 days | Brain atrophy, thin corpus callosum | Developmental delay, microcephaly, liver disease, elevated lactate | 3.5 months | p.Y144C (hom) |
| 23         | Arab    | Yes       | F             | No | NA | Liver disease, elevated lactate | 2 days | p.Y177D/p.Y144C (het) |
| 24         | Spanish | NA        | F             | NA | NA | Elevated lactate | NA | p.G309S/p.R153G (het) |
| References | Subject | Ethnicity | Consanguinity | Gender | Seizures and age of onset | Brain MRI | Other clinical phenotypes | Death of age | FARS2 variants |
|------------|---------|-----------|---------------|--------|--------------------------|-----------|---------------------------|-------------|----------------|
| Barcia et al. [15] | 25 French and Chinese | No | F | No | Mild ventriculomegaly | Axial hypotonia, developmental delay, and spastic tetraparesis | Alive at 8 years of age | p.R419H/p.S426* |
| | 26 French | No | F | Myoclonic focal and generalized seizures, 19 months | Marked ventriculomegaly, enlargement of the subarachnoid spaces due to white matter loss, especially in the Sylvian fissures, abnormal T2 hyperintensities in the lentiform nuclei and dorsal brainstem, cerebellar atrophy | Global hypotonia, psychomotor delay, mild scoliosis, spastic tetraparesis, and severe muscular atrophy predominating on inferior limbs | Alive at 16 years of age | p.R330H/p.L371F (het) |
| | 27 French | No | M | Myoclonic generalized and focal seizures, 1 year | Moderate ventriculomegaly and enlargement of the subarachnoid spaces; Dentate nuclei, brainstem and pallidal T2 hyperintensity | Severe psychomotor delay, global hypotonia and lumbar mild scoliosis | Alive at 5 years of age | p.R330H/p.L371F (het) |

M male, F female, MRI magnetic resonance imaging, het heterozygous, hom homozygous
Table 3  The clinical and genetic features of the previously reported cases with FARS2 mutations affected by juvenile-onset epilepsy

| References       | Subject | Ethnicity | Consanguinity | Gender | Seizures and age of onset                                      | Brain MRI                                                                                                                   | Other clinical phenotypes                          | Death of age | FARS2 variants                      |
|------------------|---------|-----------|----------------|--------|-----------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------|--------------|-------------------------------------|
| Walker et al. [17] | 1       | NA        | No             | F      | A prolonged generalized tonic–clonic convulsion, 8 years        | Extensive areas of abnor-mal T2 hyperintensity in the frontal lobes (right greater than left), anterior cingulate gyrus, left superior frontal gyrus, bilateral temporal lobes, and left cerebellar cortex | Motor and speech delays                               | 15 years     | p.P85A/p.H135D (het)                |
| Hotait et al. [11] | 2       | NA        | No             | F      | Brief focal aware clonic seizures semiologically characterized by twitching of the left side of the face, 16 years | Restricted diffusion in the cortical-subcortical areas of the right frontal lobe, right insula, right thalamus and to lesser extent in the right temporal, both parietal lobes and left frontal lobe | Paresis of left upper extremity                      | Alive at 17 years of age | p.V197M/exon 2 microdeletion (het) |
| Chen et al. [9]   | 3       | NA        | No             | M      | Generalized tonic–clonic convulsions, 12 years                  | Increased wandering lesions involving bilateral frontal, temporal, and parietal lobes, occipital cortex and subcortical | Increased serum lactic acid, pes cavus, mild muscular atrophy and compensatory hypertrophy | 20 years     | p.V197M/p.F402S (het)              |

* M male, F female, MRI magnetic resonance imaging, het heterozygous
| References       | Subject | Ethnicity | Consanguinity | Gender | Seizures and age of onset | Brain MRI | Other clinical phenotypes                                                                 | Death of age | FARS2 variants                        |
|------------------|---------|-----------|----------------|--------|--------------------------|-----------|------------------------------------------------------------------------------------------|--------------|---------------------------------------|
| Yang et al. [19] | 1       | Chinese   | Yes            | F      | No                       | Normal    | Progressive lower limb spasticity, pyramidal weakness with hyperreflexia, extensor plantar responses, and scissors gait | Alive at 41 years of age | p.D142Y (hom)                         |
|                  | 2       | Chinese   | Yes            | M      | No                       | Normal    | Progressive lower limb spasticity, pyramidal weakness with hyperreflexia, extensor plantar responses, and scissors gait | Alive at 30 years of age | p.D142Y (hom)                         |
|                  | 3       | Chinese   | Yes            | F      | No                       | Normal    | Progressive lower limb spasticity, pyramidal weakness with hyperreflexia, extensor plantar responses, and scissors gait | Alive at 26 years of age | p.D142Y (hom)                         |
|                  | 4       | Chinese   | Yes            | F      | No                       | Normal    | Progressive lower limb spasticity, pyramidal weakness with hyperreflexia, extensor plantar responses, and scissors gait | Alive at 23 years of age | p.D142Y (hom)                         |
| Vantoys et al. [20] | 5     | NA        | No             | M      | Convulsive seizures, 19 months | Slight cortical atrophy at 20 months; Bilateral, round, focal T2-hyperintense lesions in the anterior part of the mesencephalon at 17 years | Increased lactate, developmental delay, spastic paraplegia, neurogenic bladder, and sphincter dyssynergia | Alive at 19 years of age | p.A154V/p.P361L (het)                  |
Table 4 (continued)

| References | Subject        | Ethnicity   | Consanguinity | Gender | Seizures and age of onset | Brain MRI                                                                                           | Other clinical phenotypes                                                                 | Death of age                      | FARS2 variants       |
|------------|----------------|-------------|---------------|--------|---------------------------|----------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|-----------------------------------|---------------------|
| 6          | NA             | NA          | NA            | F      | No                        | Symmetrical T2 hyperintensities of the posterior tegmentum at 17 months; More extensive T2 hyperintense lesions at the tegmentum and periaqueductal gray matter at 6 years; Near resolution of the tegmental lesions but new T2 hyperintense lesions bilaterally in the anterior inferior thalamus and signs of cerebellar atrophy at 15 years | Delayed motor development, spastic paraplegia                                            | Alive at 15 years of age                             | p.V174del/p.P361L (het) |
| Vernon et al. [8] | 7             | NA          | NA            | F      | Seizure, 2 months         | Normal                                                                                             | Globally delayed development, mild facial dysmorphism, an elevated lactate level, truncal hypotonia with brisk extremity reflexes throughout, and an intermittent intention tremor | Alive at 5 years of age                     | p.R419C/a 116 kb microdeletion (het) |
| 8          | NA             | NA          | NA            | M      | Seizures, only within 6 weeks after birth | Two small foci of T2/FLAIR hyperintensity involving the periventricular white matter and deep white matter of the right posterior frontal lobe | Delayed development, cerebral palsy, metabolic acidosis, truncal hypotonia, dystarthric speech, and a mild intention tremor | Alive at 13 years of age                  | p.R419C/a 116 kb microdeletion (het) |
| Almannai et al. [6] | 9             | North American | No            | F      | No                        | Brain atrophy                                                                                      | Developmental delay, spastic paraplegia                                                       | Alive at 20 years of age                  | p.H159P/p.R419C (het) |
| 10         | North American | No          | F             | No     | NA                        | Developmental delay, spastic paraplegia                                                            | Alive at 17 years of age                                                                     | p.H159P/p.R419C (het) |
| References          | Subject | Ethnicity                  | Consanguinity | Gender | Seizures and age of onset | Brain MRI                                                                 | Other clinical phenotypes                                                                 | Death of age               | FARS2 variants                      |
|---------------------|---------|---------------------------|---------------|--------|----------------------------|--------------------------------------------------------------------------|------------------------------------------------------------------------------------------|----------------------------|-------------------------------------|
| Sahai et al. [21]   | 11      | Northern European and Ashkenazi Jewish | No            | M      | No                         | Abnormal signal hyperintensities in the bilateral dentate nuclei          | Spasticity in lower extremities                                                            | Alive at 9 years of age       | p.Q216X/p.P136H (het)               |
| Meszarosova et al.  | 12      | Czech Roma                | No            | M      | No                         | Normal                                                                   | Gait impairment, progressive limb spasticity, hyperreflexia, pes cavus        | Alive at 22 years of age       | p.P361L/exons 1–2 microdeletion (het) |
| Forman et al. [23]  | 13      | Irish                     | No            | M      | No                         | Normal                                                                   | Delayed walking, tremor in the upper limbs, dysphonia; Spasticity, weakness, brisk deep tendon reflexes, extensor plantar responses, and clonus in the lower limbs | Alive at 13 years of age        | p.G141E/an 75 kb microdeletion (het) |
|                     | 14      | Irish                     | No            | F      | No                         | Normal                                                                   | Delayed walking, tremor in the upper limbs; Spasticity, weakness, brisk deep tendon reflexes, extensor plantar responses, and clonus in the lower limbs | Alive at 7 years of age          | p.G141E/an 75 kb microdeletion (het) |

M male, F female, MRI magnetic resonance imaging, het heterozygous, hom homozygous
The two siblings in our study passed away soon after birth due to severe clinical manifestations and treatment abandonment, and therefore, we failed to follow the disease process. The other variant in our patients and the four Korean patients suggested that disease severity may vary, but this needed to be further evaluated and validated.

In summary, our study revealed the genetic basis and clinical features of two Chinese siblings with COXPD14 and expanded the mutation spectrum of FARS2. The two compound heterozygous variants in FARS2 are associated with the phenotypic characteristics of the patients. However, further research is essential to explore the pathogenesis of COXPD14 caused by dysfunction of mtPhRS protein resulting from FARS2 variants.

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Author contributions
LL drafted the initial manuscript, JM and JW conducted data analyses, LD revised the manuscript, and SL conceptualized and designed the study. All the authors read and approved the final manuscript.

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Availability of data and materials
The data analyzed during this study are available from the corresponding authors upon reasonable request.

Declarations
Ethics approval and consent to participate
This study was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University and Zibo Maternal and Child Health Hospital. The participants provided informed consent for participation.

Consent for publication
An informed consent for the publication has been obtained from the corresponding parents provided informed consent for participation.

Competing interests
No competing of interest are declared by the authors.

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References
1. Davis RL, Liang C, Sue CM. Mitochondrial diseases. Handb Clin Neurol. 2018;147:125–41.
2. Debray FG, Lambert M, Chevalier I, Robitaille Y, Decarie JC, Shoubridge EA, et al. Long-term outcome and clinical spectrum of 73 pediatric patients with mitochondrial diseases. Pediatrics. 2007;119(4):722–33.
3. Craven L, Alston CL, Taylor RW, Turnbull DM. Recent advances in mitochondrial disease. Annu Rev Genomics Hum Genet. 2017;18:257–75.
4. Yahata N, Matsumoto Y, Omi M, Yamamoto N, Hata R. TALEN-mediated shift of mitochondrial DNA heteroplasmy in MELAS-iPSCs with m.13513G>A mutation. Sci Rep. 2017;7(1):15557.
5. Gorman GS, Schaefer AM, Ng Y, Gomez N, Blakely EL, Alston CL, et al. Prevalence of nuclear and mitochondrial DNA mutations related to adult mitochondrial disease. Ann Neurol. 2015;77(5):753–9.
6. Almannai M, Wang J, Dai H, El-Hattab AW, Faqeh EA, Saleh MA, et al. FARS2 deficiency, new cases, review of clinical, biochemical, and molecular spectra, and variants interpretation based on structural, functional, and evolutionary significance. Mol Genet Metab. 2018;125(3):281–91.
7. Piniglione F, Conte G, Ghezzi D, Parazzini C, Righini A, Vergani R, et al. Clinical findings in a patient with FARS2 mutations and early-infantile-encephalopathy with epilepsy. Am J Med Genet A. 2016;170(1):3004–7.
8. Vernon HJ, McClellan R, Batista DA, Nadud A. Mutations in FARS2 and non-fatal mitochondrial dysfunction in two siblings. Am J Med Genet A. 2015;167A(5):1147–51.
9. Chen Z, Zhang Y. A patient with juvenile-onset refractory status epilepticus caused by two novel compound heterozygous mutations in FARS2 gene. Int J Neurosci. 2019,129(11):1094–7.
10. Almalki A, Alston CL, Parker A, Simonic I, Mehta SG, He L, et al. Mutation of the human mitochondrial phenylalanyl-tRNA synthetase causes infantile-onset epilepsy and cytochrome c oxidase deficiency. Biochim Biophys Acta. 2014;1842(1):56–64.
11. Hotait M, Naessredine W, El-Houby R, Diki M, Navfal Q, Beydoun A. FARS2 mutations: more than two phenotypes? A case report. Front Genet. 2020;11:787.
12. Cho JS, Kim SH, Kim HY, Chung T, Kim D, Jang S, et al. FARS2 mutation and epilepsy: possible link with early-onset epileptic encephalopathy. Epilepsy Res. 2017;129:118–24.
13. Richards S, Aziz N, Bale S, Bick D, Gusella JF, Kolski P, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American college of medical genetics and genomics and the association for molecular pathology. Genet Med. 2015;17(5):405–24.
14. Li Q, Wang K. InterVar: clinical interpretation of genetic variants by the 2015 ACMG-AMP guidelines. Am J Hum Genet. 2017;100(2):267–80.
15. Barcia G, Rio M, Assouline Z, Zhangarelli C, Roux C, de Lonlay P, et al. Novel FARS2 variants in patients with early onset encephalopathy with or without epilepsy associated with long survival. Eur J Hum Genet. 2021;29(3):533–8.
16. Shamseldin HE, AlShammari M, Al-Sheddi T, Salih MA, Alkalhi H, Kentab A, et al. Genomic analysis of mitochondrial diseases in a consanguineous population reveals novel candidate disease genes. J Med Genet. 2012;49(4):234–41.
17. Walker MA, Mohler KP, Hopkins KW, Oakley DH, Sweetser DA, Ibbeta M, et al. Novel compound heterozygous mutations expand the recognized phenotypes of FARS2-linked disease. J Child Neurol. 2016;31(9):1127–37.
18. Eto JM, Yadavalli SS, Euro L, Is hannoi P, Gutz A, Camoll CJ, et al. Mitochondrial phenylalanyl-tRNA synthetase mutations underlie fatal infantile Alpers encephalopathy. Hum Mol Genet. 2012;21(20):4521–9.
19. Yang Y, Liu W, Fang Z, Shi J, He C, et al. A newly identified missense mutation in FARS2 causes autosomal-recessive spastic paraplegia. Hum Mutat. 2016;37(2):165–9.
20. Vantroys E, Larson A, Friederich M, Knight K, Swanson MA, Powell CA, et al. New insights into the phenotype of FARS2 deficiency. Mol Genet Metab. 2017;122(4):72–81.
21. Sahai SK, Steiner RE, Au MG, Graham JM, Salamon N, Ibbeta M, et al. FARS2 mutations presenting with pure spastic paraplegia and lesions of the dentate nuclei. Ann Clin Transl Neurol. 2018;5(9):1128–33.
22. Meszarosova AU, Seemann P, Jenick J, Drabova J, Cibochova J, Stellmachova J, et al. Two types of recessive hereditary spastic paraplegia in Roma patients in compound heterozygous state; no ethnically prevalent variant found. Neu rosci Lett. 2020;721:134800.
23. Forman EB, Gorman KM, Ennis S, King MD. FARS2 causing complex hereditary spastic paraplegia with dysphonia: expanding the disease spectrum. J Child Neurol. 2019;34(10):621.

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