TARS2 Variants Cause Combination Oxidative Phosphorylation Deficiency-21: A Case Report and Literature Review

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Abstract

Objective The aim of the study is to explore the clinical and genetic characteristics of the combined oxidative phosphorylation defect type 21 (COXPD21) caused by the TARS2 compound heterozygous pathogenic variants, and to improve clinicians' awareness of the disease.

Methods The proband was a girl of first birth, with repeated refractory hypokalemia, hearing impairment, developmental delay, intellectual disability, developmental retardation after infection, high limb muscle tension, and increased serum lactate as the clinical phenotype. The clinical performance, diagnosis, treatment process, and gene characteristics of COXPD21 caused by TARS2 of the case were analyzed, reviewed, and compared with the literature from the CNKI, Wanfang Data, and biomedical literature database (PubMed) until November 2021.

Results The child was diagnosed with COXPD21 after two heterozygous variants in the TARS2 gene were found via whole exome sequencing. One of the variants was c.1679(exon14) A > C (p.Asp560Ala) missense, derived from the mother, and the other was c.1036(exon10)C > T (p.Arg346Cys) missense, derived from the father. The literature was searched and reviewed with the keywords "mitochondrial encephalomyopathy," "TARS2," and "combination oxidative phosphorylation deficiency type 21." A total of four complete domestic and foreign cases were collected from the literature search.

Conclusion COXPD21 onset by a complex heterozygous variant of TARS2 causes refractory hypokalemia, which is rarely reported in China and abroad.

Keywords
- combination oxidative phosphorylation deficiency-21
- TARS2 gene
- whole exome sequencing
- hypokalemia

Introduction

With an incidence rate of roughly one in every 5,000 people, mitochondrial diseases are a set of metabolic disorders caused by mitochondrial DNA (mtDNA) or nuclear DNA variations that result in mitochondrial oxidative phosphorylation dysfunction.[1] Mitochondrial dysfunction is associated with a variety of human diseases. In addition to mtDNA pathogenic variants, various nuclear gene mutations have been found to affect mtDNA maintenance and expression.[2] Mitochondrial aminoacyl-Trna synthetases (MT-ARS), encoded by the nuclear gene ARS2, generate aminoacyl-TrNA by catalyzing the specific binding of homologous tRNA to specific amino acids. It is involved in the cytoplasmic

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and mitochondrial protein translation process, which inhibits mitochondrial protein synthesis and disrupts the mitochondrial oxidative phosphorylation system. Dysfunction of this system affects energy metabolism and physiological activity in cells. Defect in the gene encoding MT-ARS has become an etiology for human mitochondrial diseases.[3] Combination oxidative phosphorylation deficiency-21. (COXPD21) is a disease caused by mitochondrial threonyl-tRNA synthetase (MT-ThrRS) gene (TARS2) mutation on chromosome 1Q21. Currently, only four foreign cases have been reported in the literature[4–6] and one case in China.[7] Inner Mongolia Maternal and Child Health Hospital confirmed one case of COXPD21 caused by TARS2 complex heterozygous variation, which was a rare case in China. The COXPD21 gene database and clinical phenotype of the disease are expanded. Herein, we present clinical and genetic characteristics of the combined oxidative phosphorylation defect type 21 (COXPD21) case caused by the TARS2 compound heterozygous pathogenic variants.

Clinical Data and Case Presentation

A female child (age 5 years and 3 months), having mental and physical retardation since childhood was presented with symptoms of recurrent loss of appetite, poor mental health, and hypokalemia. Other characteristics of the case were: (1) anorexia, psychosis, hypokalemia, milk refusal (daily intake less than 40 mL), poor mental coordination, with occasional vomiting and diarrhea. Multiple biochemical examinations have shown hypokalemia, hypomagnesemia, no obvious hyperchloremia, and the lowest monitored blood potassium is 1.61 mmol/L. Multiple blood gas analyses have suggested compensatory acidosis, and intravenous potassium supplementation and acid correction are given for symptomatic treatment. After intragluteal injection of magnesium sulfate, potassium chloride and potassium magnesium aspartate tablets were administered regularly to sustain blood potassium and magnesium within normal levels. They were stopped after 4 years of gradual reduction treatment. (2) The child has intellectual and motor development retardation since birth, which is manifested by late head raising in February, turning over in April, sitting alone in August, and walking in August. Currently, she has an unsteady gait without support, dysarthria, and can only pronounce "baba mama." No obvious improvement is seen upon multiple rehabilitation training treatments. (3) Ataxia: holding hands shaking, limbs shaking involuntarily, and aggravated after infection. (4) Hearing impairment: severe bilateral hearing impairment after birth and cochlear implantation was done at the age of 3 years. (5) Hyperlactatemia: after birth, repeated monitoring showed that blood lactate increased significantly, up to 3.9 mmol/L. (6) Previous history, personal history, family history: the child was the second birth of the fourth fetus, and had a cesarean section due to placenta previa and amniotic fluid turbidity at $37^{4/7}$ weeks of pregnancy. The birth weight was 3,100 g, and there was no history of asphyxia and birth injury. The mother had a history of threatened abortion during pregnancy. The child had no family history of epilepsy and other genetic diseases. (7) Physical examination of the nervous system: the pupils on both sides were equal in size and equal in diameter, 2 mm, sensitive to light reflection, clear breath sound in both lungs, powerful heart sound, neat in rhythm, soft abdomen, and the liver and spleen were normal. Walking instability, scissor gait, unstable holding, intentional tremor, involuntary shaking of limbs, increased muscle tension of limbs, decreased muscle tension of trunk, symmetrical knee-tendon reflex, symmetrical Achilles tendon reflex, pathological sign (-), and meningeal irritation sign (-). (8) Auxiliary examination: the child was normal on EEG examination; with bilateral widened ventricles on cranial MRI examination (►**Figs. 1** and **2**). No abnormality was found in urine gas chromatography-mass spectrometry and blood tandem mass spectrometry. No structural abnormalities were found on Color Doppler echocardiography. Otolaryngology hearing test shows severe hearing damage. Analysis of the results of the Wechsler Intelligence Scale for children in China was IQ <45.

Combined with the clinical characteristics of the child and relevant laboratory examinations, the possibility of mitochondrial disease was considered. After the guardian of the child signed the informed consent, the patient was sent for family whole exome sequencing (WES) + family whole genome CNV + mtDNA high-sensitivity sequencing analysis. Peripheral blood (4 mL) of the children and their parents was collected in EDTA anticoagulant collection tube, and DNA was extracted from the blood of the children. High-throughput sequencing (PE150) was performed by the dnbseq-t7 sequencing platform (Huada company), and the coverage of target sequence sequencing was no less than 99%. Burrows-wheeler Aligner software was used to compare the sequencing data with the Ensemble reference genome GRCh37/ HG19.[8] SNP and small fragment insertion/deletion mutation detection were performed using GATK software.[9] Mutation annotation software was used to carry out correlation annotation for the detected high-quality mutations in various major databases (such as dbSNP, 1000 genome, ExAC, ESP and other frequency databases, OMIM, HGMD, ClinVar, etc.). Using software such as Provean, SIFT, Polyphen2-HVAR, Polyphen2-HDIV, M-Cap, Revel, Mutationtaster, etc. to predict protein structural abnormalities, and using software such as MaxEntScan and SpliceAI to predict splice site abnormalities, variants that may have harmful effects on protein structure are screened. The interpretation was done according to the American College of Medical Genetics and Genomics (ACMG) guidelines.[10,11] Finally, Sanger sequencing was performed to verify the variation and confirm the existence of co-segregation. Two heterozygous mutations of the TARS2 gene were detected by WES including C. 1679(exon14) A > C (p.Asp560Ala) missense mutation, which was derived from the mother and was judged to have unknown clinical significance according to ACMG (PM1 + PM2 + PP3). Another missense variant, C.1036 (exon10) C > T (p.Arg346Cys), was derived from the father and was judged to have unknown clinical significance according to ACMG (PM2 + PP3). The parents of the child were heterozygous carriers of the two variants mentioned above, with no abnormal clinical phenotype.

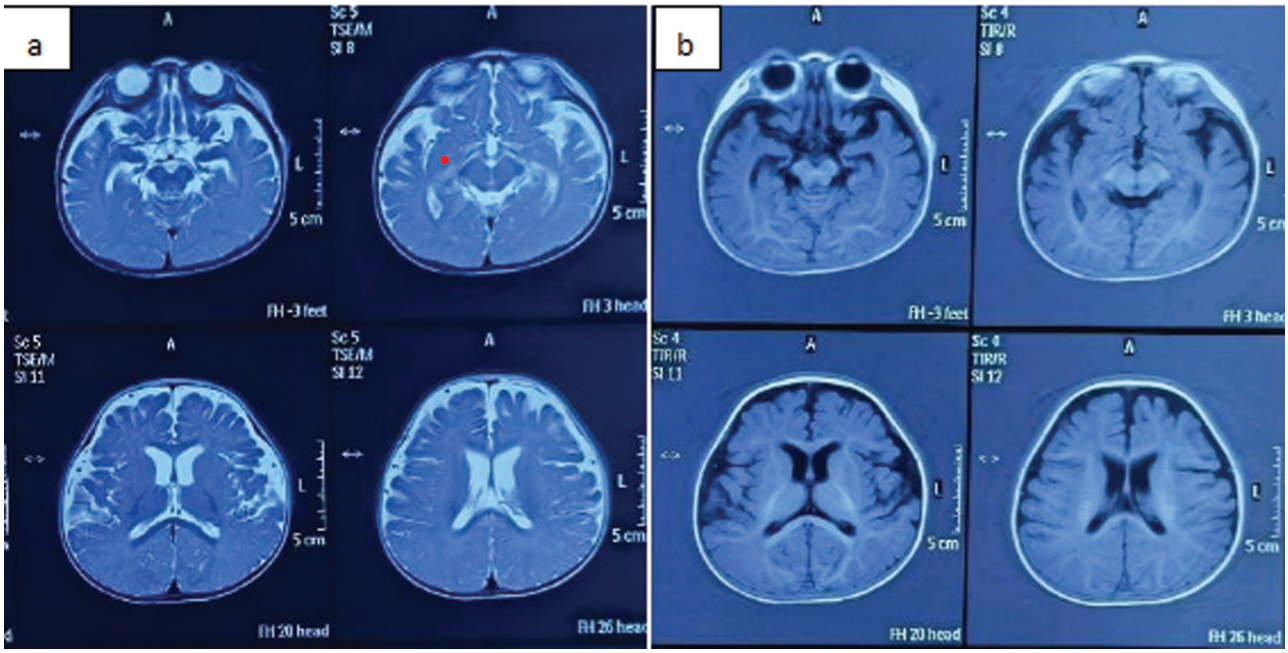

Fig. 1 MRI. (**a**) A 6-month old child with brain MRI T2 showed widening of bilateral interventricular space. (**b**) A 6-month old child, with brain MRI T1 showed widening of bilateral interventricular space.

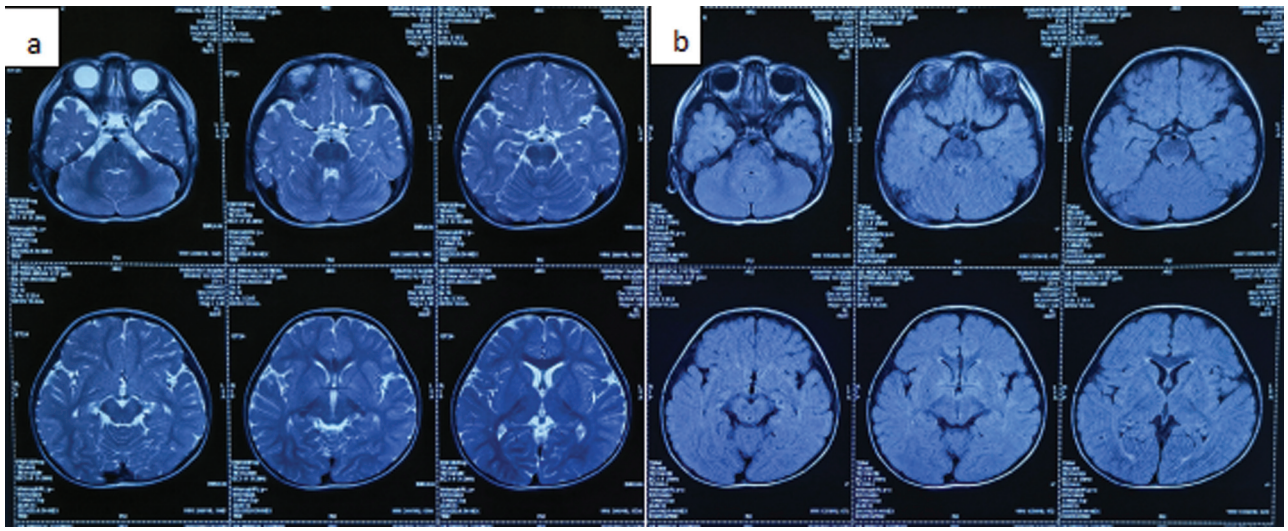

Fig. 2 MRI. (**a**) A 3-year-old child with brain MRI T2 showed widening of bilateral interventricular space. (**b**) A 3-year-old child with brain MRI T1 showed widening of bilateral interventricular space.

The child was given cocktail therapy, coenzyme Q10 10 mg/(kg d), l-carnitine 50 mg/(kg d), vitamin B2 200 mg/d, vitamin B1 500 mg/d, vitamin E 2IU/(kg d), and vitamin C 200 mg/D and symptomatic treatment. The child is now in a good general state with ongoing rehabilitation training treatment.

Discussion

The clinical manifestations of this child were mental and motor retardation with regression, repeated refractory hypokalemia, increased muscular tension in the limbs, ataxia, hyperlactatemia, and hearing impairment (extremely severe), which were consistent with the characteristics of mitochondrial disease. Wes sequencing confirmed that the child carried a compound heterozygous variant of TRAS2 gene c.1036C > T and c.1679A > C, which had not been reported in the literature before. According to ACMG, it was considered a pathogenic variant, so the child was diagnosed with COXPD21.

This disease is rare and only three cases abroad and one case in China have been reported to date. Among the threw children retrieved, two were siblings, which were first reported in 2014. Diodato et al found that two patients carried compound heterozygous variants in TARS2C.845C >T (P.P282L) and C.695 +3A > G (IVS6 + 3). The clinical manifestations were low back tension, increased limb muscle tension, severe mental and motor retardation, and hyperlactatemia. Cell function studies demonstrated that the

content of aminoacyl tRNA THR in fibroblasts of children was severely reduced. The enzyme activities of mitochondrial respiratory chain complexes I, III, IV, and V in the children patients' cells were decreased. Both children died due to metabolic crises a few months after birth.[4] Clinical manifestations of the third case were hypotonia, cerebellar atrophy, mental and motor retardation, and hyperlactatemia. Whole genome sequencing (WGS) found TARS2 shear variation and missense variation, both of which were predicted to cause the loss of MT-THRRS function, leading to mitochondrial dysfunction.[5]

The clinical manifestations in the fourth case were increased limb muscle tension, epilepsy, mental and motor retardation, and hyperlactatemia. WGS revealed two complex heterozygous variants in TARS2, c.470C >G. P. hr157Arg and c.2143G > A, P. lu715Lys, and by Sanger to verify the variation and to confirm the existence of pedigree co-separation. This child was diagnosed with combination oxidative phosphorylation deficiency type 21.[6] The fifth case was the first reported case from China in 2021. The child was a 6-month-old male, who was underdeveloped since childhood and had developed epilepsy since the age of 3 months, which was manifested as focal seizures, spasms, myoclonus, aggravated convulsions after respiratory tract infection, coma, cyanosis, shortness of breath, low and dull heart sound, large liver, and increased muscle tension in limbs. The WGS showed that the child had a complex heterozygotic variation of the TARS2 gene, c.987_988 insA and C. 470C > G, both of which were new mutations, and the child died at the age of 7 months,[7] as shown in ►**Table 1**.

Aminoacyl-trna synthetase (aaRS) is encoded by nuclear genes and catalyzes the binding of specific amino acids to the corresponding tRNAs. The resulting aminoacyl-tRNA is the raw material for the synthesis of proteins in the ribosome and is widely expressed in the cytoplasm and mitochondria. It plays an important role in protein synthesis, but its defects are tissue specific.[12] Mt-thrrs belong to Class II aaRS and contain 718 amino acids, which are composed of the N1 domain, N2 domain (redaction function), synthetic domain, and tRNA binding domain.[13] Mt-thrrs cannot accurately select and recognize homologous amino acids and are prone to incorrectly activate non-homologous serine sequences. The editing function of its N1 domain can clarify incorrectly activated serine and ensure the quality of mitochondrial translation.[14] Mt-thrrs is encoded by the TARS2 gene, which is located on chromosome 1Q21.3 and contains 18 exons with a size of 19.6 KB. The gene mutations can lead to coding protein mt - ThrRS dysfunction, amide acylation activity, reduced mitochondrial protein synthesis system disorders, and respiratory chain enzymes in various complex subunits expression levels decrease significantly. This, in turn, leads to complex I activity, resulting in hindrance in mitochondrial oxidative phosphorylation function and cells showing obvious respiration defects. Reduced mitochondrial ATP production and mitochondrial membrane potential lead to TARS2-related diseases.[15] These functions investigated the molecular pathogenesis of TARS2 mutation.

COXPD21, an autosomal recessive genetic disease caused by a mitochondrial oxidative phosphorylation defect, is caused by the TARS2 gene mutation. The disease primarily manifests in newborns, and it has the potential to cause multisystem dysfunction in the body. Clinical manifestations of COXPD21 include severe encephalomyopathy, cardiomyopathy, corpus callosum dysplasia, leukodystrophy, globus pallidus abnormal signaling, dystonia, and developmental delay, with poor prognosis and early death.[4] In this case, the clinical phenotype of the child was highly consistent. The two TARS2 variants carried by the child have very low frequency in the population. This complex heterozygous disease caused by these two TARS2 variants was consistent with the principle of familial co-separation and had the potential to manifest. The literature does not contain any reports of the two varieties listed above. This study expanded the genotype database of TARS2 gene-related diseases. At present, no recurrent refractory hypokalemia has been documented in the reported cases of diseases caused by the TARS2 variant, and this child presented with renal

Table 1 Cases and types of TARS2 mutations

Cases	Patients	Clinical symptom	Type
Case 1 and 2	Two siblings (male and female)	Low back tone, increased limb muscle tone, severe psychomotor retardation, and hyperlactatemia	Mother 845C > T (p.P282L) and father C. 695 + 3A > G (IVS6 + 3) complex heterozygous mutation
Case 3	Not clear	Low muscle tone, cerebellar atrophy, backward psychomotor development, hyperlactatemia	TARS2 shear variation and missense variation are not clear
Case 4	Male	Increased muscle tone in extremities, epilepsy, backward psychomotor development, hyperlactatemia	Mother C. 470C > G (P. hr157Arg) and father C. 2143G > A (P. lu715Lys) compound heterozygous mutation
Case 5	Male	Stunted development since childhood, epilepsy, coma, cyanosis, tachypnea, low and blunt heart sound, large liver, increased muscle tension of limbs after infection	C. 987_988 insA (p. pr33k fs*4) and c.470C > G (p.T157R)
Case 6	Female	Hypokalemia, hearing impairment, retardation of mental and motor development with regression, high muscle tone in extremities, ataxia, elevated serum lactic acid	Mother C. 1679A > C (P.app560ala) and father C. 1036C > T (P.arg346Cys) complex heterozygous mutation

tubular acidosis, expanding the clinical manifestations of TARS2 gene-related diseases.

The study found that pathogenic gene variations, in this case, were complex heterozygous variants of the TARS2 gene, enriching the clinical phenotype and genotype of TARS2-related diseases, thus providing a basis for genetic counseling.

Conflict of Interest

None declared.

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