



Short Communication

A rare homozygous mutation in the YARS2 gene presents with hypertrophic cardiomyopathy, lactic acidosis and anemia in a Chinese infant

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1. Introduction

Mitochondrial diseases (MDs) are a group of genetically and clinically diverse disorders resulting from mutations in mitochondrial DNA (mtDNA) or nuclear DNA (nDNA) that impair mitochondrial respiratory chain function and oxidative phosphorylation (OXPHOS) (El-Hattab and Scaglia, 2016; Imai-Okazaki et al., 2019). The heart is especially susceptible to mitochondrial dysfunction due to its reliance on aerobic energy production (El-Hattab and Scaglia, 2016; Yang et al., 2022). Approximately 20–40 % of MD patients experience cardiac involvement, resulting in a significantly increased mortality rate in comparison to those without cardiac involvement (Brunel-Guitton et al., 2015; Holmgren et al., 2003). Common cardiac manifestations of MDs include hypertrophic and dilated cardiomyopathy, arrhythmias, left ventricular non-compaction, and heart failure (Yang et al., 2022; Brunel-Guitton et al., 2015; Holmgren et al., 2003; Mazzaccara et al., 2021). The YARS2 gene, located at 12p11.21, encodes the mitochondrial tyrosyl-tRNA synthetase protein. This protein is crucial in mitochondrial protein synthesis by attaching tyrosine to a tRNA-like molecule in the mitochondria (Bonnefond et al., 2005). Pathogenic mutations of this gene are associated with an autosomal recessive syndrome known as mitochondrial myopathy, lactic acidosis, and sideroblastic anemia 2 (MLASA2, OMIM: 613561) (Riley et al., 2010; Nakajima et al., 2014). Patients of MLASA2 may present with hypertrophic cardiomyopathy (HCM) and respiratory failure, which are two important prognostic

factors (Sommerville et al., 2017). Early intervention is crucial for slowing disease progression and improving patient prognosis. However, significant clinical heterogeneity brings challenges to early detection and diagnosis of this disease. Here, we present a case of MLASA2 syndrome that bears a YARS2 gene mutation previously unreported in Chinese populations and summarize the clinical features of previously reported cases to provide a valuable insight to enhance knowledge of the disease.

2. Patients and methods

2.1. Clinical presentation

The proband was born to unrelated healthy Chinese parents without any birth complications. At five months old, she was diagnosed with moderate anemia and was ineffective with iron treatment. Regular blood transfusions of red blood cells were required to maintain a stable hemoglobin level. She was admitted to our center at the age of nine months with a recurrent fever and cough, as well as stunted growth. She displayed pallor and experienced shortness of breath. Additionally, she presented with skeletal muscle flaccidity. She also experienced severe acidosis (pH: 7.15), electrolyte imbalance (hypokalemia, hypophosphatemia), and hyperlactatemia (2.9–6.7 mmol/L) on admission. The Doppler echocardiography and cardiac CT revealed thickening of the left ventricular posterior wall (measuring 12.9 mm) and

Abbreviations: MDs, Mitochondrial diseases; mtDNA, Mitochondrial DNA; nDNA, Nuclear DNA; OXPHOS, Oxidative phosphorylation; MLASA2, Mitochondrial myopathy, lactic acidosis, and sideroblastic anemia 2; HCM, Hypertrophic cardiomyopathy; gnomAD, Genome Aggregation Database; MAF, Minor allele frequency; ExAC, Exome Aggregation Consortium; ACMG, American College of Medical Genetics and Genomics.

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interventricular septum (measuring 11.5 mm) (Fig. 1) as well as moderate pericardial effusion (measuring 18 mm in the left ventricular posterior wall). Pericardiocentesis was conducted during hospitalization. Based on these findings, the diagnosis of MD was suspected, and combination therapy was initiated with B vitamins, coenzyme Q10 and levocarnitine. Her blood lactate levels were decreased, but there was no significant improvement in her other symptoms. She was released from the hospital after her pulmonary infection was brought under control. Regular blood transfusion therapy was still required after discharge. According to a recent phone follow-up, she is alive and her symptoms of anemia and skeletal muscle flaccidity has resolved without specific treatment at the age of two. Regrettably, her parents declined further investigations, such as the Doppler echocardiography.

2.2. Whole-exome sequencing

We obtained approval from the Ethics Committee of Nanjing Children's Hospital and informed consent from the patient's guardian. Genomic DNA was extracted from the patient and other family members with standard procedures. To enrich the exonic regions of the DNA, we employed IDT's xGen Exome Research Panel v2.0 capture array probes. Variants with a minor allele frequency greater than 1 % were screened in public databases, including the Genome Aggregation Database (gnomAD), dbSNP, 1000 Genomes minor allele frequency (MAF) (Chinese), Exome Aggregation Consortium (ExAC), and an inhouse MAF database. To amplify exon 1 of the YARS2 gene, primer pairs were designed (F: AAGCACCTTCCCTAGGAGCTG; R: CCCCAAGGTTTAAAGGCACTTAC). The PCR mixtures contained 1.5 μ l primers, 2.0 μ l DNA, 12.5 μ l 2 \times Taq Master Mix (Vazyme Biotech Co., Ltd., Nanjing, China), and 9 μ l ddH₂O in a total volume of 25 μ l. Cycling conditions included a pre-denaturation step at 94°C for 5 min, followed by 34 cycles at 94°C for 30 s, 59°C for 30 s, and 72°C for 30 s, and a final extension at 72°C for 5 min. The PCR products were purified and then sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). A YARS2 gene variant (GenBank accession number NM_001040436) was used as a reference sequence. The pathogenicity of the variants was annotated according to the standards and

guidelines of the American College of Medical Genetics and Genomics (ACMG).

3. Results

Genetic testing revealed a homozygous missense mutation (c.156C>G; p.Phe52Leu) in the YARS2 gene, which were inherited from her healthy parents respectively (Fig. 2A), while both of her sisters are wild-type. This mutation occurred at the 156th nucleotide of the first exon of the YARS2 gene and replaces phenylalanine with leucine at position 52 of the encoded protein. This locus is located in close proximity to the catalytic center of tRNA tyrosylation and is highly conserved across species (Fig. 2C). Research indicated that F52 conducts the function of recognizing and/or stabilizing the tRNA^{Tyr} amino acid acceptor helix, which is lost in the mutant (Riley et al., 2010). According to the ACMG standards, it is classified as a likely pathogenic mutation. The bioinformatic analyses of PROVEAN and MutationTaster indicate that this mutation is pathogenic.

To date, 29 families and 37 patients have been reported with comprehensive case data involving 33 YARS2 gene mutations (Riley et al., 2010; Nakajima et al., 2014; Sommerville et al., 2017; Riley et al., 2018; Riley et al., 2013; Sasarman et al., 2012; Shahni et al., 2013; Ardisson et al., 2015; Binghua et al., 2022; Carreño-Gago et al., 2021; Rudaks et al., 2022; Smith et al., 2018). Among these, the c.156C>G (p. Phe52Leu) is the most frequently reported variant and 10 cases have been recorded (Riley et al., 2010; Riley et al., 2018; Riley et al., 2013; Shahni et al., 2013). Sideroblastic anemia is the predominant clinical symptom in MLASA2. Among the 36 reported patients, 32 exhibited signs of anemia, and 22 required transfusion therapy. Twelve cases presented with HCM. A total of 11 deaths were recorded at the time of reporting, with a median age of 21.2 years.

4. Discussion

Here we present a rare case of MLASA2 which was caused by a mutation in the YARS2 gene. The proband presented with pallor, feeding difficulties, and developmental delay at 9 months of age. Laboratory

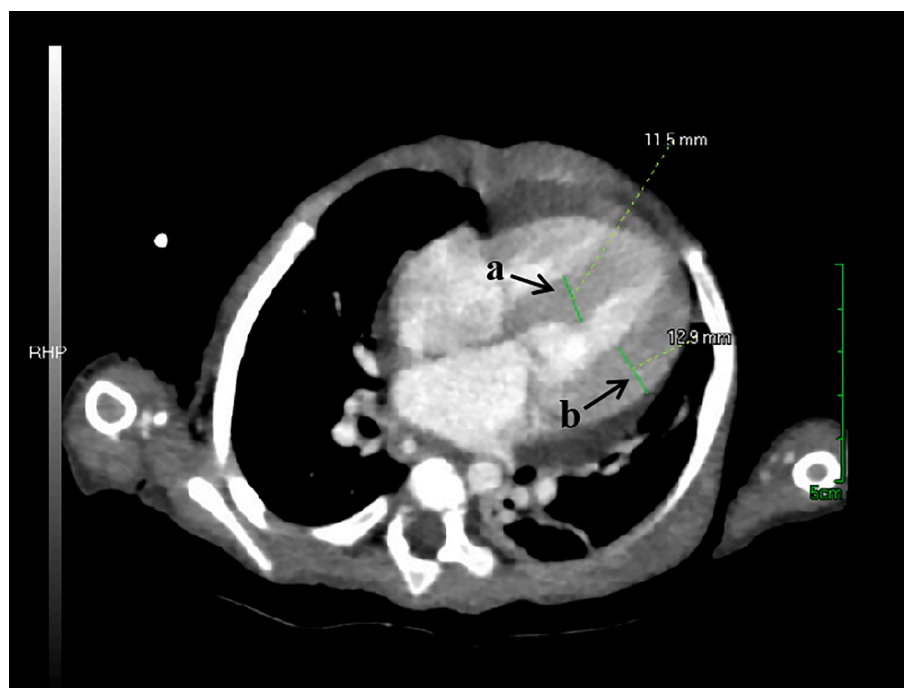


Fig. 1. The cardiac CT of the patient showed the thickness of the interventricular septum to be approximately 11.5 mm (a) and the thickness of the posterior wall of the left ventricle to be approximately 12.9 mm (b).

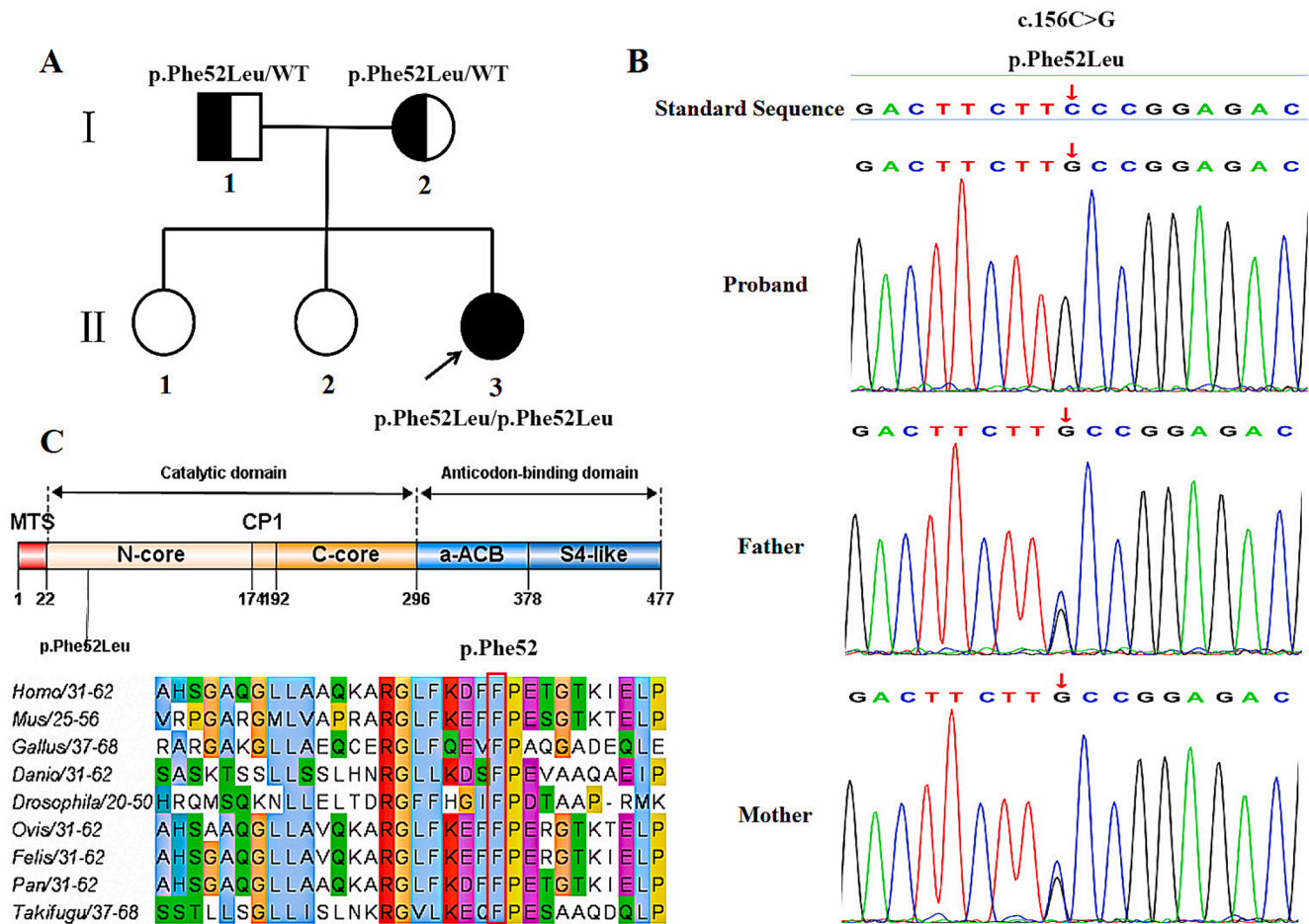


Fig. 2. (A) Genetic analysis of the YARS2 mutation in this pedigree. The arrow depicts the proband. The circles correspond to the women. The squares indicate the men. WT, wild type. (B) Representative Sanger electropherograms of the proband, father and mother illustrate the c.156C>G mutation. (C) YARS2 protein mutation localization map and conservation analysis. The mutation in this patient is indicated in black below the protein map. The F 52 sites are marked with red boxes between the different species. MTS, mitochondrial target sequence; N-core and C-core, N and C part of the catalytic domain, respectively; CP1, connective peptide; a-ACB, a-helical anticodon-binding domain; S4-like, ribosomal protein S4-like protein. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

tests revealed high lactate levels, anemia and cardiac hypertrophy, which are consistent with the typical clinical features of MLASA2 as described in the previous literature. Genetic analysis revealed a homozygous missense mutation c.156C>G (p.Phe52Leu) in the YARS2 gene, which has not been reported in the Chinese population.

MLASA2 cases have mainly been reported in Lebanese, Caucasian, and Scottish populations. There has been a total of 36 cases from 29 families reported worldwide (Riley et al., 2010; Nakajima et al., 2014; Sommerville et al., 2017; Riley et al., 2018; Riley et al., 2013; Sasarman et al., 2012; Shahni et al., 2013; Ardisson et al., 2015; Binghua et al., 2022; Carreño-Gago et al., 2021; Rudaks et al., 2022; Smith et al., 2018). Our case had an early onset of anemia at five months of age. This is consistent with previous research that MLASA2 cases typically occur during infancy or childhood. Sideroblastic anemia is the predominant clinical symptom in MLASA2, however, it is not appeared in all patients, and its severity varies among individuals. HCM is the most common cardiac phenotype in patients with MLASA2 syndrome. Of the 36 reported patients, 12 presented with HCM. A total of 11 deaths were recorded at the time of reporting, with a median age of 21.2 years. The primary causes of these deaths were respiratory muscle weakness and cardiomyopathy (Sommerville et al., 2017; Smith et al., 2018).

MLASA2 is caused by homozygous mutation in the YARS2 gene, which contains a catalytic structural domain and an anticodon binding structural domain. Most YARS2 gene mutations mentioned in literature

are situated in the catalytic structural domain. The c.156C>G (p.Phe52Leu) mutation, located in the catalytic region, is the most frequently reported mutation, with a total of 11 patients reported, including this case (Table 1). All the 11 patients were symptomatic suggesting that the double-allelic mutations at this particular locus have a significant penetrance. However, the 11 patients were not identical in clinical presentation and severity, even though they carried the same mutation. Thus, the significant phenotypic differences observed between individuals with the same genotype suggest that there may be other genetic and environmental influences that could epigenetically modify the YARS2 gene. Some researchers suggested that the background mtDNA haplotype may be responsible for the phenotypic variation (Sommerville et al., 2017; Riley et al., 2013). However, given the rarity of the reported patient cohort, it is difficult to draw definitive conclusions about genotype-phenotype correlation.

There is currently no definitive treatment available for patients diagnosed with MLASA2. Early medication, including B vitamins, coenzyme Q10 and L-carnitine, may stabilize some cases, but their overall efficacy appears to be limited. Sideroblastic anemia in MLASA2 patients does not respond to treatment with pyridoxine and erythropoietin, just like our case, regular blood transfusions are the primary treatment. However, spontaneous remission of sideroblastic anemia has been documented in certain cases (Sommerville et al., 2017). HCM also does not appear to be a persistent symptom, as demonstrated by a

Table 1
Clinical Phenotype for patients with a homozygous c.156C>G (p.F52L) mutation in the YARS2 gene

Reference	Riley et al. (2010)			Shahn et al. (2013)	Riley et al. (2013)		Riley et al. (2018)			This study	
Patient	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
Sex	M	F	F	M	F	F	F	M	F	M	F
Age at onset	10 weeks	Infancy	7y	1y	8 weeks	23y	14y	6y	4y	6y	5m
Age at death	18y	Alive at 16y	Alive at 24y	Alive at 13y	3 m	Alive at 28 y	28y	Alive at 9 y	Alive at 7 y	Alive at 10 y	Alive at 17m
Ethnicity	Lebanese	Lebanese	Lebanese	Lebanese	Lebanese	Lebanese	Lebanese/ American	Lebanese	Lebanese	Lebanese	Chinese
Family history	Yes	Yes	No	No	No	Yes	No	Yes	Yes	No	No
Sideroblastic anemia	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Transfusion-dependent	Yes	Yes	No	Yes	Yes	No	Yes	Yes	No	No	Yes
Faltering growth	Yes	Yes	Yes	Yes	Yes	NM	Yes	NM	NM	Yes	Yes
Dysphagia	Yes	Yes	No	Yes	Yes	NM	NM	NM	NM	NM	Yes
Cardiomyopathy	Yes	NM	No	Yes	Yes	NM	NM	NM	NM	No	Yes
Exercise intolerance	Yes	Yes	Yes	Yes	NM	Yes	Yes	NM	NM	Yes	Yes
Respiratory failure	17y	NM	No	12y	3m	NM	Yes	No	No	NM	No
Blood lactate (mmol/L)	3–13.7	2.5–8.4	4.1	5.6–10.1	27	3.24-5.1	9.1	NM	NM	6-13.9	2.9-6.7

Abbreviations: M = male, F = female, NM = not mentioned.

patient who had discrete myocardial hypertrophy at the age of three, which normalized by the age of five. In addition, the patient’s anemic symptoms relieved after one year of transfusion therapy (Riley et al., 2013). In addition, a 10-week-old infant with transfusion-dependent sideroblastic anemia presented with HCM at three months of age, which then subsided spontaneously without specific treatment. At 17 years of age, the patient experienced a recurrence of the cardiomyopathy and subsequently succumbed to cardiopulmonary failure (Riley et al., 2010). Therefore, it is necessary to conduct regular systematic examinations, including the Doppler echocardiography, even if the patient’s clinical symptoms have subsided.

5. Conclusion

We present a rare case of MLASA2 caused by YARS2 gene mutation in China. The proband presented with symptoms of anemia, developmental delay, elevated lactate levels and HCM at the age of nine months. Genetic analysis revealed that the proband had a homozygous missense mutation, c.156C>G (p.Phe52Leu). Our findings expand the YARS2 gene spectrum and contribute to the ongoing exploration of the genotype-phenotype correlation in YARS2-related MDs. In addition, we highlight that a thorough evaluation of cardiac structure and function is important for patients with MLASA2.

6. Ethics approval and consent to participate

The studies involving human participants were reviewed and approved by the institutional ethical committee of the Children’s Hospital of Nanjing Medical University. Written informed consent to participate in this study was provided by the participants’ legal guardian/next of kin.

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CRedit authorship contribution statement

Dandan Xiang: Writing – original draft, Methodology. **Kangkang Xu:** Writing – original draft, Supervision, Software, Formal analysis, Data curation, Conceptualization. **Mei Chen:** Data curation, Investigation, Supervision. **Zhongman Zhang:** Writing – review & editing, Validation, Supervision, Software. **Ningning Sun:** Validation, Software, Investigation. **Yuying Qi:** Validation, Supervision. **Jie Lu:** Validation, Methodology, Investigation. **Chunli Wang:** Writing – review & editing, Supervision. **Shiwei Yang:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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