ACTA MÉDICA PORTUGUESA
A Revista Científica da Ordem dos Médicos

CASO CLÍNICO

A Novel Combination of Biallelic Variants in the *VARS2* Gene: A Severe Phenotype

Uma Nova Combinação de Variantes Bialélicas no Gene *VARS2*: Um Fenótipo Grave

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Acta Med Port (In Press) ▪ https://doi.org/10.20344/amp.23831

ABSTRACT

To our knowledge, only 29 individuals have been described in the literature with biallelic pathogenic variants in the valyl-tRNA synthetase 2 (*VARS2*) gene, responsible for changes in the mitochondrial respiratory chain complex. We report two siblings with a novel combination of biallelic variants in the *VARS2* gene (c.1079C>T p.Ala360Val, likely pathogenic, and c.1258G>A p.Ala420Thr, likely pathogenic). Both presented early hypertrophic cardiomyopathy and lactic acidosis, with fatal outcomes within the first year of life. The first also presented severe fetal growth restriction and a ventricular septal defect; the second developed epilepsy, respiratory failure, and psychomotor delay. This genotype may be linked to a particularly severe cardiac phenotype. Our report broadens the clinical and genetic spectrum of *VARS2*-related mitochondrial disease, highlights the variability of phenotypic expression, and reinforces the importance of early molecular diagnosis in neonatal-onset cardiomyopathy. Genetic confirmation enables accurate genetic counselling and consideration of prenatal or preimplantation diagnosis in future pregnancies.

Keywords: Cardiomyopathy, Hypertrophic; Epilepsy; Mitochondrial Diseases; Valine-tRNA Ligase/genetics

RESUMO

Do que se conhece, estão descritos na literatura apenas 29 indivíduos com variantes patogénicas em homozigotia ou heterozigotia composta no gene valil-tRNA sintetase 2 (*VARS2*), responsável por alterações nos complexos da cadeia respiratória mitocondrial. Descrevemos dois irmãos com uma nova combinação de variantes bialélicas no gene *VARS2* (c.1079C>T p.Ala360Val, provavelmente patogénica, e c.1258G>A p.Ala420Thr, provavelmente patogénica). Ambos apresentavam cardiomiopatia hipertrófica e acidose láctica, com desfecho fatal no primeiro ano de vida. O primeiro manifestava também restrição do crescimento fetal e defeito do septo interventricular; o segundo apresentava epilepsia, insuficiência respiratória e atraso do desenvolvimento psicomotor. Este genótipo poderá associar-se a um fenótipo cardíaco grave. Estes casos ampliam o espetro clínico e genético da doença mitocondrial relacionada com o gene *VARS2*, evidenciam a variabilidade fenotípica e reforçam a importância do diagnóstico molecular precoce na cardiomiopatia. A confirmação genética permite um aconselhamento preciso e a consideração do diagnóstico pré-natal ou pré-implantação em gravidezes futuras.

Palavras-chave: Cardiomiopatia Hipertrófica; Doenças Mitocondriais; Epilepsia; Valina-tRNA Ligase

INTRODUCTION

Mitochondrial diseases represent a broad group of multisystem disorders caused by inherited or acquired pathogenic variants, in mitochondrial or nuclear DNA.[1,2] This group appears to be the leading cause of neurometabolic diseases, with an estimated prevalence of 1/5000 births.[3]

Combined phosphorylation deficiency type 20 (COXPD20) is a rare autosomal recessive mitochondrial disease characterized by alterations in the activity of mitochondrial respiratory chain complex.[1,3] This disease results from biallelic pathogenic variants, either in compound heterozygosity or homozygosity, in the *VARS2* gene (mitochondrial valyl-tRNA synthetase 2), located on chromosome 6p21, which encodes the mitochondrial valyl-tRNA synthetase (Val-tRNA).[3] A defect in Val-tRNA activity causes an alteration in oxidative phosphorylation, which consequently decreases ATP production.[4,5]

Biallelic *VARS2* variants were first described in 2014 in two unrelated patients with mitochondrial respiratory chain dysfunction.[6,7] Bruni *et al* (2018) further characterized the genetic, clinical, and biochemical features of COXPD20 in a review of 13 patients.[8] Clinical presentation is precocious and variable, including feeding difficulties, failure to thrive, psychomotor development delay, hypotonia, epilepsy, microcephaly, encephalopathy, hypertrophic cardiomyopathy, pulmonary hypertension, hyperlactatemia, metabolic acidosis and nonspecific changes on brain magnetic resonance imaging (MRI).[1,2,4,5,8,9] To our knowledge, only 29 individuals, from 24 families, with COXPD20 have been reported worldwide.[1,10]

This article aims to describe two siblings with novel biallelic variants in the *VARS2* gene associated with a severe phenotype. It also compares their clinical manifestations with those previously described.

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Revisto por/*Reviewed by*: Sandra Pereira
Recebido/*Received*: 14/08/2025 - **Aceite/*Accepted*:** 12/02/2026 - **Publicado *Online*/*Published Online*:** 18/05/2026

CASE REPORTS

Case 1

The index case was a female newborn, the first child of young, non-consanguineous Portuguese parents. Family history was notable for neonatal death in a maternal aunt, global developmental delay in a second-degree maternal cousin, and structural/conduction cardiac abnormalities in the paternal lineage.

Pregnancy was uneventful until the third trimester, when the ultrasound revealed biventricular hypertrophic cardiomyopathy, a muscular ventricular septal defect (VSD), bradycardia and severe fetal growth restriction with preeclampsia. She was delivered by elective cesarean section at 33+2 weeks. Apgar score was 2 at 1, 5 and 10 minutes, requiring advanced neonatal resuscitation and subsequent admission to the neonatal intensive care unit (NICU).

Serial echocardiograms showed progressive ventricular hypertrophy. Propranolol was initiated but did not modify disease progression. The patient developed worsening heart failure and generalized hypotonia, with no documented seizures.

An extensive etiological investigation was carried out. Blood tests revealed significantly and persistently elevated lactate levels and metabolic acidosis. Increased alanine levels were noted in amino acid screening in blood and urine, along with redox potential abnormalities, including elevated lactate and pyruvate with a normal lactate/pyruvate ratio. Other metabolic investigations, including mitochondrial genome sequencing, yielded normal results. Due to ongoing suspicion of mitochondrial disease, treatment with L-carnitine was initiated (75 mg/kg/day, later optimized to 100 mg/kg/day).

Clinical exome sequencing identified four heterozygous variants, all classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines: a variant of unknown significance (VUS) in the *AKAP9* gene (NM_005751.4): c.3751+1G>A, p.(spl); a VUS in the *KCND3* gene (NM_004980.4): c.1501C>T, p.(Arg501*); two variants in the *VARS2* gene (NM_001167734.2): c.1079C>T, p.(Ala360Val) (VUS) and c.1258G>A, p.(Ala420Thr) (likely pathogenic). Variants c.3751+1G>A in the *AKAP9* gene and c.1501C>T in the *KCND3* gene were inherited from the father and the mother, respectively, and were deemed not relevant to the patient's phenotype. On the other hand, the c.1079C>T variant in *VARS2* gene was of paternal origin and the c.1258G>A variant was maternally inherited, confirming these variants were in compound heterozygosity. After clinical discussion, the ACMG criterion PM3 was used at the strong level allowing the reclassification of variant c.1079C>T in *VARS2* as being likely pathogenic. So, the most likely diagnosis is COXPD20, as it explains the severe hypertrophic cardiomyopathy, as well as the overall phenotype.

The child died on day 40 of life due to progressive cardiac failure.

Case 2

During a subsequent pregnancy and given the 25% recurrence risk, amniocentesis was performed and the familial variants in *VARS2* gene were tested in fetal DNA. The fetus inherited both variants, confirming COXPD20. After genetic counselling, the parents declined pregnancy termination. Pregnancy was complicated by gestational diabetes. Ultrasounds were unremarkable, except for a 22-week fetal echocardiogram showing borderline right ventricular hypertrophy and mild pleural effusion.

A male infant was delivered by caesarean section at 39+1 weeks, with Apgar scores of 5, 8, and 9 at 1, 5, and 10 minutes. He required brief positive pressure ventilation. Echocardiography on day 1 revealed marked septal hypertrophy, a patent foramen ovale, and mildly reduced contractility (Fig. 1). On the third day of life, he was admitted to the NICU due to respiratory distress. Physical examination showed poor peripheral perfusion, hypotonia, palpable hepatic border and hypospadia. The initial blood gas analysis showed lactate levels of 4.4 mmol/L (maximum value of 8.3 mmol/L). Chest X-ray showed a mildly increased cardiothoracic ratio and bilateral interstitial infiltrate. Electrocardiography revealed deep R and negative T waves in the right leads, with cardiac axis between 90º - 120º. He gradually manifested significant hypotonia and muscle weakness, with compromised ventilation and need for positive airway pressure (CPAP). A new echocardiogram 15 days later demonstrated non-obstructive hypertrophic cardiomyopathy with marked concentric hypertrophy of the left ventricle and the free wall of the right ventricle, good systolic function, and mild diastolic dysfunction. The patient started therapy with propranolol, with a gradual dose increase. During hospitalization, he had convulsive episodes, which were treated with phenobarbital. Transfontanellar ultrasound and electroencephalogram were normal. He started treatment with levetiracetam and vitamin supplementation with thiamine (150 mg 24/24h), coenzyme Q10 (6 mg 8/8h), biotin (10 mg 24/24h), riboflavin (33 mg 8/8h), creatine (0.5 mg 6/6h), and levocarnitine (50 mg 8/8h).

At discharge (day 34), the patient had generalized hypotonia, a grade II/VI systolic murmur, and mild hepatomegaly. He required CPAP and nasogastric feeding, and was treated with propranolol, levetiracetam, and vitamin supplementation.

During multidisciplinary follow-up, nonspecific facial dysmorphisms were observed, namely short palpebral fissures, mild hypertelorism, short nose with a rounded tip, thin upper lip, micrognathia, and low-set ears (Fig. 2). Other clinical

manifestations emerged, such as short stature and delayed psychomotor development. He remained dependent on a nasogastric tube for feeding. The child died at 9 months-old due to a respiratory infection that aggravated respiratory failure and cardiomyopathy.

VARS2 gene variants

Appendix 1 (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/23831/15958) summarizes all the main *VARS2* variants reported to date. The most common variant seems to be c.1100C>T (p.Thr367Ile), present in 51,7% (15/29) of the published cases, consisting of a substitution of threonine for isoleucine at codon 367.[1-3,8,11] To date, only two Portuguese patients have been reported: one with the homozygous variant c.1100C>T (p.Thr367Ile) and another with the compound heterozygosity of variants c.1100C>T (p.Thr367Ile) and c.1258G>A (p.Ala420Thr).[3,12] Compound heterozygosity of variants c.643C>T (p.His215Tyr) and c.1354A>G (p.Met452Val)[2] and also of variants c.1940C>T (p.Thr647Met) and c.2318G>A (p.Arg773Gln)[4] has been associated with more severe phenotypes. Other examples are shown in Appendix 1 (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/23831/15958).

Epilepsy of variable severity appears to be a common feature of *VARS2*-related mitochondrial disease.[8] Hypertrophic cardiomyopathy is not universal; notably, the frequent c.1100C>T variant is not typically associated with cardiac involvement. Hyperlactatemia with metabolic acidosis seems to be a consistent finding.[8] Other laboratory results are usually unremarkable or nonspecific: for example, the homozygous c.1981C>A (p.Pro661Thr) variant was described in a child with persistent elevation of liver transaminases (~100 UI/L).[5] Brain MRI shows variable and nonspecific findings, namely cortical and cerebellar atrophy, loss of volume of white matter and basal ganglia, hypoplasia of the corpus callosum, enlargement of the lateral ventricles, oedema of the brainstem and frontal white matter, and bilateral periventricular white matter hyperintensities.[2,3,5,8,13]

DISCUSSION

This article describes a new combination of heterozygous variants in the *VARS2* gene, c.1079C>T p.(Ala360Val) and c.1258G>A p.(Ala420Thr), associated with a severe phenotype characterized by hypertrophic cardiomyopathy, hypotonia, failure to thrive, and lactic acidosis, with fatal outcome in infancy. The first sibling also had a muscular VSD and severe fetal growth restriction, while the second developed epilepsy, respiratory failure, feeding difficulties, hepatomegaly, and delayed psychomotor development.

The diagnosis of mitochondrial diseases remains challenging due to their heterogeneity and the constant expansion of known causative genes.[3,11] Whole-exome sequencing is fundamental in the diagnostic pathway.[3]

Clinical variability and survival are wide-ranging, probably reflecting tissue-specific expression of valyl-tRNA synthetase and epigenetic effects. Future studies using animal and cellular models of *VARS2* variants are needed to explore the mechanisms by which defective valyl-tRNA synthetase disrupts cardiac and neurological development.[2] The evidence suggests that genes involved in RNA metabolism, including tRNA synthetases, are crucial for brain development,[5] although their role in myocardial disease remains poorly understood.

Currently, therapeutic approaches remain limited. Supportive strategies like vitamin supplementation and carbohydrate restriction offer modest benefit.[4] Marquez *et al* demonstrated that valine supplementation improves cardiac function in a variant-dependent manner, in a vertebrate model.[13] Coenzyme Q10 and riboflavin have shown supportive antioxidant effects in patients with homozygous c.1100C>T (p.Thr367Ile).[11] Antiepileptic drugs are indicated when seizures are present.[11]

The present cases expand the known spectrum of COXPD20. Our findings suggest that the variant c.1258G>A (p.Ala420Thr), either in homozygosity or compound heterozygosity, may be associated with particularly severe cardiac involvement. Additional studies are required to confirm genotype-phenotype correlations and improve prognostic counselling.

ACKNOWLEDGMENTS

The authors declare that no AI tools were used during the preparation of this work.

AUTHOR CONTRIBUTIONS

JC, MR, IA: Literature search, writing of the manuscript.
MN, AR: Data collection, critical review of the manuscript.
PL, MR: Critical review of the manuscript.

All authors approved the final version to be published.

PROTECTION OF HUMANS AND ANIMALS

The authors declare that the procedures were followed according to the regulations established by the Clinical Research and Ethics Committee and to the Helsinki Declaration of the World Medical Association updated in October 2024.

DATA CONFIDENTIALITY

The authors declare having followed the protocols in use at their working center regarding patients' data publication.

PARENTAL CONSENT

Obtained.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

FUNDING SOURCES

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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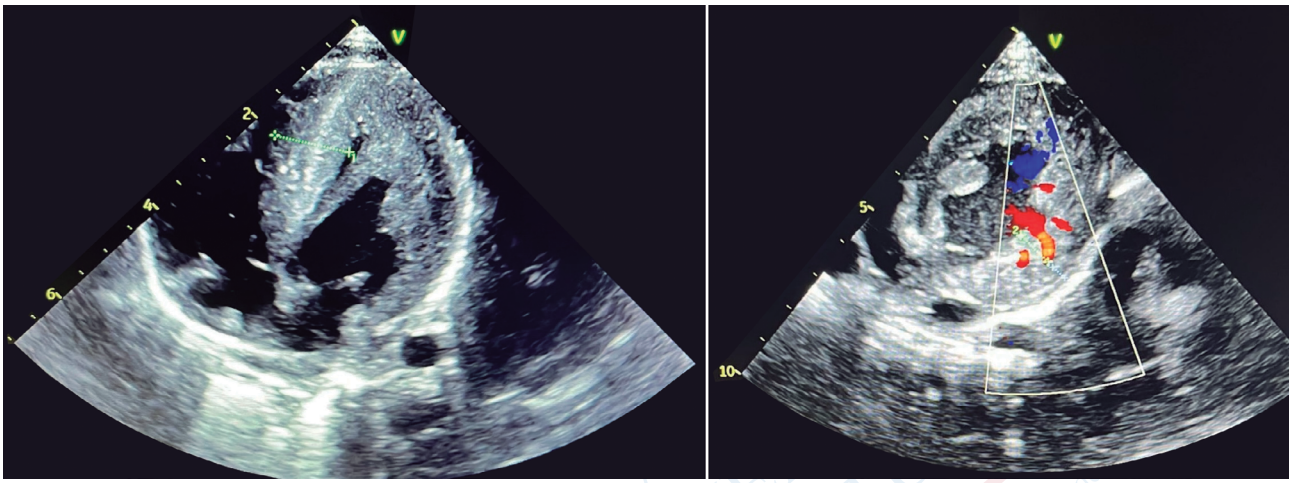

Figure 1 – Echocardiogram on the first day of life shows patent foramen ovale and interventricular septum with marked hypertrophy

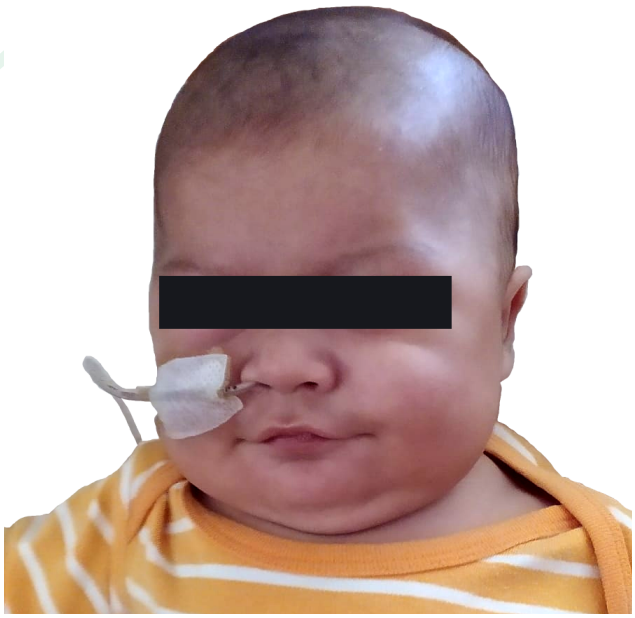

Figure 2 – Frontal photograph of the patient from Case 2 at the age of six months, showing short nose with a rounded tip, thin upper lip, and micrognathia