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CASE STUDY

# Biallelic variants in *GTPBP3*: New patients, phenotypic spectrum, and outcome

Francesca Nardecchia[1], Rosalba Carrozzo[2], Alice Innocenti[1], Alessandra Torraco[2], Valerio Zaccaria[1], Teresa Rizza[2], Francesco Pisani[1], Enrico Bertini[3] & Vincenzo Leuzzi[1]

[1]Unit of Child Neurology and Psychiatry, Department of Human Neuroscience, Sapienza University of Rome, Rome, Italy
[2]Unit of Cell Biology and Diagnosis of Mitochondrial Disorders, Laboratory of Medical Genetics, Bambino Gesù Children's Hospital IRCCS, Rome, Italy
[3]Neuromuscular Disorders Research Unit, Bambino Gesù Children's Hospital IRCCS, Rome, Italy

**Correspondence**
Francesca Nardecchia, Department of Human Neuroscience, Sapienza University of Rome, Via dei Sabelli 108, Roma 00185, Italy. Tel: +39 06 49979836; Fax: +39 06 49972908; E-mail: francesca.nardecchia@uniroma1.it

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## Abstract

**Introduction**: COXPD23 is a rare mitochondrial disease caused by biallelic pathogenic variants in *GTPBP3*. We report on two siblings with a mild phenotype. **Case reports**: The young boy presented with global developmental delay, ataxic gait and upper limbs tremor, and the older sister with absence seizures and hypertrophic cardiomyopathy. Respiratory chain impairment was confirmed in muscle. **Discussion**: Reviewed cases point toward clustering around two prevalent phenotypes: an early-onset presentation with severe fatal encephalopathy and a late milder presentation with global developmental delay/ID and cardiopathy, with the latter as, is the main feature. Our patients showed an intermediate phenotype with intrafamilial variability.

## Introduction

*GTPBP3* is a nuclear gene on chromosome 19p13.11 encoding for the mitochondrial GTP-binding protein 3, an evolutionarily conserved, multidomain protein involved in mitochondrial tRNA modification, and mitochondrial translation.[1] By impairing mitochondrial posttranscriptional protein synthesis, biallelic variants in *GTPBP3* cause derangement of oxygen consumption, ATP production, potential membrane homeostasis, superoxide accumulation, and increasing protein degradation, featuring the biochemical consequences of a combined oxidative phosphorylation deficiency (OMIM #616198, COXPD23). COXPD23 is an ultrarare mitochondrial encephalopathy[2]; in fact, less than 20 patients with biallelic pathogenic variants in *GTPBP3* have been so far reported presenting with a large phenotypical spectrum.[2–7] A genotype–phenotype correlation has been recently suggested.[3,6]

With the aim of contributing to the characterization of the phenotypic spectrum of the disease, we report on two Italian siblings with biallelic variants in *GTPBP3* and reviewed reported cases from the literature, focusing on the presentation and outcome.

## Case Report

### Patient 1 (II-3)

This 9-year-old boy (II-3), the last of three siblings from consanguineous parents, was born after a pregnancy complicated by abruptio placentae at 12 weeks of gestation and uneventful at term delivery (weight: 3650 g, 58th centile; length: 51 cm, 51st centile; head circumference 34 cm, 22nd centile). Presenting symptoms were global developmental delay (GDD) (gait unsupported at the age of 20 months; lack of language), irritability, and behavioral issues (hetero-aggressive conduct). Moreover, the parents reported frequent unexplained episodes of vomiting and a transient (few days) limping gait episode at the age of 30 months. On examination, at age 3.5 years, he showed GDD, severe language impairment, and ataxic gait. Brain MRI showed bilateral lesions of the thalamus, and multiple focal alterations of cerebral white matter. Brain $^{1}$H-MRS disclosed lactate peak in basal ganglia and semioval white matter voxels (Fig. 1). Finally, the echocardiographic evaluation revealed a slight increase in the trabeculae on the posterior wall of the left ventricle. An

extensive neurometabolic work-up was unhelpful, except for the persistent increased excretion of lactate and 3-hydroxyvaleric acid in urine as detected by gas chromatography–mass spectrometry.

After the diagnosis, a treatment was started including levocarnitine (1 g/day), thiamine (300 mg/day), ascorbic acid (1 g/day), riboflavin (100 mg/day), and idebenone (400 mg/day). During the last 5.5 years of follow-up, a progressive clinical improvement was observed with the emergence of spoken language and reduction of irritability and behavioral issues. At the age 5.5 years, Leiter-R scale IQ was 70. Nevertheless, a postural and action tremor of the upper limbs has been noticed since the age of 7 years.

### Patient 2 (II-1)

This 16-year-old girl, the older sister of Patient 1, was born after a normal pregnancy and delivery. She suffered from mild developmental delay with prevalent language impairment. During the kindergarten years, social, behavioral, and cognitive immaturity was noted by the teachers. A first formal IQ assessment was performed during primary school when a mild intellectual disability was diagnosed. She suffered from febrile seizures from 8 to 36 months of age and from drug-responsive absence epilepsy since the age of 5. At 9 years, brain MRI and $^{1}$HMRS were normal. Metabolic work-up showed a mild increase of blood lactic acid and urinary excretion of pyruvic acid and 3-hydroxyvaleric acid. A cardiological monitoring detected progressive hypertrophic cardiomyopathy (HCM). Moreover, since the age of 13 she had been suffering from recurrent myoclonic absence, in the interictal EEG, associated with paroxysmal sharp and slow wave complexes enhanced by the intermittent photo stimulation. Seizures were responsive to levetiracetam and Lamotrigine.

She is currently under treatment with the same supportive vitamin supplementation as her younger brother with a stable clinical course. Written informed consent of patients' parents was obtained. The study was approved by the local Ethics Committee.

### Genetic and metabolic studies

Next-generation sequencing, taking into account the phenotype of the patient, led to the identification of two heterozygous missense variants in the *GTPBP3* gene (NM_032620.4): c.776A > G (p.Asn259Ser) and c.964G > C (p.Ala322Pro) (Fig. 2). Both variants were reported in the gnomAD database with a frequency in the general population of 0.00006164 and 0.0001104, respectively. The first variant was maternally inherited and the second was paternally inherited. Moreover, both variants were present in the oldest sister (Patient 2), clinically affected, and absent in a healthy brother. According to ACMG criteria the p.Ala322Pro is considered likely pathogenic (PM2 supporting, PP1, PP3 moderate, PS3 moderate) whereas the p.Asp259Ser is considered VUS (PM2 supporting, PP1). The p.Asn259Ser variant was never reported in the literature, whereas the p.Ala322Pro variant was reported in three patients with more severe phenotypes[2] and in vitro studies showed deleterious effects of the p. Ala322Pro variant on the GTPase activity of the protein.[8]

The spectrophotometric determination of respiratory chain enzymatic activities[9] in muscle specimen of Patient 1 displayed a reduction of Complex IV (−61%) after normalization with the citrate synthase and compared with the lowest controls value; Complex I activity was at the lower control value. Moreover, the complex V activity measured in fibroblast mitochondria of Patient 1 using a spectrophotometric method,[10] documented a reduction with all the substrates used (succinate: −43%; malate: −38%, pyruvate/malate: −49%) (Fig. 3), suggesting an altered electron flux through the mitochondrial respiratory chain and consequently a mitochondrial disorder.

## Discussion and Review of the Literature

Table 1 (and Table S1 for more details) shows the main clinical characteristics of patients affected by COXPD23 reported by the literature so far. Twenty cases have been described[2–7] clustering around two prevalent phenotypes: an early-onset presentation with severe fatal encephalopathy, cardiogenic shock, and decompensated metabolic acidosis (in 8 patients) and a milder presentation (12 patients) with developmental delay leading to intellectual disability, with (5) or without (8) drug-responsive epilepsy, and exercise intolerance (3). Bilateral thalamic lesions, suggesting metabolic edema, is a possible neuroimaging clue of this condition occurring in 2 out of 2 patients with severe and in 6 out of 10 patients with a milder phenotype undergoing a brain MRI. Increase of lactate in biological fluids and brain (when examined) is the most constant metabolic alteration. In the present cases the biochemical study in muscle tissue and fibroblasts confirmed the relevant impairment of OXPHOS and complex V activity.[2]

Concerning the outcome of patients presenting mild phenotype, in 7 out of 12, the progression of hypertrophic cardiomyopathy (HCM), rather than brain lesions, appeared to affect the outcome of the disease. The oldest subjects reported so far, died at 27 years of age for poly-organ failure.[7]

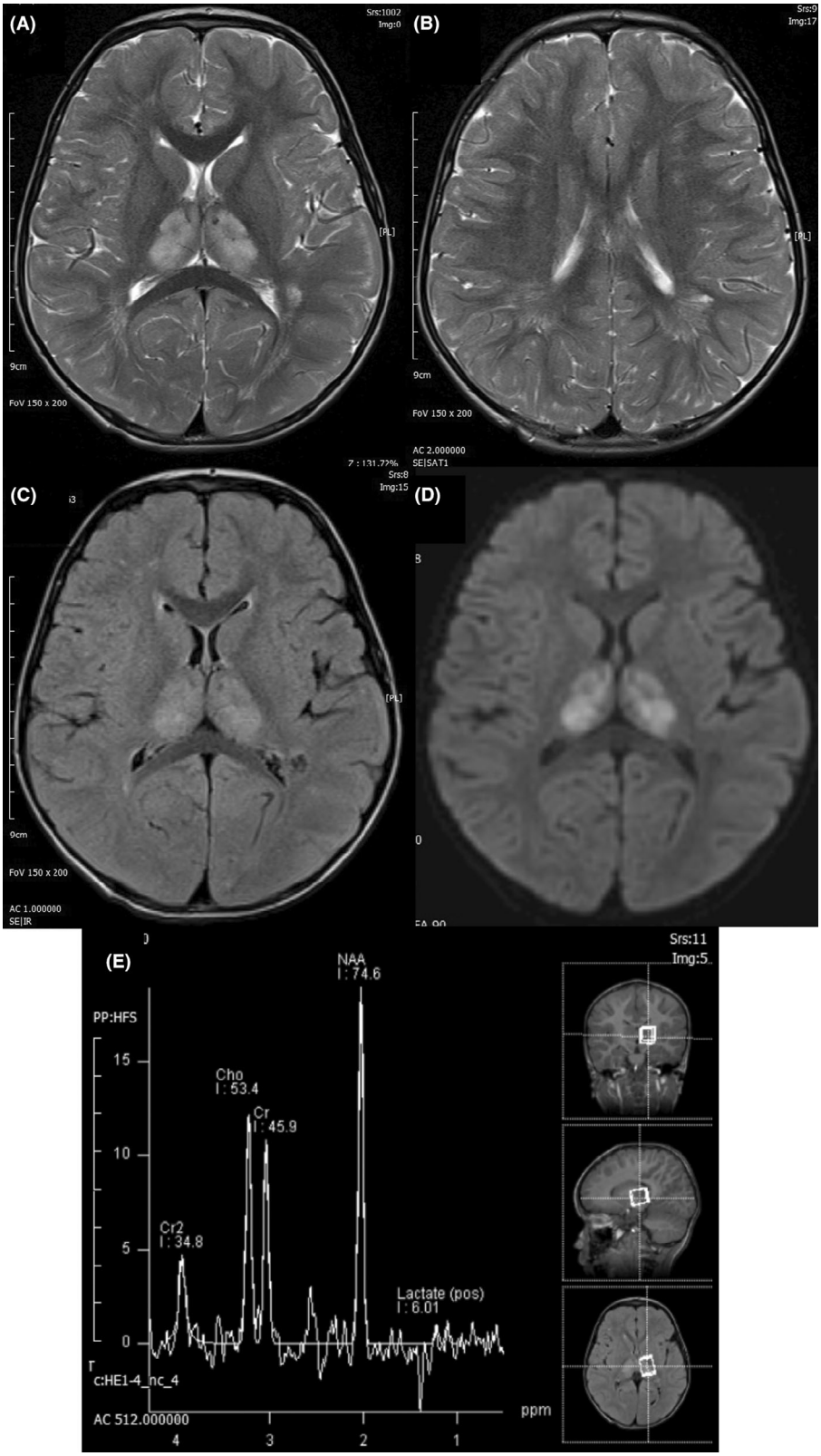


**Figure 1.** Brain MRI of proband (Patient 1). Brain MRI showing axial T2 (A, B), T2-flair (C), DWI (D) bilateral thalamic hyperintensity, and multiple bilateral focal lesions in periventricular white matter. MR spectroscopy reveals lactate peaks (E).

 

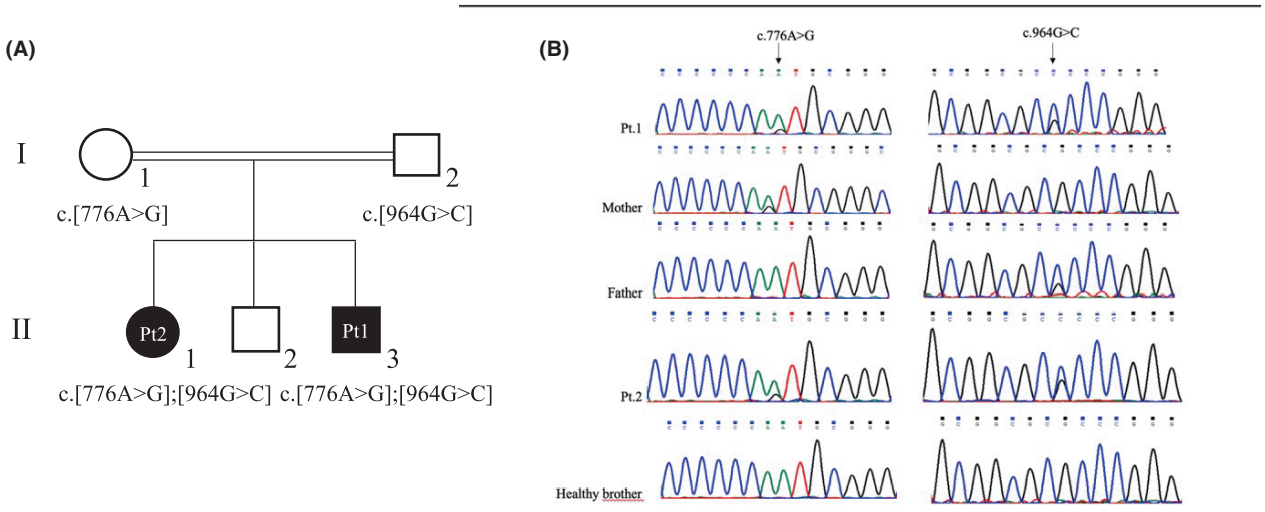


**Figure 2.** Patients' pedigree and variant localization. (A) Pedigree of the investigated family; (B) gDNA electropherograms (Sanger sequencing) of the variants identified in patients. Variants are indicated by black arrows.

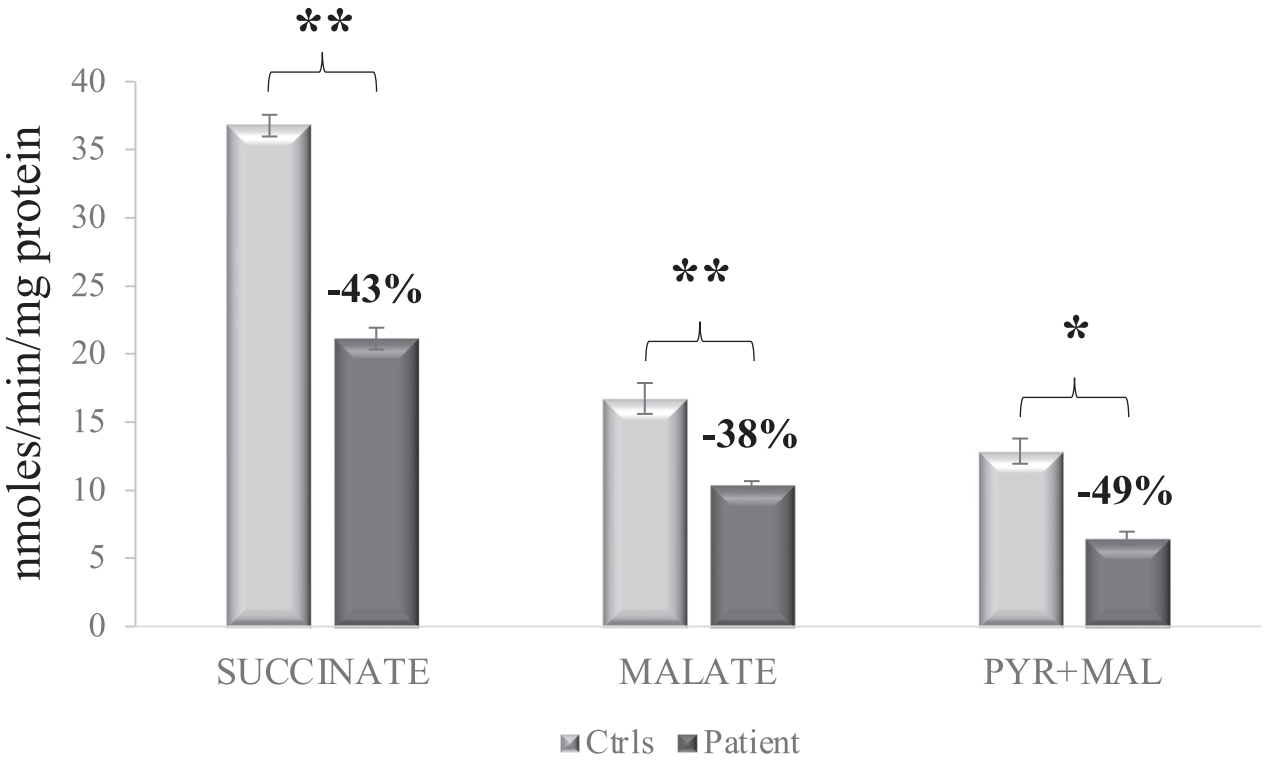


**Figure 3.** ATP synthesis analysis on mitochondria isolated from skin biopsy. Spectrophotometric determination of complex V activity. ATP synthesis in fibroblasts mitochondria of patient 1 was reduced with either substrate used; succinate: −43%; malate: −38%; pyruvate+malate: −49%. Data are presented as mean ± SD. Student's *t* test was used for the analysis of statistical significance. All reported differences are significant (**$p < .001$; *$p < .01$).

The literature review shows that neither the lactate levels nor the MRI findings seem to predict the severity of the disease. Patients with a milder phenotype may not show clinical symptoms until late infancy or early childhood, suggesting that the late onset is the most reliable prognostic factor. A recent review suggested a possible

**Table 1.** Clinical and genetic characteristics of patients with *GTPBP3* pathogenic variants.

| Patient ID | Sex | Age at onset | Presenting symptoms | Plasma lactate | TTE | Brain MRI | Outcomes[a], last follow-up | GTPBP3 mutations |
|---|---|---|---|---|---|---|---|---|
| 1[2] | F | 3.5 months | Poor feeding, failure to thrive, hypoactivity | + | DCM | ND | Died, 8 months, CHF | c.[484G > C]; [673G > A]; [964G > C] p.[Ala162Pro]; [Glu225Lys]; [Ala322Pro] |
| 2[2] | F | Birth | Poor feeding, hypotonic, respiratory failure | + | HCM | ND | Died, 1 day, asystolia | c.[1009G > C]; [1009G > C] p.[Asp337His]; [Asp337His] |
| 3[2] | M | Birth | Poor feeding, hypotonic, CHF, metabolic acidosis | + | HCM | Bilateral hyperintensities in thalamus | Died, 10 months, CHF | c.[665-2delA]; [665-2delA] p.[Ala222Gly]; [Asp223_Ser270del]; [Ala222Gly]; [Asp223_Ser270del] |
| 4[2] | M | 4 weeks | Hypothermia, recurrent apnea metabolic acidosis | + | HCM | Hyperintensities in subthalamic nuclei | Died, 5 weeks, acidosis | c.[424G > A]; [424G > A] p.[Glu142Lys]; [Glu142Lys] |
| 5[2] | F | 1 week | Cardiogenic shock, metabolic acidosis | + | DCM | ND | Died, 9 months, CHF | c.[32_33delinsGTG]; [32_33delinsGTG] p.[Gln11Argfs*98]; [Gln11Argfs*98] |
| 6[2] | F | Birth | Cardiogenic shock, metabolic acidosis | ND | DCM | ND | Died, 10 days, CHF | c.[32_33delinsGTG]; [32_33delinsGTG] p.[Gln11Argfs*98]; [Gln11Argfs*98] |
| 7[3] | M | 17 h | Hypothermia, poor response, respiratory failure, cardiogenic shock, metabolic acidosis | + | Normal | ND | Died, 5 days, CHF | c.[413C > T]; [509_510del] p.[Ala138Val]; [Gln170Glyfs*42] |
| 8[2] | M | 10 years | Intellectual disability, fatigability, visual impairment, slight dyspnea with climbing stairs | + | HCM | Brain H-MRS: lactate peaks | Alive, 14 years | c.[1291dupC]; [1375G > A] p.[Pro430Argfs*86]; Glu459Lys] |
| 9[2] | M | No data | Intellectual disability, fatigability, visual impairment, slight dyspnea with climbing stairs | ND | HCM | Brain H-MRS: lactate peaks; lactate | Alive, 17 years | c.[1291dupC]; [1375G > A] p.[Pro430Argfs*86]; Glu459Lys] |
| 10[2] | M | 2 years | Sudden respiratory failure, CHF | ND | HCM | ND | Alive, 5 years | c.[476A > T]; [964G > C] p.[Glu159Val]; [Ala322Pro] |
| 11[2] | F | 2 years | Developmental delay, epileptic seizures | + | ND | Bilateral hyperintensities in thalamus | Alive, 5 years | c.[770C > A]; [770C > A] p.[Pro257His]; [Pro257His] |
| 12[2] | F | 1 year | Developmental delay, epileptic seizures, hypotonia | + | Normal | Bilateral hyperintensities in thalamus | Alive, 2 years | c.[8G > T]; [934_957del] p.[Arg3Leu]; [Gly312_Val319del] |
| 13[4] | F | 3 weeks | Mental motor retardation, seizure, hearing disability, thrombocytopenia | ND | Normal | Delayed myelination | Alive, 10 years | c.[932C > T]; [932C > T] p.[Pro311Leu]; [Pro311Leu] |
| 14[3] | F | 1 year | Developmental delay, hypotonia | + | ND | Bilateral hyperintensities in thalamus | Alive, 3 years | c.[544G > T]; [c.785A > C] p.[Gly182X]; [Gln262Pro] |

(*Continued*)

 

**Table 1** Continued.

| Patient ID | Sex | Age at onset | Presenting symptoms | Plasma lactate | TTE | Brain MRI | Outcomes[a], last follow-up | GTPBP3 mutations |
|---|---|---|---|---|---|---|---|---|
| 15[3] | F | 1 years | Developmental delay, intellectual disability, fatigability | + | HCM | Bilateral hyperintensities in thalamus | Alive, 3 years | c.[424G > A]; [c.785A > C] p.[Glu142Lys]; [Gln262Pro] |
| 16[5] | F | 5 years | ND | ND | HCM | ND | Alive, 5 years | c.[1289G > A]; [545G > A] p.[Cys430Tyr]; p.[Gly182Glu] |
| 17[6] | M | 3 days | Feeding difficulties, developmental delay, intellectual disability, seizures, visual impairment | + | HCM | Bilateral hyperintensities in thalamus | Alive, 9 years | c.[1102dupC]; [689A > C] p.[Arg368Profs*22]; [Gln230Pro] |
| 18[7] | M | 21 years | Ventricular tachycardia, syncope, CHF | ND | HCM | ND | Died, 27 years, poly-organ failure | c.[181G > C]; [1199C > T] p.[Ala61Pro]; [Thr400Met] |
| 19 (present case 1) | M | 1.6 years | Developmental delay, ataxia, behavioral difficulties | + | Normal | Bilateral hyperintensities in thalamus, multiple WM lesions in cerebral white matter; H-MRS: lactate peak | Alive, 9 years | c.[872A > G]; [1060G > C] p.[Asn291Ser]; [Ala354Pro] |
| 20 (present case 2) | F | 5 years | Developmental delay, intellectual disability, epileptic seizure, behavioral difficulties | + | HCM | normal brain MRI and HMRS | Alive, 15 years | c.[872A > G]; [1060G > C] p.[Asn291Ser]; [Ala354Pro] |

CHF, congestive heart failure; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; H-MRS, high-resolution mass spectrometry; ND, no data; TTE, transthoracic echocardiography.
[a]When the patient deceased, the cause of death has been reported.

predictive value of the genotype.[6] Although ACMG criteria do not allow to consider the variants found in our patient as pathogenic, multiple lines of evidence point toward the deleterious effects of both variants. First of all both variants are very rare in the general population; second the p.Ala322Pro has been previously reported in combination with a p.Glu159Val and functional studies demonstrated the absence of GTPBP3 protein in the patient that was replenished after cDNA transduction of the WT form.[2] In addition, in vitro studies performed on a set of *GTPBP3* variants displayed deleterious effect for the p.Ala322Pro.[8] Lastly, both variants segregate with the disease in family members, being compound heterozygous only in the two affected siblings, whereas the parents were carriers of only one variant and both variants were absent in a healthy brother.

The two siblings here reported support the association between pathogenic variants in compound heterozygosity and milder phenotype, although with intrafamilial variability: indeed, the sister suffers from HCM and epilepsy, while the younger brother has a more severe but isolated neurological phenotype. In patients with biallelic variants in *GTPBP3*, no data are available about the effect of the supplementation of vitamins implied in mitochondrial energetic machinery. At follow-up, after 7 years, we could observe a tangible neurological and neurodevelopmental improvement in our younger patient, while in the older sister, the stabilization of her cardiological picture (HCM). Although unexpected, this positive outcome can hardly be ascribed to vitamin supplementation due to the lack of possible biochemical markers denoting improvement or robust natural history studies.

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## Conflict of Interest Statement

The authors report that there are no competing interests to declare.

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## Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Supplementary Table 1.**

 