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# Whole exome sequencing identifies a homozygous *POLG2* missense variant in an infant with fulminant hepatic failure and mitochondrial DNA depletion

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ABSTRACT

Mitochondrial DNA (mtDNA) depletion syndrome manifests as diverse early-onset diseases that affect skeletal muscle, brain and liver function. Mutations in several nuclear DNA-encoded genes cause mtDNA depletion. We report on a patient, a 3-month-old boy who presented with hepatic failure, and was found to have severe mtDNA depletion in liver and muscle. Whole-exome sequencing identified a homozygous missense variant (c.544C > T, p.R182W) in the accessory subunit of mitochondrial DNA polymerase gamma (*POLG2*), which is required for mitochondrial DNA replication. This variant is predicted to disrupt a critical region needed for homodimerization of the POLG2 protein and cause loss of processive DNA synthesis. Both parents were phenotypically normal and heterozygous for this variant. Heterozygous mutations in *POLG2* were previously associated with progressive external ophthalmoplegia and mtDNA deletions. This is the first report of a patient with a homozygous mutation in *POLG2* and with a clinical presentation of severe hepatic failure and mitochondrial depletion.


## 1. Introduction

Mitochondrial DNA (mtDNA) depletion syndrome (MDS) comprises a genetically and clinically heterogeneous group of autosomal recessive disorders. These disorders are characterized by a profound decrease in mtDNA content that progressively impairs energy production in multiple organ systems [Poulton and Holt, 2009]. MDS has three major clinical presentations that include myopathic, encephalomyopathic, and hepatocerebral forms [Finsterer and Ahting, 2013]. Onset can be in early infancy or childhood, or less frequently in adulthood [El-Hattab and Scaglia, 2013; Suomalainen and Isohanni, 2010].

Nuclear DNA-encoded genes maintain the mtDNA copy number, and mutations in several genes lead to mtDNA depletion. These genes are broadly classified into 2 categories: nuclear genes encoding for enzymes involved in mitochondrial nucleotide synthesis (*TK2*, *SUCLA2*, *SUCLG1*, *RRM2B*, *DGUOK*, and *TYMP*) or those required for mtDNA replication (*POLG* and *C10orf2*) [El-Hattab and Scaglia, 2013]. MtDNA replication is accomplished by DNA polymerase gamma, a heterotrimer consisting of one catalytic subunit of DNA polymerase encoded by *POLG* and a dimer of accessory subunits encoded by *POLG2, a* processivity factor for the DNA polymerase [Copeland, 2014]. Mutations in *POLG* are a common cause of MDS, and lead to broad and variable phenotypes including progressive external ophthalmoplegia (PEO) with both autosomal dominant and recessive inheritance, myopathy, neurological and hepatocerebral forms [Copeland, 2014]. In contrast to *POLG* mutations, only rare cases of heterozygous mutations in *POLG2* causing

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mtDNA depletion have been described in association with autosomal dominant PEO and muscle weakness (MIM 610131, PEOA4) [Longley et al., 2006; Walter et al., 2010; Young et al., 2011].

Here, we report a 3-month-old boy who presented with fulminant neonatal hepatic failure. We found severe mtDNA depletion in liver and muscle, and partial depletion in blood lymphocytes. Whole-exome sequencing (WES) revealed a homozygous missense variant in *POLG2* that is predicted to be deleterious. There was no history of consanguinity, but both parents were heterozygous for this variant. This patient is the first report of *POLG2* homozygous mutation, with associated mtDNA depletion and hepatic failure in infancy.

## 2. Clinical description

The infant was a male born at 37 weeks via cesarean section, after an uncomplicated pregnancy with a birth weight of 3.5 kg. Parents were healthy: both father (age 43) and mother (age 36 year) were from the same town but non-consanguineous. The patient has a healthy 5-year-old brother. The mother had 2 early first trimester miscarriages (6 and 8 weeks), and a stillbirth at 8 months from likely placental insufficiency based on autopsy. Family history in parent's siblings, sibling's children, and grandparents was negative for liver disease, metabolic disorders, ophthalmoplegia, or any other major illness. He was discharged without complications. The infant was healthy until 3 months of age when he presented to another institution with a one-week history of decreased oral intake, difficult breathing, and abdominal distention. On admission he was found to have profound metabolic acidosis with an anion gap of 21 mEq/L (normal 3–11 mEq/L), moderate hyperkalemia, and increased lactate 14 mmol/L (normal 0.5–1.6 mmol/L). Coagulopathy with elevated INR of 3.0 (normal 0.9–1.2) was noted in the setting of elevated liver enzymes (AST 244 U/L, normal 12–38 U/L and ALT 151 U/L, normal 7–41 U/L), elevated total bilirubin level of 8.0 mg/dL (normal 0.3–1.3 mg/dL) with direct bilirubin of 4.6 mg/dL (normal, 0–0.4 mg/dL), elevated bile acids at 229 μmol/L (normal 1–10 μmol/L), normal GGT and decreased albumin 2.8 g/dL (normal, 3.5–5.5 g/dL). He was transferred to our institution for fulminant hepatic failure after intubation for impending respiratory failure. He was subsequently stabilized, metabolically compensated and extubated. At initial consultation, the infant was breathing spontaneously and was alert. He had mild jaundice. Other than an upturned nose and low nasal bridge, there were no dysmorphic features. The abdomen was distended with a firm liver edge felt 1–2 cm below the rib cage; the spleen was not palpable. Subsequent neurological examination was unremarkable with normal muscle tone. Brain MRI showed thinning of the corpus callosum. Myelination pattern was within normal limits for the patient's age. There were subtle diffusion abnormalities involving bilateral hippocampal formations but no evidence of abnormal enhancement or signal abnormalities to suggest an infectious or inflammatory process. In addition, MR spectroscopy demonstrated small lactate peaks in the left basal ganglia and left frontal white matter.

## 3. Methods

### 3.1. Histo-chemical studies

Analysis of muscle using 8-μm-thick sections were carried out as previously described [Tanji and Bonilla, 2001].

### 3.2. Electron microscopy

An ultra-thin section of liver was examined with a JEOL 1011 electron microscope (JEOL, Tokyo, Japan) using magnifications that ranged from 5000 to 60,000X.

### 3.3. Mitochondrial DNA copy number determination

Multiplex Real-time PCR was employed to determine the copy number of mitochondrial DNA in blood leukocytes, muscle, and liver tissues using a TaqMan assay as previously described [Cossarizza et al., 2003] with minor modifications. Briefly, short segments of the ND2 gene in mtDNA and the FASLG gene in nuclear DNA (nDNA) were simultaneously amplified in the presence of fluorescently labeled probes. All amplifications were carried out on a 7500 Fast Real-Time PCR System (Applied Biosystems, USA) and data analyses were performed using 7500 V.O.6 software. To ensure an accurate determination of mtDNA and nDNA copy number, different concentrations of a single reference plasmid that contained one copy of each of the appropriate regions of mtDNA and nDNA were included in the assay, and copy numbers were calculated from the standard curve. The control DNA was from 2 females, aged 14 and 16 years and 1 male, age 6 years, these controls did not have any evidence of mitochondrial DNA depletion syndrome.

### 3.4. Whole-exome sequencing (WES)

WES was performed on whole genome-amplified DNA obtained from the blood of the patient using Illumina Hiseq2500. Hybrid capture method was used to isolate 70 megabases of targeted DNA that included the 16 kb mtDNA and all coding and untranslated exons of 1423 nuclear genes, including 1013 mitochondrial genes from the MitoCarta databases [Pagliarini et al., 2008]. De novo, homozygous or compound heterozygous variants with allele frequencies of less than 1% in the general population were further analyzed. Clinically significant variants were confirmed using Sanger sequencing of the proband and both parents.

### 3.5. Molecular modeling

The human apo POLG2 structure (PDB: 2G4C) [Fan et al., 2006] was examined using PyMol (The PyMOL Molecular Graphics System, Version 1.7.4 Schrödinger, LLC). The mutagenesis wizard was utilized to mutate R182 to W and the rotamer with the fewest clashes was chosen.

## 4. Results

Mitochondrial and other metabolic disorders were in the differential diagnosis based on the clinical presentation and initial laboratory findings. Further work-up included infectious screen, complete metabolic panel, liver and muscle biopsies, mitochondrial DNA studies on peripheral blood, muscle and liver tissue, and WES. An infectious etiology for the liver failure was ruled out by testing for Adenovirus, Parvovirus, CMV, EBV, RPR, HSV 1 + 2, Hepatitis A, Hepatitis B, Hepatitis C and HIV 1 + 2. Extensive metabolic work-up was conducted. Ammonia was normal but pyruvate was elevated at 0.23 mM/L (normal 0.04–0.13 mM/L). Although the total and free carnitine levels were normal, elevations of many long chain and long chain 3-hydroxyacylcarnitine were consistent with mitochondrial dysfunction. Plasma amino acid analysis showed increased levels of methionine at 558 μM (normal 10–60 μM) and tyrosine at 328 μM (normal 30–140 μM) in the absence of plasma succinylacetone, consistent with significant hepatic dysfunction. Also, urinary organic acid analysis showed increased levels of lactate and pyruvate, likely secondary to impaired mitochondrial function. Presence of normal levels of plasma very long chain fatty acids reduced the likelihood of many peroxisomal disorders and the