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# A new mutation in *MT-ND1* m.3928G>C p.V208L causes Leigh disease with infantile spasms

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ABSTRACT

New mutations in mitochondrial DNA encoded genes of complex I are rarely reported. An infant developed Leigh disease with infantile spasms. Complex I enzyme activity was deficient and response to increasing coenzyme Q concentrations was reduced. Complex I assembly was intact. A new mutation in *MT-ND1* m.3928G>C p.V208L, affecting a conserved amino acid in a critical domain, part of the coenzyme Q binding pocket, was present at high heteroplasmy. The unaffected mother did not carry measurable mutant mitochondrial DNA, but concern remained for gonadal mosaicism. Prenatal testing was possible for a subsequent sibling. The ND1 p.V208L mutation causes Leigh disease.



## 1. Introduction

Subacute necrotizing encephalomyelopathy, also known as Leigh disease, is a progressive neurodegenerative disorder named after Denis Archibald Leigh, who in 1951 first described the neurodegenerative condition with characteristic pathological lesions including capillary proliferation (Leigh, 1951). These lesions occur in variable combinations in the basal ganglia, thalami, dentate nucleus, and brain stem gray matter. The variable clinical presentation of Leigh disease includes movement disorders, rigidity, tremor, chorea, hypokinesia, myoclonus, loss of appetite, vomiting, irritability, continuous crying and seizures. The disorder is caused by energetic failure on a cellular level. Multiple genetic causes have been described in Leigh disease involving all aspects of energy generation including deficiencies of pyruvate dehydrogenase or of subunits of respiratory chain complexes, defects in mitochondrial DNA, its transcription and translation, and the assembly of the components of the respiratory chain complexes. Mutations in mitochondrial DNA (mtDNA) can cause Leigh disease when sufficiently abundant or severe enough to impair oxidative phosphorylation early in life (DiMauro and Schon, 2008). Enzymatically, the most common cause of Leigh disease is an isolated deficiency of complex I (Rahman et al., 1996). Complex I is a large complex composed of 39 nuclear-encoded subunits and seven mtDNA-encoded subunits (Brandt, 2006). Causes for complex I deficiency include defects in mtDNA, its transcription and translation, defects in the individual components including most of the nuclear-encoded subunits, and in multiple assembly factors (Hoefs et al., 2012; Janssen et al., 2006; Triepels et al., 2001; Tucker et al., 2011). Mutations in each of the seven mtDNA-encoded subunits have been reported. Together, they account for 20 to 30% of the causes of isolated complex I deficiency (Malfatti et al., 2007; Swalwell et al., 2011).

Despite this frequency, only a few mutations are known for each of the individual subunits. The ND1 gene harbors a common mutation at position 3260 that causes Leber hereditary optic neuropathy (LHON). Beyond LHON, only eight missense mutations and two mutations affecting the initiator methionine codon have been recognized in ND1 (Supplementary material Table 1) (Blakely et al., 2005; Blakely et al., 2006; Campos et al., 1997; Caporali et al., 2013; Horváth et al., 2008; Kirby et al., 2004; Malfatti et al., 2007; Morava et al., 2006; Musumeci et al., 2000; Spruijt et al., 2007; Wong, 2007). This contrasts to the

*Abbreviations:* EEG, electroencephalogram; PAGE, polyacrylamide gel electrophoresis; mtDNA, mitochondrial DNA; LHON, Leber hereditary optic neuropathy; MELAS, mitochondrial encephalomyopathy with lactic acidosis and stroke like episodes; HRM, high resolution melt; PCR, polymerase chain reaction.

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substantial genetic heterogeneity observed in most of the nuclear genes (Tucker et al., 2011). Identifying a new mutation as pathogenic in a protein-coding gene on mtDNA is difficult given the large number of polymorphisms that can occur. For this reason, criteria for proof of pathogenicity have been developed (Mitchell et al., 2006; Wong, 2007). The identification of a mutation as pathogenic is clinically important. It provides a diagnosis for the affected family. We describe here a child who presented with Leigh disease and infantile spasms, caused by a newly identified pathogenic mutation in *MT-ND1*. Identification of the pathogenic nature was complicated by apparent homoplasmy on Sanger sequencing and the apparent conservative nature of the amino acid change. Careful review allowed the recognition of its pathogenic nature.

## 2. Case report

This boy was the second child of non-consanguineous parents, born at 36 weeks following an uncomplicated pregnancy and uneventful neonatal period. In the third month of life, he became irritable, but developmentally was smiling. In his fourth month, his irritability, oral intake and head control all worsened. Microcephaly, failure to thrive and hypotonia were noted. He developed stridor with feeds. At age five months, his demeanor changed to quiet and lethargic, with intermittent fits of screaming. He stopped smiling, crying, and visual tracking, and his feeding ability continued to decline. At age seven months his eyes began to oscillate and he became minimally responsive. He had axial hypotonia, exaggerated patellar reflexes, absent Achilles tendon reflexes, a positive Babinski sign, dystonic posturing of the hands, rigidity in his arms, and little spontaneous movements. He also developed daily non-suppressible twitching of his left foot, then his right leg, then both legs lasting for 10 sec. An electroencephalogram (EEG) at five months of age had shown diffuse slowing, but no epileptiform discharges. A new EEG at seven months of age showed further slowing of the background and appearance of multifocal epileptiform discharges. He was started on levetiracetam. Over the next two weeks, the seizures evolved, occurring multiple times daily and involving both arms jerking forward every 8 sec in 4–8 minute clusters. On a follow-up study, the interictal EEG showed hypsarrhythmia. His seizures at that time correlated with a high amplitude slow wave followed by a 1–2 second electrodecrement. He was switched from levetiracetam to topiramate, with some reduction in the spasms. At seven months, biopsies of skin, muscle and liver were obtained. He was unable to be extubated postoperatively, and died at age eight months following withdrawal of care after recognition of the rapid progression of disease.

Serum lactate was increased at 10.3 mM (normal 0.5–2.2), and pyruvate was 0.53 mM (normal 0.08–0.16), yielding a lactate/pyruvate ratio of 19.4. Serum amino acids showed increased alanine at 625 μM (normal 143–439), and the other amino acids were normal. Urine organic acids showed increased lactate, ketones, and Krebs cycle metabolites. Normal results included serum hepatic enzymes, carnitine levels, thyroid hormone studies, and enzyme activities of biotinidase and pyruvate dehydrogenase complex. Minor elevations were noticed in the levels of serum creatine kinase at 249 IU/L (normal 24–174), serum α-fetoprotein at 62.5 ng/mL (normal 0–15), and serum ammonia at 103 μM (normal 0–37). Mitochondrial genetic studies included a normal common mtDNA mutation panel and normal sequencing of the *PDHA1*, *SURF1*, *POLG1*, and *DGUOK* genes.

Magnetic resonance imaging of the brain at four months showed increased T2 signal with restriction of diffusion bilaterally in the thalami, caudate heads, globi pallidi, putamen, midbrain, middle cerebral peduncles and red nuclei (Fig. 1A). At age eight months, progressive volume loss in the basal ganglia and thalami was seen, and new symmetrically increased T2 signal was present in the cortical and white matter of the frontal and anterior temporal lobes bilaterally (Fig. 1B).

## 3. Methods

All subjects were consented on an IRB approved protocol. Respiratory chain enzymes were measured in frozen skeletal muscle homogenate in Ghent as described (Seneca et al., 2005). Respiratory chain enzyme activities in cultured skin fibroblasts were measured in Colorado as described (Kendrick et al., 2011; Rahman et al., 1996). The natural log of the ratios of control samples was normally distributed and the results were expressed as Z-scores. To investigate the mechanism of pathogenicity, complex I was assayed when incubated with increasing concentrations of coenzyme Q1 from 20 to 500 μM spectrophotometrically on a Cary 300 spectrophotometer at 30 °C in post 600 g supernatants of fibroblasts from the proband and two controls. The intactness of the assembly of complex I was evaluated in skeletal muscle and in a fibroblast pellet using blue native PAGE gel analysis followed by in gel activity staining as described (Smet et al., 2005, 2011; Van Coster et al., 2001).

The mtDNA was Sanger sequenced and the amount of mtDNA was quantified by qPCR (Bai and Wong, 2005; Landsverk et al., 2012). The percentage mutant mtDNA for the m.3928G>C mutation was quantified by high resolution melt analysis (HRM). HRM is usually used to screen for the presence of point mutations. However it can also be used to determine mutant load in a mosaic genotype by performing melt-curve analyses of serial dilutions of a mutant sample diluted with a control sample (Pichler et al., 2009). Serial dilutions were made of a 100% mutant load sample (or as close to 100% as possible) with a control sample in 10% increments from 0% to 100%. These calibration samples, a no-DNA control and the patient samples were used as templates in the HRM reaction. Each reaction mixture contained 1 μL of DNA solution (50 ng), 200 nmol/L of each primer [m.3839F 5′TCATGACCCTTGGCCATAAt3′ and m.4063R 5′CAGGGGAGAGTGCGTCATA3′], 10 μL of LightCycler LC480 High Resolution Melting Master (Roche), 3 mmol/L $MgCl_2$, and water to a final volume of 20 μL (Pichler et al., 2009). PCR conditions were: 95 °C for 10 min, followed by 30 cycles of 10 sec at 95 °C, 10 sec at 55 °C, and 30 sec at 72 °C. After amplification, the PCR product was denatured at 95 °C for 1 min and cooled down to 40 °C to allow heteroduplex formation. The final HRM step was performed from 60 °C to 95 °C with an increase of 1 °C per second. The HRM curve analysis was performed using Gene Scanning software (Roche). For sample analysis, melting curves were normalized, temperature-adjusted, and, finally, a difference plot was generated using the 0% sample as baseline. The accuracy and sensitivity of the method is dependent on the increments of the dilution and the clarity of the melt curves traces observed. For this assay the standard deviation was determined at 2.5%, and the sensitivity was below 2% heteroplasmy. The degree of heteroplasmy was measured in skeletal muscle, liver, blood, and cultured skin fibroblasts of the proband (II-2), in blood and urine of the mother (I-1), in blood of the brother (II-1), and in chorionic villus and blood of the subsequent sibling (II-3) (Fig. 2).

## 4. Results

Biopsies of the patient's muscle and liver showed normal histology using both light and electron microscopy and also showed normal COX staining. Enzyme assays of the proband's respiratory chain complexes revealed an isolated deficiency of complex I activity in both skeletal muscle and cultured skin fibroblasts (Table 1). To identify the pathogenetic mechanism of the deficient activity of complex I, its assembly was evaluated by blue native PAGE. This showed an intact complex stained with nitroblue tetrazolium (Fig. 3). In contrast, the enzyme kinetics with coenzyme Q1 were abnormal and the activity did not increase with a Vmax at 77 μM (Fig. 4).

Quantification of mtDNA in skeletal muscle from the proband was 109% of control (1908, control 1746 ± 361 copy number of mitochondrial DNA per haploid copy number of nuclear DNA), and in liver 70% of control (2187 control 3134 ± 733 copy number of