Case Report

Mitochondrial Ultrastructural Defects in NDUFS3-Related Disorder

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Complex I, the largest multisubunit enzyme complex of the respiratory chain, has a vital role in the energy production of the cell, and the clinical spectrum of complex I deficiency varies from severe lactic acidosis in infants to muscle weakness in adults. Pathogenic variants of NDUFS3 (constitutes the catalytic core of the complex I) have been reported in a small number of patients with variable phenotypes. We describe a girl with a history of infantile-onset nonepileptic myoclonus, who developed myopathy at the age of 2 years. Next-generation sequencing revealed compound heterozygous for two variants in the NDUFS3 gene. The electron-microscopic study of the skeletal muscle showed an increase in the number of mitochondria inside the myofibers; mitochondria were variably enlarged with some irregularity and were aligned perpendicular to the myofibrils in a stacked-up manner. This is the first description of mitochondrial ultrastructural abnormality in an individual with NDUFS3-related disorder.

**KEYWORDS:** Complex I, mitochondria, myoclonus, myopathy, NDUFS3

**INTRODUCTION**

Pathogenic variants in NDUFS3 (NADH dehydrogenase iron-sulfur protein 3) that codes for a subunit of complex I complex have been reported in a small number of individuals with variable phenotypes. Previous reports did not describe histopathological features of NDUFS3-related disorder, including ultrastructural defects in skeletal muscles. We report clinicopathological features of a 2-year-old girl with novel compound heterozygote variants in the NDUFS3 gene.

**CASE REPORT**

She was born at 38 weeks of gestation to a para two mother via vaginal delivery with no perinatal complications. Parents are nonconsanguineous. Her growth parameters were normal. At 3 months of age, she presented with intermittent episodes of generalized whole body jerking lasting for 5–10 s at a time over the course of 1 hour. Her neurological examination was normal, and a video electroencephalogram captured multiple events without epileptiform activity. These repetitive trains of jerks during wakefulness, without definite activation, were consistent with generalized intermittent myoclonus. Reflex or action myoclonus was not present. Extensive workup at that time, including brain magnetic resonance imaging (MRI) and single nucleotide polymorphism microarray, were normal. She had been treated with levetiracetam for the nonepileptic myoclonic activity for 1 year without significant benefit and was subsequently weaned off without any adverse effect. She continued to have serial head and shoulder jerking episodes approximately once per day.

Parents also noticed that she was dragging her feet and was struggling to climb stairs at the age of 2 years. Furthermore, she also had frequent choking during feeding. Her neurological examination was significant for mild generalized weakness of limb, neck, and facial muscles. Mild generalized truncal and appendicular hypotonia was noted with more involvement of the upper extremities than the lower. Deep tendon reflexes were hypoactive. The examination did not reveal ptosis, nystagmus, optic atrophy, vision loss, muscle wasting.
dystonia, or ataxia. Repeat neuroimaging and metabolic workup, including lactate (plasma and CSF), pyruvate, amino acids (plasma and urine), and urine organic acids, were unremarkable. Targeted next-generation sequencing with copy number analysis of a panel of genes based upon phenotype was performed as a trio with samples of both parents utilized. The patient was noted to be compound heterozygous for two variants in the NDUSF3 gene (a mitochondrial complex I gene): c.424 C > T (p.R142C) [inherited from mother] and c.540 C > A (p.N180K) [inherited from father]. Neither variant was observed at a significant frequency in large patient cohorts. In-silico analysis, including protein predictors and evolutionary conservation, supported a deleterious effect.

A muscle biopsy was then performed due to symptoms of weakness and hypotonia, as well as to correlate with the finding of the genetic testing. Light microscopic examination of the skeletal muscle was unremarkable [Figure 1]. Specifically, no significant myofiber size variation, inflammation, abnormal accumulations or inclusions, ragged-red fibers, ragged-blue fibers, or COX-negative fibers were seen. Oil red O stain was unremarkable with no significant lipid accumulation. Myofibers type and distribution, and NADH-TR reaction were unremarkable. No neurogenic changes were identified. However, the electron-microscopic examination of the skeletal muscle showed an increase in the number of mitochondria inside the myofibers [Figure 2]. The mitochondria were also variably enlarged with some irregularity, but no abnormal shapes, inclusions, or accumulations were identified. The mitochondria had lost their usual orientation and were aligned perpendicular to the myofibrils in a stacked-up manner. A few lipid droplets were present,

Figure 1: Light-microscopic findings: All major histochemical stains and reactions, including hematoxylin and eosin (A), Gomori trichrome (B), ATPase at pH 4.6 (C), NADH-TR (D), succinic dehydrogenase (E), and cytochrome oxidase (F) are unremarkable. (Original magnification: ×200)
with no increase in the lipid content. Mitochondrial electron transport chain enzymes revealed 65% of mean activities in complex I activity. However, other complex activities were 82–118% of the control [Table 1]. A mitochondrial DNA depletion syndrome was excluded by a quantitative PCR examination.

**Discussion**

Although mutations in *NDUFS3* were first reported in 2004, less than five patients have been reported so far, and no report exists regarding presented features here such as nonepileptic myoclonus and myopathy [Table 2]. Benit et al. first reported a child with compound heterozygote variants of *NDUFS3*. He had late-onset Leigh syndrome and developed a persistent stiff neck at the age of 9 years, which subsequently progressed to severe axial dystonia with oral and pharyngeal motor dysfunction, dysphagia, optic atrophy, and tetraparesis. Brain MRI revealed high T2 signal intensity in the putamen, white matter, and the brain stem. CSF lactate was elevated, and a complex I deficiency was identified in the skeletal muscle biopsy. During the second decade, he died from a rapid multisystem dysfunction. Pagniez-Mammeri et al. reported a novel heterozygous missense transition in *NDUFS3* in a patient with unspecified encephalomyopathy. Haack et al. identified another patient with developmental delay, hypotonia, lactic acidosis, and rapid progression of the disease who had homozygous *NDUFS3* pathogenic variants. Complex I activity in muscle and fibroblast was noted to be 28% and 36% of the control, respectively. Lou et al. recently reported an infant who had a typical onset of Leigh syndrome at the age of 7 months. Clinical and radiologic deterioration was evident with lactic acidosis, and the patient died at 2 years of age. The patient’s lymphoblastoid cells showed diminished NDUFS3 and complex I assembly compared to the control.

Early-onset myopathy secondary to defects involving Complex I is extremely rare. Additionally, diagnosis by muscle biopsy is difficult by conventional staining due to the absence of typical ragged-red fibers of mitochondrial myopathy. This suggests the importance of electron microscopical examination of the muscle specimen. Although mitochondrial proliferation was a significant clue for the ultimate diagnosis for this patient, it is a nonspecific finding for mitochondrial myopathies.

In summary, this is the fifth patient with a reported *NDUFS3* mutation, and for the first time, we reported morphological changes of mitochondria, which we suspect are due to the identified variants. This case also describes a novel phenotype with myoclonus and early onset myopathy. This patient still remains at risk

![Figure 2: Electron-microscopic findings: Enlarged and increased numbers of mitochondria stacked up amongst the myofibrils (arrows) are seen. No significant subsarcolemmal accumulation of mitochondria is identified (block arrow). Only a few lipid droplets (arrowheads) are present. (Original magnification: ×2500)](#)

| ETC complexes | Patient (% of mean) | Control ± SD* |
|---------------|--------------------|---------------|
| NADH: Ferricyanide dehydrogenase | I | 234 (65) | 360.4 ± |
| NADH: cytochrome c reductase (total) | I + III | 26.9 (95) | 28.4 ± 6.1 |
| NADH: cytochrome i reductase (Rotenone sensitive) | I + III | 8.87 (102) | 8.7 ± 3.9 |
| Succinate dehydrogenase | II | 10 (104) | 9.6 ± 3.0 |
| Succinate: cytochrome c reductase | II + III | 4.97 (118) | 4.2 ± 1.2 |
| Cytochrome c oxidase | IV | 21.1 (52) | 40.3 ± 15.5 |
| Citrate synthase | | 234 (80) | 293.1 ± 68.0 |

*nmol/min/mg protein
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for neuroregression as delayed, and a later-onset with rapid progression of the disease has been described. Although the patient remains on several high dose supplements (“mitochondrial cocktail”), a definitive preventive or curative therapy for these conditions is still elusive.

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Conflicts of interest
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Table 2: Phenotype and genotype spectrum associated with NDUFS3-related disorder

| Publication          | Age  | Sex | Phenotype                          | Genotype (NDUFS3 gene)                  | Mitochondrial morphology |
|----------------------|------|-----|------------------------------------|----------------------------------------|--------------------------|
| Benit et al.         | 9 yrs|M    | Late-onset Leigh syndrome. Death at 13 years of age | Compound heterozygotes (R199W and T145I mutations) | Not available |
| Pagniez-Mammeri et al | UNK  | UNK | Unspecified encephalomyopathy      | Heterozygous missense (P223L) variant Homozygous R199W mutation. | Not available |
| Haack et al.         | <3 yrs old | UNK | Developmental delay, hypotonia, lactic acidosis, rapid progression of the disease | Homozygous R199W mutation. | Not available |
| Lou et al.           | 7 months | M   | Early-onset Leigh syndrome at 7 months and died at 2 years of age | Compound heterozygotes (R140W and R199W) | Not available |
| Samanta et al. (This publication) | 18 days | F   | Neonatal onset nonepileptic myoclonus and early myopathic features at the age of 2 years | Compound heterozygous (R142C and N180K) Increase in the number of mitochondria inside the myofibers. The mitochondria were also variably enlarged with some irregularity, but no abnormal shapes, inclusions, or accumulations were identified. The mitochondria were aligned perpendicular to the myofibrils in a stacked-up manner. | Not available |

UNK = unknown; M = male; F = female