

Widening the Heterogeneity of Leigh Syndrome: Clinical, Biochemical, and Neuroradiologic Features in a Patient Harboring a *NDUFA10* Mutation

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Abstract Leigh syndrome (LS) is an early-onset progressive neurodegenerative disorder, characterized by a wide clinical and genetic heterogeneity, and is the most frequent disorder of mitochondrial energy production in children. Beside its great variability in clinical, biochemical, and genetic features, LS is pathologically uniformly characterized by multifocal bilateral and symmetric spongiform degeneration of the basal ganglia, brainstem, thalamus, cerebellum, spinal cord, and optic nerves. Isolated complex I deficiency is the most common defect identified in Leigh syndrome. In 2011, the first child with a mutation of *NDUFA10* gene, coding for an accessory subunits of complex I, was described. Here, we present an additional description of a child with Leigh syndrome harboring a homozygous mutation in *NDUFA10*, providing insights in clinical, biochemical, and neuroradiologic features for future earlier recognition.

Introduction

Defects in mitochondrial energy production are the most frequent group of inherited metabolic disorders, with an incidence of at least 1 in 5,000 live births (Skaldal et al. 2003). The most common clinical manifestation of mitochondrial disease in children is Leigh syndrome (Leigh 1951) (LS), an early-onset progressive neurodegenerative disorder, characterized by a wide clinical and genetic heterogeneity. Clinical presentation frequently includes psychomotor delay or regression, acute neurological or acidotic episodes, hypotonia, ataxia, spasticity, movement disorders, and corresponding anomalies of the basal ganglia and brainstem on MRI (Tetreault et al. 2015). Up to now, more than 75 disease genes have been linked to LS, involving the oxidative phosphorylation (OXPHOS) system, the pyruvate dehydrogenase complex (PDHc), and multiple other enzymes mostly linked to OXPHOS system or to a broader pathway of energy generation (Lake et al. 2016).

Isolated complex I deficiency is the most common biochemical defect identified in LS, accounting for approximately 25% of all children with OXPHOS deficiencies (Smeitink et al. 2001). Complex I (NADH: ubiquinone oxidoreductase) is the first and the largest multiheteromeric respiratory chain (RC) enzyme complex being composed of 45 protein subunits (7 of which encoded by mtDNA), with a pivotal role in ATP synthesis, transferring electrons from reduced NADH to coenzyme Q10, and pumping protons across the inner mitochondrial membrane. Moreover, its maturation, assembly, and stability rely on a number of ancillary proteins. Up to now, pathogenic mutations have been reported in all the seven mtDNA-encoded subunits

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and in several nuclear DNA-encoded subunits or assembly factors, including NADH dehydrogenase 1 α subcomplex 10 (NDUFA10) (Lake et al. 2016). NDUFA10 is located in the hydrophobic protein fraction and is homologous to deoxyribonucleoside kinases, but it has no enzymatic activity (Elurbe and Huynen 2016). The function of this accessory subunit is still not clear, but it has been suggested to have a role in the transfer of protons across the inner membrane (Hoefs et al. 2011). Until now, only two cases of pathogenic *NDUFA10* mutations have been reported (Hoefs et al. 2011; Haack et al. 2012), both presenting with LS and complex I deficiency. In the present paper, we further characterize the clinical, biochemical, and neuroradiologic features of a child with LS due to *NDUFA10* mutation.

Case Report: Biochemical and Molecular Analyses

Case Report

We present the case of an Italian boy, the third child of consanguineous parents (third degree cousins), born at 41 weeks of gestation after a normal pregnancy. Family history is remarkable for a paternal cousin who died at the age of 4 months for an unspecified cardiomyopathy, two maternal cousins manifesting an unspecified myopathy, and another cousin affected by an early-onset epileptic encephalopathy. In the proband, birth weight was 3,460 g and length 52 cm. The neonatal period was uneventful, but nystagmus was reported since the first month of life. He reached all his milestones but with a slight delay. At the age of 2 years and 8 months, after a febrile illness, he presented acute-onset ataxia with severe hyposthenia and inability to stand and walk. This condition lasted for approximately a week and was followed by a slow and partial neurological improvement but without complete recovery. A first brain MRI was performed at that age and showed rather symmetrical lesions of the putamina and globi pallidi, characterized by swelling, necrotic changes, and mixed cytotoxic and vasogenic edema. Asymmetric focal lesions of the caudate nuclei were also noted, as well as focal involvement of the left cerebral peduncle (Fig. 1). MR spectroscopy detected a lactate doublet peak.

The child was then referred to our attention for further evaluation. On clinical examination, we observed a generalized hypotonia with muscular hypotrophy especially in the lower limbs; tendon reflexes were slightly increased, and oscillatory nystagmus was present. Gait ataxia and clumsiness were also observed. The psychomotor evaluation showed a moderate delay in his development. Blood and urine tests were unremarkable with the exception of levels of plasma lactate slightly above normal range (39.2 mg/dl, normal range 8–22 mg/dl). The clinical,

neuroimaging, and biochemical features strongly suggested a LS, and a defect of PDHc or OXPHOS pathway was considered. At first, on the basis of his clinical presentation and mostly of intermittent ataxia, molecular analysis on PDHc genes was performed and thiamine-biotin therapy was started.

In the next year, the child presented a slight improvement in neurological development with a partial recovery of gait and language. However, severe difficulties in oculomotor abilities persisted with extrapyramidal signs such as dystonic features, nystagmus, and ataxia. Parents reported intermittent relapses, usually associated with febrile illness, with only partial recovery after each episode, resulting in a progressive neurologic deterioration. Cardiac evaluations were always unremarkable. A second brain MRI, performed at the age of 3 years and 6 months, showed the chronic evolution of the putaminal lesions and the presence of new symmetric lesions of the caudate heads and Meynert basal nuclei and asymmetric lesions at the level of the right globus pallidus and cerebral peduncle. MR spectroscopy, performed on the right caudate nucleus head, revealed a lactate doublet peak (Fig. 2).

Biochemical and Molecular Analyses

Routine morphology, histochemical stains for oxidative metabolism, and spectrophotometric determination of mitochondrial RC complexes in muscle homogenate were assayed according to standard methods.

Deltoid muscle biopsy was performed under the hypothesis of a mitochondrial disease: histological examinations showed only a slight reduction of cytochrome oxidase activity. Biochemical analyses of the respiratory chain revealed a reduction of complexes I (1.12 mmol/min/g tissue; normal range 1.56–2.60), I + II (0.08 mmol/min/g tissue, normal range 0.11–0.25), and II + III (0.02 mmol/min/g tissue, normal range 0.05–0.08) (details available on request).

Genomic blood and muscle DNA from the patient were purified by standard methods and analyzed by Sanger sequencing to exclude pathogenic variants in mitochondrial DNA (mtDNA) and *PDHA1* gene. Then, we used a customized targeted resequencing panel able to investigate the coding regions of 168 genes linked to mitochondrial disorders applying standard methodologies as outlined elsewhere (Mignarri et al. 2016). The identified pathogenic variants in *NDUFA10* (NM_004544.3) were confirmed by Sanger sequencing and were tested for segregation in the family.

Molecular analysis identified the homozygous mutation c.296G>A in exon 3 of the *NDUFA10* gene (Fig. 3a), causing a substitution of the extremely conserved throughout evolution glycine 99 for glutamate (p.G99E) (Fig. 3b). Both parents carried the heterozygous c.296G>A variant

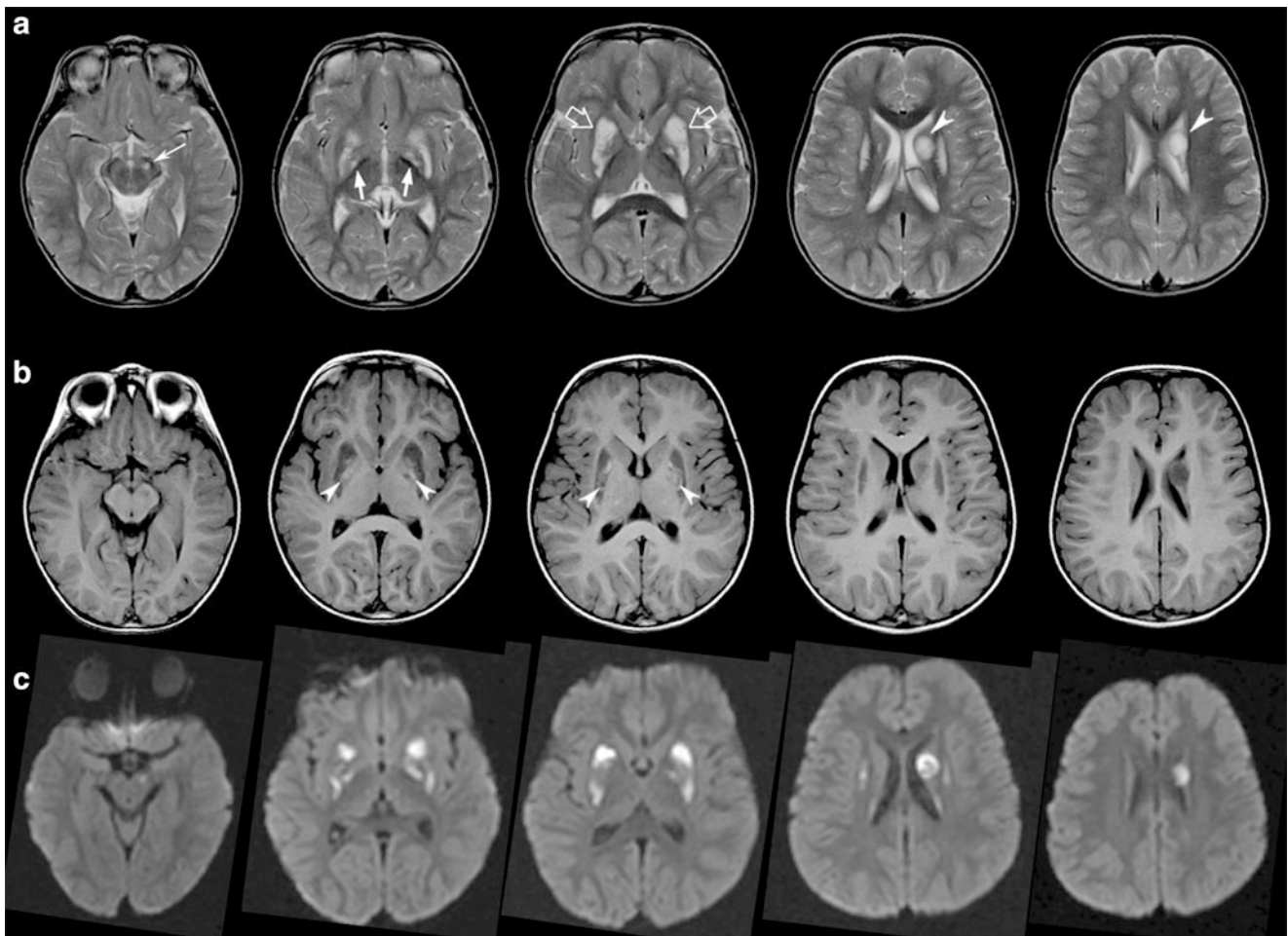


Fig. 1 Brain MRI performed at 2 years and 8 months. **(a)** Axial T2-weighted images demonstrate symmetric swelling and T2 hyperintensity of the putamina (*empty arrows*) and asymmetric involvement of globi pallidi (*arrows*), left cerebral peduncle (*thin arrows*), and left caudate nucleus (*arrowheads*). Note that the lesion of the left caudate

head is round and well demarcated, with moderate mass effect. **(b)** Hyperintense necrotic foci are present at the level of the medial part of the putamina and globi pallidi on T1-weighted images (*arrowheads*). On diffusion-weighted images **(c)**, most of the lesions are characterized by restricted diffusion in keeping with cytotoxic edema

which was predictably damaging when examined in silico using Polyphen2. The mutation has been already reported in a child presenting with severe complex I deficiency (Haack et al. 2012).

Discussion

The case described here further supports the role of *NDUFA10* mutations as a rare cause of LS with reduced complex I activity, providing new data on the related clinical, biochemical, and neuroradiologic features and contributing to the genotype-phenotype correlation in LS.

LS is the most frequent disorder of mitochondrial energy production in children, as the result of a heterogeneous group of defects in the OXPHOS or in the PDHC systems. Therefore, it is unsurprising that a wide variety of multi-

systemic symptoms may be reported, including cardiac, hepatic, gastrointestinal, and renal dysfunction (Lopez et al. 2006; Monlleo-Neila et al. 2013). The age of presentation is typically set within 2 years of age (Lake et al. 2016), but there is a wide range of disease onset. As in the case here presented, affected children generally have an acute presentation of symptoms, often during an infection, after an initial period of normal development. Progression is typically episodic, with relapses commonly related to febrile illnesses; often, there may be some initial recovery, but never back to the baseline (Lake et al. 2016; Rahman et al. 1996; Sofou et al. 2014). The overall survival is variable, but most patients die after few years from diagnosis. Poor prognosis predictors include age at disease onset less than 6 months, failure to thrive, seizures resistant to pharmacologic treatments, brainstem lesions on neuroimaging, and intensive care unit admission (Lopez et al.

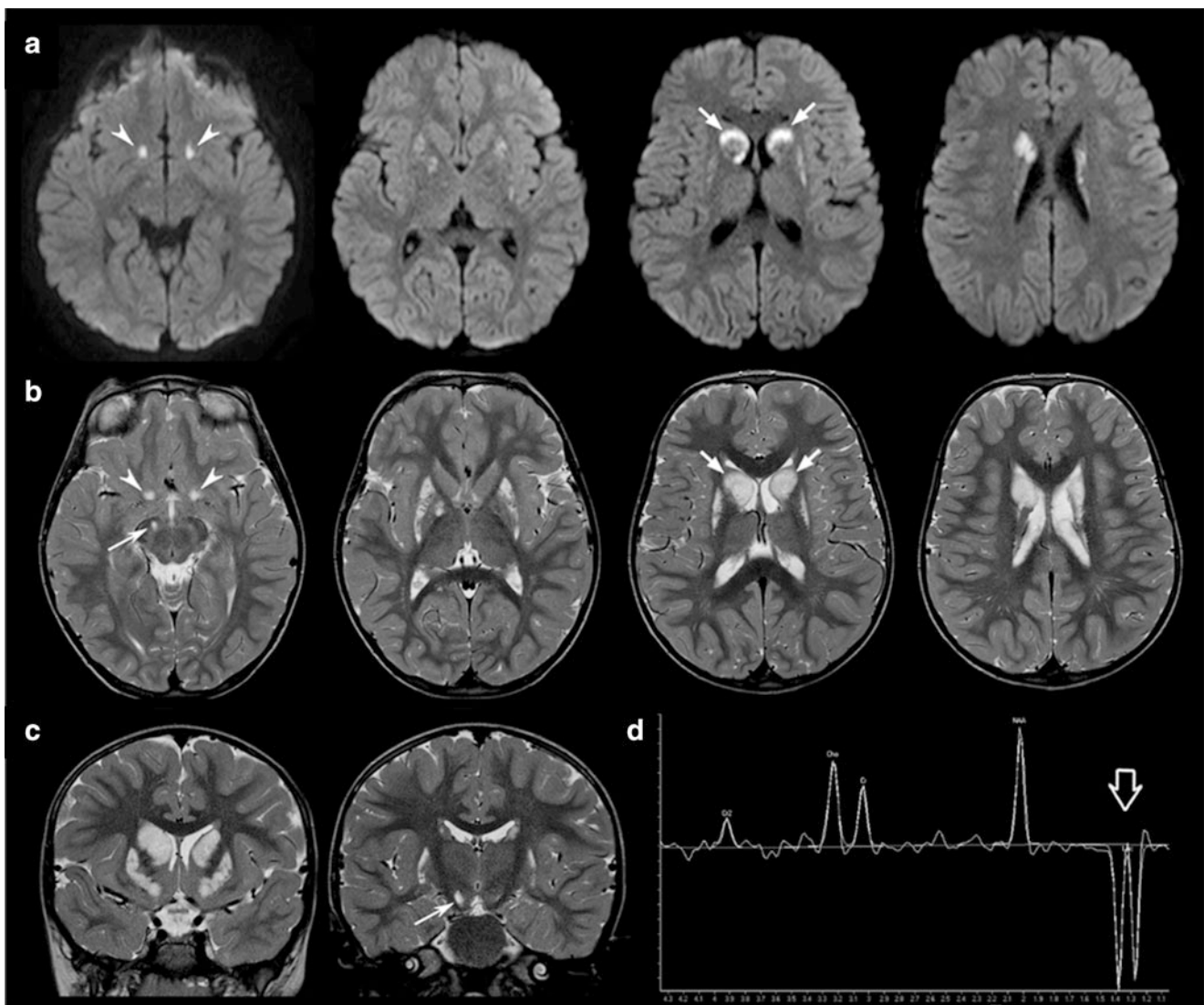


Fig. 2 Follow-up brain MRI and MR spectroscopy performed at 3 years and 6 months. Axial diffusion-weighted images (**a**) and T2-weighted images (axial, **b** and coronal, **c**) reveal new acute lesions characterized by T2 hyperintensity and restricted diffusion due to cytotoxic edema at the level of the basal nuclei of Meynert (*arrow-heads*), right cerebral peduncle (*thin arrow*), and caudate nuclei (*arrows*). Note the peculiar involvement of the heads of caudate

nuclei, with prevalent restricted diffusion in the anterior peripheral portions and increased diffusion in the central portions. The putamina are markedly reduced in size with prevalent increased diffusion, in keeping with chronic evolution of the previous lesions. (**d**) The MR spectroscopy performed at the level of the right basal ganglia demonstrates slightly reduced NAA peak and markedly elevated lactate peak (*empty arrow*)

2006; Monlleo-Neila et al. 2013; Rahman et al. 1996; Sofou et al. 2014; Fassone et al. 2011; Benit et al. 2003; Ruhoy and Saneto 2014).

Hoefs et al. firstly described a patient harboring two heterozygous *NDUFA10* mutations, one disrupting the start codon in exon 1 (c. c.1A>G) and the other resulting in an amino acid substitution in exon 3 (p.Gln142Arg). The disease was characterized by an early onset, with hypotonia and developmental delay, and by an aggressive course with severe lactic acidosis, progressive respiratory failure, convulsions, hypertrophic cardiomyopathy, and exitus at 23 months of age (Hoefs et al. 2011). Conversely, in the

present patient harboring a homozygous mutation c.296G>A in exon 3 of the *NDUFA10* gene, the clinical course was less severe with later age of onset, longer survival, and absence of hypertrophic cardiomyopathy. In particular, the developmental delay was very mild, and the prevailing neurological features were the episodes of ataxia with initial intercritical recovery. Interestingly, hypertrophic cardiomyopathy, a potential life-threatening finding in many patients with complex I deficiency (Fassone et al. 2011; Benit et al. 2003; Fassone and Rhaman 2012), was absent also in the patient reported by Haack et al., who harbored the same homozygous *NDUFA10* mutations in exon 3,

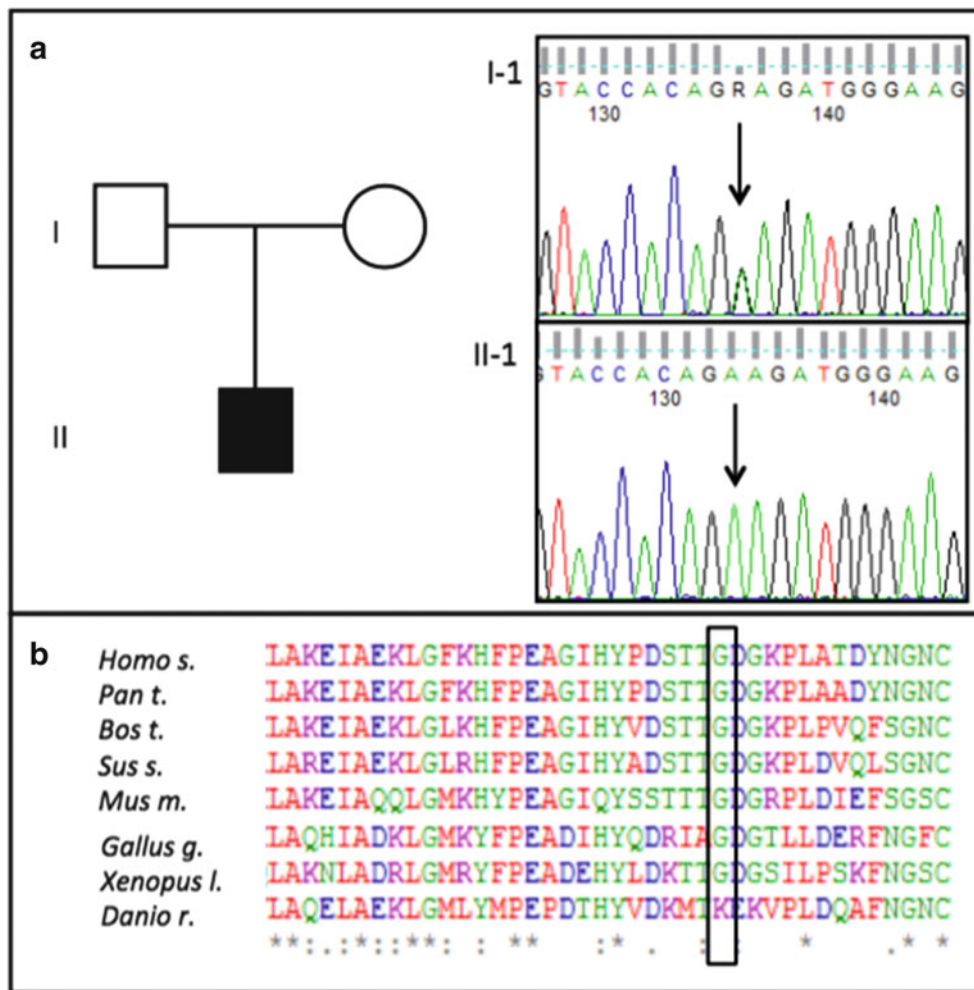


Fig. 3 Molecular analysis of *NDUFA10* gene. Electropherograms present the nucleotide change in fibroblasts of the patient (II-1); arrows point out the nucleotide substitution c.296G>A in exon 3 (a).

Bottom figure show the high conservation of the altered amino acid (p.G99E) throughout evolution (b)

suggesting the hypothesis of different effects of specific *NDUFA10* mutations in brain and cardiac muscle (Haack et al. 2012).

Of note, the relapsing-remitting course of the disease, and the first positive response to thiamine, initially led us to consider a PDH complex defect as the most likely diagnostic hypothesis (Giribaldi et al. 2012). However, this diagnosis was dismissed by the results of the analysis of RC enzyme activity revealing a defect in the OXPHOS system, subsequently confirmed by molecular assays. In particular, we noticed a clear reduction in complex I activity, associated to a milder decrease in complex I + III and II + III activities. These results are similar to those described by Hoefs et al., underlying the importance of screening for the presence of disease-causing *NDUFA10* mutations not only in patients with isolated complex I deficiencies but also in patients with a reduced complex I deficiency combined with a reduction in complex II or III

activity. From a histopathologic point of view, similarly to Hoefs et al., we did not find major abnormalities in the patient’s muscle biopsy. Indeed, ragged red fibers, a typical sign of many mitochondrial disorders, are rare in LS, and muscle biopsy histology may be completely normal (Sofou et al. 2014; Gerards et al. 2016; Finsterer 2008).

Besides its wide clinical, biochemical, and genetic heterogeneity, LS is uniformly characterized by the presence of multifocal bilateral and symmetric spongiform degeneration of the basal ganglia, thalamus, cerebellum, brainstem, spinal cord, and optic nerves, variably associated to demyelination and gliosis (Rahman et al. 1996; Ruhoy and Saneto 2014). These pathological changes, also described in the autopsy of the child with *NDUFA10* mutation reported by Hoefs et al., are well related to the abnormalities commonly identified on brain MRI.

MR imaging in LS usually reveals symmetrical hyperintense lesions of the basal ganglia and brainstem on T2-

weighted images. Cerebellar dentate nuclei are typically abnormal, while spinal cord, hemispheric white matter, and cerebral cortex changes are less frequently found (Lake et al. 2016; Gerards et al. 2016; Lee et al. 2009; Arii and Tanabe 2000). Acutely affected areas may show restricted diffusion, and MR spectroscopy typically reveals elevated lactate, most prominent within the lesions, due to the impairment of the mitochondrial function with secondary energetic failure and cytotoxic edema (Ruhoy and Saneto 2014; Saneto et al. 2008). Interestingly, beside the classical putaminal lesions, the present patient showed a rather unusual asymmetric involvement of the caudate nuclei, globi pallidi, and cerebral peduncles. In particular, peculiar asynchronous round lesions were noted at the level of the caudate heads, characterized by mass effect and mixed restricted and increased diffusion, consistent with the presence of both cytotoxic and vasogenic edema (Fig. 1). Of note, asymmetric involvement of the basal ganglia and brainstem may be found in other mitochondrial diseases. For instance, mutations in *MTFMT*, encoding a mitochondrial methionyl-tRNA formyl-transferase, may cause variably asymmetric Leigh encephalopathy and cystic or multifocal leukoencephalopathy (Haack et al. 2014; Baertling et al. 2016). Brain MRI images are not provided in the two reported patients with *NDUFA10* mutations, but the basal ganglia involvement is described as symmetric in both cases. Further studies are therefore needed to establish if *NDUFA10* mutations may cause a specific and recognizable neuroradiologic pattern. In literature, several attempts have been made to correlate the brain MR findings to a specific mutation in LS. For instance, the subthalamic nuclei and brainstem are consistently abnormal in patients with *SURF1* mutation (Rossi et al. 2003). On the other hand, abnormalities in the subthalamic nuclei are identified also in patients with other genetic defects in LS, demonstrating the complexity and wide variability of neuroimaging features in these conditions. Indeed, despite its typical features, MRI imaging alone may lack specificity in LS detection, as other mitochondrial conditions and non-mitochondrial diseases can show similar basal ganglia alterations on MRI. Therefore, LS diagnosis is currently based on a combined set of clinical, biochemical, and neuroimaging findings (Rahman et al. 1996; Baertling et al. 2014).

In conclusion, our description adds further evidence on the pathogenic role of *NDUFA10* in LS, expanding the spectrum of clinical and brain MRI manifestations. Our data suggest that even in patients apparently sharing the same genotype, the variability of single mutations may lead to different clinical, biochemical, and neuroradiologic phenotype.

Short Running Title (Synopsis)

New Clinical and Neuroradiologic Features of *NDUFA10* Mutation.

Authors Contribution

We confirm that all listed authors have provided a significant contribution in the manuscript preparation, in intellectual revision, and in patient's care.

Conflict of Interest

Francesca Minoia, Marta Bertamino, Paolo Picco, Maria-savina Severino, Andrea Rossi, Chiara Fiorillo, Carlo Minetti, Claudia Nesti, Filippo Maria Santorelli, and Maja Di Rocco declare that they have no conflict of interest related to the present manuscript.

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Details of Ethical Approval

Specific ethics committee was not requested for anonymous data report.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

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