Molecular Genetics and Metabolism 97 (2009) 185–189

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

# NDUFS4 mutations cause Leigh syndrome with predominant brainstem involvement

E. Leshinsky-Silver [a,b,e,*], Anne-Sophie Lebre [c], Limor Minai [c], Ann Saada [d], Julie Steffann [c], Sarit Cohen [a,b], Agnes Rötig [c], Arnold Munnich [c], Dorit Lev [e,f], Tally Lerman-Sagie [e,g]

[a] Molecular Genetics Laboratory, Wolfson Medical Center, Holon, Israel
[b] Sackler School of Medicine, Tel Aviv University, Israel
[c] INSERM U781 and Service de Génétique, Hôpital Necker Enfants Malades, Unversité René Descartes, Paris, France
[d] Metabolic Unit, Hadassah Medical Center, Jerusalem, Israel
[e] Mitochondrial Disease Center, Wolfson Medical Center, Holon, Israel
[f] Institute of Medical Genetics, Wolfson Medical Center, Holon, Israel
[g] Pediatric Neurology Unit, Wolfson Medical Center, Holon, Israel

ARTICLE INFO

*Article history:*
Received 11 February 2009
Received in revised form 4 March 2009
Accepted 4 March 2009
Available online 11 March 2009

*Keywords:*
Mitochondria
Respiratory chain
Leigh syndrome
NDUFS4
Complex I
Assembly

ABSTRACT

Complex I deficiency is a frequent cause of Leigh syndrome. We describe a non-consanguineous Ashkenazi–Sephardic Jewish patient with Leigh syndrome due to complex I deficiency. The clinical and neuroradiological presentation showed predominant brainstem involvement. Blue native polyacrylamide gel electrophoresis analysis revealed an impaired assembly of complex I.

The patient was found to be compound heterozygous of two mutations in the NDUFS4 gene: p.Asp119His (a novel mutation) and p.Lys154 fs (recently described in an Ashkenazi Jewish family). These findings support the suggestion that the p.Lys154 fs mutation in NDUFS4 should be evaluated in Ashkenazi Jewish patients presenting with early onset Leigh syndrome even before enzymatic studies.

Our results further demonstrated that NDUFS4 presents a hotspot of mutations in the genetic apparatus of oxidative phosphorylation and the correct assembly of the subunit it encodes is essential for completion of the assembly of complex I.



## Introduction

Complex I of the respiratory chain is the first, largest and most complicated of the five complexes of the oxidative phosphorylation (OXPHOS) system that produces cell energy [1–3]. It is one of the entry points of the OXPHOS system. It contributes to the formation of the proton electrochemical gradient across the inner mitochondrial membrane by coupling proton translocation to electron transfer from NADH to ubiquinone. The proton gradient provides the driving force for ATP synthesis, ion transport, and maintenance of antioxidant defenses. Complex I consist of 45 different subunits, flavoproteins (Fp), iron proteins (Ip) and hydrophobic proteins (Hp), together having a molecular weight of close to one MDa. The catalytic core consists of 14 conserved proteins: two Fps (encoded by the NDUFV1 and NDUFV2), five Ips (NDUFS1, NDUFS2, NDUFS 3, NDUFS 7 and NDUFS 8) and seven Hps (the mitochondrial DNA-encoded ND1-6 and ND4L) [1–3]. Assembly and maintenance of this large multi-protein complex requires assistance of specific factors such as NDUFAF1, B17.2L and Ecsit, C6ORF66 and Apoptotic Inducing Factor (AIF) [1–4]. Isolated complex I deficiency is the most commonly identified biochemical defect in mitochondrial OXPHOS disorders, but it is probably under-diagnosed, since both lactate levels and muscle morphology may be normal. It is usually caused by autosomal recessive mutations involving subunits encoded by the nuclear genome. So far, mutations have been demonstrated in the NDUFV1, NDUFV2, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS6, NDUFS7, NDUFS8, NDUFA11 and the assembly factors: B17.2L and NDUFAF1 and C6ORF66 [1–3]. Nonetheless, molecular diagnosis is still unavailable for more than 50% of the patients.

Leigh and Leigh-like syndromes (LS), are the most frequent clinical presentations of Complex I deficiency. Leigh syndrome is a neurodegenerative pediatric disorder characterized by bilateral, symmetric focal hyperintensities in the basal ganglia, thalamus, substantia nigra, and brainstem, demonstrated by MRI, $T_2$ images [5]. The clinical symptoms include developmental deterioration, hypotonia, ophthalmoplegia, nystagmus, ataxia and dystonia. The disease is usually lethal within the first years of life due to medullary involvement culminating in respiratory failure. The heart and gastrointestinal tract may also be involved. Some patients may develop predominant cerebellar atrophy or a leukoencephalopathy. The basal ganglia are usually affected before the brainstem. In most cases, the inheritance is autosomal recessive, but at least 25% of

* Corresponding author. Address: Molecular Genetic Laboratory and Mitochondrial Disease Center, Wolfson Medical Center, Holon, Israel. Fax: +972 3 5028543.
*E-mail address:* leshinsky@wolfson.health.gov.il (E. Leshinsky-Silver).

1096-7192/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.ymgme.2009.03.002

complex I deficient Leigh patients harbor a heteroplasmic mtDNA mutation [6] and a minority may demonstrate X-linked inheritance.

We describe an Ashkenazi–Sephardic Leigh syndrome patient with partial isolated complex I deficiency and severely impaired complex I assembly, harboring two heterozygous mutations in the NDUFS4 gene.

## Material and methods

### *Patient*

The patient was the product of a normal 40 week pregnancy and delivery. Birth weight was 4030 g. The family history was unremarkable. The parents are non-consanguineous, Ashkenazi and Sephardic Jews.

Development was normal until 8 months of age when he presented with myoclonus during a febrile illness. The neurological and developmental exams were normal; weight was at the 10th percentile. An EEG did not demonstrate epileptic activity. At the age of 12 months he presented with sudden onset of left exotropia and the neurological exam also demonstrated increased reflexes and right Achilles clonus. MRI at this age showed bilateral bright lesions in the medulla (Fig. 1). The strabismus disappeared after a month. At the age of 19 months the parents complained of delayed walking. The exam demonstrated excellent cognitive development, mild hypotonia and a wide based unstable gait.

At 26 months, following varicella he developed a sudden onset of complete left ophthalmoplegia, ataxia and tremor. Neurological exam also demonstrated pyramidal signs in the right leg. Speech was mildly dysarthric but comprehension was above age level. MRI showed lesions in the midbrain, and dorsal pons extending into the medulla. He deteriorated rapidly and 6 weeks later succumbed from respiratory insufficiency.

### *Methods*

#### *Biochemical studies*

*Mitochondrial isolation and respiratory chain (RC) analysis.* Mitochondria were isolated from fresh muscle tissue or fibroblasts by homogenization and differential centrifugation in sucrose buffer [7]. The activities of citrate synthase (CS), ferricyanide reductase (C-I), rotenone sensitive NADH-cytochrome *c* reductase (C-I+III), rotenone sensitive NADH coenzyme Q reductase (C-I), succinate cytochrome *c* reductase (C-II+III), succinate dehydrogenase (C-II), cytochrome *c* oxidase (C-IV), MgATP ase (C-V) and pyruvate dehydrogenase complex activities were determined by spectrophotometry [7–9].

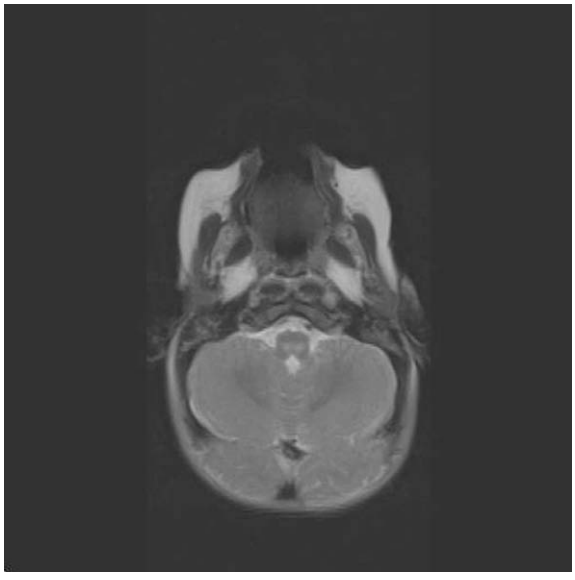

**Fig. 1.** Axial T2 weighted image shows bilateral bright lesions in the medulla.

#### *Western blot analysis*

Western blot of Blue native polyacrylamide gel electrophoresis (BN-PAGE) was performed by hybridization with monoclonal antibodies raised against GRIM19 subunit of complex I subunits, 70 KDa complex II subunit, core 2 subunit of complex III and COX2 subunit of complex IV (Mitoscience) [10,11].

#### *Molecular analysis*

Total DNA was extracted from blood, muscle, using the Puregene kit (Gentra, Minneapolis, USA), according to the manufacture's instructions.

The mtDNA Leigh mutations: 8993g-c and 9176t-c, 10158t-c, 11777c-a, 11778g-a, 13513g-a, 13514a-g, 5537int, 8344a-g and 10191t-c were analyzed as previously reported [12–15].

mtDNA sequencing was performed using 24 overlapping primer pairs [13], purified with the ExoSapIT kit (Amersham Pharmacia biotech purified PCR product. Amersham, Buckinghanshire, UK), according to the manufacturer's instructions, and sequenced with fluorescently labeled dideoxynucleotide terminators and an Applied Biosystem 373A automated sequencer.

Amplification of the coding sequence on genomic DNA of the nuclear encoded subunits of the complex I was performed using a set of primers that amplified all the exons with flanking intronic sequences. The reaction was performed in a 50 μl volume containing 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 250 μM dNTPs, 1 μM of each primer, 100 ng of genomic DNA and 1.25 U of AmpliTaq Gold DNA polymerase (Perkin Elmer Applied Biosystem) with an initial denaturation step of 10 min at 95 °C to activate the polymerase followed by 35 cycles of 94 °C; 15 s, 60 °C; 45 s, 72 °C; 45 s and a final elongation of 10 min at 72 °C. Predicted amplicon sizes were confirmed by agarose gel electrophoresis. The amplified PCR products were purified and sequenced as described above.

## Results

Muscle histochemistry and electron microscopy demonstrated normal morphology.

Respiratory chain activity analysis revealed a partial reduction in complex I activity in muscle homogenate but normal activities in cultured fibroblasts (Table 1). In fibroblasts, RC activities were within the normal values.

The maternally inherited mtDNA mutations were first ruled out: 8993g-c and 9176t-c, 10158t-c, 11777c-a, 11778g-a, 13513g-a, 13514a-g, 5537int, 8344a-g and 10191t-c both in blood and in DNA extracted from urine sediments. Further sequencing of the whole mtDNA did not reveal pathogenic mutations.

Nuclear genes encoding complex I subunits were then sequenced. We first sequenced NDUFS7 and NDUFS8 because of the phenotype similarity and found normal sequences. We then sequenced NDUFV1 and NDUFS1, the X-linked NDUFA1, NDUFB11 genes and the assembly gene B17.2L. All revealed normal sequences.

Blue native gel electrophoresis revealed an impaired assembly of complex I (Fig. 2). The migration profile of the patient's complex I indicated that the complex is slightly smaller than the fully assembled one, implying for a possible mutation in the NDUFS4