Leigh Disease with Brainstem Involvement in Complex I Deficiency due to Assembly Factor NDUFAF2 Defect

Authors
M. Herzer[1,8], J. Koch[2,8], H. Prokisch[1], R. Rodenburg[3], C. Rauscher[2], W. Radauer[2], R. Forstner[4], P. Pilz[5], B. Rolinski[6], P. Freisinger[7], F. Madignier[1], J. A. Mayr[2], W. Sperl[2]

Affiliations
Affiliation addresses are listed at the end of the article

Key words
- NDUFAF2
- complex I deficiency
- Leigh disease
- assembly factor
- brainstem
- involvement
- mitochondrial encephalomyopathy

Abstract

Mitochondrial NADH: ubiquinone oxidoreductase (complex I) deficiency accounts for most defects in mitochondrial oxidative phosphorylation. Pathogenic mutations have been described in all 7 mitochondrial and 12 of the 38 nuclear encoded subunits as well as in assembly factors by interfering with the building of the mature enzyme complex within the inner mitochondrial membrane. We now describe a male patient with a novel homozygous stop mutation in the *NDUFAF2* gene. The boy presented with severe apnoea and nystagmus. MRI showed brainstem lesions without involvement of basal ganglia and thalamus, plasma lactate was normal or close to normal. He died after a fulminate course within 2 months after the first crisis. Neuropathology verified Leigh disease. We give a synopsis with other reported patients. Within the clinical spectrum of Leigh disease, patients with mutations in *NDUFAF2* present with a distinct clinical pattern with predominantly brainstem involvement on MRI. The diagnosis should not be missed in spite of the normal lactate and lack of thalamus and basal ganglia changes on brain MRI.

Introduction

With a minimum birth prevalence of at least 1:5000 [16] disorders of the mitochondrial respiratory chain account for a main part of inherited metabolic diseases. Embedded within the inner mitochondrial membrane, the respiratory chain is made up of 5 enzyme complexes which produce ATP by oxidative phosphorylation. Especially high energy-dependent organs like brain, heart and skeletal muscle are vulnerable to defects of the aerobic energy metabolism.

Isolated complex I deficiency encompasses about one third of these hereditary defects in the mitochondrial energy generating system [2,9]. Being the largest complex of the respiratory chain, complex I contains 45 subunits, which are under genetic control of both mitochondrial and nuclear genes [4]. Pathogenic mutations causing complex I defects have been described in all 7 mitochondrial encoded subunits and 12 nuclear genes, namely NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS6, NDUFS7, NDUFS8, NDUFV1, NDUFV2, NDUFA1, NDUFA2 and NDUFA11, which are highly conserved and account for a large number of different phenotypes in the affected patients [2,3,5]. However, these mutations explain pathogenicity only for a small part of the entire complex I deficiency patients. For more than 50% of the patients molecular diagnostics fail to detect the cause of the disease. In previous reports assembly factor mutations have been shown to cause complex I deficiency.

Ogilvie and colleagues showed first that the NDUFAF2 gene, which shares similarities in amino acid sequence with subunit NDUFA12, serves as complex I assembly factor [12], as well as NDUFAF1, C6ORF66, C8ORF38, C20ORF7 and NDUFAF3, for which mutations have been described [1,6,7,12–15,17]. Herein, we describe a patient with complex I deficiency in both muscle and fibroblasts harbouring a new mutation in the NDUFAF2 gene. We compare the clinical picture with previously published cases and summarize the unique neuropathological pattern of the known patients with NDUFAF2 mutations.

Case Report

The patient was the first child of non-consanguineous Austrian parents and was born after an uneventful pregnancy. Early psychomotor development was moderately delayed. He was able to

received 30.12.2009
accepted 25.05.2010

Bibliography
DOI http://dx.doi.org/10.1055/s-0030-1255062
Neuropediatrics 2010; 41: 30–34

ISSN 0174-304X

Correspondence
Prof. Dr. Wolfgang Sperl
Department of Pediatrics
University Hospital Salzburg
Paracelsus Medical University
Muellner Hauptstraße 48
5020 Salzburg
Austria
Tel.: +43 662 44822600
Fax: +43 662 44822604
w.sperl@salk.at

walk at the age of 22 months. Horizontal nystagmus was noticed at the age of 14 months and improved with orthoptist therapy. At the age of 26 months, during pneumonia, he was found to be apnoeic and needed respiratory assistance. The CT scan of the brain was normal, cerebrospinal fluid (CSF) showed no signs of central nervous system infection. He recovered promptly and was discharged from hospital after 8 days. Metabolic screening, virological investigations and lactate measurements in plasma and CSF were normal.

3 weeks later, with respiratory infection, he was found to be apnoeic, cyanotic and comatose. On admission he was mechanically ventilated and presented with foot drop position and bilateral positive Babinski signs. Neuroimaging at the age of 27 month (**Fig. 1**) revealed on T_2-weighted images lesions in the upper cervical spinal cord, the medulla oblongata, in the pontine tegmentum, in the midbrain in the substantia nigra and the periaqueductal region (**Fig. 1a**). In the cerebral white matter patchy and not completely confluent lesions, mainly located in the frontal and parietal deep white matter, are seen. The corpus callosum, internal capsule and basal ganglia and thalami are spared (**Fig. 1b**).

On admission lactate was elevated (serum 4.2 mmol/L, reference value < 2.2 mmol/L; CSF 6 mmol/L, reference value < 2.1 mmol/L), subsequently lactate levels were within the normal range. CSF protein was normal. Fundoscopy showed pale optic discs. Auditory evoked potentials disclosed signs of lesions in the rostral pontine auditory pathway. 3 weeks later the patient died under mechanical ventilation of cardiac arrest at the age of 27 months.

Histological findings

Autopsy and biopsies of skin, muscle and liver were performed. Neuropathological examination of CNS revealed softening and brown discolouration of the substantia nigra and the tegmentum of the brainstem (**Fig. 1c**) and typical signs of Leigh disease with necrosis, spongy state and capillary proliferation (**Fig. 1d**) predominantly in the brainstem (substantia nigra, tegmentum of mesencephalon, pons and medulla oblongata), the optic nerves and chiasma. Some less pronounced changes were found in nucleus dentatus cerebelli and the uppermost spinal cord, and mild involvement of the medial thalamus and hypothalamus. The mammillary bodies were not affected. Dorsal spinal tracts, spinocerebral tracts and dorsal spinal nerve roots displayed Wallerian degeneration. There were no signs of hypoxic damage.

Measurement of respiratory chain enzyme activities in fibroblasts and muscle

Activities of the respiratory chain complexes were determined in isolated fibroblast mitochondria and in muscle homogenate by standard spectrophotometric methods [10]. The complex I activity was clearly reduced with 12% residual activity of the lowest control value in muscle and 59% in fibroblasts. In muscle and fibroblast complexes II and III were also reduced (presumably secondary effects of the complex I deficiency) (see **Table 1**).

Mutation analysis

Genomic DNA was extracted from skeletal muscle homogenate by proteinase K treatment followed by phenol/chloroform extraction. Mutation screen in candidate genes including NDUFAF2 was performed by high-resolution melting analysis using Idaho LightScanner Technology as described by Meisinger et al. in 2009 [11]. All exons of NDUFAF2 were PCR amplified by intronic primers (available on request) and altered melting curves compared were directly sequenced with BigDye Cycle sequencing kit (Applied Biosystems). Sanger sequencing of the entire mitochondrial DNA failed to detect a mutation. High-resolution melting profile analysis of exonic sequences from NDUFAF2 revealed a novel homozygous nonsense mutation c.9G > A introducing an early stop codon at position 3 of NDUFAF2 (p. Trp3X, **Fig. 2**).

Discussion

Patients with complex I deficiencies present with a heterogeneous spectrum of clinical phenotypes, reflecting the complex structure and its function within the respiratory chain. So far, in more than 50% of the patients molecular diagnosis failed to detect the cause of disease. There are very few patients published with complex I deficiency due to mutations in assembly factors and only 4 patients published with a defect of *NDUFAF2*. We present a new case with a novel mutation in this assembly factor and point out the common clinical and neuropathological patterns of all so far known patients.

Ogilvie et al. (2005, **Table 2**) [12] reported on a girl with a severe childhood-onset progressive encephalopathy caused by mutation the NDUFAF2 gene. The clinical course was characterized initially by nystagmus and a wide-based gait at the age of 14 months. At the age of 3 years, a crisis during an infection was marked by acute ataxia, lethargy, decreased reflexes and signs of

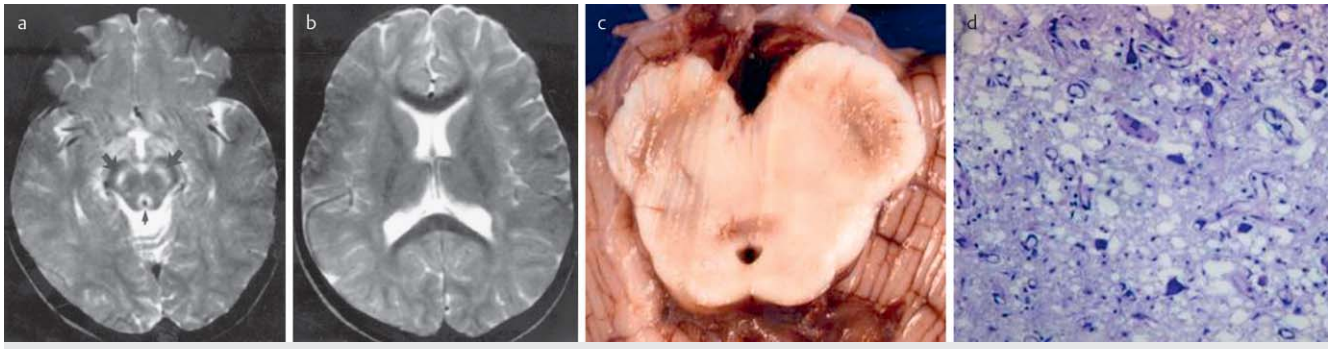

Fig. 1 MRI of brain (T_2-weighted images) shows increased signal in the midbrain in the substantia nigra (**a**: gross arrow) and the periaqueductal region (**a**: fine arrow), corpus callosum, internal capsule and basal nuclei are spared (**b**). Autopsy shows softening and brown discolouration of substantia nigra and subaqueductal area of the midbrain (**c**), histopathology: spongy state, necrosis and capillary proliferation of pontine tegmentum (cresyl violet ×100) (**d**).

Table 1 Respiratory chain enzyme activities in fibroblasts and muscle.

Muscle	Patient*	Normal Range*	Patient**	Normal Range**
complex I (CI)	2	30–65	0.02	0.17–0.26
complex I + III (C13)	7	27–58	0.05	0.13–0.19
complex II (CII)	39	53–100	0.29	0.30–0.36
complex II + III (C23)	12	41–75	0.09	0.20–0.28
complex III (CIII)	81	230–490	0.61	1.30–1.96
complex V (CV)	108	78–180	0.81	0.42–0.70
cytochrome c oxidase (COX)	113	205–739	0.85	1.24–2.38
citrate synthase (CS)	133	160–310		
Fibroblasts	**Patient***	**Normal Range***	**Patient****	**Normal Range****
complex I (CI)	6	15–53	0.02	0.05–0.09
complex I + III (C13)	16	102–343	0.05	0.24–0.58
complex II (CII)	99	103–285	0.34	0.37–0.48
complex II + III (C23)	161	167–314	0.55	0.39–0.72
complex III (CIII)	298	283–1174	0.98	1.17–1.99
complex V (CV)	45	36 – 167	0.15	0.15–0.39
cytochrome c oxidase (COX)	381	392–939	1.35	1.30–1.68
citrate synthase (CS)	297	242–590		

* Units/g protein

** Units/unit citrate synthase

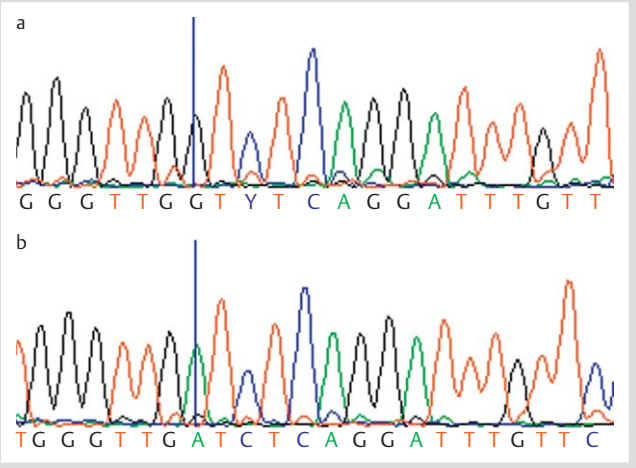

Fig. 2 DNA sequence analysis of the patient revealed a homozygous missense mutation (c.9G>A) resulting in a premature stop codon instead of a tryptophan at position 3 (p.Trp3X). **a**: control; **b**: patient.

optic atrophy. At the age of 8 years she worsened with dysphagia, sleep apnoea and respiratory failure. She remained comatose and died at the age of 13 years. Neuropathology proved Leigh disease. Cerebral cMRI exhibited symmetric lesions in the mamillothalamic tracts, substantia nigra, medial lemniscus, medial longitudinal fasciculus and spinothalamic tracts. Basal ganglia and thalamus were not affected. Blood lactate, liver function and echocardiogram were normal almost all the time, so the authors stated that clinical presentation, laboratory and cMRI findings did not resemble typical Leigh syndrome.

Barghuti et al. (2008) added 2 more patients who were identified by homozygosity mapping [1]. The clinical course (see **Table 2**) was marked by apnoea, nystagmus, optic nerve atrophy (observed in 1 patient) and early death 4 and 13 months after the first crisis, respectively. Both patients had normal blood lactate levels and very similar cMRI changes of the brainstem as the patient from Ogilvie et al. Therefore, Barghuti et al. postulated that patients with NDUFAF2 mutations display a unique pattern of degenerative changes in neuroradiology.

Our case resembles the findings in the above-mentioned patients. There was a moderate delay in psychomotor development and nystagmus prior to the first crisis with severe apnoea during an infection. The second crisis within 3 weeks led to death due to cardiorespiratory arrest. The patient had normal or close to normal blood lactate levels (in CSF lactate was considerable increased) and cMRI findings were very similar to those of the so far described patients. The diagnosis of Leigh disease was proven by neuropathology (**Fig. 1c, d**). It can be speculated that other clinically similar patients with NDUFAF2 mutations might have been missed for diagnosis on intensive care ward units. The severe clinical picture with acute fulminant and lethal respiratory failure without lactate elevation and the atypical neuroradiological presentation might have misled to other diagnostic considerations than mitochondrial encephalopathy.

Very recently, Hoefs et al. (2009) published a girl with an NDUFAF2 mutation [7], who presented with nystagmus at the age of 3 months, followed by feeding difficulties, renal tubular acidosis, muscular hypotonia and dyskinetic movements. She died at age of 1 year due to respiratory failure (**Table 2**). Plasma lactate levels were moderately elevated and cMRI revealed high signal abnormalities in thalamus, brainstem and spinal cord. This case shows that neuroradiology findings in patients with NDUFAF2 mutations are not always as unique as postulated by Barghuti et al. [1].

All NDUFAF2 patients harboured loss of function mutations with a truncated protein useless in complex I assembly [7,8,12].

Median complex I residual activity was 31% of the lowest control value with a range of 12–55% in the 5 patients suggesting that mutated NDUFAF2 has a great influence on proper complex I function, although the residual activity does not correlate to the patient's clinical course. Nevertheless, there are specific features which have been observed in patients with NDUFAF2 mutations.

Patients expired at a median age of 2 years (range from 1 up to 13 years). Clinical hallmarks are nystagmus, optic nerve atrophy, apnoea and respiratory failure as signs of the mainly affected

Table 2 Information of patients with complex I deficiency due to mutations in NDUFAF2.

Reference	This report	Ogilvie et al. 2005	Barghuti et al. 2008	Barghuti et al. 2008	Hoefs et al. 2009
gender	male	female	female	male	female
mutation	c.9G>A, p.W3X homozygous	c.182C>T, p.R45X homozygous	c.1A>T, p.M1L homozygous	c.1A>T, p.M1L homozygous	c.114C>G, p.Y38X homozygous
consanguinity	no	no	yes	yes	yes
age of onset	14 months	12 months	20 months	8 months	3 months
age of death	24 months	13 years	2 years	21 months	12 months
death by	apnoea	respiratory failure	apnoea	not commented	respiratory failure
first symptoms	nystagmus, motor delay	nystagmus, wide-based gait	apnoea	motor delay, nystagmus	nystagmus
following symptoms	apnoea, pale optic disc, optic atrophy (histology)	ataxia, absent deep tendon reflexes, optic atrophy, weakness, dysphagia, sleep apnoea, respiratory failure, coma	myoclonic seizures, muscular hypotonia, horizontal and rotatory nystagmus, dysmetria, ataxia	optic atrophy; age 18 months: episodes of flaccidity and apnoea, remained ventilator dependent	muscular hypotonia, motor delay, renal tubular acidosis, feeding difficulties
lactate (serum)	normal – 4.2 mmol/L (<2.1 mmol/L)	normal	normal	normal	3–5 mmol/L (<2.3 mmol/L)
lactate (CSF)	6 mmol/L (<1.8 mmol/L)	4.2 mmol/L (1.4–3.9 mmol/L)	normal	normal	n.d.
complex I activity*	12%	55%	49%	31%	21%
cMRI findings (T_2-w):					
telencephalon (including basal ganglia)	basal nuclei spared, patchy lesions in cerebral white matter	cortex, subcortical white matter relatively spared	cortex, subcortical white matter relatively spared	no changes reported	no changes reported
diencephalon including thalamus	spared	mamillothalamic tract	mamillothalamic tract	mamillothalamic tract	symmetrical, bilateral lesions of thalamus
mesenzephalon, pons, cerebellum, medulla oblongata, spinal cord	substantia nigra, periaqueductal region, pontine tegmentum, spinal trigeminal nucleus	substantia nigra, medial lemniscus, medial longitudinal fasciculus, spinothalamic tracts, cerebellar white matter	substantia nigra, medial lemniscus, medial longitudinal fasciculus, spinothalamic tracts, cerebellar white matter	substantia nigra, periaqueductal grey matter, medial lemniscus, medial longitudinal fasciculus, spinothalamic tracts, cerebellar white matter	cerebral peduncles, brainstem, spinal cord

* Percentage residual activity of lowest control value in skeletal muscle

Abbreviations: n.d. = no data

brainstem. The clinical course in all patients was marked by an early onset and progressive course. Furthermore, 4 of the 5 patients presented with acute episodes of encephalopathy during infections and were stable in the interval. On cMRI, brainstem lesions without changes in thalami and basal ganglia on T_2-weighted images are found in 4 out of 5 patients. Plasma lactate was normal in 3 of 5 patients and in the remaining only slightly elevated. CSF lactate levels were normal in 2 and elevated in 2 patients.

In conclusion, the diagnosis of a complex I assembly defect due to an NDUFAF2 mutation should not be missed. The lack of cMRI changes in thalamus and basal ganglia and normal or close to normal plasma and CSF lactate levels might mislead to other than mitochondrial diseases.

Acknowledgements

We are thankful to Marjo van der Knaap for helpful comments on the neuroimaging of our patient and Eugen Boltshauser for providing important literature. This work was funded by the Oesterreichische Nationalbank-Jubiläumsfonds Grant Number 12568 and the Vereinigung zur Pädiatrischen Forschung und Fortbildung Salzburg. This work was supported by the Bundesministerium für Bildung und Forschung (BMBF) funded German Network for Mitochondrial Disorders (mitoNET #01GM0862) and Systems Biology of Metabotypes (SysMBo #0315494A).

Affiliations

[1] Institute of Human Genetics, TU Munich and Helmholtz Zentrum München, Munich, Germany
[2] Department of Pediatrics, University Hospital Salzburg, Paracelsus Medical University, Salzburg, Austria
[3] Nijmegen Center for Mitochondrial Disorders, Radboud University, Nijmegen Medical Centre, Nijmegen, The Netherlands
[4] Department of Radiology, University Hospital Salzburg, Paracelsus Medical University, Salzburg, Austria
[5] Department of Neuropathology, University Hospital Salzburg, Paracelsus Medical University, Salzburg, Austria
[6] Institute of Clinical Chemistry, Klinikum München, Technical University, Munich, Germany
[7] Children's Hospital Schwabing, Technical University Munich, Munich, Germany
[8] These authors contributed equally to the work

References

1 *Barghuti F, Elian K, Gomori JM et al.* The unique neuroradiology of complex I deficiency due to NDUFA12L defect. Mol Genet Metab 2008; 94: 78–82
2 *Bénit P, Lebon S, Chol M et al.* Mitochondrial complex I deficiency in humans. Current Genomics 2004; 5: 137–146
3 *Berger I, Hershkovitz E, Shaag A et al.* Mitochondrial complex I deficiency caused by a deleterious NDUFA11 mutation. Ann Neurol 2008; 63: 405–408
4 *Carroll J, Fearnley IM, Skehel JM et al.* Bovine complex I is a complex of 45 different subunits. J Biol Chem 2006; 281: 32724–32727
5 *Distelmaier F, Koopman W, van den Heuvel L et al.* Mitochondrial complex I deficiency: from organelle dysfunction to clinical disease. Brain 2009; 132: 833–842
6 *Dunning CJ, McKenzie M, Sugiana C et al.* Human CIA30 is involved in the early assembly of mitochondrial complex I and mutations in its gene cause disease. EMBO J 2007; 26: 3227–3237
7 *Hoefs SJ, Dieteren CE, Rodenburg RJ et al.* Baculovirus complementation restores a novel NDUFAF2 mutation causing complex I deficiency. Hum Mutat 2009; 30: 728–736
8 *Janssen RJ, Distelmaier F, Smeets R et al.* Contiguous gene deletion of ELOVL7, ERCC8 and NDUFAF2 in a patient with a fatal multisystem disorder. Hum Mol Genet 2009; 18: 3365–3374
9 *Loeffen JL, Smeitink JA, Trijbels JM et al.* Isolated complex I deficiency in children: clinical, biochemical and genetic aspects. Hum Mutat 2000; 15: 123–134
10 *Mayr JA, Paul J, Pecina P et al.* Reduced respiratory control with ADP and changed pattern of respiratory chain enzymes as a result of selective deficiency of the mitochondrial ATP synthase. Pediatr Res 2004; 55: 988–994
11 *Meisinger C, Prokisch H, Gieger C et al.* A genome-wide association study identifies 3 loci associated with mean platelet volume. Am J Hum Genet 2009; 84: 66–71
12 *Ogilvie I, Kennaway NG, Shoubridge EA.* A molecular chaperone for mitochondrial complex I assembly is mutated in a progressive encephalopathy. J Clin Invest 2005; 115: 2784–2792
13 *Pagliarini DJ, Calvo SE, Chang B et al.* A mitochondrial protein compendium elucidates complex I disease biology. Cell 2008; 134: 112–123
14 *Saada A, Edvardson S, Rapoport M et al.* C6ORF66 is an assembly factor of mitochondrial complex I. Am J Hum Genet 2008; 82: 32–38
15 *Saada A, Vogel RO, Hoefs SJ et al.* Mutations in NDUFAF3 (C3ORF60), encoding an NDUFAF4 (C6ORF66)-interacting complex I assembly protein, cause fatal neonatal mitochondrial disease. Am J Hum Genet 2009; 84: 718–727
16 *Schaefer AM, Taylor RW, Turnbull DM et al.* The epidemiology of mitochondrial disorders – past, present and future. Biochim Biophys Acta 2004; 1659: 115–120
17 *Sugiana C, Pagliarini DJ, McKenzie M et al.* Mutation of C20orf7 disrupts complex I assembly and causes lethal neonatal mitochondrial disease. Am J Hum Genet 2008; 83: 468–478

Notice

This article was changed according to the following erratum on: 14.09.2010

Erratum

Some author of this article was not mentioned. The name of the author should be mentioned here:
Florence Madignier, Institute of Human Genetics, TU Munich and Helmholtz Zentrum München, Germany

Addendum to Acknowledgement:

This work was supported by the Bundesministerium für Bildung und Forschung (BMBF) funded German Network for Mitochondrial Disorders (mitoNET #01GM0862) and Systems Biology of Metabotypes (SysMBo #0315494A).