



## CASE STUDY

# Early-onset leukoencephalomyelopathy due to a biallelic *NDUFV1* variant in a mid-forties patient

Markus Gschwind<sup>1,2,3,\*</sup> , Nuria Garcia Segarra<sup>4,\*</sup>, André Schaller<sup>5</sup>, Ramona Bolognini<sup>5</sup>, Jean-Marc Nuoffer<sup>6</sup>, Raphael Hourez<sup>7</sup>, Manuel Deprez<sup>8,†</sup>, Benoit Lhermitte<sup>9</sup>, Philippe Maeder<sup>10</sup>, Christel Tran<sup>4,‡</sup>  & Thierry Kuntzer<sup>3,‡</sup>

<sup>1</sup>Clinic of Neurology, Kantonsspital Aarau, Aarau, Switzerland

<sup>2</sup>Department of Neurology, Geneva University Hospital and University of Geneva, Geneva, Switzerland

<sup>3</sup>Department of Neurology, Lausanne University Hospital CHUV, Lausanne, Switzerland

<sup>4</sup>Center for Molecular Diseases, Division of Genetic Medicine, Lausanne University Hospital, University of Lausanne, Switzerland

<sup>5</sup>Division of Human Genetics, Department of Pediatrics, Inselspital, Bern University Hospital, University of Bern, Switzerland

<sup>6</sup>University Institute of Clinical Chemistry, Inselspital, Bern University Hospital, Bern, Switzerland

<sup>7</sup>Department of Neurology, Centre Hospitalier Universitaire Brugman, Brussels, Belgium

<sup>8</sup>Department of Pathology, Lausanne University Hospital CHUV, Lausanne, Switzerland

<sup>9</sup>Department of Pathology, Hautepierre University Hospital, France

<sup>10</sup>Department of Neuroradiology, Lausanne University Hospital CHUV, Lausanne, Switzerland

## Correspondence

Markus Gschwind, Klinik für Neurologie, Kantonsspital Aarau, Tellstrasse 25, CH-5011 Aarau, Switzerland. Tel. +41-62-838-3961; Fax: +41 62 838 6674; E-mail: markus.gschwind@ksa.ch

## Funding Information

No funding information provided.

Received: 11 May 2021; Revised: 11 March 2022; Accepted: 26 March 2022

doi: 10.1002/acn3.51556

\*Authors contributed equally.

†Deceased.

‡Shared last-authorship.

## Abstract

We present a patient who developed, after an early-onset, a stable course of spastic paraplegia and ataxia for 4 decades and eventually succumbed to two episodes of postinfectious lactic acidosis. Diagnostic workup including muscle biopsy and postmortem analysis, oxymetric analysis, spectrophotometric enzyme analysis, and MitoExome sequencing revealed a necrotizing leukoencephalomyelopathy due to the so far unreported biallelic variant of the *NDUFV1* gene (p.(Pro122Leu)). This case extends our understanding of *NDUFV1* variants with a 14-fold longer lifetime than so far reported cases, and will foster sensitivity toward respiratory chain disease also in adult patients with sudden deteriorating neurological deficits.

## Background

Complex I deficiency (OMIM #252010) is the most common biochemical defect among the genetic mitochondrial disorders. However, its genetic basis is heterogeneous, and so is the clinical spectrum that ranges from lethal neonatal disease to adult-onset conditions. Among the frequent presentations are neonatal cardiomyopathy, leukoencephalomyelopathy, and fatal lactic acidosis.<sup>1</sup> Here, we describe an adult individual in whom the diagnosis of a mitochondrial disorder has been made only post-mortem (with the first biallelic *NDUFV1* variant c.365C>T p.(Pro122Leu)), in spite of having presented

since early childhood with developmental delay, progressive spastic paraplegia, and cerebellar ataxia. In his mid-forties, he suddenly developed a postinfectious metabolic acidosis with muscle weakness and rapidly progressive leukoencephalomyelopathy with a fatal course over a few months.

## Case

An institutionalized man in his mid-forties was admitted twice in our neurology department following new episodes of mutism, coma, and flaccid tetraparesis, 6 months apart.

Medical history revealed that he was the second child of consanguineous parents at the 3<sup>rd</sup> degree. At the age of 4 months, motor development became delayed; generalized hypotonia and brisk reflexes were noticed, followed by slowly appearing progressive spastic paraplegia and signs of cerebellar ataxia during his first two decades. At the age of 20, the extent of his intellectual disability was considered significant (IQ around 60), he could walk only with assistance or drive his electric wheelchair. He talked in simple grammar and participated in the institution's activities. There were no dysmorphic features and no seizures. During the next two decades the only medical contact was the orthopedic surgeon for bilateral pes equinovarus treatment. His spasticity was regularly treated with oral baclofen.

The first admission was 2 weeks after recovery from a viral gastroenteritis with fever, after he suddenly had presented with fatigue, dysarthria, difficulties lifting his upper limbs, and drowsiness. On clinical examination, excessive sweating, severe dysarthria, generalized muscle weakness, and areflexia were observed. He then received iv methylprednisone 1 g/d for 6 days for a suspected acute disseminated encephalomyelitis, but recovered only very slowly.

The second admission was 6 months later, 1 week after a urinary infection. He presented with abdominal pain, confusion, anarthria, and finally coma. Again generalized muscle weakness and areflexia with bilateral Babinski sign were the prominent signs. Laboratory workup showed leukocytosis at 22.7 G/L with polymorphonuclear leukocytes, lactic acidosis (pH 7.3, lactate 14.3 mmol/L, pCO<sub>2</sub> 18.4 mmHg, bicarbonate 8.9 mmol/L). Lactate/ pyruvate ratio, amino acids profile, and urine organic acids were normal. The CSF protein content and lactate were elevated to 2 g/L (normal <0.45), and to 7.9 mmol/L (normal <1.8) respectively, but no pleocytosis was found.

MRI of the head and neck during the first episode (Fig. 1A. a-e) showed in the T2-weighted image an inhomogeneous hypersignal in the posterior cervical region, extending up to the anterior horn. The brain was of normal size and not atrophic, however all ventricles were dilated symmetrically. The white matter showed diffuse T1-hypointensities and T2-hyperintensities, mostly in the frontal periventricular regions, sparing gray matter, and U fibers. Cysts with partially enhancing walls and hyperintense areas gave a heterogeneous appearance to the thinned diseased white matter.

During the second episode 6 months later (Fig. 1B. a-b), the lesions of the periventricular white matter in the frontal and occipital regions were confluent, with new T2-hyperintensities in the medulla, pons, the posterior limb of the internal capsule, and both thalami. The frontal white matter and centrum semiovale appeared to be

swollen and contrast enhancement was more prominent and with new irregularities in the frontal periventricular white matter and the internal capsule. After corticoid treatment, contrast enhancement disappeared (Fig. 1B. c). MR spectroscopy found highly elevated lactate peak within the frontal white matter lesions, as well as a moderately decreased peak of N-acetylaspartate (NAA) (Fig. 1B. d).

The comatose patient continued to deteriorate slowly. He was finally transferred to palliative care, and passed away 4 months later from acute pneumonia.

Postmortem findings are described in Figure 1C and D. Extra-cerebral autopsy showed multiple lesions of acute bronchopneumonia that was mostly responsible for the acute death, as well as hepatic microvacuolar steatosis. The other organs, including the heart, were unremarkable.

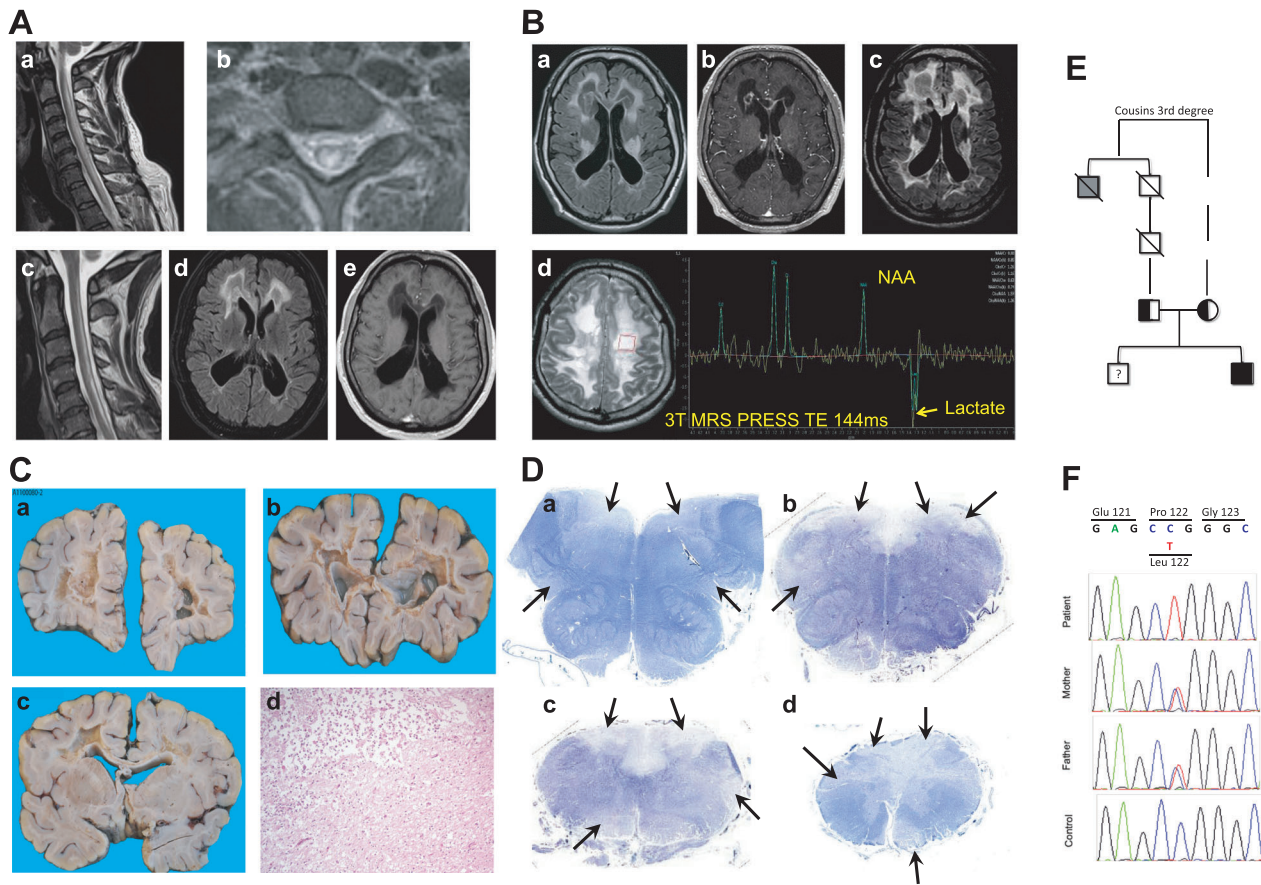
The brain's external examination was unremarkable, it weighed 1280 gr after formalin fixation. Coronal sections revealed large, bilateral, and symmetrical lesion occupying the entire bilateral centrum semiovale. These lesions were characterized by a marked yellowish discoloration of the white matter with pronounced secondary cavitation (Fig. 1C). The surrounding cortex was unremarkable as were the basal ganglia nuclei. The cerebellum, the brainstem, and the upper cervical cord showed no macroscopic abnormality.

Microscopically, lesions of the centrum semiovale were infarct-like, characterized by large areas of axonal and myelin damage, and numerous resorptive vacuolated macrophages. A scattered mild perivascular lymphocytic infiltrate was observed. Similar but much less extensive infarct-like lesion were occasionally found in the basal ganglia nuclei. These lesions were not clearly delineated and did not follow a specific vascular pattern.

The brainstem and upper cervical cord were included in paraffin in totality. The midbrain and the pons were unremarkable. The medulla showed axonal and myelin damage in the anterior and lateral spinothalamic tracts. Also, the upper cervical cord showed axonal and myelin damage associated with foci of vacuolated macrophages especially in the anterior, lateral, and posterior tracts (Fig. 1D).

In view of the clinical picture of the patient with central and peripheral neurological involvement, lactic acidosis and lactate peak as well as a decreased peak of NAA on MR spectroscopy, mitochondrial disease was suspected.

On a biopsy of the vastus lateralis muscle, oxymetric analysis, and spectrophotometric enzyme analysis of the respiratory chain (RC) in fibroblasts were performed as previously described.<sup>2</sup> In summary, analysis in fibroblasts was performed in isolated mitochondria, and in skeletal muscle homogenates (600 g supernatants) as described.<sup>3,4</sup> The activities of the individual respiratory chain



**Figure 1.** (A) MRI of the first episode of degradation. a: T2 of spinal cord showed a hypersignal of the posterior two thirds of the spinal cord over its entire extent, but predominantly in the cervical region. – b: Axial plane of the cervical MRI showing that the lesions were inhomogenous and affected the anterior horn. – 3 weeks later, a second MRI was done. – c: T2 of the spinal cord showed that the abnormalities regressing only partially after 3 weeks. – d and e: FLAIR and T1 post-gadolinium of the brain showed markedly dilated lateral ventricles, especially in the posterior regions, due to white matter volume loss. In the remaining white matter extensive diffuse T1-hypointense and T2-hyperintense abnormalities were found, most prominent in the frontal periventricular regions, sparing gray matter and U fibers. Cysts with partially enhancing walls and hyperintense areas gave an heterogenous appearance to the thinned diseased white matter. This picture was interpreted as a mix of old and more recent lesions. The corpus callosum was thinned, brainstem and cerebellum were normal. (B) MRI during second episode of degradation, 6 months later. a: New brain MRI revealed progression of the confluent lesions of the periventricular white matter in the frontal and occipital regions. The frontal white matter and centrum semiovale appeared to be more swollen than 6 months before. – b: Contrast enhancement was much more prominent with new irregularities in the frontal periventricular white matter and in the internal capsule. – c: Two weeks later the confluent cystic lesions were even more advanced. – d: MR point resolved spectroscopy showed a highly elevated lactate level and a moderately decreased peak of N-acetylaspartate (NAA) in frontal white matter lesions. (C) Postmortem brain: Coronal brain sections showed a severe leukoencephalopathy affecting both hemispheres with relative sparing of the temporal lobes (a-c). Large infarct-like lesions were found with axon-myelinic loss, numerous vacuolated macrophages, and swelling of the vascular endothelium (d). (D) Postmortem brainstem and spinal cord: The brain stem and upper cervical cord (segment C1-C4) were sectioned and included in paraffin in totality. Hematoxylin–eosin and Luxol fast blues stains revealed extensive damage (a) in the cerebellar peduncles, (b) the medulla oblongata and the upper spinal cord (c and d) in the anterior, lateral and posterior tracts (marked by arrows). (E) The pedigree chart illustrates that father and mother of the patient were cousins of 3<sup>rd</sup> degree and were healthy heterozygous carriers of the *NDUFV1* variant. The patient has a healthy brother. A brother of the father's grandfather had an unspecified learning disability. (F) MitoExome sequencing on the muscle tissue revealed a biallelic variant in *NDUFV1* (exon 4) c.365C>T p.(Pro122Leu) in the patient. The patient's mother and his father were healthy monoallelic carriers.

complexes and the mitochondrial matrix marker enzyme citrate synthase were measured spectrophotometrically with an UV-1601 spectrophotometer (Shimadzu) thermostatically maintained at 30°C. Values are estimated by the difference in activity levels measured in the presence and

absence of specific inhibitors, and are expressed as ratio to the mitochondrial marker enzyme citrate synthase (mU/mU citrate synthase). The complex I (CI) activity was rotenone sensitive and the enzymatic activities of isolated complex II, IV, and V were normal. In fibroblasts

all the CI-related enzymatic activity ratios were normal, as well as the CI dependent and succinate-related pyruvate ratio (SRPR) analyzed with high-resolution respirometric analysis using an OROBOROS system. In muscle homogenates, all activity ratios were however clearly indicative of an isolated CI defect.

Complete mtDNA sequencing from the skeletal muscle biopsy revealed no pathogenic variants. Subsequently, MitoExome sequencing comprised of 1476 nuclear genes (including 1013 genes coding for mitochondrial proteins according to the MitoCarta supplemented with genes from extensive literature search) using an in-solution hybridization capture method (NimbleGen Madison, WI, USA) was performed with paired-end sequencing on a HiSeq2500 (Illumina) to an average 178x coverage. Sequence alignment and variant calling were done with CLC Workbench v.7.0.4. CLC bio, Aarhus, Denmark).<sup>5</sup> Variants with a higher frequency than 0.1% (gnomAD) were excluded from the analysis. Only the homozygous variant in *NDUFV1* (exon 4) c.365C > T p.(Pro122Leu) remained to be compatible with an autosomal-recessive pattern of inheritance and a pathogenic prediction (SIFT, Mutationtaster, and PolyPhen2). Additionally, according to the ACMG-guidelines,<sup>6</sup> this variant is being classified as likely pathogenic. Both, the patient's father and his mother were identified as being healthy heterozygous carriers of this variant (Fig. 1E and F).

## Conclusions

Mitochondrial complex I deficiency is a relatively non-specific finding that has both a variable clinical presentation and a large genetic heterogeneity. The clinician must think about the possibility of mitochondrial disease; and the task of the laboratory is difficult because of the many different genes involved. Thus, securing a **diagnosis** still remains **challenging**. Of note, the diagnosis in our patient was suspected only during the final stage of his clinical course, and confirmed only by molecular analysis after biochemical analysis was doubtful.

The NADH Dehydrogenase (Ubiquinone) Flavoprotein 1 (*NDUFV1*) is a core subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I), which binds to the flavin mononucleotide using ubiquinone as an electron acceptor. It is a nuclear gene that encodes the 51 kDa subunit and forms the NADH- and FMN-binding site of complex I.<sup>7</sup>

The *NDUFV1* variant found in the homozygous/biallelic state in our patient has previously been reported at the compound heterozygous state in four patients: In one patient<sup>8</sup> (the variant is referred to as p.(Pro113Leu) with reference to transcript variant 2 (NM\_001166102), onset was at age 1 year, with stroke, exercise intolerance, and

leukodystrophy. Two others patients were sisters,<sup>9</sup> 15 and 13 years of age, who showed mild learning disability and decreased visual acuity due to optic nerve atrophy. The fourth patient presented with early-onset (3-month) and progressively deteriorating leukoencephalopathy.<sup>10</sup> Our patient had an early-onset, but the course was considered as stationary for over two decades, finally terminated by a rapidly evolving leukoencephalopathy apparently triggered by recurrent infectious episodes with metabolic decompensations.

Our patient extends substantially the clinical profile of known pathogenic *NDUFV1* variants including a detailed description of the biochemical, radiological, tissue histopathology, and postmortem autopsy. According to the HGMD database (HGM Professional 2021.4, total), there are 47 *NDUFV1* variants reported until now, 33 of which are missense or nonsense, five splicing, seven small deletions, one small insertion/duplication, and at least one large deletion. No clear genotype–phenotype correlation has been observed.<sup>1</sup>

A non-exhaustive review of the literature on PubMed revealed at least 43 reported patients with *NDUFV1* variants description (Supplementary Table). The median age of onset was 9 months (range 0–18 years), nine of them have already died at an average age of 14 months (range 4–36 month), and the remaining patients have a current median age of 45.5 months (range 1–480 months). Eighteen of the 43 reported patients were homozygous, with a median onset age of 8 months, and only three already died from infantile-onset leukoencephalopathy (median onset age 3.5 months). Therefore, the life span of more than 4 decades in our patient following the early childhood-onset of leukoencephalopathy is very unusual in patients with *NDUFV1* variants (Supplementary Figure). The next oldest surviving patient, aged 40 at the time of publication, presented not earlier than at young adult age with spasticity and ataxia, and was compound heterozygous c.1268C>T/c.175C>T for variants in *NDUFV1*.<sup>11</sup>

To conclude, it is therefore important to consider complex I deficiency not only in young children, but also in adult patients with leukoencephalomyelopathies. Because of the large number of candidate genes, whole exome sequencing is currently widely used as a tool to determine the molecular diagnosis of complex I deficient patients. Muscle biopsy and biochemical testing in tissues analysis should be considered when genetic testing cannot confirm a diagnosis in clinically suspected complex I deficiency. In our case, an earlier diagnostic may have had an impact on the final (fatal) outcome, as preventive measure aimed at limiting catabolic triggers and antioxidant therapies such as multiple vitamins, co-enzyme Q10, and carnitine are sometimes clinically effective.<sup>12</sup>

## Acknowledgments

The authors would like to thank the Next Generation Sequencing Platform of the University of Bern, Switzerland, for performing the high-throughput sequencing on the captured DNA. The authors would also like to thank Professor Marjo van der Knaap, Amsterdam, Netherland, for guiding, fruitful discussion, and the search of a *DARS2* mutation which could therefore be excluded. The authors would like to thank Prof. Andrea Superti-Furga for his thorough review of the manuscript and comments.

## Author Contributions

MG, RH, and TK cared for the patient and confirmed the diagnosis. MG, NGS, CT, and TK drafted the manuscript. AS and RB performed the genetic analysis. JMN did the enzymatic analysis. PM analyzed the radiologic images. MD and BL performed the postmortem analysis. CT contributed important knowledge. All authors contributed to revising the manuscript. Written consent was obtained from the parents of the deceased.

## Conflict of Interest

MG, NGS, AS, RB, JMN, RH, MD, BL, PM, CT, and TK have no conflict of interest to disclose.

## References

1. Koene S, Rodenburg RJ, van der Knaap MS, et al. Natural disease course and genotype-phenotype correlations in Complex I deficiency caused by nuclear gene defects: what we learned from 130 cases. *J Inher Metab Dis*. 2012;35(5):737-747.
2. Jackson CB, Nuoffer JM, Hahn D, et al. Mutations in *SDHD* lead to autosomal recessive encephalomyopathy and isolated mitochondrial complex II deficiency. *J Med Genet*. 2014;51(3):170-175.
3. Rustin P, Chretien D, Bourgeron T, et al. Biochemical and molecular investigations in respiratory chain deficiencies. *Clin Chim Acta*. 1994;228(1):35-51.
4. Birch-Machin MA, Turnbull DM. Assaying mitochondrial respiratory complex activity in mitochondria isolated from human cells and tissues. *Methods Cell Biol*. 2001;65:97-117.
5. Haack TB, Jackson CB, Murayama K, et al. Deficiency of *ECHS1* causes mitochondrial encephalopathy with cardiac involvement. *Ann Clin Transl Neurol*. 2015;2(5):492-509.
6. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424.
7. Spencer SR, Taylor JB, Cowell IG, Xia CL, Pemble SE, Ketterer B. The human mitochondrial NADH: ubiquinone oxidoreductase 51-kDa subunit maps adjacent to the glutathione S-transferase P1-1 gene on chromosome 11q13. *Genomics*. 1992;14(4):1116-1118.
8. Lieber DS, Calvo SE, Shanahan K, et al. Targeted exome sequencing of suspected mitochondrial disorders. *Neurology*. 2013;80(19):1762-1770.
9. Björkman K, Sofou K, Darin N, et al. Broad phenotypic variability in patients with complex I deficiency due to mutations in *NDUFS1* and *NDUFV1*. *Mitochondrion*. 2015;21:33-40.
10. Zhang J, Liu M, Zhang Z, et al. Genotypic spectrum and natural history of cavitating leukoencephalopathies in childhood. *Pediatr Neurol*. 2019;94:38-47.
11. Baertling F, Sanchez-Caballero L, van den Brand MAM, et al. A Heterozygous *NDUFV1* variant aggravates mitochondrial complex I deficiency in a family with a homoplasmic *ND1* variant. *J Pediatr*. 2018;196:309-313.e3.
12. Grad LI, Lemire BD. Mitochondrial complex I mutations in *Caenorhabditis elegans* produce cytochrome c oxidase deficiency, oxidative stress and vitamin-responsive lactic acidosis. *Hum Mol Genet*. 2004;13(3):303-314.

## Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Data S1.** Early-onset leukoencephalomyelopathy due to a biallelic *NDUFV1* variant in a mid-forties patient.