Supplemental Material

Case report: P1

Female with childhood-onset *DNAJC30*-associated LS

This 24-year-old female was born as the second child from healthy unrelated parents. She has one healthy brother and no family history of neurological or ophthalmological disease. The pregnancy was uneventful with a spontaneous uncomplicated delivery. She had normal development during the first four years of life with no major disease.

At 4 years-of-age she started to fall easily and to show spastic movements of the right hand. She experienced a progressive decrease of walking distance and was unable to stand up independently from horizontal and sitting position.

A first brain MRI at the age of 4-years and 3-months showed bilateral signal intensities of the putamina and the pedunculi cerebelli typical for Leigh syndrome (LS). CSF and plasma lactate were not elevated.

Her symptoms slowly progressed with increasing dysarthria and loss of gait. Her cognitive development was normal.

At age 7-years a muscle biopsy (fresh muscle) revealed a mitochondrial complex I deficiency (0.08 mU/mUCS, normal range 0.14-0.35). A brain MRI at this time confirmed the bilateral putaminal necrosis and the lesions in the pedunculi cerbelli without progression compared to the MRI at 4-years of age. Magnetic resonance spectroscopy (MRS) revealed a moderate lactate peak in the lesions. Myelinization was normal for age. There were no abnormalities of the optic nerve.

The patient was treated with coenzyme Q10 (10 mg/kg/d), L-carnitin 50 mg/kg/d, riboflavin 5 mg/kg/d, and baclofen 2 x 30 mg/d. She had a fat rich diet (about 60% of caloric intake from lipids).

Several cardiological, ophthalmological, and ENT-examinations were normal.

During the following years she has developed severe spasticity mainly of the extremities and lost most of her motor capacities. She is currently wheelchair-dependent and unable to walk. She communicates with the help of an eye-controlled computer. Her cognitive development seems adequate.

Case report: P2

Female with childhood-onset *DNAJC30*-associated LS

This 17-year-old female patient is the only child of healthy, unrelated Polish parents. She was born at term following an uncomplicated pregnancy by spontaneous vaginal delivery with an Apgar score of 10. The weight at birth was 3.5kg and the head circumference was 32cm. The developmental milestones were within the normal range. At the age of 2 years, strabismus, which appeared unexpectedly, was operated on. The result of the brain MRI performed before the surgery was normal.

At the age of 4 years, left-sided hemiplegia accompanied by nasal speech following a severe upper respiratory tract infection. At this time, the brain MRI displayed (SE/T2 and FLAIR) hyperintensive symmetrical lesions involving the middle and posterior part of the putamen and the globus pallidus, the anterior right side of the thalamus, substantia nigra, and the right brain peduncle. Magnetic resonance spectroscopy (MRS) showed lactate peaks within the lesions.

She stopped walking and talking when she was 4.3 years of age. She was diagnosed with extrapyramidal syndrome with asymmetric tetraplegia, ataxia, and hypotonia of probable mitochondrial origin. Neurological examination revealed central hypotonia and increased muscle tone in the upper and lower extremities. Deep tendon reflexes were absent. No improvement was observed after treatment with Biperiden.

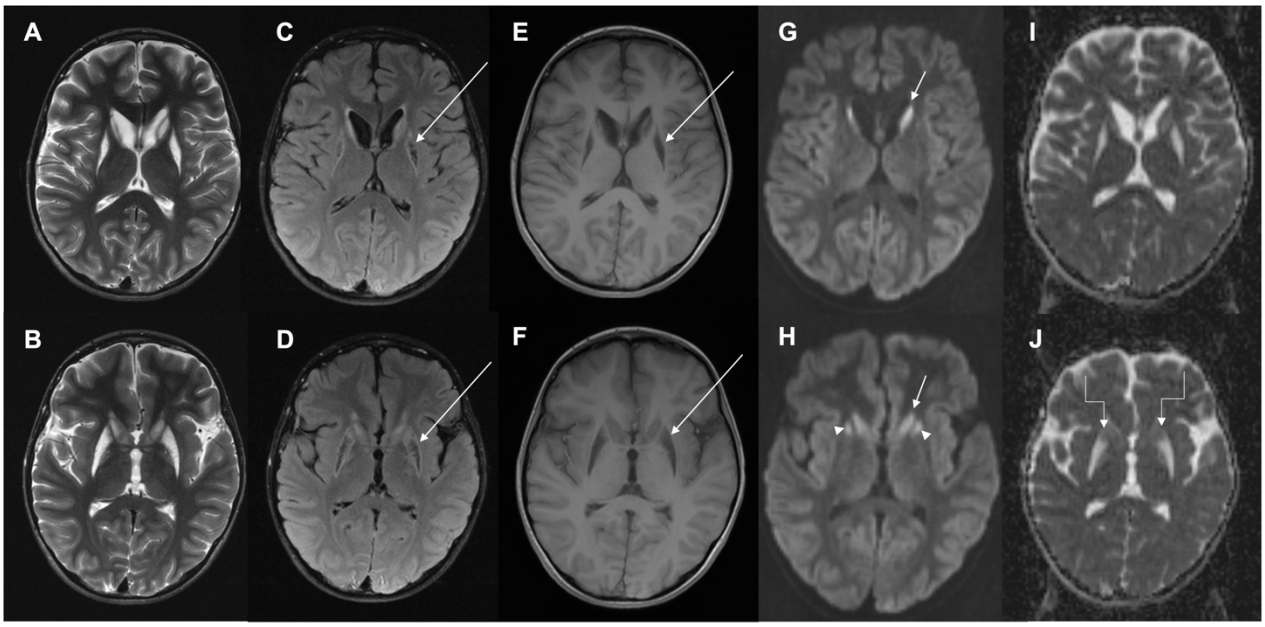
The results of blood tests were as follow: pH 7.34 (7.35-7.45); PCO2 40.5 mmHg (35.0-48.0); PO2 44.3 mmHg; (83.0-108.0) HCO3 21.0 mmol/l (22.0-26.0); SBE -3.6 mmol/l (-3.0- +2.0); SO2 79.5 % (95.0-99.0); creatine kinase activity was 303 U/l (14-171); free carnitine 29 (35-75), total carnitine 50 (42-80); lactic acid (in blood) 10.7 mg/dl (5.7-22.0); lactic acid in CSF was 11.0 mg/dl (11.0-19.0).

A skeletal muscle biopsy was performed when she was 5 years of age. The histochemical and immunomorphological examination of the muscle tissue displayed no abnormalities. Enzymatic activity of OXPHOS in the muscle homogenate was also normal.

The course of the disease was mildly progressive. Excessive global dystonia resulted in the left hip dislocation at the age of 12 years. Feeding problems have led to malnutrition and cachexia. Her weight at the age of 16 year was 24 kg. A gastrostomy tube was implemented, and the patient's general status improved.

The next MRI brain revealed progressive changes (comparing with the previous examination) distinctive for Leigh syndrome. Hyperintensive changes previously seen in the brainstem, the brain peduncle, and thalamus (seen in T2) had resolved. The previously described putaminal changes turned into the spongio-necrotic areas. There were new symmetrical hyperintensive areas in the anterior part of the putamen, the caudal nuclei. The structure of the putamen and caudal nuclei decreased, which resulted in the atrophic expansion of the volume of the lateral ventricles, especially in the anterior horns and the central parts.

Molecular genetic study revealed a heterozygous variant c.457T>C in the eighth exon of the *NDUFS8* gene, inherited from the mother. Biochemical tests of the activity of OXPHOS enzymes in fibroblasts showed decreased activity of complex I of the respiratory chain complexes.



MRI brain examination at the age of 12 years. **A-B,** T2-weighted images; **C-D,** FLAIR sequence; **E-F,** T1-weighted images; **G-H,** DWI - diffusion weighted images; **I-J,** ADC - apparent diffusion coefficient maps. Decreased volume and bilateral symmetrical, abnormal signal of the putamina and heads of caudate nuclei. Increased signal intensity on T2-weighted images. Decreased signal intensity on FLAIR sequence and T1-weighted images. Abnormal lucency of the putamina visible on FLAIR and T1-weighted images representing areas of necrosis (long arrow on **C-F**). DWI with b=1000 demonstrating punctate hyperintense signal in in the anterior part of putamina (arrow head on **H**) and caudate nuclei (short arrow on **G-H**) with slight noticeable low signal on ADC maps (broken arrow on **J**) - restricted diffusion.

Case report: P3

Male with adult-onset *DNAJC30*-associated LS

This patient is a 28-year-old male, who was born at full-term via C-section due to prolonged labour to a 25-year-old woman and a 25-year-old man. He has no similarly affected family members. The patient is the oldest of four brothers. The others are 25, 22 and 21 years of age. Near sightedness is reported in the 25- and 22-year-old brothers. No other vision issues reported. No strokes or stroke-like symptoms reported. No consanguinity reported. Pregnancy was complicated by extreme hyperemesis. Birth weight 3.6kg. No developmental problems or major medical problems prior to initial presentation.

In 2013, at 19 years of age, the patient experienced an episode of abrupt decline after waking up with blurry vision and "balance problems" without an apparent reason. These symptoms worsened over the next several months. On examination he had nystagmus, ophthalmoplegia, and dysarthria. LHON was initially suspected, however, he was ultimately diagnosed with LS based on his brain MRI findings, indicating an LHON/LS overlap syndrome. Lactate 2.0, pyruvate normal, urine organic acids, and plasma amino acids normal. EMG/NCS demonstrated a mild myopathy.

In 2014, about a year after his initial presentation, he had a febrile illness and "crashed" with development of worsening vision, nystagmus, ophthalmoplegia, dysarthria, severe fatigue, shortness of breath and central apnoea (mixed central and obstructive hypopnea) requiring BiPAP, gastrointestinal dysmotility/reflux, dysphagia, left-sided weakness, and ataxia. Over the next few months, he was able to wean off respiratory support and recovered some function but never returned to baseline. He also developed hypertension during this time. Following this the patient has been chronically fatigued, and dysarthria has remained.

Genetic testing in 2016 (whole exome sequencing) revealed variants of uncertain significance (VUS) in *ADORA1, DNAJC30, NDUFS2, SCN2A, UGGT1,* and *WFS1*. The variant in *NDUFS2,*

a known LS-associated gene, was heterozygous and a second rare variant to be in-keeping with the autosomal recessive mode of inheritance of the disease was not identified. Similarly, the variant in *WFS1,* a known autosomal recessive disease gene associated with optic atrophy, was heterozygous. No pathogenic variants were found in the mitochondrial DNA. This exome included samples from both parents and two unaffected brothers. Exome reanalysis in 2018 was unrevealing. Exome reanalysis in 2021, however, reprioritized the homozygous variants in *DNAJC30* c.152A>G; p.Tyr51Cys, given recent publication of autosomal recessive LHON and Leigh syndrome due to biallelic variants in *DNAJC30* (Stenton et al., 2021). Given the patient's symptoms, this homozygous variant was thought to be pathogenic. The 21-year-old unaffected brother was also found to be homozygous for the variant in *DNAJC30*. The 22-year-old unaffected brother was heterozygous for the variant.

A follow-up brain MRI in 2017 demonstrated i) slight progression of the left basal ganglia lesions with small foci of restricted diffusion, indicating acute or active disease, ii) interval development of signal changes within the ventral lower brainstem, representing gliosis and Wallerian degeneration from prior injury, and iii) mild volume loss and residual signal changes of the bilateral putamen and periaqueductal regions, compatible with encephalomalacia and sequelae of LS. An MRS demonstrated elevated lactate in the left basal ganglia and periaqueductal regions compatible with the provided clinical history of LS.

At last examination in 2021 he remains independent for activities of daily living. He eats everything by mouth and denies chewing or swallowing problems. He denies episodes of choking and has not had aspiration pneumonia. His examination was notable for pronounced dysarthric speech that is somewhat difficult to understand, mild bilateral ptosis, and delayed response to questions. His continues to experience a poorly regulated breathing pattern and is only able to speak two to three words at a time without needing to catch his breath. Symmetrical reflexes were present without clonus and symmetric facial movements were present. He has a left facial droop and is hemiplegic on the left with decreased left arm swing and marked spasticity with circumduction of the left leg resulting in an unsteady gait (left-side dominant prior onset).

He takes vitamin B-complex 100, 20 mg of biotin, 1000 mg CoQ10, some vitamin E and C (patient uncertain of doses). He subjectively notices worsening symptoms when he misses his doses of biotin.