

## Supplemental Data

### ***NAXE* Mutations Disrupt the Cellular NAD(P)H**

### **Repair System and Cause a Lethal**

### **Neurometabolic Disorder of Early Childhood**

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## Supplemental Case Reports

**Individual #1-1** (c.[177C>A];[177C>A]; p.[Tyr59\*];[Tyr59\*]), a boy, was born to healthy consanguineous parents from Gambia. Among his siblings, one girl was similarly affected (Individual #1-2, see below), one was a premature infant who died at the age of three months, and three are healthy. The index case was considered normal at birth (birth weight 3540 g, lengths 55 cm) and medical follow-up during the first 1.5 years of life was unremarkable. In particular, no neurological problems or developmental delay were noted.

At the age of 20 months, he suffered from a febrile tonsillitis (temperatures up to 40°C), which was treated with oral antibiotics. Shortly after the start of fever, he developed a torticollis and was admitted to the hospital. Initial examination additionally revealed ataxia. He received intravenous fluids and antibiotics. An initial brain MRI was without obvious pathological findings. During the following days his situation gradually improved. However, about two weeks after admission, the boy again developed fever and his neurological status deteriorated with severe ataxia and nystagmus. In the following, he suddenly suffered from respiratory insufficiency, requiring intensive care treatment and mechanical ventilation. Brain MRI showed severe cerebellar edema and spinal MRI revealed extensive myelopathy (see Figure 2 as well as Figure S1). Laboratory investigations of blood and cerebrospinal fluid demonstrated transiently elevated lactate levels (in blood up to 4.6 mmol/L, NR <1.6; in CSF up to 2.69 mmol/L, NR <2.24 mmol/L). Extensive metabolic, immunological, and microbiological investigations were without any specific findings. During the further course, the boy developed massive Lyell-like bullous skin lesions of unclear aetiology. Under the suspicion of a mitochondrial disorder, a muscle and skin biopsy was performed. Biochemical examination of fresh muscle tissue revealed mild

mitochondrial complex I deficiency (64 mU/U citrate synthase [CS]; NR 70-251 mU/U CS) and a globally reduced ATP production rate (15.5 nmol/h.mU CS; NR 42.1-81.2 nmol/h.mU CS). Investigation of skin fibroblasts was normal. During the further clinical course the boy was comatose und suffered from repeated seizures. Finally, at the age of 21 month he died due to cardiovascular failure.

**Individual #1-2**, a girl, was born as the third child of the parents. As described for her brother (individual #1-1), pregnancy, birth and postnatal development were unremarkable.

At the age of 19 months, the child suffered from a febrile respiratory infection (temperatures up to 39.9 °C). Two days after onset of symptoms her clinical condition deteriorated. She vomited repeatedly and developed ataxia. Despite emergency hospital admission and application of intravenous fluids as well as antibiotics neurological signs were rapidly progressive and the girl developed a flaccid tetraparesis, requiring mechanical ventilation. Brain MRI demonstrated symmetrical signal alterations of cerebellar white matter and spinal MRI revealed extensive myelopathy (C2-Th10; see Figure 2). Laboratory investigations showed transiently elevated liver enzymes (GOT up to 790 U/L, NR <52 U/L; GPT up to 785 U/L, NR <29 U/L). Analysis of cerebrospinal fluid revealed moderately increased lactate levels (up to 3.2 mmol/L, NR <2.24 mmol/L). Immunological, microbiological and viral investigations were without specific findings. Under the suspicion of an autoimmune disease (e.g. transverse myelitis), intravenous cortisone and immunoglobulins were administered without therapeutic effect. About 14 days after onset of neurological signs, transient ichthyosiform skin changes were noted mainly at the abdomen and the inguinal region.

For the next 2 months, the child was continuously treated at the intensive care unit and required mechanical ventilation. However, her condition was rather stable and even slightly improved until she suddenly developed fever, abdominal distension and repeated seizures. In addition, massive Lyell-like bullous skin lesions developed as observed in her brother (see Figure 2). A skin biopsy was taken, which showed intraepidermal bullae without signs of inflammation. Based on the histology results, a staphylococcal Lyell's syndrome or a toxic epidermal necrolysis appeared to be unlikely. The neurological status of the girl further deteriorated, and examination revealed absence of pupil reactions to light, of pharyngeal and tracheal reflexes and of spontaneous breathing. Follow-up MRI demonstrated severe global brain atrophy (see Figure S1). While mechanical ventilation was sustained, she finally died at the age of 24 months due to cardiovascular failure. Autopsy was performed after parental consent and revealed extensive areas of necrosis affecting brain stem, as well as cervical and thoracic spine.

**Individual #2** (c.[196C>T];[516+1G>A]; p.[Gln66\*];[?]), a girl, was born as the first child of healthy and unrelated parents in Croatia. A previous pregnancy of the mother had ended by early miscarriage of unknown cause. The index case was born on term, after normal pregnancy and uneventful delivery (birth weight 3740 g, lengths 48 cm). Development was normal until the second half of the first year when she failed to thrive and had mild psychomotor delay. Parents also reported fatigue, occasional tremor and convergent strabismus, which were more pronounced after exertion. At the age of 15 months the child experienced psychomotor regression during febrile illness, but fully recovered during the following few weeks.

At the age of 17 months she had respiratory infection with high fever. On the third day of illness, acute deterioration with respiratory failure occurred. She was resuscitated in hospital and had normal oxygenation and heart rate during the whole process. Upon admission to hospital her GCS was 8, blood gases, CBC, glucose, electrolytes, aminotransferases, urea, creatinine, CK and protein electrophoresis were normal. CSF lactate was high (4.1 mmol/L; NR < 2.2 mmol/L), other CSF findings (cells, proteins, glucose) were normal and blood lactate was slightly increased (1.9 mmol/L; NR < 1.33). Metabolic work-up (plasma and CSF amino acids, organic acids in urine) was unremarkable except for mildly increased excretion of pyruvate in urine. Electroencephalogram showed slower brain activity (4-5 Hz), but without epileptic discharges. Brain MRI showed extensive signal abnormalities in the cortex, subcortical cerebral white matter, caudate nucleus, putamen, pons, middle cerebellar peduncles and medulla oblongata. Moreover, cerebellar edema and cervical myelopathy were seen (Figure 2). Diffusion-weighted images showed diffusion restriction in large areas of the cerebral cortex. Due to clinical presentation and symmetrical basal ganglia involvement, treatment with a mitochondrial cocktail was started (L-arginine, coenzyme Q10, vitamins C, B1 and B2). Muscle biopsy showed myopathic changes, while respiratory chain enzyme and pyruvate dehydrogenase activities in fresh frozen muscle were normal. During the following month she was in coma and had tracheostomy. After one month of mechanical ventilation she was successfully weaned from ventilator. At that point she reacted on painful stimuli, but still had disturbed consciousness with hyperkinesia of face muscles, extremity tremor, nystagmus and rigidity of upper limbs. Control MRI performed in one month intervals showed global brain atrophy with thin cerebral cortex, reduced white matter and signal abnormalities in the caudate nucleus and

putamen. The brainstem and cerebellum were relatively preserved (Figure 2 and Figure S1). Since thiamine transporter deficiency was suspected, thiamine was increased to 200 mg bid per day (previously 100 mg), biotin was added to the cocktail, and antiepileptic treatment was modified (clonazepam for the treatment of hyperkinesia), thereafter she markedly improved. In the following days hyperkinesia almost disappeared, she started to fixate objects and respond to her parents. During the next weeks her muscle tone was improved and she could sit unsupported. About three months after first crisis, her condition worsened again, she became hypotonic with more pronounced strabismus, tremor and psychomotor restlessness. She had short episodes of fever, and erythematous rash on intertriginous and perigenital areas, hands, and feet. MRI performed at that point showed thin cerebral cortex with diffusely abnormal signal, cerebral white matter atrophy, and mild signal abnormalities in the caudate nucleus and putamen. MRS showed increased choline and mildly increased lactate in deep white matter and normal metabolite peaks in basal ganglia. Soon, she again developed respiratory insufficiency and a deep coma from which she never recovered. CT scan revealed hypodensity of mesencephalon, pons, and medulla oblongata with brain edema, herniation of cerebellar tonsils with enlarged IV ventricle and hypertensive hydrocephalus. External ventricular drain was placed. CSF lactate was elevated (5.0 mmol/L; NR < 2.2 mmol/L) while blood lactate was normal or close to normal. In the following days skin changes worsened and she had sharply demarcated edematous and erythematous rash on feet, hands, limbs, and areas around mouth and eyes (Figure 2). Skin biopsy showed focal keratosis and normal dermis without inflammation. Clinically, she had signs of systemic inflammatory reaction. Immunological and microbiological work-up was unremarkable. She received intravenous immunoglobulins (2g/kg) and pulse doses of

steroids but there was no obvious benefit. She lost all her hair. After the skin peeled off the skin changes resolved. Further clinical course was complicated with *C. difficile* colitis and sepsis. Control brain MRI three weeks after deterioration showed diffuse brain edema, especially in cerebellum and brain stem as well as cervical myelopathy (Figure 2). After 6 weeks ventricular drainage was taken out and there was no need to place ventriculoperitoneal shunt. Further course was complicated by episodes of hemodynamic instability probably of central origin and sepsis. Last brain MRI was performed two months after deterioration and showed diffuse atrophy, reduced diffusion due to cytotoxic edema of brain stem and cerebellar white matter, and extensive cystic alterations of pons, cerebellar peduncles, pontomesencephalic and pontomedullary junctions. The girl died at the age of 24 months due to sepsis which led to irreversible circulatory failure. Autopsy showed periventricular and cortico-subcortical encephalomalacia with massive brain edema.

**Individual #3** (c.[804\_807delinsA];[ 804\_807delinsA]; p.[Lys270del];[Lys270del]), a boy, was born after normal pregnancy and labor at 41 weeks of gestational age (birth weight 3510 g) in Germany. He had a mild developmental delay. He was able to crawl at 10 months. He started to sit at 11 months and remained slightly unstable when sitting. At 12 months he was able to speak single words.

At the age of 16 months he developed a bilateral ptosis, a left-sided abducens palsy and a vertical gaze palsy. To exclude a myasthenic syndrome, an edrophonium test was done which was negative and the boy was hospitalized for further diagnostic workup. Three days after admission to the hospital he showed progressive ataxia of trunk and limbs as well as an upgaze nystagmus. Tendon reflexes were normal, and there was no central paresis. He was irritable but mental function was otherwise

normal. He had a mild upper respiratory tract infection. Brain MRI was normal. Ventricles were not enlarged and there was no increased signal intensity in basal ganglia or brain stem. Metabolic screening (organic acids in urine, amino acids in plasma and CSF, carnitine and acylcarnitines) were normal. Nerve conduction velocities were in the normal range. Following anesthesia for the MRI the child remained very sleepy. At day 8 he became progressively somnolent. CSF examination showed an increase of lactate (3.6 mmol/L) but was otherwise normal (cells, protein, glucose). In the following night high fever (39.9°C), central bradypnea, and coma (GCS 4-6) developed. At admission to the ICU, blood gases were normal, but 2 hours later he showed progressive respiratory failure with a pCO<sub>2</sub> of 59 mm Hg and he was intubated. A thorax scan showed signs of beginning pneumonia. On day 11, a CT scan of the brain showed severe brain edema with absent grey and white matter differentiation, beginning herniation of the cerebellum but widely enlarged ventricles I, II and III. Histological, histochemical and ultrastructural examination of a vastus lateralis muscle biopsy was largely normal but showed some lipid and glycogen accumulation as well as aggregated mitochondria without paracrystalline inclusions in single fibers. Functional investigation of intact mitochondrial from a fresh muscle biopsy showed decreased oxidation of pyruvate-containing substrates while acetylcarnitine substrates were normally oxidized (Table S1). Complexes I –V of the oxidative phosphorylation and pyruvate dehydrogenase had normal activity. On day 12 pupils dilated and became unresponsive. The child died 12 days after admission.

**Individual #4-1** (c.[653A>T];[743delC]; p.[Asp218Val];[ Ala248Glufs\*26]), a boy, was born after uneventful pregnancy and labor at 38 weeks of gestational age (birth weight was 3100 g) in Poland. His neonatal and infantile period was uneventful. At

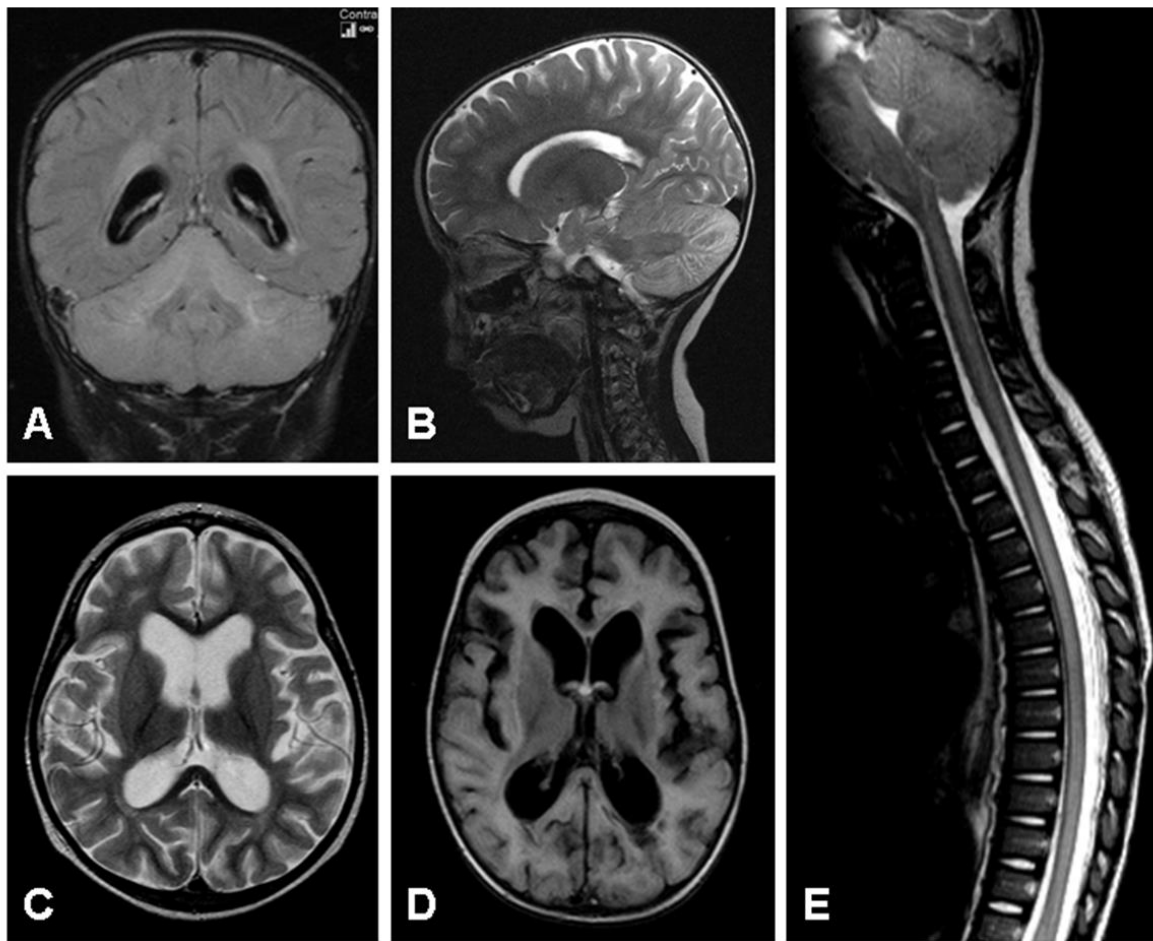
the age of 16 months he presented with an acute episode of ataxia. Emergency CT scan was without obvious pathology. Lumbar puncture was performed and CSF analysis was normal, although lactate was slightly elevated (2.6 mmol/L, NR < 2.2 mmol/L). Ataxia resolved after 3 weeks of steroid treatment. The next episode of ataxia occurred at the age of 29 months, with nystagmus, muscle hypotonia, and dysarthria. Brain MRI showed no pathologies. Electroencephalogram showed diffuse and slow activity (2.7-5 Hz). Lactate in CSF was 3.7 mmol/L (NR < 2.2 mmol/L). This time, disease progressed quickly. Two days after beginning of symptoms the boy became comatose, and developed respiratory insufficiency due to bradypnea. He required intubation and mechanical ventilation. After 7 days the child was still in coma. Electroencephalogram showed diffuse slow waves (1.5-2 Hz, 160-400  $\mu$ V). One focal reddish, psoriatic skin change on neck was observed. MRI of the brain revealed severe cerebellum and brain stem edema with T2 hypo/hyperintense changes in cerebellum and the cervical spine (see Figure 2 and Figure S1). Intensive antiedema therapy was introduced without clinical improvement. As the disease resembled acute viral encephalitis, virological tests were performed, which were negative, as well as were metabolic tests. Level of protein in CSF was normal. On the 14<sup>th</sup> day of the episodes CT scan revealed massive brain edema and isoelectric line in electroencephalogram was found. The next day the child died. Autopsy showed massive brain edema and lack of Purkinje cells in cerebellum.

**Individual #4-2** (c.[653A>T];[743delC]; p.[Asp218Val];[ Ala248Glufs\*26]) the brother of individual #4-1, was born after uneventful pregnancy at 41 weeks of gestational age (Birth weight 3190 g). His psychomotor development was delayed due to muscular hypotonia.

At the age of 8 months he presented with myoclonic seizures, which were treated with valproate (VLP). He developed transient respiratory insufficiency requiring mechanical respiratory support. Later he was discharged home with gradual clinical improvement. After rehabilitation therapy and withdrawal of VLP he began to walk with support. At the age of 20 months the boy developed a further episode of acute deterioration. He rapidly became hypotonic, comatose and again suffered from seizures. Disease progressed and during one week he developed coma, respiratory insufficiency and acute hydrocephalus, requiring a ventriculoperitoneal shunt. In CSF increased lactate levels were found. Consecutive CT scans revealed progressive brain atrophy, especially of the brain stem. The child died at the age of 24 months, after 4 months hospitalization and intensive care treatment.

## SUPPLEMENTAL FIGURES

Figure S1. Additional neuroimaging findings in patients with *NAXE* mutations



A) Brain MRI (coronal view, T2-weighted) of individual #1-1, showing diffuse cerebellar edema. B) Brain MRI (sagittal view, T2-weighted) of individual #4-1, demonstrating diffuse signal abnormalities and edema of the cerebellum. C) Brain MRI (axial view, T2-weighted) of individual #1-2 after several months after onset of symptoms showing severe global brain atrophy. D) Brain MRI (axial view, T2 TIRM dark fluid) of individual #2 after long term intensive care treatment showing a similar degree of brain atrophy as seen in C). E) Spinal MRI (sagittal view, T2-weighted) of patient 1-2 demonstrating affection of nearly the complete spinal cord.

## SUPPLEMENTAL TABLES

**Table S1. Biochemical analysis in muscle biopsy of individual #3**

<b><i>I. Enzyme investigations</i></b>	<b>mUnit/mg protein</b>	<b>mUnit/mUnit CS</b>
Citrate synthase (CS)	95 (150 - 338)	
Complex I	24 (28 - 76)	0,25 (0,14 - 0,35)
Complex I+III	63 (49 - 218)	0,67 (0,24 - 0,81)
Complex II	18 (33 - 102)	0,19 (0,18 - 0,41)
Complex II+III	34 (65 - 180)	0,36 (0,3 - 0,67)
Complex III	164 (304 - 896)	1,73 (1,45 - 3,76)
Cytochrome c oxidase	190 (181 - 593)	2,00 (0,91 - 2,24)
Complex V	90 (86 - 257)	0,95 (0,42 - 1,26)
Pyruvate dehydrogenase	8,3 (5,3 - 19,8)	0,087 (0,026 - 0,079)
<b><i>II. Substrate oxidation</i></b>	<b>nmol/h/mg protein</b>	<b>nmol/h/mUnit CS</b>
[1-14C]Pyruvate+malate	122 (263 - 900)	1,29 (1,54 - 3,55)
[1-14C]Pyruvate+carnitine	139 (302 - 856)	1,47 (1,65 - 3,66)
[U-14C]Malat+pyruvate+malonate	132 (282 - 874)	1,40 (1,56 - 3,87)
[U-14C]Malat+acetylcarn.+malonate	165 (273 - 678)	1,74 (1,16 - 2,82)
[U-14C]Malat+acetylcarn.+arsenite	83 (156 - 378)	0,88 (0,57 - 1,52)
[U-14C]Glutamat+acetylcarnitine	34 (86 - 209)	0,36 (0,35 - 1,06)

normal ranges in brackets