Supplementary Online Content


**eAppendix.** Clinical Data

**eFigure.** Muscle cDNA Analysis in Patient IV Confirms that the Variant c.107377+1G>A Causes a Misplicing

This supplementary material has been provided by the authors to give readers additional information about their work.
**eAppendix: Clinical Data**

**Patient I**

Patient I was a man in his late 50s. He was born from non-consanguineous parents and his family history was negative for neuromuscular disorders. The patient presented first symptoms at the age of 40 years, complaining of progressive distal weakness in the lower limbs. Over the years he showed progressive involvement of axial muscles and later onset of proximal weakness initially at upper limbs and subsequently also at lower limbs. Muscle MRI confirmed severe fatty degeneration of gluteus, quadriceps, femoral biceps, and paraspinal muscles. Cardiac function was normal. Respiratory evaluations showed a restrictive respiratory pattern. At the last assessment, the patient showed severe weakness and hypotrophy of the proximal muscles in the upper limbs (MRC 3), especially of deltoid and infraspinatus. He was not able to raise his arms over the head. Distal strength in the hands was normal. At lower limbs he showed proximal weakness (MRC iliopsoas 3 and quadriceps 4) and severe distal weakness (MRC dorsiflexion 0 and plantarflexion 3). He also had ankle contractions and a waddling gait with bilateral foot-drop. Muscle biopsy showed a dystrophic pattern with severe connective tissue increase and fiber necrosis.

**Patient II**

Patient II was a man in his mid-50s presenting with an adult onset distal myopathy. Since 2008, he had complained of myalgia in the lower limbs and exercise intolerance. At the last assessment, the patient showed a severe weakness of the neck flexors, peroneal and distal upper limb muscles and a mild to moderate involvement of pelvic muscles. The patient displayed no heart involvement, except for very frequent supraventricular heart beats. Pulmonary function tests were only minimally impaired. Creatine kinase (CK) was slightly increased (2-3X). A mixed pattern was observed on EMG. Muscle biopsy revealed myopathic findings, including cytoplasmic bodies in a few fibers and
rare autophagic vacuoles. His mother died in her late 60s from an ischemic heart attack, but had gait
difficulties since age 40.

Patient III

Patient III has been extensively described in Evilä et al. 2016 (ref.33 – family E).

Patient IV

Since his childhood the patient has difficulty in running, fatigability and toe-walking. He was first
referred when he was a child and limb girdle muscular weakness was observed. A muscle biopsy
was performed showing myopathic changes: fiber size variation, internal nuclei, degenerating-
regenerating fibers, rare rimmed vacuoles and mild fibrosis. A follow-up in his late teens revealed
mild worsening of muscle weakness and presence of dilated cardiomyopathy without arrhythmias.
He underwent bilateral surgical correction of Achilles’ tendon contractures. On the latest physical
examination, mild weakness of elbow flexors (4/5), arm extensors (3/5), thigh flexors (4/5),
adductors (3/5) and extensors (3/5) was observed. Elbows, fingers and right Achilles’ tendon
contractures were also present. Muscle MRI showed a fatty infiltration mainly in adductor magnus,
biceps femoris and semimembranosus in the thigh and medial gastrocnemius in the legs, with less
severe changes in adductor longus, rectus femoris and vastus intermedius.

Patient V

At birth, the proband showed mild hypotonia, hyporeflexia and macrocephaly. Since childhood, he
had slowly deteriorating gait and frequent falling. At the latest examination, he presented with
diminished exercise capacity and normal CK levels. Strength was progressively diminishing in both
hands with more difficulties to e.g. work with a computer. Also rising up the stairs became more
difficult and walking was restricted to approximately 300 m. MRI showed pseudo-hypertrophy and
fatty infiltration of lumbar muscles, bilateral fatty infiltration of gluteus maximus, vastus
intermedius, rectus femoris, biceps femoris, a pseudo-hypertrophy of adductor. A diffuse fatty
infiltration of lower leg extensors and flexors (tibialis anterior, soleus, gastrocnemius and caput medialis) was also observed. Muscle biopsy showed increased variability of fiber size and internal nuclei, necrotic fibers and phagocytosis. Electron microscopy revealed the presence of inclusion bodies.

**Patient VI**

Patient VI was a woman in her mid-50s with her first symptoms in her early 30s. Family history was negative. First neurological examination revealed mild plantar and dorsal ankle flexion weakness associated with both posterior and anterior compartment lower leg muscle hypotrophy, mild weakness of knee flexion and extension, bilateral pes cavus and Achilles tendon contractions. CK was 395 U/L (normal value <195). A muscle biopsy in her early 40s showed considerable variability in fiber size, presence of cores in most fibers, and a few autophagic vacuoles. Weakness progressed over the years with need of a stick for the last 5 years. The latest examination showed steppage and waddling gait, severe (MRC 0/5) tibial, peroneal and knee flexor muscle weakness and hypotrophy, moderate knee extension, hip flexion and extension muscle weakness. Axial and upper limb muscle power and cranial muscles were normal. Echocardiography in her early 50s showed mild left ventricular hypokinesia and mildly reduced ejection fraction (43%), progressing to 35% after 4 years. In her first 50s, spirometry was normal.

**Patients VIIa and VIIb**

Patient VIIa was a woman in her early 50s with an onset (walking difficulty) in her early 30s, mainly due to distal lower limb muscle weakness. She was first admitted for examinations in her mid-30s and showed marked (MRC 2/5) tibialis anterior muscle weakness and hypotrophy, mild (MRC 4/5) knee flexor muscle weakness and severe Achilles tendon contractions, predominant on the right side. Cranial muscles, upper limb and axial muscle power were normal. A muscle biopsy revealed high variability of fiber size, internalized nuclei, increased endomysial connective tissue,
autophagic vacuoles in numerous fibers, a few degenerating fibers, and focal reduction of oxidative enzyme activity. Muscle MRI in her mid-45 showed almost complete fatty replacement of bilateral tibialis anterior, peroneal, soleus and left medial gastrocnemius muscles, with sparing of tibialis posterior and lateral gastrocnemius. In the thigh only biceps femoris showed fatty infiltration. She underwent tendon lengthening surgery in her late 30s. Electromyography in her late 40s showed high frequency discharges in proximal and distal muscles. Latest neurological examination revealed steppage, marked (MRC 1/5) foot dorsiflexion, mild (MRC4/5) distal upper limb and proximal lower limb muscle weakness, the latter slightly more pronounced on the right side. No feature suggestive of respiratory or cardiac involvement was detected during the follow-up period.

Similar clinical (onset in early adulthood and moderate progression) and histological (myopathic findings) features were observed in patient VIIb, sister of the proband. In her early 60s, pulmonary function tests showed the first sign of a restrictive disease which so far does not require ventilatory support. The patient also presented with paroxysmal sinus tachycardia and frequent ventricular extrasystole.

Patient VIII

The patient had presented with difficulties in running and Achilles’ tendon contractures since the preteen years. Weakness in lower extremities worsened in the early 30s. CK was elevated (between 400 and 700 UI/l). No signs of cardiomyopathy were detected on heart ultrasound. Muscle biopsy showed myopathic changes with prominent internal nuclei and fiber type disproportion. Some fibers displayed likely autophagic vacuoles and core-like areas were also reported.

At the latest examination, the patient showed hypotrophy of lower limb muscles, mild hyperlordosis and Achille’s tendon contractures. He walked with a waddling and steppage gait. Weakness was mainly present in hip adductors and flexors (MRC 2), knee flexors and hip extensors (MRC 3), tibialis anterior, iliopsoas, biceps and triceps brachii (MRC 4). Muscle MRI showed asymmetric
fatty replacement in paraspinal, obturator externus and glutei muscles, diffuse involvement of thigh muscles (including semitendinosus, gracilis and sartorius) with peculiar relative sparing of adductor magnus, rectus femoris and vastus lateralis and widespread fatty replacement of lower leg muscles with sparing of flexor and extensor digitorum longus, tibialis posterior and to a lesser extent soleus.

**Patient IXa and IXb**

The proband was a teenage boy who had hypotonia and congenital torticollis at birth. He had delayed motor milestones and frequent falls. He was referred to the neuromuscular unit as a child because of a proximal and distal weakness. At the examination in 2015, he presented winged scapulae, pectus excavatum and pes cavus. He had a waddling gait, was unable to rise from the floor, and presented proximal weakness in the upper limbs, proximo-distal weakness in the lower limbs, neck flexor weakness and mild facial weakness. Muscle biopsy showed myopathic changes with internal nuclei, fibers presenting mini-multicores, and type 1 fiber predominance.

A similar phenotype was observed in his affect sister (IXb).

**Patient Xa and Xb**

Both the affected siblings show a slowly progressive distal myopathy with an onset in the second decade. At the latest neurological examination, the patients walked with a waddling gait and bilateral steppage. Distal weakness in both upper and lower limbs (tibialis anterior) was observed. Muscle MRI showed fatty infiltration in all muscles of thigh (IIb and III) as well as in tibialis anterior (III-IV) and in soleus (IIb – III). Myopathic changes were seen at EMG. A muscle biopsy showed severe increase of connective tissue, muscle fiber variability, several rounded hypotrophic and rare rounded hypertrophic fibers, numerous internal nuclei and the presence of rare vacuoles. CK was normal and no heart or respiratory involvement was observed.
**eFigure.** Muscle cDNA analysis in patient IV confirms that the variant c.107377+1G>A causes a misplicing

The variant c.107377+1G>A, identified in patient IV, actives two different exonic cryptic acceptor sites with the subsequent activation of cryptic branch sites. Two altered transcripts were identified, one missing the last 69 nucleotides of exon 362 and the second one missing the last 7 nucleotides of the same exon.