Supplementary Information

SNUPN deficiency causes a recessive muscular dystrophy due to RNA mis-splicing and ECM dysregulation

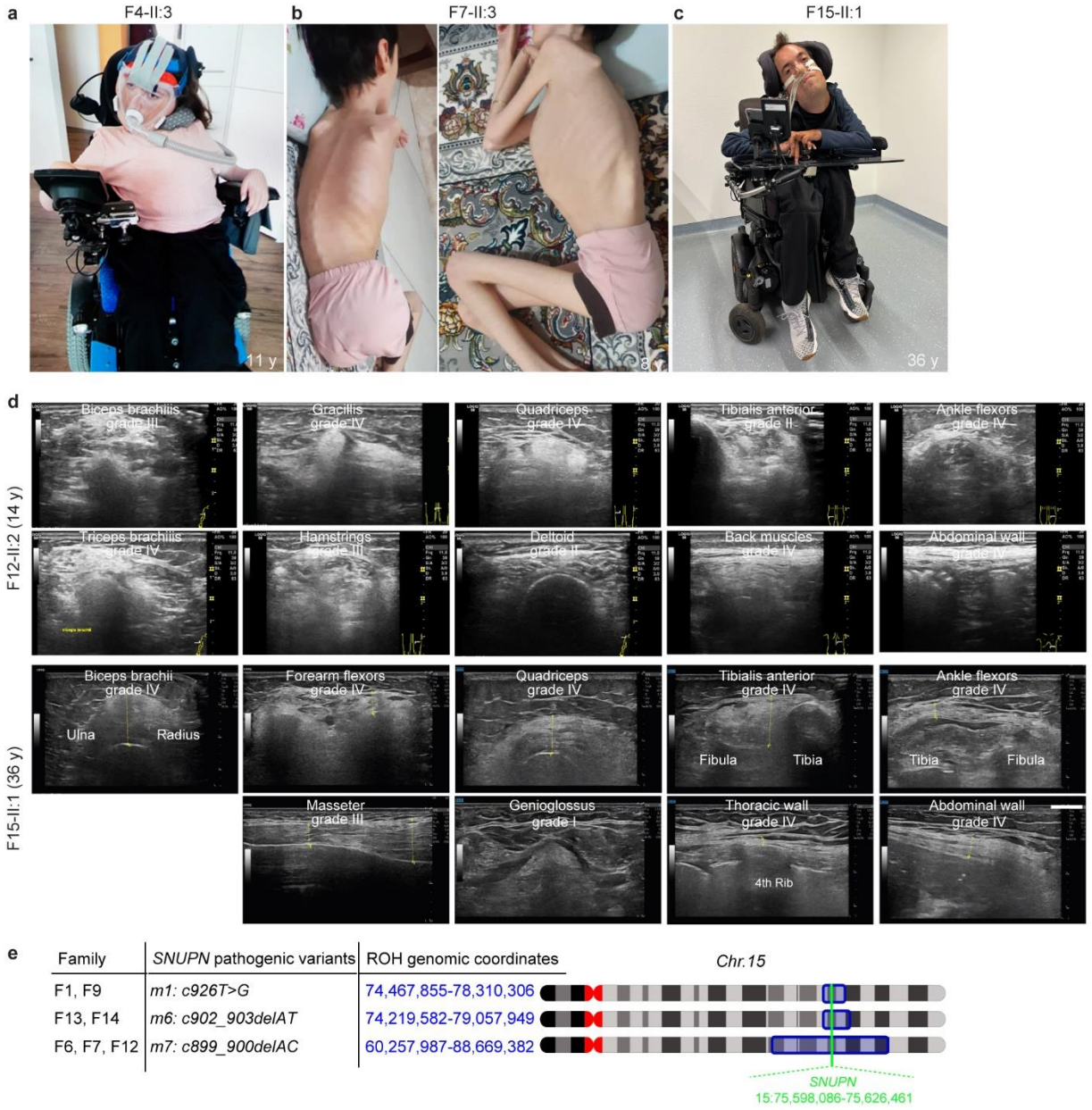
Supplementary Information contains:

Supplementary Figures (1 to 6)

Supplementary Tables (1 to 2)

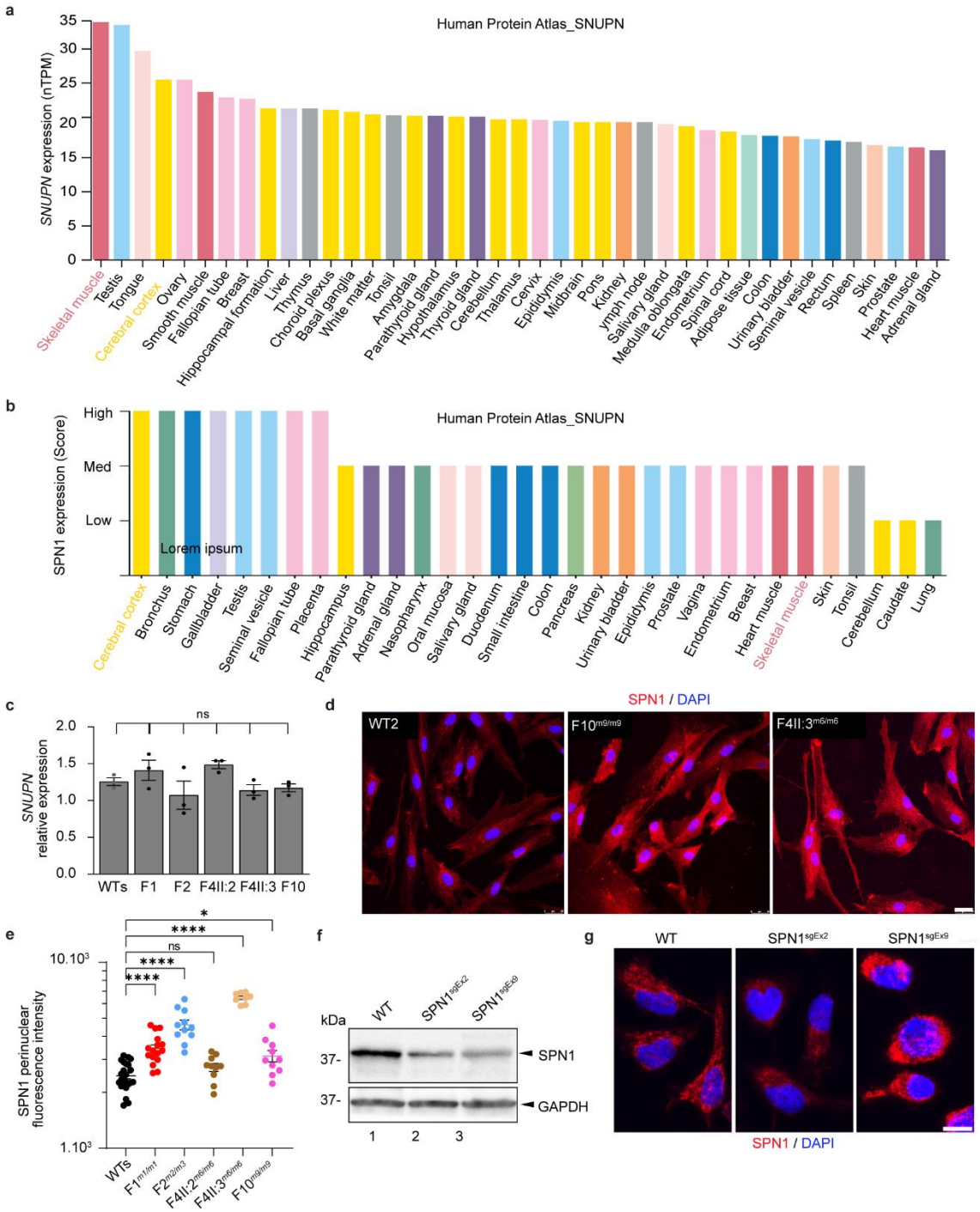
Supplementary Notes 1

Supplementary Figures



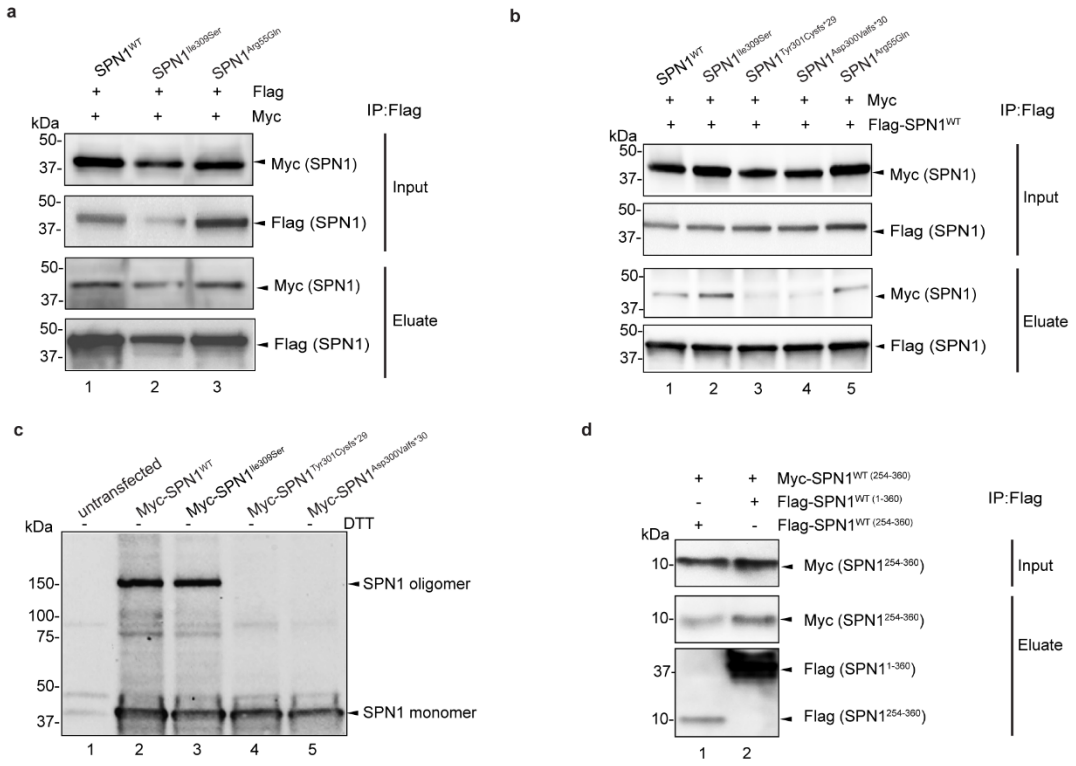
Supplementary Figure 1. Further clinical and genetic characterization of *SNUPN* mutants. a, Proband from family 4 (F4-II:3; 16-year-old) permanently bound to wheelchair and artificial respiratory devices, showing severe disability. **b,** 8-year-old affected from family F7 (F7-II:3) showing severe scoliosis. **c,** a 36-year-old affected man from family F15 (F15-II:1) is confined to a wheel-chair due to severe disability **d,** Muscular ultrasounds of patients from families F12-II:2 and F15-II:1. Scans showing severe general muscle atrophy with homogeneously increased echogenicity indicative

of fibrous remodeling. In line with preserved bulbar function (speaking, chewing, swallowing) masseter and genioglossus were relatively spared. **e,** Runs of homozygosity (ROH) analysis results for *m1*, *m6* and *m7* pathogenic variants. The ROH genomic coordinates and *SNUPN* gene are indicated in blue and green, respectively.



Supplementary Figure 2. Level of snurportin-1 in human tissues and cells. **a,b,** *SNUPN* RNA (**a**) and SPN1 protein (**b**) level in different tissues as retrieved from the human protein atlas. nTPM = number of transcripts per million. **c,** RT-qPCR graph showing no significant differences of *SNUPN* transcripts in five mutant fibroblasts ($F1^{m1/m1}$, $F2^{m2/m3}$, F4-II:$2^{m6/m6}$, F4-II:$3^{m6/m6}$ and $F10^{m9/m9}$) compared to WTs (3 distinct WT lines). Fold change relative to WT is plotted as mean ± SEM, (n = 3 biological replicates).

ns, non-significant (two-tailed unpaired t-test). **d,** Representative images of immunofluorescence showing endogenous SPN1 (red) accumulation in the cytoplasm particularly around the nuclei in two patients' fibroblast lines (F10$^{m9/m9}$, and F4-II:3$^{m6/m6}$) compared to WT2. Nuclei were labeled with DAPI (blue). Scale bar, 10 µm. n = 3 independent staining. **e,** Column scatter graph showing significant increase in perinuclear SPN1 fluorescence intensity in patients' fibroblasts compared to WTs cells. n = 28 (WTs), 16 (F1$^{m1/m1}$), 11 (F2$^{m2/m3}$), 10 (F4-II:2$^{m6/m6}$), 8 (F4-II:3$^{m6/m6}$ and 10 (F10$^{m9/m9}$) cells. ns, non significant; *P = 0.0230; ****P<0.0001 (two-tailed unpaired *t*-test). **f,** Western blot analysis using whole cell lysates of HeLa WT and mutant cell lines showing significant SPN1 reduction in HeLa SPN1^{sgEx2} and SPN1^{sgEx9} mutant cell lines compared to HeLa WT. SPN1^{sgEx2} harbors two mutations at exon 2, while SPN1^{sgEx9} carries three mutations in exon 9. GAPDH served as a loading control. n= 3 independent experiments. **g,** Immunofluorescence images representing aggregation of endogenous SPN1 (red) in the cytoplasm of SPN1^{sgEx9} cell lines but not SPN1^{sgEx2} and HeLa WT cells. Nuclei were labeled with DAPI (blue). Scale bar, 10 µm. n = 2 independent staining. Source data are provided as a Source Data file.



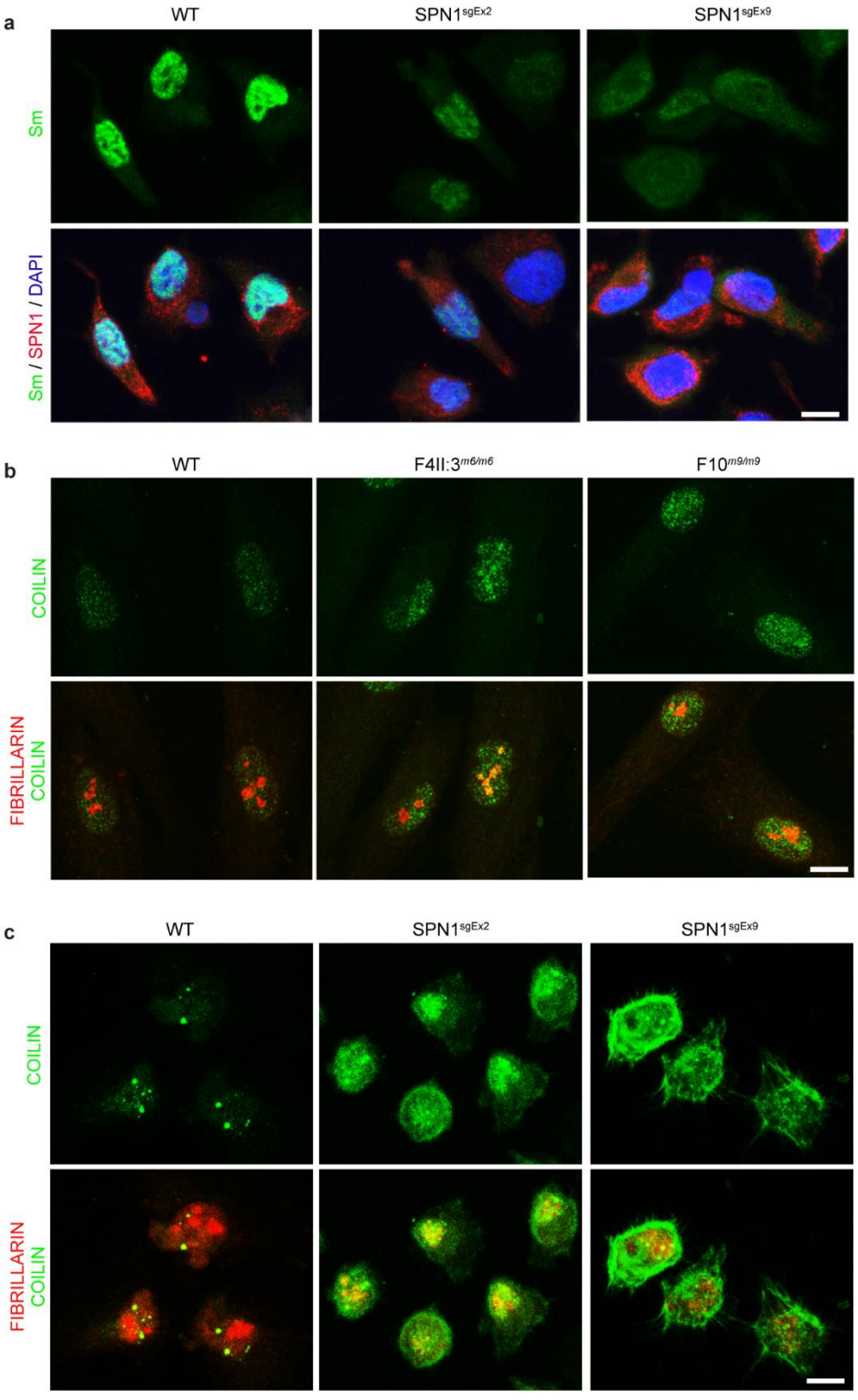
e

Residue number	Residue name	heptad Position	Packing Angle(Deg)	Residue number	Residue name	heptad Position	Packing Angle(Deg)	Residue number	Residue name	heptad Position	Packing Angle(Deg)	Residue number	Residue name	heptad Position	Packing Angle(Deg)
Chain A				Chain B				Chain C				Chain D			
305	GLN	d	64.249	323	LYS	g	64.194	320	MET	a	155.868	305	GLN	c	113.298
309	ILE	a	61.52	320	MET	d	50.049	317	LYS	e	141.6	306	LEU	d	100.142
312	HIS	d	97.048	317	LYS	a	96.638	316	GLN	d	123.495	309	ILE	g	108.563
313	LYS	a	12.788	313	LYS	d	58.854	315	SER	c	53.395	312	HIS	c	54.5
316	GLN	d	65.06	312	HIS	g	54.169	313	LYS	a	29.55	313	LYS	d	48.979
320	MET	e	19.868	309	ILE	d	80.438	312	HIS	c	54.909	315	SER	f	52.313
				306	LEU	a	122.32	309	ILE	g	114.072	316	GLN	a	147.643
								308	GLN	g	52.287	320	MET	e	145.348
								305	GLN	d	119.324				

Supplementary Figure 3. SPN1 C-terminus is essential for tetramerization.

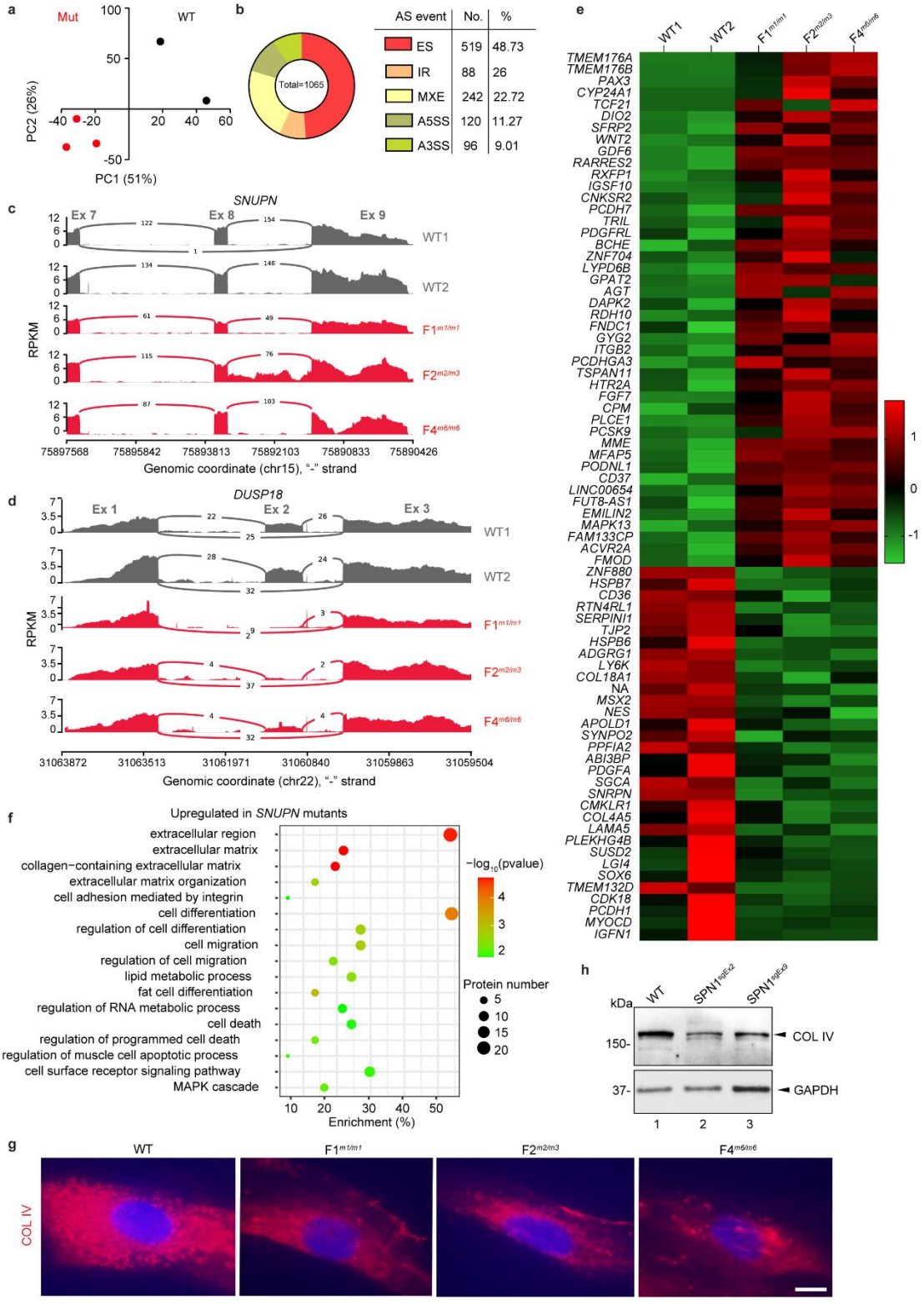
a, Co-immunoprecipitation (co-IP) assay in HEK293T cells co-transfected with Flag- and Myc-tagged SPN1 constructs using Flag beads. Input and eluate samples blotted with anti-Myc (top) or anti-Flag (bottom) antibodies show no defect in SPN1 self-interaction in SPN1^{Ile309Ser} (Lane 2) and SPN1^{Arg55Gln} (Lane 3) compared to SPN1^{WT} (lanes 1). n = 2 independent experiments. **b,** Co-immunoprecipitation (co-IP) assay in HEK293T cells co-transfected with Flag-SPN1^{WT} and WT or mutant Myc-tagged SPN1 constructs using Flag beads. Input and eluate samples blotted with anti-Myc or anti-Flag antibodies show

interaction between Flag-SPN1^{WT}/Myc-SPN1^{WT} (Lane 1), Flag-SPN1^{WT} / Myc-SPN1^{Ile309Ser} (Lane 2) and Flag-SPN1^{WT} / Myc-SPN1^{Arg55Gln} (Lane 5). As expected, the SPN1 self-interaction was disrupted between Flag-SPN1^{WT} / Myc-SPN1^{Tyr301Cysfs*29} (Lane 3) and Flag-SPN1^{WT} / Flag-SPN1^{Asp300Valfs*30} (Lane 4). n = 3 independent experiments. **c,** Western blot analysis of HeLa cells cytoplasmic fraction transfected with WT or mutant Myc-tagged SPN1 constructs. Two main bands at 41 and 150 kDa were observed in Myc-SPN1^{WT} and Myc-SPN1^{Ile309Ser}. The bands 150 kDa completely disappeared in the cytoplasmic fractions of Myc-SPN1^{Tyr301Cysfs*29} and Myc-SPN1^{Asp300Valfs*30} transfected samples. n = 2 independent experiments. **d**, Co-immunoprecipitation (co-IP) assay performed in HEK293T cells co-transfected with Flag- and Myc-tagged SPN1 C-termini fragments using Flag beads. Input and eluate samples blotted with anti-Myc or anti-Flag antibodies revealed interaction between Myc-SPN1$^{WT(254-360)}$ and Flag-SPN1$^{WT(254-360)}$ (Lane 1) or Flag-SPN1$^{WT(1-360)}$ (Lane 2). n = 3 independent experiments. **e**, Table listing C-terminal residues involved in SPN1 tetramer formation predicted by Socket2 coiled-coil analysis. Source data are provided as a Source Data file.


a
WT
SPN1sgEx2
SPN1sgEx9
Sm
Sm / SPN1 / DAPI
b
WT
F4II:3m6/m6
F10m9/m9
COILIN
FIBRILLARIN
COILIN
c
WT
SPN1sgEx2
SPN1sgEx9
COILIN
FIBRILLARIN
COILIN

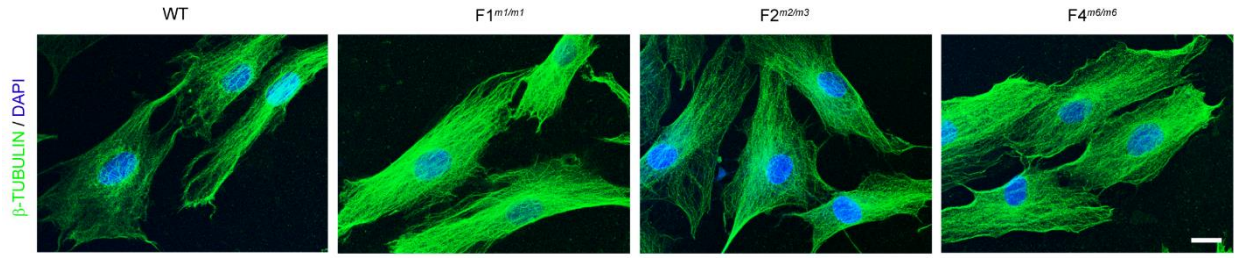
Supplementary Figure 4. Cajal bodies are disrupted in SPN1 mutant cells.

a, Representative immunofluorescence images of HeLa WT and SPN1 mutant cell lines showing significant reduction in the nuclear levels of Sm proteins (green) in SPN1^{sgEx2} and SPN1^{sgEx9} mutant cell lines compared to WT. Note that SPN1 (red) in the cytoplasm is reduced in SPN1^{sgEx2} and accumulated around nuclei in SPN1^{sgEx9} mutant cells. Nuclei are labeled with DAPI (blue). Scale bar, 10 µm. n = 3 independent stainings. **b** and **c,** Representative immunofluorescence images showing co-localization of COILIN (green) and FIBRILLARIN (red), a nucleolar marker in **b,** patients F4II:3$^{m6/m6}$ and F10$^{m9/m9}$ compared to WT fibroblasts (n = 3 independent stainings). and **c,** in SPN1^{sgEx2} and SPN1^{sgEx9} mutant cell lines compared to WT (n = 3 independent stainings). Scale bar, 10 µm.


a
Mut
WT
PC2 (26%)
PC1 (51%)
b
Total=1065
AS event | No. | %
ES | 519 | 48.73
IR | 88 | 26
MXE | 242 | 22.72
A5SS | 120 | 11.27
A3SS | 96 | 9.01
c
SNUPN
Ex 7
Ex 8
Ex 9
WT1
WT2
F1m1/m1
F2m2/m3
F4m6/m6
RPKM
Genomic coordinate (chr15), "-" strand
d
DUSP18
Ex 1
Ex 2
Ex 3
Genomic coordinate (chr22), "-" strand
e
WT1
WT2
F1m1/m1
F2m2/m3
F4m6/m6
TMEM176A
TMEM176B
PAX3
CYP24A1
TCF21
DIO2
SFRP2
WNT2
GDF6
RARRES2
RXFP1
IGSF10
CNKSR2
PCDH7
TRIL
PDGFRL
BCHE
ZNF704
LYPD6B
GPAT2
AGT
DAPK2
RDH10
FNDC1
GYG2
ITGB2
PCDHGA3
TSPAN11
HTR2A
FGF7
CPM
PLCE1
PCSK9
MME
MFAP5
PODNL1
CD37
LINC00654
FUT8-AS1
EMILIN2
MAPK13
FAM133CP
ACVR2A
FMOD
ZNF880
HSPB7
CD36
RTN4RL1
SERPINI1
TJP2
HSPB6
ADGRG1
LY6K
COL18A1
NA
MSX2
NES
APOLD1
SYNPO2
PPFIA2
ABI3BP
PDGFA
SGCA
SNRPN
CMKLR1
COL4A5
LAMA5
PLEKHG4B
SUSD2
LGI4
SOX6
TMEM132D
CDK18
PCDH1
MYOCD
IGFN1
f
Upregulated in SNUPN mutants
extracellular region
extracellular matrix
collagen−containing extracellular matrix
extracellular matrix organization
cell adhesion mediated by integrin
cell differentiation
regulation of cell differentiation
cell migration
regulation of cell migration
lipid metabolic process
fat cell differentiation
regulation of RNA metabolic process
cell death
regulation of programmed cell death
regulation of muscle cell apoptotic process
cell surface receptor signaling pathway
MAPK cascade
Enrichment (%)
−log10(pvalue)
Protein number
h
kDa
WT
SPN1sgEx2
SPN1sgEx9
150-
37-
COL IV
GAPDH
g
WT
F1m1/m1
F2m2/m3
F4m6/m6
COL IV

Supplementary Figure 5. *SNUPN* pathogenic variants lead to RNA dysregulation.

a, 2D scatter plot of the principal component analysis (PCA). Black dots represent WT samples (n = 2) while the red dots represent mutant (Mut) samples (n = 3). **b,** Ring graph displaying the distribution of significant alternative splicing (AS) events in the patients compared to WT using RNA-seq data. ES = Exon Skipping; IR = Intron Retention; MXE = Mutually Exclusive Exon; A5SS = Alternative 5' Splice Site; A3SS = Alternative 3' Splice Site. **c,** Sashimi plot showing *SNUPN* intron 8 retention only in F2$^{m2/m3}$ patient and not in the WTs (WT1 and WT2) and the other two patient fibroblasts (F1$^{m1/m1}$ and F4$^{m6/m6}$). **d,** *DUSP18* exon 2 skipping in all three patients (F1$^{m1/m1}$, F2$^{m2/m3}$ and F4$^{m6/m6}$). The genomic coordinates are represented on the x-axis and the RPKM value on the y-axis. RPKM = Reads per kilobase of transcript per million reads mapped. **e,** Heatmap representing differentially expressed genes between WTs (WT1 and WT2) and patients (F1$^{m1/m1}$, F2$^{m2/m3}$ and F4$^{m6/m6}$). Green and red colors represent lower and higher relative expression levels, respectively. **f,** Bubble plot depicting the gene ontology (GO) enrichment analyses of the upregulated genes in three *SNUPN* mutant fibroblasts compared to two WTs. The top affected biological pathways are primarily involved in collagen and extracellular matrix biology. Circle size indicates the number of differentially expressed genes enriched in each pathway. Circle color represents the enrichment significance. Red shows highly statistically significant. **g,** Immunofluorescence staining of primary fibroblasts showing decreased amount of endogenous COL IV in F1$^{m1/m1}$, F2$^{m2/m3}$ and F4$^{m6/m6}$ mutant cells compared to WT. Nuclei are labeled with DAPI (blue). n = 3 independent stainings. Scale bar, 10 µm. **h,** Immunoblot analysis indicating reduced levels of COL IV in SPN1^{sgEx2} and SPN1^{sgEx9} mutant cell lines compared to WT. Source data are provided as a Source Data file.



Supplementary Figure 6. ***SNUPN*** **pathogenic variants lead to cytoskeleton defects.** Immunofluorescence staining of cytoskeleton using anti-β-TUBULIN (green) in WT and patients' primary fibroblasts (F1$^{m1/m1}$, F2$^{m2/m3}$ and F4$^{m6/m6}$) showing an increased fluorescence signals in the three mutants compared to the WT cells. Nuclei are labeled with DAPI (blue). n = 3 independent stainings. Scale bar, 20 µm.

Supplementary tables

Supplementary Table 1 List of primers

Gene	Primer Name	Primer Sequence (5′– 3′)
Genotyping Primer Sequences		
SNUPN	SNUPN-m1 (F1)-F	GAAAAAGGAGTCGGACCACA
SNUPN	SNUPN-m1 (F1)-R	GGGAACCTCCCCATAACTGT
SNUPN	SNUPN-m2/m3 (F2)-F	AGCTGCCCTTGTGTGTATAG
SNUPN	SNUPN-m2/m3 (F2)-R	TCCTTAAGGAGGCTTCTCTC
SNUPN	SNUPN-m9 (F10)-F	CTCTCCATCCCTGTCCAGTC
SNUPN	SNUPN-m9 (F10)-F	TACTGTGGAAGCTGAGGTGG
Cloning Primer Sequences		
SNUPN	SNUPN-ORF-F	ATCGGATCCGGGAAGATGGAAGAGTTGAGTCAGGCCCT
SNUPN	SNUPN-ORF-R	TAGCTCGAGTTAATTCTCCATGAGGCATCCAGG
SNUPN	SNUPN-ORF-N-FLAG-F	ATCGGATCCGGGAAGATGGACTACAAGGACGACGATGACAAGG AAGAG TTGAGTCAGGCCCT
SNUPN	SNUPN-ORF-N-MYC-F	ATCGGATCCGGGAAGATGGAGCAAAAGCTCATTTCTGAAGAGG ACTTGGAAGAGTTGAGTCAGGCCCT
SNUPN	SNUPN 1-253-R	TAGCTCGAGTTACTCAAAAGGGAAATCCATAGATAGCA
SNUPN	SNUPN 254-360-MYC-F	ATCGGATCCATGGAGCAAAAGCTCATTTCTGAAGAGGACTTGGTAGAT GGACTTCTCTTCTACCACA
SNUPN	SNUPN 254-360-FLAG-F	ATCGGATCCATGGACTACAAGGACGACGATGACAAGGTAGATGGACTT CTCTTCTACCACA
Site-Directed Mutagenesis Primer Sequences		
SNUPN	SNUPN-m1 (926 T>G) F	CACCAGCTCCAGCAGAGTATGGAGCACAAGAAG
SNUPN	SNUPN-m1 (926 T>G) R	CTTCTTGTGCTCCATACTCTGCTGGAGCTGGTG
SNUPN	SNUPN-m6 (902-903DEL) F	GACCACCAAGCCAGACTGCTGGGCAC
SNUPN	SNUPN-m6 (902-903DEL) R	GTGCCCAGCAGTCTGGCTTGGTGGTC
SNUPN	SNUPN-m7 (899-900DEL) F	GACCACCAAGCCAGTATGCTGGGCACCA
SNUPN	SNUPN-m7 (899-900DEL) R	TGGTGCCCAGCATACTGGCTTGGTGGTC
SNUPN	SNUPN-m9 (164G>A) F	GGTTCACATAATCCAGCTGCTTGGATTTCTGCAGT
SNUPN	SNUPN-m9 (164G>A) R	ACTGCAGAAATCCAAGCAGCTGGATTATGTGAACC
RT-qPCR Primer Sequences		
SNUPN	SNUPN-qPCR-F1	TTCCTTGGAGCAGAGTGAGC
SNUPN	SNUPN-qPCR-R1	AGTCTTCTGGCATGGTTCACA
SNUPN	SNUPN-qPCR-F2	CAAGCGGCTGGATTATGTGAA
SNUPN	SNUPN-qPCR-R2	AGGAACGTCAATTAACCACTCAG
LAMA5	LAMA5-qPCR-F	CCCACCGAGGACCTTTACTG
LAMA5	LAMA5-qPCR-R	GGTGTGCCTTGTTGCTGTT
SGCA	SGCA-qPCR-F	AGACCACGCTACACCCACTT
SGCA	SGCA-qPCR-R	GACAGCGACATGCTCAGGA
SFRP2	SFRP2-qPCR-F	ACGGCATCGAATACCAGAACA
SFRP2	SFRP2-qPCR-R	CTCGTCTAGGTCATCGAGGCA
ITGB2	ITGB2-qPCR-F	TGCGTCCTCTCTCAGGAGTG
ITGB2	ITGB2-qPCR-R	GGTCCATGATGTCGTCAGCC

Supplementary Table 2 List of sgRNAs

Gene	sgRNA name	Targeted exon	sgRNA Sequence (5´– 3´)
SNUPN	sgEx2	Exon 2	ACTTGGACTTGTACTGGGAT
SNUPN	sgEx9	Exon 9	CCACCAAGGGAGTGCTTCCG

Supplementary Note 1

Case reports

Family 1 from Kosovo

9-year-old girl who was born by C/S at term with normal birth measurements after an uneventful pregnancy. Fetal intrauterine movements were reported as normal by the mother, and her psychomotor development was compatible with her chronological age. Her initial sign, frequent falling due to the weakness of her lower limbs, occurred at the age of 5. It was followed by difficulty climbing stairs due to the progression of her lower limb weakness, and difficulty lifting her head up due to the weakness of her neck muscles around 6 years of age. Her serum creatine kinase (CK) was detected as elevated more than 10x (3133 U/L) while the EMG demonstrated mild myogenic involvement. Muscle biopsy performed from her right deltoid muscle showed dystrophic changes with no specific histopathological diagnosis.
Her first physical examination was done at the age of 61/2, and revealed a proximal muscle weakness of her lower and upper extremities as well as a prominent weakness in her neck flexors and abdominal muscles. Her weak muscles and their MRC scales were as follows: neck flexors 2/5, abdominal muscles 2/5, deltoid 4/5, triceps 4/5, iliopsoas 3/5, tibialis anterior 4/5, extensor hallucis brevis 4/5. Bilateral mild calf muscle pseudohypertrophy and a mild S-shaped scoliosis were also detected.
Previous external genetic testing, results negative: 31 myositis gene Panel, 79 exon Multiplex Ligation-dependent Probe Amplification (MLPA) dystrophin (DMD) gene screening, CGH array, SMN1/2 genes sequencing, and clinical exome sequencing on 4811 OMIM genes.
Her second physical examination which was done at the age of 8 years 5 months revealed the progression of the muscle weaknesses, in particular proximal weaknesses, as follows: neck flexors 2/5, neck extensors 4/5, abdominal muscles 1-2/5, deltoid 2-3/5, pectoral muscles 4/5, biceps 4/5, triceps 3-4/5, iliopsoas 2/5, gluteus maximus 3/5, hip abductors 4/5, hip adductors 2/5, hamstrings 4/5, tibialis anterior 4/5, extensor hallucis brevis 4/5. Mild contractures were also detected in her elbows and ankles, and her scoliosis had progressed compared to the previous examination. Decreased sensitivity to pain was also detected as a remarkable additional finding. She was able to walk independently but with a hyperlordotic waddling gait. She was not able to climb stairs even with support. The physical examination of the other systems was in normal limits, and the ophthalmological examination revealed no pathological findings.

Family 2 from Italy

Muscular biopsy performed on quadriceps femoris (December 2013):
Desmin, αβ crystallin, actin showed areas of intra-cytoplasmic accumulation of numerous fiber; Merosin pattern was normal; α-Dystroglycan staining was reduced in numerous fibers compared to adult control samples; Acid phosphatase showed normal activity, ORO and PAS showed normal glycolipid pattern; ATPase activity (ph 4.3, 4.45, 9.45) showed normal fiber type differentiation but with predominance of type I fibers. NADH-TR, SDH, COX/SDH showed that oxidative activity is reduced or absent in areas of cytoplasmic eosinophilia. No COX negative fibers were observed. Hematoxilin-eosin and Gomori trichrome staining showed presence of a few hypotrophic fibers, some of them were angulated, close to normotrophic fibers or mildly hypertrophic fibers. No necrotic fibers or fiber splittings were observed. There were rare nuclear centralizations. Some fibers present optical empty micro vacuolization, intra-cytoplasmic or sub-sarcolemmal. In some muscle fascicles, numerous fibers with areas of cytoplasmic eosinophilia or intense trichrome staining are observed. No ragged red fibers or interstitial cellular infiltrates are present. Vessels and connective tissue are normal. Conclusions: The analysis of the muscular biopsy has revealed the presence of a primary myopathy characterized by the accumulation inside the fibers of aggregates of myofibrillar

proteins. The reduction of the expression of alpha-dystroglycan has to be considered in the clinical context of the patient.

Family 3 from USA

Family history noncontributory, three unaffected siblings. Patient early history was significant for prematurity (33 weeks) and a vascular ring which was surgically repaired along with an aortopexy. He also has a history of bronchomalacia. He was discharged home on oxygen and CPAP. He was admitted at age 4 months when he was noted to have difficulties feeding, breathing and a frequent cough. He was intubated for increasing respiratory acidosis and desaturation. Fluoroscopy testing, at that time showed decreased movement of the right diaphragm. After failing extubation a tracheostomy was placed. A swallow study revealed poor coordination with swallowing requiring placement of a Gastrostomy tube. He was found to have generalized weakness upon examination. Serum Creatine Kinase level at that time was elevated at 1500 U/L. Differential diagnosis included a congenital muscular dystrophy or a congenital inflammatory myopathy. Neuromuscular examination at age 11 months showed generalized weakness (Medical Research Council (MRC) 2/5 and 3/5 range) in both proximal and distal muscles. He was able to maintain head control when propped in a sitting position. He was noted to have a tremor. He had contractures at the hips, mild knee and ankle, and some tightness of the iliotibial bands. He required ventilation throughout most of the day. He showed mild clinical improvement in both respiratory status and strength following a corticosteroid trial. Age 13 months he was diagnosed with cataracts. He never achieved ambulation and passed away at age 15 yrs.

Muscle biopsy was suggestive of a mild dystrophy and showed scattered small basophilic-appearing muscle fibers with mildly increased endomysial connective tissue. Some occasional immature muscle fibers with larger nuclei. No fiber type grouping. Alkaline phosphatase stained scattered smaller fibers. Metabolic staining and dystrophin immunohistochemistry were unremarkable.

EMG studies were suggestive of an irritable myopathy with small polyphasic motor units and a marked increase of insertional activity in the form of fibrillation potentials and positive sharp waves. Nerve conduction studies were normal.

MRI at age 18 months showed moderate diffuse cerebral atrophy with prominence of the ventricular system and sulci. There was absence of the inferior vermis and of the cerebellar tonsils bilaterally.

Mutations: chr15:75890860G>A; c.922C>T p.Gln308Ter (maternal) & 15:75890934G>C; c.848C>G p.Ser283Ter (paternal).

Family 4 from Switzerland

Patient II:2 is the index case of Family 4 was born to non-consanguineous healthy Swiss parents. He is the second of 3 children of family 4. His older brother is healthy, his younger sister is affected by the same muscle disease (Patient 2, Family 4). Family history does not provide evidence of other family members affected by a neuromuscular disease.

Pregnancy was normal apart from reduced fetal movements. Birth at term by emergency cesarean section because of bradycardia (Apgar 6/7/7, umbilical cord arterial pH 6.94) followed by treatment in the neonatal intensive care unit for asphyxia and neonatal infection. The patient showed first motor symptoms in the neonatal period with muscular hypotonia and poor head control especially in prone. Thereafter, his motor development was delayed: The patient achieved the ability to sit without support at age 8 months but could not sit up by

himself. He started to bottom-shuffle at age 11 months. At the age of 2 years, he was able to walk a few steps without support, but lost the ability to walk without support after only a few weeks. Until age 5, he was able to walk with support and used a walker for mobility. He started to use an electric wheelchair at age 4 years and became fully non-ambulant at age 6 years. Until the end of the first decade, the disease was relentless. Weakness initially affected the upper limbs more than the lower limbs and was more pronounced proximal and axial. Deteriorating motor functions were in addition compromised by severe, rapidly progredient contractures, which started already in the second year of life in the ankles, fingers and the cervical vertebral column leading to torticollis. The patient lost almost all motor functions except for finger movements and minor movements with hands and feet by age 10 years. Importantly, facial, ocular and masticatory muscle strength was quite well preserved until last follow up. At last follow-up at age of 18 years, the patient was able to use his electric wheelchair over short distances and his mobile phone or playstation when hands and devices were placed in an optimal position. He was fully dependent on the care and support of his family or assistants.

During the course of the disease, contractures progressed and finally included all relevant joints, e.g., hips, knees, ankles, shoulders, elbows, wrist, fingers. Achillotenotomy was done at age 8 years. Contractures of the jaw joints restricted oral feeding and made interventions under intubation anesthesia impossible. The limited mouth opening and dysphagia made tube feeding necessary, gastrostomy placement at age 8 years. The patient was fed partially by mouth until age 16 years. The patient developed a rigid spine and myopathic scoliosis. Scoliosis was initially treated with a brace which was not tolerated by the patient anymore at a later stage of the disease. Due to the severity of the disease and the impossibility of intubation, the patient was deemed unsuitable for invasive interventions requiring intubation anesthesia including scoliosis surgery.

Respiratory muscle weakness in combination with rigid spine and thorax resulted in a severe restrictive ventilatory dysfunction. Starting at age 3 years, the patient acquired numerous respiratory tract infections and was regularly hospitalized for treatment. Non-invasive nighttime ventilation was started at age 6 years. The ventilation had to be extended to daytime at age 7 years. From age 10 years, the patient was ventilated at least 18 hours per day, and tolerated only short interruptions of the ventilation. The patient and his family refused tracheostomy, which is the reason why the patient was still under non-invasive ventilation using a full-face mask at last follow up. The long-term use of the full-face mask resulted in an impressive deformation of the mid-face and compression of the auditory canals which led to mild to moderate hearing loss on both sides.

The patient had congenital cataracts on both sides. Lensectomy and posterior chamber lens implantation was done at age 9 years. Speech development was delayed, but several developmental tests documented normal cognitive abilities (SON-R nonverbal IQ 100 at age 3 years and HAWIVA IQ 105-110 at age 5 years). Heart function was repetitively investigated and was normal until last follow-up. Brain MRI at age 2 years was normal. CK levels were investigated repeatedly and were always elevated (2000-7000 U/L).

Muscle biopsy was done twice at age 2 and age 6 years and showed a dystrophic pattern with increased endomysial fibrosis and muscle fiber necrosis. Immunohistochemistry did not show abnormalities. The later muscle biopsy showed eosinophilic inclusions and increased fatty storage.

Patient II:3 is the younger sister of patient 1, family 4 of this cohort. She was born at term via vaginal delivery at term, APGAR 8/9/10. Apart from reduced fetal movements the pregnancy was normal. Muscular hypotonia was noticed very early, but otherwise neonatal period was reported as normal. The patient then developed a muscular disorder which was phenotypically very similar to the disease of her brother. In early infancy severe proximal and axial weakness as well as lack of head control were noticed. The patient achieved the ability to sit without

support at age 14 months, was able to turn from prone to supine at age 17 months, and to stand with support at age 2 years. She started to shuffle at age 2 years and to walk without support at age 4 years but lost the ability to walk at age 5 years. Because of the progredient weakness and development of significant contractures the girl lost virtually all motor functions until the end of the first decade, when she was only still able to move her hands, fingers and feet against gravity.
Contractures started at the ankles, fingers and the cervical vertebral column resulting in torticollis in infancy. Later, contracture affected all relevant larger joints at both the lower and upper extremities. Achillotenotomy was done at age 6 years. The girl developed rigid spine and severe myopathic scoliosis (Cobb angle 152° at last follow up).
A severe restrictive ventilatory dysfunction made the introduction of non-invasive night-time ventilation necessary at age 3 years. The ventilation had to be expanded to daytime at age 9 years. From age 13 years on, ventilation was necessary for at least 20 hours per day. As in her brother, full time non-invasive ventilation was done with a full-face mask, because of refusal of tracheostomy, and resulted in an impressive deformation of the midface. Oral feeding was compromised because of reduced mouth opening, malocclusion, and dysphagia. Gastrostomy was placed at the age of 8 years for supplementary tube feeding. This patient did not have congenital cataracts. Her speech and cognitive development were normal. Heart function was repetitively investigated and was normal until last follow-up. CK levels were elevated (around 1000 U/L).

Family 5 from Iraq

4th child of healthy parents with unremarkable development of siblings. The family is from Iraq, the parents are not consanguineous. At the time of this documentation, the boy is 3 years and 2 months old. At the age of 11 months, the pediatrician noticed a significant motor developmental delay with inconspicuous cognitive development during a screening examination. At this time, the child's physical growth was regular and the head circumference was borderline microcephalic (3rd percentile). In addition to the muscular hypotonia, a funnel chest and a weakness of the foot lifter on the left > right side were noticed. At 20 months CK elevation was elevated 5459 U/l. An emergency card regarding malignant hyperthermia was issued. Lensectomy was performed for cataract. Strabismus sursoadductorius is suspected. There is no echocardiographic suspicion of cardiomyopathy. A sleep EEG was unremarkable on September 21 and EMG was not performed.
In July 2022 a percutaneous myofasciotomy of the gatrocnemius muscles was performed. Since wearing orthoses, the gait pattern is increasingly stable (status 09/22). Buckling feet are described on both sides.
The brain MRI at the age of 22 months showed mild nonspecific cerebral findings. The corpus callosum was relatively short and clumsy. The cerebellar fissures of the vermis, as well as the fissures of both cerebellar hemispheres, were enlarged, indicating mild cerebellar volume loss. Supratentorial, perivascular spaces were diffusely accentuated. Right temporopolar, a focal arachnoidal cyst was visible. A muscle biopsy has not been done so far because the parents refused.
Trio exome analysis identified the homozygous variant c.899_900del p.(Asp300Val) in the *SNUPN* gene. The patient's parents each carry the variant in heterozygous form. The patient's three siblings are not affected and have not been tested.

Family 6 from Iran

The proband is the third child of related parents. The parents and two older sisters are apparently healthy. Pregnancy and delivery were uneventful. Birth weight and length were

2800 grams (25th centile) and 51 cm respectively (50th centile). Congenital hypotonia was noted. Milestones were delayed, held head at 5 months, rolled over at 11 months, walked at 18 months but had poor balance and frequent falls from the beginning. First words were said at 12 months and examination at 3 years he could only say 3-4 words. He had difficulty in running and climbing stairs, and gradually lost the ability to walk by the age of 6 years. Neurological evaluation was performed because of delay in milestones. Brain CT scan performed at 1 year was normal. Electrodiagnostic testing was compatible with myopathic processes. Creatine phosphokinase and lactate dehydrogenase measured at 2 years were elevated, 3806 U/L (normal range 24-195 U/L) and 1694 U/L (normal range for 1-12 years <650 U/L) respectively. Examination at 3.5 years revealed general muscle weakness with sparing of facial muscles. He had mild pseudohypertrophy of calves, scoliosis, and flat feet.

Family 7 from Iran

Patient II:3, the proband is the second child of apparently healthy related parents. Pregnancy was uneventful. Delivery was by natural vaginal delivery at 9 months. Birth weight, length and head circumference were 3400 grams (50th centile), 50 cm (50th centile) and 33 cm (25th centile) respectively. Mild congenital hypotonia was noted from birth. Motor milestones were achieved on time, held head at 2-3 months, sat at 7 months, stood at 12 months, and walked at 14 months. Walking was with frequent falls and he had waddling gait from the start and difficulty getting up from a sitting position. Assessment at 4 years, she had general muscle weakness with sparing of facial muscles. She developed respiratory insufficiency from the age of 8 years and became oxygen dependent. Laboratory tests were as follows: Creatine phosphokinase was elevated 2961 U/L (normal range for 24-195 U/L), LDH 973 U/L (normal range 1-12 years <650 U/L). Scoliosis and contractures of the elbows and knees developed from the age of 7 years.

Patient II:1, the older brother, had a similar clinical phenotype with congenital hypotonia, delayed motor milestones, and onset of muscle weakness at the age of 2 years. Milestones were delayed. Recurrent respiratory infection was noted in childhood. Onset of muscle weakness was noted at 24 months with frequent falling and waddling gait. The child was treated with corticosteroid for 5 years (ages 2-7). Surgery for cataract was performed at age of 7 years which was thought to be the result of corticosteroid therapy and treatment was discontinued. Onset of scoliosis and contractures of elbows and knees was at the age of 7 years. He became wheelchair-bound at the same age. Cognition was normal. One month before his death at the age of 10 years, the parents noted cyanosis of lips, but further study to evaluate the cause was not performed. Cause of death was reported as cardiac arrest.

Family 8 from Macedonia

31/2-year-old-boy who was born by C/S at 36 weeks of the gestation with a birth weight of 2270 g (-1.06 SD) after an uneventful dizygotic twin pregnancy. His height was 45 cm (-0.89 SD) and the head circumference was 35 cm (+1.58 SD) at birth. His twin pair was also a male with normal birth measurements. His motor development was delayed compared to his twin pair. He started sitting without support at the age of 9 months, while his twin pair started at 6 months. He also started walking independently at 17 months, while his twin pair started at 11 months. He was initially evaluated by a neurologist due to the weakness and motor delay at 12 months, and his serum CK was found as elevated more than 10x (3690 U/L). EMG was

reported as normal, however it was suboptimal due to the too young age of the patient. DMD MLPA analysis revealed no deletions/duplications.

Physical examination at the age of 31/2 years revealed normal muscle strength of the upper extremities, and proximal muscle weakness of the lower extremities as well as a prominent weakness in his neck flexors and abdominal muscles (MRC scale was 4/5 for proximal lower limb muscles, 2/5 for neck flexors, and 2/5 for abdominal muscles). Gowers sign was positive, and calf muscle pseudohypertrophy was also present. He was able to walk independently but with a waddling gait pattern. He was not able to climb stairs without support.

Family 9 from Romania

Patient II:2

The index is a 14-year-old male born to healthy parents from Romania. The patient experienced the first symptoms at 4-year-old (2010), with pelvic girdle involvement. He presented difficulty in climbing stairs, frequent falls. A positive Gowers sign and a high level of CPK (CPK = 191 IU/L) were detected. At 5 years old the CPK levels reached 5750 IU/L and the electromyography aspect was suggestive of myopathy. Symptoms showed progressive worsening, and between 7 and 9 years old the patient presented difficulty walking on toes. Deep tendon reflexes were diminished and the patient presented hyperlordosis and important tendon retractions. When the patient was 9 years old, the shoulder girdle also started to be affected. In addition, he presented various equine feet, for which he underwent surgical correction. In 2020 the patient had severe impairment of the pelvic girdle (he walked only with support), moderate impairment of the shoulder girdle, and severe restrictive ventilatory dysfunction. At the last hospitalization in our clinic (2022) the patient was non-ambulatory with important thoraco-lumbar scoliosis and lumbar hyperlordosis. The neck flexor muscles were impaired. He has important axial deficit, superior limbs with low muscle force proximal and distal, proximal muscle atrophies, bilateral shoulder retractions; the abduction at the level of scapulo-humeral joint was limited, the extension at the elbow joint level was limited, inferior limbs with low muscle force proximal and distal, bilateral coxo-femural muscle atrophies and retractions, globally absent deep tendon reflexes. He has severe restrictive ventilatory dysfunction and uses non-invasive support ventilation. PUL = 24 / 42 pts, EK2 = 20/51 pts. Supplementary tests: October 2020 respiratory studies : Severe restrictive ventilatory dysfunction: FVC = 15%, FEV1 = 17%, low MIP/MEP. March 2022 - EKG: minor right bundle block with secondary ST-T changes, normal abdominal ultrasound, respiratory studies with low MIP/MEP, spirometry with FVC = 17% and FEV1 = 18% of predicted values. Exome sequencing performed at Centogene in 2019 identified the same homozygous variant identified in his similarly affected cousin: SNUPN NM_001042581.1:c.926T>G, p.(Ile309Ser).

Patient II:1

The patient is a 18-years-old female born to healthy parents, she has a similarly affected cousin. She experienced the first symptoms at 9.5 years old (2012). At that time, CK was 2529 IU/L. The pelvic girdle was affected as she reported difficulty in walking longer distances and in running. She had Achile's tendon retractions for which she underwent surgical correction in 2017. During the year 2021 the shoulder girdle also started to be affected. During the last hospitalization in our clinic (March 2022), when she was 18.3 years old, she presented low muscle tone and muscle force at the level of pelvic and shoulder girdle, positive Gowers sign, globally absent deep tendon reflexes, hypotrophic calves. NSSA = 18/34 pts, PUL = 40/42 pts, 6MWT = 400 meters. Supplementary performed tests: Feb 2019 - nerve conduction studies were normal NCV for right and left tibial nerve, normal NCV for right and left peroneal nerve. Respiratory studies - low MIP/MEP, spirometry with moderate restrictive ventilatory dysfunction (FVC = 69%, FEV1 = 73%). June- July 2021 – normal echocardiography, respiratory studies – low MIP/MEP, spirometry with moderate restrictive ventilatory

dysfunction (FVC = 64%, FEV1 = 72%). March 2023 - EKG was normal for age, normal abdominal ultrasonography. Exome sequencing performed at Centogene in 2019 identified the same homozygous variant identified in her similarly affected cousin: SNUPN NM_001042581.1:c.926T>G, p.(Ile309Ser). Sanger sequencing performed on the parent's DNA revealed they were carriers.

Family 10 from Guatemala

The proband is a 13-year-old female with short stature, microcephaly, developmental delay, and progressive neuromuscular disease.
She was born full-term without complications but came to medical attention due to speech and motor developmental delays. At age 3 she had a brain MRI demonstrating a bilateral opercular migrational disorder. Muscle biopsy performed due to her persistent weakness and fatigue was suggestive of dystroglycanopathy. Her CK measurements ranged from 480 to 6601. She also received growth hormone for short stature but did not respond to the treatment.
At age 7, she underwent serial casting for Achilles tendon contractures, which then led to a regression in her mobility. She later exhibited ataxia and intermittent torticollis, as well as worsening scoliosis for which she underwent a posterior lumbar fusion. She was subsequently hospitalized for pneumonia and found to have severe obstructive sleep apnea requiring BiPAP. By age 11, her myopathic weakness and contractures progressed such that she was no longer ambulatory.
The proband has a current weight of 27.8 kg (z = -3.40), height of 119 cm (z = -5.58), and microcephaly. She has developed chronic lung disease complicated by recurrent pneumonia. She is G-tube dependent secondary to persistent dysphagia. She has also been diagnosed with intellectual disability.
She is the first liveborn child of a nonconsanguineous Guatemalan couple. Her family history is negative for any similarly affected individual.

Muscle histopathological report

	Date		
Muscle biopsy	10/3/12	Sparrow, then UM, then Iowa	1. Marked variation in fiber size with some large hypertrophic fibers for the patient's age and some small rounded atrophic fibers. 2. Several fibers with increased number of internalize nuclei, ring or whorl fibers. 3. Several fibers undergoing degeneration and regeneration. 4. Focal fatty replacement. 5. Small foci of inflammation is seen which appears to be predominantly small T-cells (CD3) and occasional macrophages (CD68). 6. Trichrome staining highlights increased endomesial fibrosis without any abnormal inclusions. PAS stain sections showed no abnormal glycogen accumulation and several fibers are depleted of glycogen. 7. Antibodies for dystrophin (3 domains), sarcoglycans (alpha, beta, delta, gamma), beta-dystroglycan, merosin, laminin A/C, laminin B1, myotilin, emerin, caveolin 3, and dysferlin were performed at University of Michigan Health System. The panel was normal except for staining of dysferlin and caveolin 3 which showed sarcoplasmic rather than membranous staining. Also, alpha-dystroglycan antibodies were not available in this laboratory. 8. Muscle specimen was sent to Dr. Steven Moore in Iowa on 1/22/2013. His report states that 2 different alpha dystroglycan antibodies were used and there was reduced immuno staining more prominent with VIA4-1 than with IIH6 antibody. He suggested a fibroblast assay of alpha dystroglycan which may be more helpful identifying the mutant gene.

Family 11 from Kosovo

Pregnancy and delivery**:** Unremarkable. Postnatal adaptation without problems.
Early psychomotor development: Walking around her first birthday. The father noticed between the second and third year of life that the daughter was never able to climb the stairs well and also getting up was always with the assistance of the arms. Initial presentation at 6 years of age. When walking longer distances, the patient complains of calf pain or she says she is tired. She often walks on tiptoes, she has never been able to jump, and she walks the stairs in a postural step with holding. A first laboratory control showed a clear hyperCKemia around 6000. Unremarkable cognition.
Examination age 6 years**:** Mental development: age appropriate, normal speech, age appropriate behavior, normal concentration span.Cranial nerves: Oculomotor function regular, no strabismus. Pupillary reaction lateral, direct and indirect. Facial expressions are variable and symmetrical. No dysarthria. Coordination: no dysmetria. Serial finger movements unremarkable. No ataxia. Skeletal system: No scoliosis. All joints are active and passive movable. Clear muscle relief. Motor function: Walking with speed possible, no running. Climbing stairs with a staggered step. Rising from the ground with Gowers maneuver. Tiptoe walk possible, heel walk with difficulty and little elevation of the toe. One-legged stance only implied possible, no hopping. Slight weakness of neck flexors. Strength of lower extremity reduced 3-4/5, distal 4/5. upper extremity proximal 4/5. Climbing stairs with a staggered step. Rising from the ground with Gowers maneuver. Tiptoe walk possible, heel walk with difficulty and only little elevation of the toe. One-legged stance only implied possible, no hopping. Slight weakness of neck flexors. Strength proximal to lower extremity reduced 3-4/5, distal better 4/5. Upper extremity proximal 4/5. Fine motor skills of hands are normal. Sensitivity: touch sensation unremarkable on all sides. MER: triggerable, reflex zones not widened. Abdominal skin reflexes are regular. No Babinski phenomenon.
Examination age 13 years: Good general condition and very slim nutritional condition. Somatogram: length: 152 cm (7th percentile), weight: 26 kg (1st percentile, related to length), BMI: 11,3. Cognition and psyche: friendly, very good cooperation, no depressive mood. Coordination: no longer possible to assess due to paresis. Motor skills: Comes in a wheelchair, which is being pushed. Transfer from wheelchair to chair/examination could not be possible without help. Walking is only possible with the support of the father. Free standing is only possible for a few seconds. Sitting only possible with leaning against the backrest or wall. The left hand can be brought to the face, the right hand above the head. Strength: in the area of the arms 3-4/5. fingers spread 3-4/5. iliopsoas 2/5. gastrocnemius 3-4/5. Skeletal system: rigid spine. Long right convex scoliosis. Flexion contracture elbow about 50° bilaterally. Contracture of the neck extensors. Extension contracture in the knee joint, the 90° angle in the knee joint is just reached.

Family 12 from Iraq

Patient: male, currently 11 years old. Pregnancy and delivery: Unremarkable. Postnatal adaptation without problems. GG 3880 g. Family history: Consanguineous (Degree 2), Iraqi parents. no known neuromuscular diseases. two siblings: one younger sister (7 years old) and one older brother (13 years old). Both are healthy. Early psychomotor development: normal development until 2 years of age. At the age of 2 he could walk freely up to 2 km without any problems and participated in longer walks. From the age of 2 ½ years he started to cry when he was tired and also to refuse to walk. From the age of 3 ½ years, he had lost the function of walking freely and could only walk with the support of a rollator. From the age of 6, he had also lost the ability to walk with assistance, and from the 7th year of life lost free sitting. Currently, the highest motor function is turning from supine to lateral position. The impairment of the upper extremities was noticeable from the 5th/6th year of life. At 3 1⁄2 yrs: beginning of contractures lower extremity. At 6 yrs: contractures ankle and knee. At 6 years: Scoliosis- rigid spine, upper extremities worse and onset contractures upper extremity (elbow). At 7 years: massive contractures especially elbow, rigid spine especially cervical spine (no flexion/rotation of cervical spine possible!). First words/vocabulary/grammar regular. From 3 ½ speech tempo slower. Pediatric audiology at 3y: pathological, hearing aids provided at age 3.5 yrs. As the disease progressed, speech became more dyslexic and dysarthric, swallowing function problematic. He develops severe cachexia. At the age of 9 years becomes a gastric tube /gastrostomy. Respiration: from the age of 8 years showed deterioration of his respiratory functions and was dependent on non-invasive ventilation. Nephrolithiasis: At the age of 9 years was diagnosed with nephrolithiasis.

Examination age 5,5 years: Myopathic facies. Dyslalia, dysgrammatismus, good speech comprehension. Generalized muscle weakness: arms cannot be raised above shoulder height, head control only possible for a short time. No free sitting, no weight bearing in standing position (due to pain after tibia fracture). Contractures of ankle and knee joints, hip (adductors), rigid spine. Reflexes not triggerable.

Examination age 7,5 years: Myopathic facies, good tongue motor function, no fasciculations. Dyslalia, dysgrammatismus, dysarthria, good speech comprehension. Generalized muscle weakness, turning only possible to the side, no sitting, no prone position, arms can be raised to elbow height, no head control No free sitting, no weight bearing in standing position. Contractures of the ankle and knee joints, the hip (adductors), rigid spine in the cervical spine, elbow, wrist joint. Reflexes not triggerable. Generalized muscular atrophy. Hallux valgus bds, clinodactyly 4 toe left side. Cognition and psyche: friendly, very good cooperation, no depressive mood. Coordination: no longer possible to assess due to paresis. Strength: in the area of the arms 2/5, fingers spread 2/5, iliopsoas 1-2/5. gastrocnemius 1-2/5. Skeletal system: rigid spine. Flexion contracture elbow about 50° bilaterally. Contracture of the neck extensors. Extension contracture in the knee joint and angle. length 119 cm (6. Perz., SDS - 1,73)1, weight 13,6 kg (< 1. Perz., SDS -5,79)2, head circumference: 51,7 cm (10. Perz., SDS -1,27)3, BMI 9,6 kg/m² (< 1. Perz., SDS -7,61).

Pathological report summary from patient muscle biopsy (3 years):

Microscopically, skeletal muscle fibers with a significantly broadened calf spectrum can be seen transversely in the cryostat sections, and in the paraffin-embedded material also obliquely to longitudinally. In addition to normal-sized muscle fibers, there are numerous partially and completely atrophic or hypotrophic muscle fibers that are disseminated and have a rounded configuration. Clearly hypertrophic fibers, on the other hand, do not occur. Individual fresh muscle fiber necrosis, myophagic reactions and basophilic, apparently regenerating muscle fibers are found. Clusters of pyknotic nuclei in completely atrophic muscle fibers are recurrent. Central cores are also often found. While no conspicuous lipid deposits can be detected in the muscle fibers, distribution disorders of PAS-positive material are often found. Congophilic deposits are not detectable in the examined tissue. Few "Rag red fibers' are detected. Furthermore, increased fuchsinophilic material can also be seen in the other muscle fibers, subsarcolemmal and intermyofibrillar. Accordingly, there is often an increased NADH reaction in the muscle fibers in these areas, and a less pronounced SDH reaction as well. In some muscle fibers, the SDH response appears somewhat reduced in the central sections. Clearly COX-negative muscle fibers cannot be seen, although the assessment of the partly artificially weakened reaction is now possible to a limited extent. The MAD response is mostly normal. In the myophagic reactions, as well as in the inflammatory infiltrates, a clearly increased number of acid phosphatase-positive cell elements can be seen. There is only a small amount of punctiform acid phosphatase-positive material in the subsarcolemma. The endomysial connective tissue is slightly to moderately increased. In addition, there is quite a lot, especially in the paraffin-embedded material perimysial adipose tissue included. Occasionally, the endomysial inflammatory infiltrates described above can be found.

Immunohistochemistry reveals a moderately pronounced numerical predominance of type I fibers, with frequent co-expression of fast and slow myosin being detectable. A fiber type grouping cannot be identified. Although the atrophic muscle fibers can be assigned to both main fiber types, the type II fibers are significantly more affected. An increased expression of desmin is found in smaller or regenerating muscle fibers as well as in individual larger muscle fibers. Muscle fibers that are disseminated or partially located in smaller groups with low to moderate overexpression of MHC1 were found repeatedly. Immunohistochemically, CD45-positive cells were repeatedly detected in the endomysial in the myophagic reactions. In addition to numerous smaller, but often also larger, neonatal myosin-expressing muscle fibers, there are also repeated embryonic myosin-expressing muscle fibers. After incubation with antibodies against proteins of the dystrophin-glycoprotein complex, cell nuclei and other muscle proteins, numerous muscle skeletal fibers show an uneven and sometimes increased expression of caveolin-3, dysferlin and sometimes also utrophin, whereby these are predominantly regenerating muscle fibers. On the other hand, although the positive control is also relatively weak, a significantly reduced reaction can be achieved with the antibody against alpha-dystroglycan. The other immunohistochemical reactions, including dystrophin, were largely normal.

Muscle biopsy shows a distinct myopathy with numerous immature, embryonic and neonatal myocyte-expressing muscle fibers, repeatedly de- and regenerating muscle fibers as well as inflammatory infiltrates and myofibrillar architectural disorders. In addition, there are additional non-specific myopathic changes such as cytoplasmic bodies and autophagic vacuoles. Although "ragged red fibers"also occasionally occur, the typical picture of a mitochondrial myopathy does not appear. According to the additional immunohistochemical studies, a congenital muscular dystrophy with abnormal glycosylation of alpha-dystroglycan cannot be

excluded, while no evidence of another form of muscular dystrophy associated with defects in the proteins studied can be found.
Diagnostics: A first laboratory control (3y) showed a clear hyperCKemia around 3500. Brain MRI at the age 3 years and 7,5 years: cerebellar atrophy, volume reduction supratentorial and corpus callosum. Muscle biopsy at age 3 years: marked myopathy with numerous immature, embryonic and neonatal myosin, expressed muscle fibers, repeatedly degenerated as well as regenerated muscle fibers, inflammatory infiltrates and myofibrillar architectural defects in addition, unspecific myopathic changes such as cytoplasmic corpuscles and autophagic vacuoles occasionally also ragged red fibers without typical picture of a mitochondrial myopathy.

Family 13 from Columbia

The index is a 5-year-old female patient born to healthy, nonconsanguineous parents from Colombia. This is the only child, negative family history. During pregnancy the mother presented gestational diabetes and preeclampsia. Multiple prenatal ultrasounds were normal. Fetal movements were present since the 18th week with good intensity. The patient was born at 36.4 weeks, by Cesarean section due to severe preeclampsia. She was admitted at birth for jaundice due to Rh incompatibility, treated with phototherapy for 2 days. The motor milestones were as follows: cephalic support - 5 months, rolling - 6 months, sitting - 6 months, crawling - 1 year, ambulation - 23 months. She required physical therapy from the age of 8 months due to hypotonia and motor delay. Language development: bi-syllables - 1 year, currently makes sentences, knows body parts, knows animals. Physical examination: alert, conscious, oriented, unsteady gait, axial hypotonia, ROT ++/++++. Bilateral plantar flexor response. Studies performed: EMG: Negative for motor neuron disease, suggestive of myopathy, CK: 2348 (2019). Normal pediatric echocardiography, chest X-ray: increased AP diameter, rest normal. Oscillometry: normal central resistances, increased peripheral resistances with significant response to bronchodilator (2021). CK 3866 (2022). Pediatric Echocardiography: Mild tricuspid valve insufficiency, accessory papillary muscle in left ventricle, cardiac cavities of normal size. 6-meter walk test: 223 mts which corresponds to 40% of expected (2023). Physical examination: Head and Neck: Normocephalic - discrete cephaloparesis, no craniofacial anomalies, discrete micrognathia, normal primary dentition. Chest: Pectus carinatum with asymmetry in the sternum, mammary hypertelorism, non- pathological preserved RsRS. RsCsRs ejection murmur. Spine and Extremities: Hyperlordosis with hypertrophy of gastronemii, no minor limb anomalies. Neurological: Alert, conscious, oriented, speech, unsteady gait with some rocking, hyperlordosis, hypotonia axial level, ROT
++/++++. Bilateral flexor plantar response. Pelvic and shoulder girdle weakness. Positive Gowers. Flexible flat feet. No tongue fasciculations. Genetic testing: NGS and MLPA for DMD- negative. Exome sequencing with heterozygous VUS paternally inherited in MYPN c. 2683G>T, p. Asp895Tyr and RYR1 c. 12283-7C>T (2020). Genome sequencing at Centogene (2022) identified no relevant variants in known diagnostic genes, and a homozygous variant in SNUPN c.902_903delAT, p.Tyr301fs.

Family 14 from Columbia

The patient is a 5-year-old female. She is the only child of consanguineous parents from Colombia, with a history of focal onset ictal events with secondary generalization at 18 months, associated with global neurodevelopmental delay and mild elevation of transaminases. She received phenobarbital for one year, discontinued in January/20 with absence of seizures.

Surgical removal of cataracts (right eye) in 2018. On physical examination, normocephalic, winged ears. Axial hypotonia, with appendicular hypertonia predominantly in lower limbs, gait disorder, walks on tiptoes with support, no autonomous gait, hyperreflexia in lower extremities, bilateral Babinski, tremor, dysmetria, hypotrophic extremities. Expressive language disorder. Paraclinicals: Brain MRI (2019) normal, in 2022 with cerebellar atrophy with vermian hypoplasia. Echocardiogram (2021) with EF 69%, tricuspid valve slightly dysplastic, mild tricuspid valve insufficiency. Total abdominal echo – normal (2021). PEA stem (2021) - normal hearing. Ophthalmological exam – normal (2021). Metabolic screening - normal. CPK is very elevated, and slightly increased transaminases. EMG with a report of myopathy. Genetic studies included: Ataxia panel – negative and mitochondrial genome – negative (Centogene), exome sequencing (2022) identified a homozygous variant of unknown significance in TRIP4 NM_016213.4:c.1138G>A p.(Val380Ile) with poor overlap with the index phenotype and a homozygous variant in SNUPN c.902_903delAT, p.Tyr301fs (Centogene).

Family 15 from Germany

The 36-year-old male patient is a single child of non-consanguineous parents without any history of neuromuscular diseases. Pregnancy and birth proceeded without abnormalities. At the age of three to four months head retention weakness was first noted during a routine pediatric examination. Motor development was henceforth delayed whereas the intellectual development was unaffected and even above average. The patient was not able to sit freely until the age of 14 months, free walking was achieved at 18 months. Gowers sign was clearly positive at the age of 2.5 years. Diagnostics revealed elevated CK levels (465-1300 U/l) and mild myopathic changes in electromyography (polyphasia, M. quadriceps femoris and M. deltoideus). Muscle biopsies were performed at the age of 1.5, 2.5 (both M. quadriceps femoris) and 8.5 (paravertebral) years but showed only mild and unspecific myopathic changes incongruent with progressive muscle weakness. Muscle weakness and atrophy primarily affected the trunk and proximal lower limbs but extended to the proximal upper extremity. Loss of ambulation occurred at 6.5 years; an electrical wheelchair was used from 8 years on. During childhood the patient developed severe neuromuscular scoliosis requiring orthopedic surgery at the age of 8.5 years and progressive contractures of the elbow, hip, knee, and ankle joints. From the age of 12 years on, ventilatory insufficiency required first intermittent, later continuous non-invasive ventilation via a nasal mask. To date, there is no evidence of cardiac involvement (repeated echocardiography since early childhood) or any other extramuscular symptoms. Generalized myopathy progressed over the years leaving the patient with only some preserved motor function of the hands and minimal lower leg movement. Apart from his intellectual abilities the patient's phenotype reminded physicians of Duchenne muscular dystrophy throughout his childhood and adolescence but immunohistochemistry (biopsies 1989 and 1995) and genetic testing was negative (multiplex PCR in 1991, whole exome sequencing in 2021).