Supplemental materials and methods

Supplemental Methods

Targeted sequencing

A custom-made SureSelect oligonucleotide probe library was designed to capture the coding and flanking intronic sequences of 820 genes for different muscular disorders (MDSureSelectXT, NMD-Chip Consortium, www.nmd.chip.eu). Sample preparation, sequence capture, enrichment, and elution were performed according to the manufacturer's instructions (SureSelect, Agilent). Enriched sequences were then sequenced on a HiSeq2000 instrument (Illumina) at IntegraGen (Evry, France) and paired-end read of 100 bp length were produced. Image analysis and base calling was performed using Illumina Real Time Analysis Pipeline version 1.10 with default parameters. The Burrows-Wheeler algorithm was applied to align sequence reads to the UCSC Genome Browser hg19 version of the human genome and variants were called via the GATK software package.[1] The variants were filtered according to their quality, functional class (non-synonymous and/or affecting splicing), putative pathogenicity (PolyPhen-2, SIFT, OMIM) and frequency (≤1% in available genomic databases: IntegraGen, 1000 Genomes, dbSNP, NHLBI Exome Sequencing Project, and Exome Variant Server).

Morphological and immunofluorescence analyses

For myofibrillar proteins analysis, we used antibodies against desmin (D33, DAKO), αBcrystallin (G2JF, Novocastra) and myotilin (RSO34, Novocastra). For titin N-ter staining (Millipore MAB1553; dilution 1:10), tissue sections were fixed twice 10 min in 100% acetone at 4°C and incubated with the primary and then secondary antibodies (1:500, Cy3 conjugated goat anti-mouse IgG, Jackson Immunoresearch). For titin C-ter studies, sections were fixed

with 4% paraformaldehyde in PBS for 10 min and blocked for 1h with 3% BSA IgG-free a room temperature. Sections were incubated with primary antibodies, rabbit anti-Titin M10-1 polyclonal (1:100) or mouse anti-α-actinin monoclonal (1:1000, Sigma-Aldrich A7811) and then with secondary antibodies, Alexa fluor 488 goat antirabbit (1:500, Life Technologies) or Cy3 conjugated goat anti-mouse IgG (1:500, Jackson Immunoresearch) for 45 min at room temperature. Sections were imaged on an Axioplan 2 epifluorescence microscope (Carl Zeiss).

Reference

1. DePristo MA, Banks E, Poplin R, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nature genetics 2011;43:491-498.

Supplemental Table e-1: Primer for amplification of exons of the M-line Titin

	Upper primer	**Lower primer**
Exons screening		
Mex1-2a	TTATTAACTTGGGGGTGGAGG	TGCTTTCAAATGATTCATGGAG
Mex1-3b	TGGAATTGTCCATCGTTGTG	CCTCCTTCTTCACCAACTGC
Mex1-4c	GCAATTCGATCTCAGAAGGG	GGACAGTGGCTGACCATCTT
Mex1-5	TACTGGCAAATGCAGAATGC	CTCTTCTTCAAGACGCAGCC
Mex1-6d	GAGATAGTGAGACCAGCCCG	TGAAAGGCTGCTGACTCAAA
Mex1-7	ACTCCAGAGAGAACTCGCCC	ACGCTGTAATTGCCCTCATC
Mex1-8b	TAAGTACTTCTGCCCGCCAC	TGGCCTGTAGAATGCAAATG
Mex2	CTGCCATCTGGACAAAAGATG	CTCAAAATCTCCCAAATCCAC
Mex3	AAAAGGTGGGGGTCTCTTTC	TCTTCAGATGTGGAAGACATGG
Mex4	ATCCCCTGAAATCGAATGGT	ACATCAGTTGGCTGTCCCTC
Mex5	GGGTTATGCTGCTGTGTGTG	TCAGAAAGATTAGTCCGTGTGAAA
Mex6a	AGGGCCTGTGCCCTTATACT	CCAGGTTTTTCAGGTGCAAT
3'UTR	GGAGTGCCTGAATAGCTTGG	GCATGGGCTGTTTTGAACTT
cDNA analysis		
Mex1-8F	CGTGACAGGAGGGGATTATACC	Mex 5-6B	TCAGAAAGATTAGTCCGTGTGAAA
qPCR		
TTNMex1	AAGGCATGGGAGCAGTTCAT	TTGCCACTGAAAGGAATCTTGA
P0	CTCCAAGCAGATGCAGCAGA	ATAGCCTTGCGCATCATGGT

Supplemental Table e-2: Medical Research Council score

Muscle	**Patient 1**	**Patient 2**	**Patient 3**
Scapular girdle	2	2	2+
Biceps brachialis	3-	2 left, 2- right	3
Triceps brachialis	3+		3-
Wrists flexors	4+	4	5
Wrists extensors	3+	4	4
Finger extensors	3+	3+	3+
Finger flexors	4+	+2 left, 3 right	5-
Hip adductors	1	0	3-
Psoas	2	0	2
Glutei	2	0	1 (max) /2 (min)
Knee flexors	3-	0	2+
Knee extensors	3	2+ left, 2- right	3 left, 3- right
Feet elevators	4+	1 left, 0 right	2
Feet flexors	3+	2	3-
Neck flexors	5	Not scored	2
Neck extensors	5	Not scored	4
Abdominal muscles	1	1	1

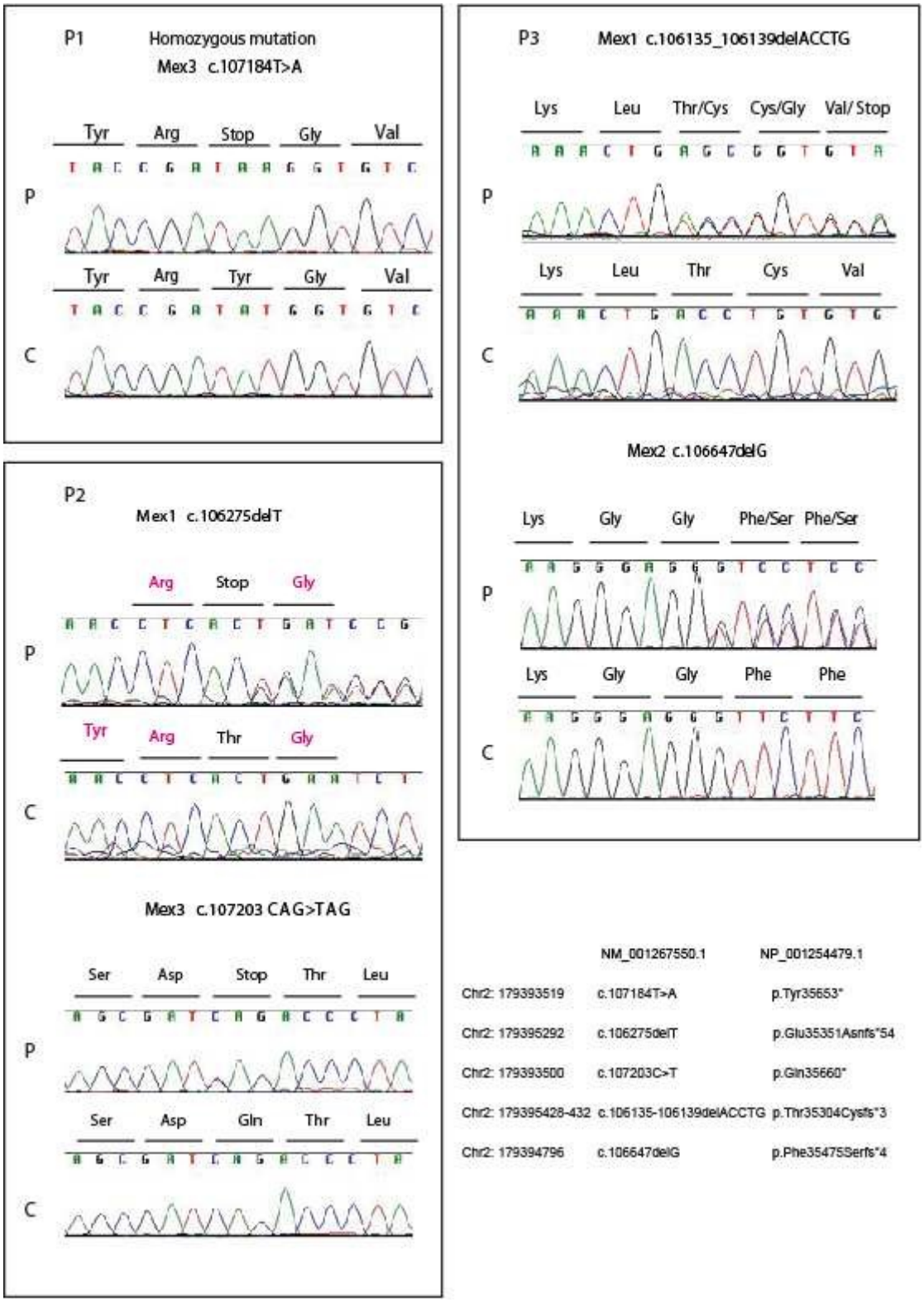
Supplemental Table e-3: Details of the contracture presentation of the patients. ND: not determined.

	P1	**P2**	**P3**
Jaw	ND	absent	present
Spine	Neck and spine were spared	Diffuse rigid spine including cervical spine	Rigid
Elbow	-30°	-50°	-60°
Wrists	-10°	Present not scored	Extensors, -80°, flexors -60°
Fingers	Flexors	Flexors	Flexors
Hips	ND	Present but not scored	Abduction -60°
Knee	-50°	-20°	-50°
Ankle	-30°	-15°	-5°

Supplemental Table e-4: Molecular analysis performed in the patients. Only the WB blot for calpain 3 showed abnormality.

	Histo-immunofluorescence analyses	Western blot	DNA or RNA analysis
Patient 1	emerin, collagen VI, dystrophin, α-sarcoglycan, βsarcoglycan, γ-sarcoglycan, δ-sarcoglycan, spectrin, laminin-α2, dysferlin	collagen VI calpain 3	*CAPN3,FKRP, ANO5, LMNA, COL6A1, A2A3*
Patient 2		calpain 3	*LMNA*, *SEPN1*
Patient 3	dystrophin, sarcoglycans, telethonin, αB-cristallin, desmin dysferlin, α-dystroglycan, collagen VI	calpain 3	*CAPN3,LMNA ACTA1, TPM2 TPM3*

Supplemental Figure e-1: Chromatograms for the identified mutations of patient 1, patient 2 and patient 3 versus controls. The correspondences at genomic, coding and protein sequences are indicated bottom right.



	NM_001267550.1	NP_001254479.1
Chr2: 179393519	c.107184T>A	p.Tyr35653*
Chr2: 179395292	c.106275delT	p.Glu35351Asnfs*54
Chr2: 179393500	c.107203C>T	p.Gln35660*
Chr2: 179395428-432	c.106135-106139delACCTG	p.Thr35304Cysfs*3
Chr2: 179394796	c.106647delG	p.Phe35475Serfs*4

Supplemental Figure e-2: A/ RT-PCR across Mex1 and Mex6 showed normal splicing of the region in the patients (P2 and P3). **B/** qRT-PCR using Mex1 primers showed decrease level of messenger probably due to NMD (expressed in 2-ΔΔCt).