Clinical and morphological phenotype of the filamin myopathy: a study of 31 German patients

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Mutations in the filamin C gene (*FLNC*) cause a myofibrillar myopathy (MFM), morphologically characterized by focal myofibrillar destruction and abnormal accumulation of several proteins within skeletal muscle fibres. We studied 31 patients from four German families to evaluate the phenotype of filaminopathy. All patients harboured the same p.W2710X mutation in *FLNC*. Haplotype analysis suggested a founder mutation in these German filaminopathy families. The mean age at onset of clinical symptoms was 44 +/− 6 years (range, 24–57 years). Slowly progressive muscle weakness was mostly pronounced proximally, initially affecting the lower extremities and involving the upper extremities in the course of disease progression, similar to the distribution of weakness seen in limb-girdle muscular dystrophies (LGMD). Patients frequently developed respiratory muscle weakness. About one-third of the patients showed cardiac abnormalities comprising conduction blocks, tachycardia, diastolic dysfunction and left ventricular hypertrophy indicating a cardiac involvement in filaminopathy. Serum creatine kinase levels varied from normal up to 10-fold of the upper limit. Magnetic resonance imaging studies showed a rather homogenous pattern of muscle involvement in the lower extremities differing from that in other types of MFM. Myopathological features included perturbation of myofibrillar alignment, accumulation of granulofilamentous material similar to that seen in primary desminopathies and abnormal intracellular protein deposits typical of MFM. Decreased activities of oxidative enzymes and fibre hypertrophy seem to be early features, whereas dystrophic changes were present in advanced stages of filaminopathy. Rimmed vacuoles were detected in only a few cases. The intracellular aggregates were composed of a variety of proteins including filamin C, desmin, myotilin, Xin, dystrophin and sarcoglycans. Therapy is so far limited to symptomatic treatment. The German filaminopathy cohort, the largest group of patients studied so far, shares phenotypic features with LGMD and presents with characteristic histopathological findings of MFM.

Introduction

Myofibrillar myopathies (MFM) are a genetically heterogeneous group of chronic neuromuscular disorders morphologically characterized by focal disintegration of myofibrils and abnormal accumulation of several proteins within skeletal muscle fibres. Clinical phenotype and age of onset vary. In most patients, progressive muscle weakness affecting proximal as well as distal muscles begins in adulthood. Life expectancy may be shortened by associated cardiomyopathy or due to involvement of respiratory muscles. Cataract and peripheral neuropathy occur in a subset of patients indicating a multi-system involvement (Selcen et al., 2004). In a minority of cases, mutations in desmin (DES, OMIM 125660) (Goldfarb et al., 1998), myotilin (TTID, OMIM 604103) (Selcen and Engel, 2004), ZASP (LDB3, OMIM 605906) (Selcen and Engel, 2005) and αB-crystallin (CRYAB, OMIM 123590) (Vicart et al., 1998) are associated with MFM. In 2005, we identified the first pathological mutation in the filamin C gene (FLNC, OMIM 102565) in members of an extended family of German origin with progressive muscle weakness and morphologic features of MFM (Vorgerd et al., 2005).

Filamins are a family of large cytoskeletal proteins that crosslink F-actin filaments into either parallel bundles or dynamic three-dimensional meshwork. Moreover, they interact directly with a plethora of cellular proteins of great functional diversity, indicating that they are multifunctional signalling adapter proteins (Stossel et al., 2001; van der Flier et al., 2002 Feng and Walsh, 2004; Popowicz et al., 2006;). The human filamin family consists of three isoforms, filamins A, B and C. Mutations in filamin A and B have been reported to cause a broad range of congenital malformations, affecting brain, bone and other organs

(Robertson et al., 2003; Bicknell et al., 2007). Filamin C (formerly also called filamin 2, γ-filamin or ABP-L) is the muscle-specific filamin isoform. Its upregulation during the initial stages of myocyte differentiation, its localization predominantly at the periphery of Z-disks and its direct interaction with myotilin (van der Ven et al., 2000b) and FATZ/calsarcin/myozenin (Faulkner et al., 2000;Takada et al., 2001) imply an important role of filamin C during myofibrillogenesis (Chiang et al., 2000;van der Ven et al., 2000a;van der Ven et al., 2000b). At the sarcolemma, filamin C interacts with γ- and δ-sarcoglycan in costameres (Thompson et al., 2000) and with Xin in myotendinous junctions (van der Ven et al., 2006). The p.W2710X mutation in FLNC identified in our large German family results in the inability of the mutant protein to dimerize and in the development of large filamin C-containing aggregates within the skeletal muscle fibres (Vorgerd et al., 2005).

Here we report on the clinical, genetic, radiological and myopathological features in 31 patients from four German families with MFM associated with the p.W2710X mutation in FLNC.

Patients and methods
Patients

Data of a total of 31 patients from four German families were evaluated (Fig. 1, Table 1). 23 individuals (family 1: 11 patients, family 2: six patients, family 3: two patients, family 4: four patients) with the p.W2710X mutation in FLNC were examined clinically. Clinical data of eight deceased patients from families 1 (Table 1, ID 1–6), 3 (ID 24) and 4 (ID 27) were obtained from attending physicians and relatives. Genetic and preliminary clinical data of family 1 have recently been reported (Vorgerd et al., 2005).
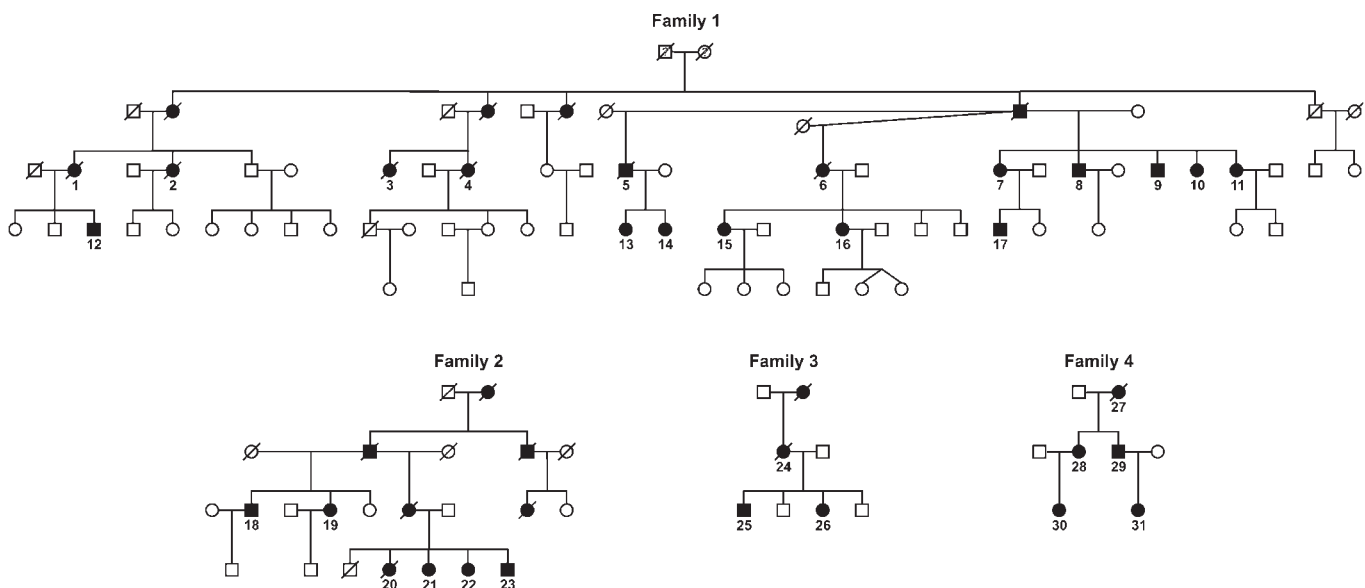
Fig. 1 Pedigrees of four German families with MFM associated with p.W2710X mutation in FLNC. Individuals with proven mutation and deceased family members who had suffered from muscle weakness are represented by filled symbols.

Table I Clinical data of 31 patients harbouring the p.W2710X mutation in *FLNC*

ID; sex; age at onset/examination/death	Initial symptoms/distribution of muscle weakness and muscle atrophy	CK increase	Cardiac findings	Respiratory insufficiency	EMG/NCS
Family 1					
1; w; 40/?/61	Waddling gait/P>D, LL>UL	n.k.	n.k.	Yes	n.k.
2; w; 52/?/73	Unsteady gait/P>D, LL>UL	n.k.	n.k.	Yes	n.k.
3; w; ?/?/69	Waddling gait/P>D, LL>UL, para-spinal muscles	No	Cardiomyopathy (n.s.)	Yes	n.k.
4; w; 42/?/64	Unsteady gait/P>D, LL>UL	<2-fold	n.k.	Yes	n.k.
5; m; 43/?/68	Weakness when climbing stairs, lower back pain/P>D, LL>UL, paraspinal muscles	6-fold	n.k.	Yes	n.k.
6; w; 45/49/64	Weakness when climbing stairs, leg pain/P>D, LL>UL	3-fold	Cardiomyopathy (n.s.)	Yes	n.k.
7; w; 37/64/alive	Weakness when climing stairs, back pain/P>D, LL>UL, winged scapula	2-fold	Normal	Yes	n.d./n.d.
8; m; 45/62/alive	Weakness when climbing stairs, lower back pain/P>D, LL>UL, winged scapula, mild ptosis	6-fold	Normal	Yes	sA/normal
9; m; 45/58/alive	Waddling gait, lower back pain/P>D, LL>UL, winged scapula	8-fold	Normal	No	mP/normal
10; w; 49/54/alive	Waddling gait, lower back pain/PLL, mild ptosis	3-fold	Normal	No	mP/normal
11; w; 44/48/alive	Weakness in legs, lower back pain/PLL>DLL, PUL	2-fold	Normal	No	mP/normal
12; m; 57/60/alive	Weakness when climbing stairs or walking uphill/P>D, LL>UL, abdominal and paraspinal muscles, winged scapula	2-fold	RBBB, decreased EF	No	mP/normal
13; w; 43/50/alive	Weakness when climbing stairs and walking uphill/foot extensor muscles>gluteal muscles & dorsal thigh	4-fold	Normal	No	n.d./n.d.
14; w; 46/48/alive	Waddling gain, weakness when climbing stairs, lower back pain/proximal-dorsal and distal-anterior lower extremities	2-fold	Normal	No	n.d./n.d.
15; w; 50/52/alive	Weakness when climbing stairs/P>D, LL>UL, abdominal and paraspinal muscles	3-fold	Normal	No	n.d./n.d.
16; w; 49/54/alive	Weakness when climbing stairs/P>D, LL>UL	5-fold	Atrial flutter, TA	Yes	n.d./n.d.
17; m; -/28/alive	Asymptomatic	< 2-fold	Normal	No	n.d./n.d.
Family 2					
18; m; 45/49/alive	/P>D, LL>UL, wheelchair-bound	3-fold	Normal	No	sA/normal
19; w; 42/45/alive	/P>D, LL>UL	2-fold	Normal	No	sA/n.d.
20; w; 44/54/54	Weakness when climbing stairs or walking uphill/P>D, LL>UL, abdominal and paraspinal muscles, winged scapula, facial muscles (slight), wheelchair-bound at the age of 52	3-fold	Normal	Yes	sA/normal
21; w; 44/52/alive	Back pain/P>D, LL=UL, neck muscles	2-fold	RBBB, DD, grade II MG	No	sA/normal
22; w; 42/49/alive	Weakness when climbing stairs or walking uphill/P>D, LL=UL, abdominal and paraspinal muscles, winged scapula, facial muscles (slight)	2-fold	LVH, DD, grade I AG	No	sA/normal
23; m; 39/45/alive	Weakness when climbing stairs/P>D, LL>UL, paraspinal muscles	10-fold	LVH	No	sA/normal
Family 3					
24; w; 47/-/70	Waddling gait/P>D, LL>UL, wheel-chair-bound at the age of 52	n.k.	n.k.	Yes	n.d.
25; m; 48/55/alive	Waddling gait/PLL	n.k.	n.k.	No	n.d.

(continued)

Table I Continued

ID; sex; age at onset/examination/death	Initial symptoms/distribution of muscle weakness and muscle atrophy	CK increase	Cardiac findings	Respiratory insufficiency	EMG/NCS
26; w; 43/50/alive	Waddling gait, myalgia PLL/PLL > PUL	n.k.	Normal	Yes	sA/normal
Family 4					
27; w; 48/?/75	?/?, at the age of 65 respirator dependent	No	RBBB	Yes	sA/normal
28; w; 45/56/alive	Back pain, weakness when climbing stairs/P > D, LL > UL, wheelchair-bound, paraspinal muscles, neck muscles, facial muscles (slight)	3-fold	LVH	Yes	sA/normal
29; m; 38/48/alive	Back and neck pain/PUL > PLL, neck muscles	3-fold	Normal	No	sA/normal
30; w; 24/24/alive	Back and neck pain/head flexors	No	Normal	No	sA/n.d.
3l; w; -/25/alive	Asymptomatic	No	Normal	No	n.d./n.d.

ID = patient identification number; CK = creatine kinase; EMG = electromyography; NCS = nerve conduction studies; w = woman; m = man; P = proximal; D = distal; UL = upper limbs; LL, lower limbs; PUL = proximal upper limbs; PLL = proximal lower limbs; DLL = distal lower limbs; EF = ejection fraction; RBBB = right bundle branch block; DD = diastolic dysfunction; MG = mitral regurgitation; AG = aortic regurgitation; LVH = left ventricular hypertrophy; sA = spontaneous activity; mP = myogenic potentials; n.s. = not specified; n.k. = not known; n.d. = not done.

Patients underwent echo- and electrocardiographic evaluations following standard protocols. Examinations obtained left ventricular dimensions, volumes and analysis of systolic, diastolic and valvular function. Neurophysiological studies included routine electromyographic measurements and standard nerve conduction studies. In 10 patients (family 1: seven patients, family 2: two patients, family 4: one patient), a skeletal muscle biopsy was performed. One patient (ID 9) was biopsied twice at the age of 52 and 62 years.

Genetic analysis

Blood samples from all family members were obtained after written informed consent. Genomic DNA was purified from peripheral blood following standard methods using the QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany). The presence of the p.W2710X mutation was analysed in all family members using the Big Dye Terminator v 1.1 Cycle Sequencing Kit on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Haplotype studies were performed on eight DNA samples of affected members from the four apparently unrelated German families. Six polymorphic microsatellite markers on chromosome 7q32.1 flanking the *FLNC* gene and the c.8130G>A *FLNC* mutation were used for haplotyping: D7S635, D7S504, D7S1875, D7S530, D7S2544 and D7S2519. Genomic locations are from the UCSC reference database *via* http://www.genome.ucsc.edu/. Haplotype analysis was performed as described previously (von der Hagen *et al.*, 2006).

Histochemical and immunofluorescence analysis

For conventional histochemical studies, 6 μm thick frozen sections of unfixed skeletal muscle were processed for haematoxylin-eosin (H&E), Gomori trichrome, acid phosphatase, periodic acid-Schiff (PAS), NADH dehydrogenase, succinate dehydrogenase (SDH), cytochrome c oxidase (COX), Congo red, adenosine triphosphatase (ATPase) at pH 4.3, 4.6, 9.4 and oil red O-staining using standard procedures. The ATPase 9.4 staining was used for the quantification of fibre types.

Immunofluorescence studies were performed on 4 μm thick frozen serial sections. Primary antibodies used in this study are listed in Table 2. Isotype-specific secondary antibodies conjugated with fluorescein isothiocyanate or biotinylated sheep anti-mouse IgG followed by Cy2-labelled streptavidin (Amersham Biosciences) were applied according to the recommendations of the manufacturer. Adjacent sections in the series were stained with Gomori trichrome.

Electron microscopy

Electron microscopy was performed on the second muscle specimen of Patient 9. The specimen was fixed in 2.5% glutaraldehyde and processed for electron microscopy by standard methods. Ultrathin skeletal muscle sections were analysed using a Zeiss 900 electron microscope at an accelerating voltage of 80 kV. Desmin immunogold staining was performed as described previously (Schröder *et al.*, 2002).

MRI of skeletal muscle

Seven patients from families 1 and 3 (ID 9–12, 21–23) underwent MRI of the lower extremities with a 1.5 Tesla MR unit (MAGNETOM Symphony QUANTUM, Siemens, Erlangen, Germany; maximum gradient 30 mT/m, slew rate 125 T/m/s). A four-channel phased array body coil was used. After acquiring T1-weighted localizers of the lower extremities, axial T2-weighted Turbo Inversion Recovery Magnitude (TIRM) images (TR 4020/3040 ms, TE 68/27 ms, TI 150 ms, slice thickness 10 mm) and T1-weighted non-fat-saturated Spin Echo images (TR 500 ms, TE 20 ms, slice thickness 10 mm) were obtained.

Results

p.W27I0X mutation in filamin C: a founder mutation in the German filamin myopathy population

Sequencing the *FLNC* gene identified a recently reported p.W2710X mutation (Vorgerd *et al.*, 2005) in all clinically

Table 2 Primary antibodies used in immunofluorescence studies

Antigen	Clone	Source	Company/reference	Dilution
Filamin C	RR90	mouse (mAb)	van der Ven et al. (2000a)	I/20
Desmin	D33	mouse (mAb)	DAKO	I/500
Myotilin	RS034	mouse (mAb)	Novocastra	I/20
αB-crystallin	G2JF	rabbit (pAb)	Novocastra	I/100
Xin	XRIB	mouse (mAb)	van der Ven et al. (2006)	I/5
α-sarcoglycan	AdI/20A6	mouse (mAb)	Novocastra	I/100
β-sarcoglycan	βSarc/5BI	mouse (mAb)	Novocastra	I/100
γ-sarcoglycan	35DAG/2IB5	mouse (mAb)	Novocastra	I/100
δ-sarcoglycan	δSarc3/I2CI	mouse (mAb)	Novocastra	I/100
Dystrophin	Dy8/6C5	mouse (mAb)	Novocastra	I/20
Spectrin	RBC2/3D5	mouse (mAb)	Novocastra	I/70
Caveolin 3	26	mouse (mAb)	Transduction	I/200
Merosin (80 kd)	5H2	mouse (mAb)	Chemicon	I/150
Merosin (300 kd)	Mer3/22B2	mouse (mAb)	Novocastra	I/100
Collagen VI	3C4	mouse (mAb)	Chemicon	I/500
α-actinin	RBC2/IB6	mouse (mAb)	Novocastra	I/1000
Slow myosin	WB-MHCs	mouse (mAb)	Novocastra	I/40
Fast myosin	WB-MHCf	mouse (mAb)	Novocastra	I/20
Titin	9DI0	mouse (mAb)	Chemicon	I/50
Filamin A	mABI680	mouse (mAb)	Chemicon	I/300

mAb = monoclonal antibodies; pAb = polyclonal antibodies.

affected patients in the four German families (Fig. 1, Table 2) and in two asymptomatic individuals (ID 17, 31) from families 1 and 4. Haplotype studies revealed that the carriers of the p.W2710X mutation share a common haplotype stretching from marker *D7S635* to *D7S2519* including the *FLNC* gene region. In one patient (ID 26), a recombination event occurred between markers *D7S1875* and *D7S530* with the assumed breakpoint proximal to the p.W2710X *FLNC* mutation (Table 3). These findings indicate a founder *FLNC* mutation in the German filaminopathy families.

Clinical spectrum of patients

The mean age of onset was 44 ± 6 years (range 24 to 57 years, Table 1). Nine affected persons (ID 1–6, 20, 24, 27) died at a mean age of 66 years (range 54 to 75 years). The distribution of muscle weakness was evaluated in 28 symptomatic patients either by clinical examination or anamnestic data. One patient had only weakness of the neck flexors (ID 30). The other 27 patients suffered from slowly progressive skeletal muscle weakness initially affecting the lower extremities and mostly involving the upper extremities in the course of disease progression. In 25/28 symptomatic patients, the weakness was more pronounced proximally than distally (Fig. 2). One patient (ID 14) showed distal and proximal weakness of the leg muscles, and in one patient (ID 13) the weakness was clearly distal. A slight involvement of facial muscles was seen in five patients (ID 8, 10, 20, 22, 28). It is noteworthy that 10/28 patients (ID 7–11, 14, 21, 28–30) reported on back pain as an initial symptom. Serum creatine kinase levels varied from normal values up to 10-fold of the upper normal limit.

Neurophysiological studies were performed in 16 patients (Table 1). In 12 patients, EMG revealed spontaneous activity like fibrillation potentials, positive sharp waves and complex repetitive discharges. Myogenic low amplitude and polyphasic potentials were detected in four patients (ID 9–12). One patient (ID 21) reported on hypaesthesia in distal lower extremities but neurographic measurements did not indicate a peripheral neuropathy in any of the patients.

Fourteen out of 31 patients including 9/9 deceased patients suffered from weakness of respiratory muscles at time of examination or at the end of lifetime (Table 1). At least two patients (ID 6, 20) died because of severe respiratory complications. Two patients (ID 7, 8) with a disease duration of more than 15 years required nocturnal ventilation.

Echocardiographic and electrocardiographic evaluation showed abnormalities in 9/25 patients (Table 1). Findings included right bundle branch block (3/25; ID 12, 21, 27), atrial flutter (ID 16) and left ventricular hypertrophy (3/25; ID 22, 23, 28). One patient (ID 12) presented with decreased ejection fraction and two patients (ID 21, 22) with diastolic dysfunction. Cardiomyopathy with left ventricular dysfunction was observed in two patients during follow-up (ID 3, 6). The majority of patients had normal ventricular volumes, dimensions and ejection fraction. Findings of minor regurgitations of aortic or mitral valve were observed in 2/25 patients (ID 21, 22). Haemodynamical relevant valve dysfunctions and pericardial effusion were not present.

Histological features

The histological findings in eleven skeletal muscle biopsies taken from 10 patients at different stages of the disease

Table 3 Haplotype analysis of selected filaminopathy patients from four German families

Marker	Type	Location (bp)	ID 11 (family 1)		ID 23 (family 2)		ID 26 (family 3)		ID 28 (family 4)	
D7S635	MS	126 960 896	**4**	6	**4**	1	4	5	**4**	1
D7S504	MS	127 302 461	**2**	1	**2**	3	1	3	**2**	4
D7S1875	MS	127 437 907	**4**	3	**4**	1	3	1	**4**	1
FLNC c.8130G>A (p.W2710X)	Mutation	128 257 719	**A**	G	**A**	G	**A**	G	**A**	G
D7S530	MS	128 889 656	**3**	1	**3**	1	**3**	4	**3**	3
D7S2544	MS	129 595 304	**2**	1	**2**	3	**2**	2	**2**	2
D7S2519	MS	129 650 934	**3**	2	**3**	2	**3**	4	**3**	3

MS = microsatellite markers. Columns represent individual haplotypes of the analysed patients. Each row represents the genotypes of the MS and the FLNC mutation. Markers are listed from centromeric to telomeric. The values in bold represent haplotype cosegregating with the p.W2710X mutation (representing a putative founder haplotype).
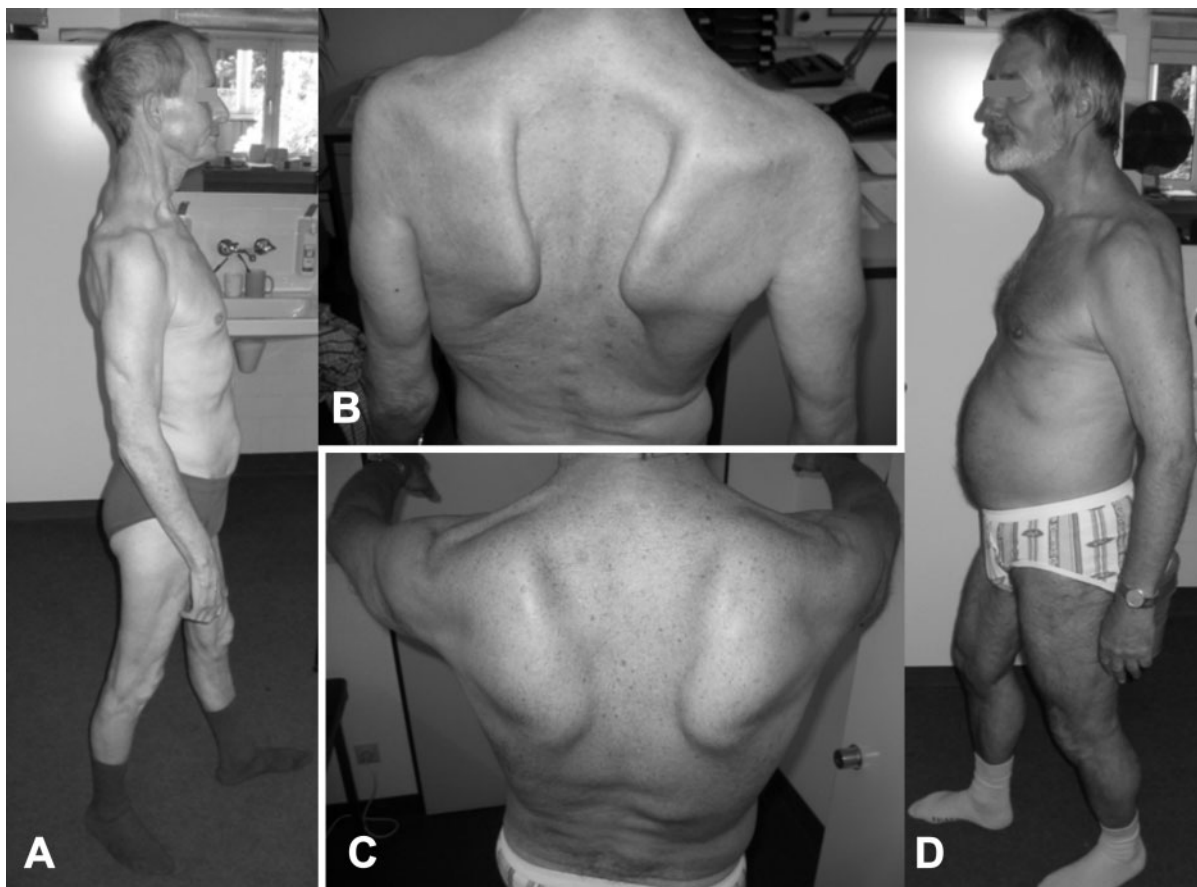
Fig. 2 Pictures of patient 9 (**A**, **B**) and patient 12 (**C**, **D**). Note the predominantly proximal atrophies. Both patients present with winged scapula (**B**, **C**).

varied from slightly unspecific changes to distinct muscular dystrophy-like features (Table 4, Fig. 3). In Patient 10, a muscle biopsy taken 4 years after clinical manifestation only showed a small increase of endomysial collagen, internal nuclei in four percent of muscle fibres, a few COX-negative fibres and slightly hypertrophic type 1 and type 2 muscle fibres. Similar changes were present in the first biopsy of Patient 9 taken 7 years after clinical onset. In addition, a few muscle fibres with core-like features were detected

(similar to findings in patient 28 shown in Fig. 3F, I, L). The second biopsy in this patient taken 10 years later showed a drastic increase of myopathic changes comprising pronounced fibre size variability, fibre splitting, muscle fibre necrosis and regeneration (like in Fig. 3A–E), rimmed and non-rimmed vacuoles (like in Fig. 3B–E), endomysial fibrosis and fatty replacement. Many fibres harboured multiple internal nuclei. Core-like features were found in about 20% of muscle fibres, predominantly in those with

Table 4 Histological findings in ten filaminopathy patients

Family	I								2		4
Patient ID	6	9	9 (re-biopsy)	10	11	12	15	16	19	23	28
Age at biopsy (years)	54	52	62	53	48	61	52	50	46	42	46
Site of biopsy	D	VL	GN	VL	VL	GN	GN	VL	VL	VL	BB
Morphological changes											
Necrosis	-	-	+	-	(+)	+	+	+	+	+	+
Fibre splitting	-	-	+	-	-	+	++	+	-	-	(+)
Internal nuclei	++	(+)	+++	+	++	+++	++	+	(+)	-	++
Rimmed vacuoles	-	-	+	-	(+)	+	-	-	-	-	+
Core-like features	(+)	(+)	+++	-	++	+++	++	++	+	++	++
COX-negative fibres	(+)	+	+	(+)	(+)	(+)	-	-	-	-	(+)
Fibre regeneration	(+)	-	+	-	(+)	++	-	-	-	-	+
Increase of endomysial collagen	Moderate	Mild	Moderate	Mild	Moderate	Severe	Moderate	Severe	Mild	Mild	Moderate
Neurogenic changes	No	No	Yes	No	No	Yes	Yes	No	No	Yes	Yes
Morphometrical studies											
Type 1 [%]/Typ 2 [%] fibres	80/20	57/43	52/48	48/52	61/39	74/26	70/30	49/51	46/54	58/42	76/24
Typ 1 fibres											
Mean diameter [μm] (range)	51 (20–120)	69 (41–102)	72 (21–144)	65 (51–85)	52 (16–92)	64 (11–160)	77 (28–132)	61 (33–108)	59 (28–116)	82 (30–126)	41 (6–117)
Hypotrophic fibres [%]	10	-	15	-	3	15	2	1	1	2	26
Hypertrophic fibres [%]	8	21	34	32	8	23	56	29	28	54	2
Typ 2 fibres											
Mean diameter [μm] (range)	53 (28–110)	80 (47–108)	44 (7–180)	64 (40–80)	66 (17–89)	59 (13–157)	79 (25–140)	69 (30–105)	45 (26–93)	61 (33–113)	46 (10–90)
Hypotrophic fibres [%]	5	-	66	-	3	24	2	2	3	3	14
Hypertrophic fibres [%]	15	49	24	23	42	20	54	48	2	6	2

D = deltoideus muscle; VL = vastus lateralis muscle; GN = gastrocnemius muscle; BB = biceps brachii muscle; (+)/+/++/+++, existent in <1/1 up to 5/6 up to 20/more than 20% of muscle fibres.
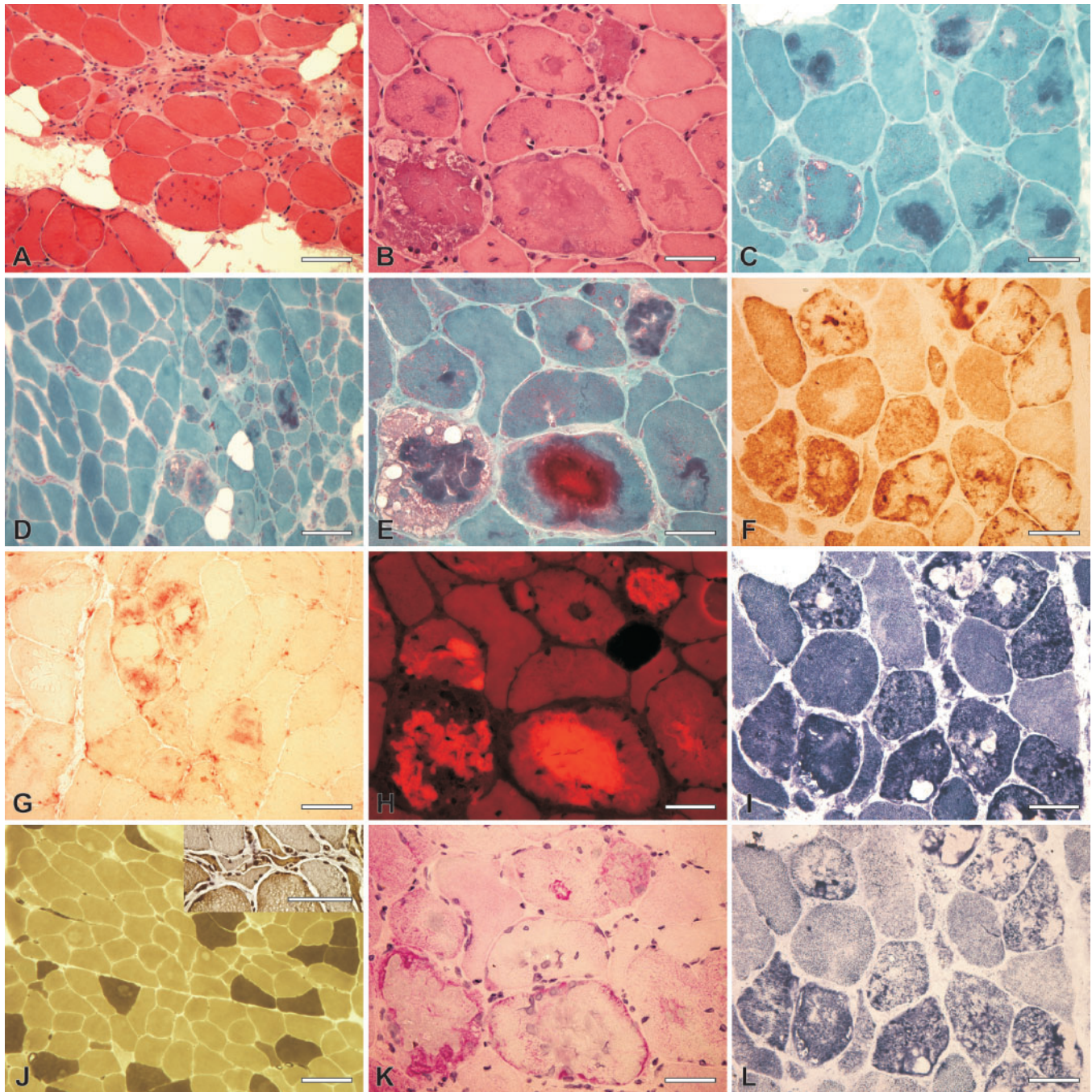
Fig. 3 Histochemical findings in muscle specimen of patient I2 (**A**) and patient 28 (**B–L**). (**A**) Dystrophic pattern with pronounced fibre size variability, internal nuclei, fibre splitting, muscle fibre necrosis and regeneration, endomysial fibrosis and fatty replacement. (**B**), (**E**), (**H**), (**K**) Serial consecutive sections with MFM-typical histological findings, congophilic deposits and PAS-positive vacuols. (**C**), (**F**), (**I**), (**L**) Serial sections show sharply circumscribed decreases of oxidative enzyme activity. (**D**) Unequal distribution of abnormal fibres across the fascicles. (**G**) Increased acid phosphatase activity in abnormal fibres. (**J**) Predominance of type I fibres. Insert: cluster of atrophic fibres of both fibre types. (**A**, **B**): HE (**C–E**): Gomori trichrome; (**F**): COX; (**G**): acid phosphatase; (**H**): Congo red; (**I**): NADH dehydrogenase; (**J**): ATPase 9.4; (**K**): PAS; (**L**): SDH. Bar in (**J**) (also applies to **A, D**) = I00 μm, bar in (**L**) (also applies to **B, C, E–I, K**) = 50 μm.

abnormal areas in trichrome staining as described below. Dystrophic changes were even more pronounced in Patient 12 (Fig. 3A). A predominance of type 1 fibres was detected in most biopsies (Table 4, Fig. 3J). Possible neurogenic changes such as denervation atrophy (Fig. 3J) were present in 5/11 biopsies (ID 12, 15, 23, 28, second biopsy of ID 9, Table 4).

In all muscle specimens except the one from Patient 10, muscle fibres with light microscopic features of abnormal protein aggregation were observed at different degrees.

These fibres typically appeared in uneven distribution across the fascicles (Fig. 3D). In trichrome-stained sections, abnormal fibres harboured a variety of pleomorphic hyaline, granular and amorphous deposits ranging from red-blue to dark-blue in colour. These inclusions varied in size, quantity and localization within the affected fibres (Figs 3C–E and 4). Some fibres harboured vacuoles in variable size and location partly rimmed or filled with basophilic membranous material (Fig. 3B–E). Non-rimmed vacuoles frequently showed strong PAS-positivity (Fig. 3K). Most inclusions in abnormal fibres were congophilic (Fig. 3H). Activities of oxidative enzymes and ATPase were decreased or lacking in abnormal fibre regions (Fig. 3F, I, L). Core-like features were detected in fibres of 10/11 biopsies (Table 4). Those fibres were often inconspicuous in trichrome-stained sections. Many abnormal fibres showed increased acid phosphatase levels (Fig. 3G).

Immunofluorescence studies

Immunofluorescence analysis indicated strong immunoreactivities for filamin C, desmin, αB-crystallin and Xin in areas appearing abnormal in trichrome-stained sections (Fig. 4). Ectopic cytoplasmic expression of γ-sarcoglycan, dystrophin (Fig. 4), caveolin-3, α-, β-, δ-sarcoglycan, merosin (80 and 300 kDa), spectrin and collagen VI were present in many abnormal fibres. Abnormal areas showed no immunoreactivity for α-actinin, myosin, titin or filamin A. Small intracellular protein accumulations were detected sporadically in the first biopsy of Patient 9 but were more prominent and very abundant in the second one taken 10 years later. Remarkably, the biopsy of Patient 10 did not show any pathological protein aggregates (Fig. 4).

Ultrastructural features

Ultrastructural analysis of the second skeletal muscle biopsy of Patient 9 showed widespread myofibrillar pathology comprising rod formation (also visible in trichrome-stained sections), Z-disc remnants, Z-disc streaming and myofibrillar lysis. Multiple muscle fibres displayed subsarcolemmal and intermyofibrillar pathological protein aggregates consisting of filamentous or desmin-positive granulofilamentous material. In this context, it is noteworthy that the granulofilamentous material observed in filaminopathies is morphologically indistinguishable from the one seen in primary desminopathies and αB-crystallinopathies. In addition, single fibres contained sarcoplasmic paired helical filaments (Fig. 5).

MRI findings

MRI showed progressive lipomatous changes of the lower limb muscles in all seven patients with high signal intensities of the affected muscles on non-fat-saturated T1-weighted images. The lipomatous alterations exhibited an almost identical, symmetrical involvement of muscle groups in the lower limbs in comparison of the patients (Fig. 6).

Predominantly, the following muscles were affected: gluteal muscles, semimembranosus, adductor magnus and longus, biceps femoris, vastus intermedius and medialis, soleus, medial head of gastrocnemius, tibialis anterior, extensor hallucis longus and extensor digitorum longus. The abnormal findings included reticular patterns of hyperintensity on T1-weighted images in less affected patients (ID 10), whereas homogeneous areas of T1w hyperintensity were visible in individuals with progressive disease (Fig. 6). Typically, the sartorius and gracilis, the superficial parts of the quadriceps femoris and the lateral head of the gastrocnemius appeared almost normal (arrowheads in Fig. 6) even in patients with more advanced clinical course. Muscular signal intensities on T2-weighted TIRM images were only mildly elevated in the examined patient group primarily affecting the muscles mentioned above, indicating absence of distinct intramuscular oedema (Fig. 6).

Discussion

In the present study, we report a large series of patients with genetically proven filamin C mutation. Filaminopathy in these patients is characterized by an onset of symptoms at a mean age of 44 years, a prominent proximal weakness more in lower than upper extremities, resembling the typical phenotype of limb-girdle-muscular dystrophy, with involvement of respiratory muscles in the disease course and hints suggesting cardiac involvement in a subset of patients. In all the patients, we found the same p.W2710X mutation in the *FLNC* gene described before in an extended German family (Vorgerd *et al.*, 2005), which is transmitted in an autosomal dominant manner. Even though a relationship between the families was not apparent or known to the families, haplotype analyses strongly indicate a founder mutation.

Filaminopathy shows typical histopathological findings of MFM and decreased activities of oxidative enzymes indicate an early mitochondrial dysfunction

Selcen et al. defined minimal criteria required for the histopathological diagnosis of MFM: (i) pleomorphic abnormal fibre regions in trichrome stained sections, (ii) vacuolar changes in some fibres, (iii) immunolocalization of multiple proteins in most pleomorphic fibre regions, and (iv) congophilia of some hyaline deposits (Selcen, 2006). Nine out of ten filaminopathy patients biopsied fulfilled these MFM criteria and showed a perturbation of myofibrillar alignment including disintegration of Z-disks at the ultrastructural level. Similar features have been reported in desminopathy (Bär *et al.*, 2005; Goldfarb *et al.*, 2004), αB-crystallinopathy (Selcen and Engel, 2003), myotilinopathy (Olive *et al.*, 2005; Selcen and Engel, 2004), ZASPopathy (Selcen and Engel, 2005), and MFM patients with unknown mutation (Selcen *et al.*, 2004).
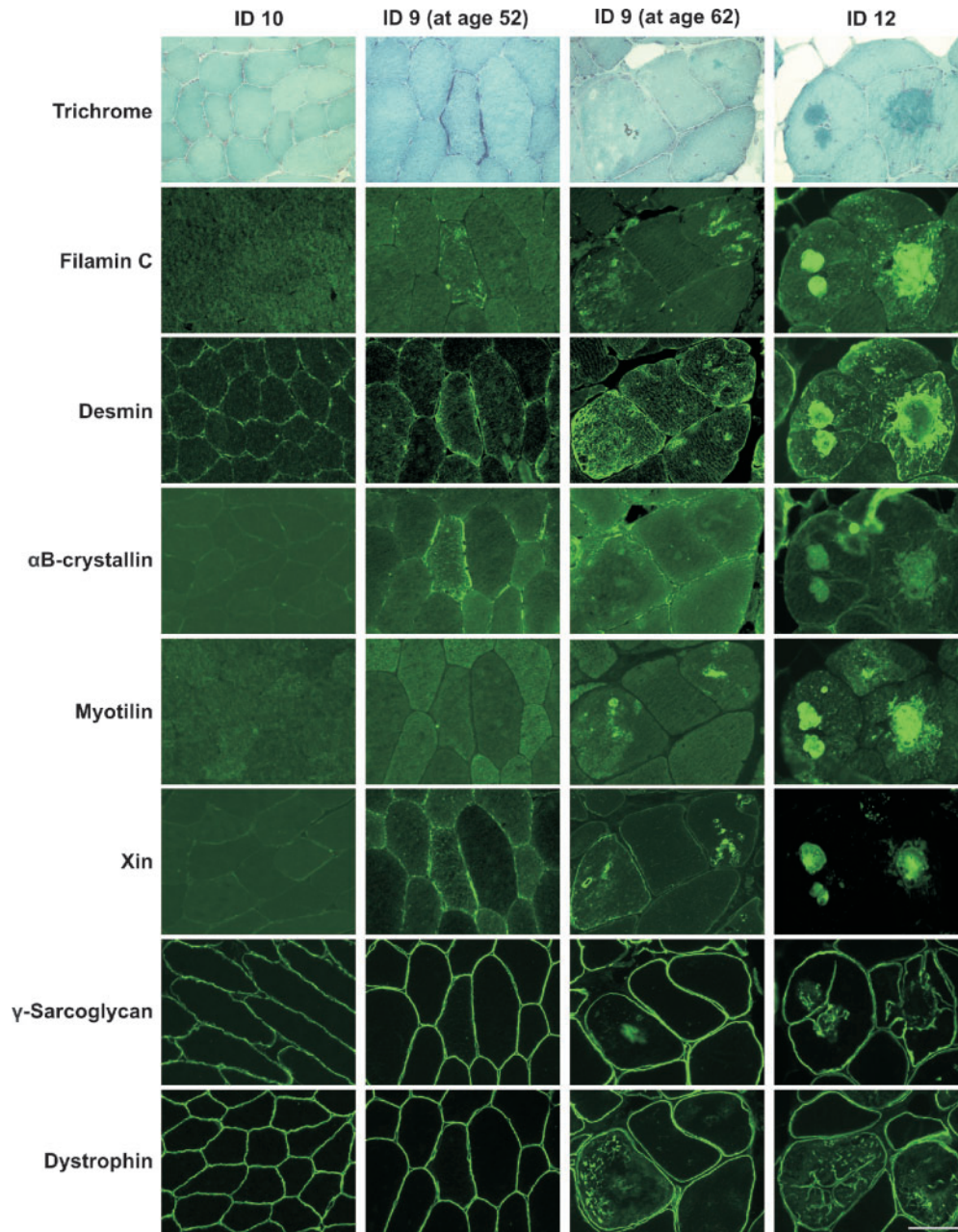
Fig. 4 Immunofluorescence findings in filaminopathy. Studies on muscle specimen of clinically affected patient 10 indicate neither abnormal fibre regions in trichrome-stained section nor immunoreactivity for any protein tested. In patients 9 and 12, serial consecutive sections are shown. Note accumulation of filamin C, desmin, αB-crystallin, myotilin and Xin (all three series) as well as ectopic expression of γ-sarcoglycan and dystrophin (patient 9 at age 62, patient 12) in fibres that appear abnormal in trichrome-stained sections.

Therefore a differentiation between subtypes of MFM on the basis of histological findings alone is not possible and needs molecular genetic studies.

Interestingly, COX-negative fibres and core-like lesions without any typical MFM changes in trichrome staining or immunofluorescence studies were early signs of filaminopathy. In analogy to a previously described case of primary desminopathy (Schröder *et al.*, 2003), these findings indicate that mitochondrial changes may be an early histological sign in MFM. In patients with muscle weakness

and focally decreased oxidative enzyme activities, beside a primary mitochondriopathy MFM should also be considered even if typical histological MFM features are lacking.

Within the clinical diversity of MFM, filaminopathy resembles a limb-girdle muscular dystrophy phenotype

Clinical presentation of MFM has been very variable. Onset may be in childhood or in the teens with sudden death
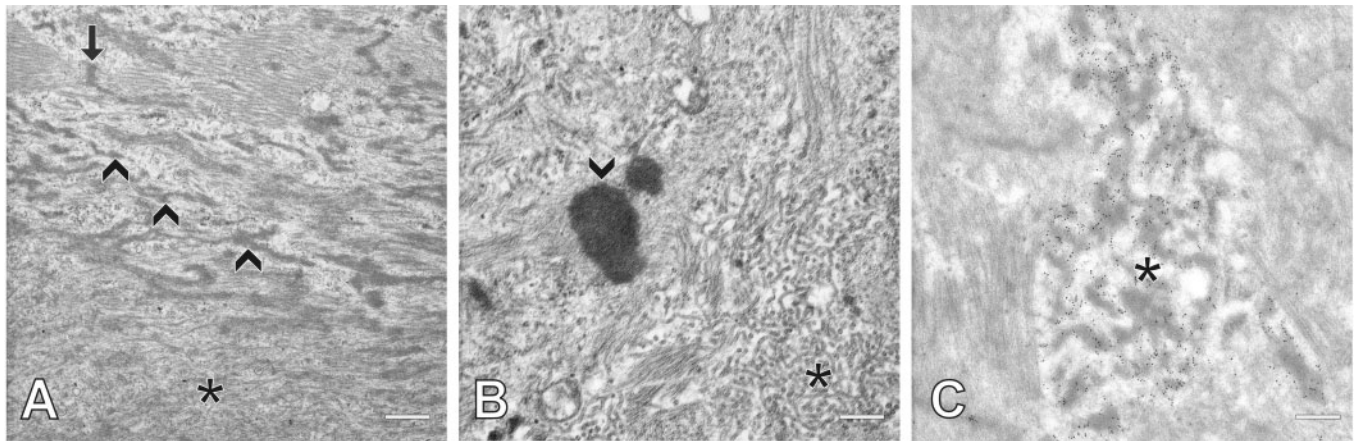
Fig. 5 Ultrastructural analysis of skeletal muscle from patient I2. (**A**) Note the presence of a myofibrillar Z-disc (arrow), Z-disc remnants (arrowheads) and adjacent filamentous deposits (asterix) (Bar = 0.6 μm). (**B**) Illustration of myofibrillar rods (arrowhead) and paired helical tubulofilamentous inclusions (Bar = 0.6 μm). (**C**) Immunogold electron microscopy using a monoclonal anti-desmin antibody (mAb, clone D33) in conjunction with a secondary antibody coupled to I0 nm gold particles revealed a dense labelling of intermyofibrillar granulofilamentous material (Bar = 0.4 μm).
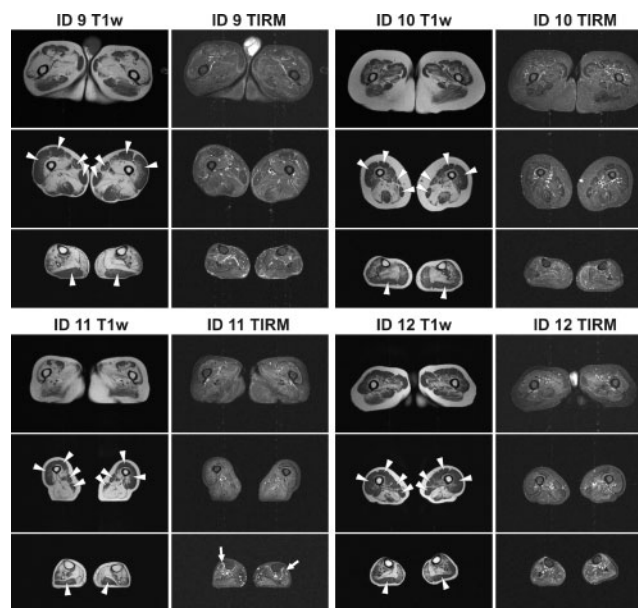
Fig. 6 Magnetic resonance imaging in four filaminopathy patients. The images show slices of the proximal and distal upper legs and of the proximal lower legs. TIw signal hyperintensities denote lipomatous changes to complete fatty degeneration of affected muscles. TIRM hyperintensities represent oedematous changes. Patient 9 shows symmetrical TIw hyperintense signal changes of the upper legs in the extensor muscle groups, principally of the vastus medialis, proximal vastus intermedius and lateralis with less pronounced involvement of the rectus femoris and distal vastus lateralis (arrowheads), and the complete adductor muscle groups; hamstring involvement is primarily restricted to the biceps femoris with relative sparing of the semitendinosus and semimembranosus muscles. The gracilis and sartorius muscles are basically spared (arrowheads) in all patients. Lower leg involvement is almost complete with sparing of the lateral heads of the gastrocnemius muscles (arrowheads) and faint remaining signal in the peroneal groups and tibialis posterior muscles. Slight hyperintense TIRM signal changes are seen in the vastus lateralis and gastrocnemius muscles. Patient I0 displays far less pronounced lipomatous changes. Upper leg involvement comprised mainly of adductor and hamstring muscles. The lower legs also show relative sparing of the lateral gastrocnemius muscles (arrowheads) with reticular fatty changes of the medial gastrocnemius muscles, peroneal and anterior tibial groups and complete fatty degeneration of the deep flexor and tibialis posterior groups. TIRM images indicate discrete signal changes in the medial gastrocnemius heads. Patient II has pronounced lipomatous changes of the upper legs with complete involvement of the adductors and hamstrings. The lower legs show a pattern similar to Patient 9. Epifascial TIRM hyperintensities of the tibialis anterior muscles (arrow) are presumably secondary to exertion. The upper legs in patient I2 show TIw hyperintensities in the adductor and hamstring groups, the latter with semitendinosus sparing; the lower legs are similarly affected as in patient I0.

from cardiac complications as observed in desminopathy with autosomal recessive inheritance (Goldfarb *et al.*, 2004), but most often the first clinical symptoms appear in adulthood (Selcen *et al.*, 2004). A predominant involvement of distal muscles is seen in the majority of patients with myotilinopathy (Olive *et al.*, 2005; Selcen and Engel, 2004) (Table 5). In ZASPopathy, patients with the p.A165V or p.R268C mutations presented with distal myopathy, whereas some patients with the p.A147T mutation showed proximal or proximal and distal muscle weakness (Griggs *et al.*, 2007; Selcen and Engel, 2005) (Table 4). In αB-crystallinopathy, patients harbouring the p.R120G missense mutation had proximal and distal weakness (Fardeau *et al.*, 1978; Fardeau *et al.*, 2000) whereas two patients with the 464delCT and p.Q151X mutation presented with prominent distal weakness (Selcen and Engel, 2003). Clinical features in desminopathy are very heterogeneous (Goldfarb *et al.*, 2004) and more than 30 different mutations have been identified so far (Olive *et al.*, 2007; Walter *et al.*, 2007). Members of a large Ashkenazi Jewish family with p.L345P desmin mutation suffered from distal weakness predominantly in the lower extremities (Horowitz and Schmalbruch, 1994; Sjöberg *et al.*, 1999). In a series of 12 patients with 7 different desmin mutations, 6 patients had distal and 6 patients proximal as well as distal weakness (Dalakas *et al.*, 2000). In a recently reported series of 15 German patients with the p.R350P desmin mutation, a limb-girdle phenotype was described in 10 of them, whereas a scapuloperoneal phenotype and a distal myopathy was seen in two patients each (Walter *et al.*, 2007) (Table 5). This indicates that the clinical phenotype in MFM not only depends on the gene affected but also on the precise mutation and additionally large interindividual variation may occur in patients with identical mutations.

In this series of filaminopathy patients presented here, proximal weakness was found in 25 out of 28 affected individuals and a clearly distal myopathy was only seen in one patient. Therefore, filaminopathy associated with p.W2710X mostly leads to a limb-girdle muscular dystrophy (LGMD)-like phenotype and can not be distinguished from a muscular dystrophy by clinical examination alone. The presence of marked winged scapula (Fig. 2) can be similar to that frequently seen in facioscapulohumeral muscular dystrophy (FSHD) but facial involvement was only present in a subset of filaminopathy patients and not as pronounced as in typical FSHD.

As we only studied patients with the same p.W2710X founder mutation the comparatively homogeneous clinical phenotype of filaminopathy has to be proven in patients with different *FLNC* mutations. So far, only two further patients with filaminopathy have been identified in a cohort of 70 MFM patients investigated at the Mayo Clinic (Selcen, 2006) and the mutation was identical to that in our patients (Selcen, personal communication). It would be interesting to investigate by haplotype analysis whether these two patients share the German founder haplotype.

MRI findings are homogenous in filaminopathy (p.W2710X) and differ from other MFM subtypes

Muscle-imaging studies presented here show a rather homogenous pattern of muscle involvement. When these MRI data were compared with other types of MFM, the alterations in proximal leg muscles were significantly more pronounced in filaminopathy compared to myotilinopathy and ZASPopathy (Fischer *et al.*, submitted for publication). In primary desminopathies, the sartorius, gracilis and semitendinosus muscles are more affected than in filaminopathy whereas involvement of vastus intermedius and medialis, adductor magnus, semimembranosus and biceps femoris muscles was more pronounced in filaminopathy. These findings indicate that muscle-imaging studies may be helpful to distinguish between the different MFM subtypes.

Respiratory muscle weakness is a frequent complication in filaminopathy

In our four filaminopathy families, all nine deceased patients evaluated and five still living patients suffered from weakness of respiratory muscles. Restrictive respiratory failure has also been documented in αB-crystallinopathy (Fardeau *et al.*, 1978; Selcen and Engel, 2003), myotilinopathy (Olive *et al.*, 2005) and desminopathy (Goldfarb *et al.*, 2004; Olive *et al.*, 2007; Walter *et al.*, 2007), whereas in ZASPopathy respiratory muscle involvement has not been described so far (Table 5). This indicates that respiratory insufficiency occurs frequently in MFM including the filaminopathy and may reduce life expectancy markedly. Therefore, a careful and regular respiratory follow-up in the disease course is essential.

Is there evidence for a cardiac and peripheral nerve involvement in filaminopathy?

Cardiac involvement is a frequent complication in MFM (Table 5). For example, patients with the p.R120G mutation in the αB-crystallin gene typically present signs of hypertrophic cardiomyopathy (Vicart *et al.*, 1998). Five out of 18 patients with myotilinopathy had cardiac findings including left and right bundle branch block and congestive heart failure leading to death in one patient (Selcen and Engel, 2004; Olive *et al.*, 2005). In ZASPopathy, cardiac involvement in terms of conduction defects and arrhythmia was reported in 3 out of 10 patients harbouring the missense mutations p.A147T and p.A165V in *ZASP* exon 6 (Selcen and Engel, 2005) (Table 5). Exon 6 is expressed in cardiac transcripts of *ZASP* and mutations in this exon have previously shown to be associated with cardiomyopathy (Vatta *et al.*, 2003). Desminopathy has the highest prevalence of cardiac involvement in all types of MFM with known mutations described so far. About half of the patients investigated in studies listed in Table 5 were affected. In a more recent study, six out of seven patients

Table 5 Phenotypic characteristics of patients with genetically proven myofibrillar myopathy

Study	N	Type of inheritance	Mutation(s)	Onset age	Prominent muscle weakness		Peripheral neuropathy	Cardiac involvement	Respiratory insufficiency	CKactivity	EMG findings	
Myotilinopathy												
Selcen et al. (2004)	6	AD (1/6)	p.S60C (3/6), p.S60F (1/6), p.S95I (1/6), p.S55F (1/6)	50–77	2/5 3/5	Proximal LL Distal LL	6/6	3/5	n.r.	Normal– 2-fold	1/5 4/5	Myopathic Myopathic-neurogenic
Olive et al. (2005)	13	AD (5/13) De novo (7/13)	p.K36E (1/13), p.S55F (6/13), p.S60C (4/13), p.S60F (1/13), p.Q74K (1/13)	42–77	2/13 1/13	Proximal LL Proximal-distal	1/13	2/13	3/13	Normal– 4-fold	10/11 1/11	Myopathic Myopathic-neurogenic
ZASPopathy												
Selcen et al. (2005)	11	AD	p.A147T (7/11), p.A165V (3/11), p.R268C (1/11)	44–73	2/11 3/11 6/11	Proximal Proximal-distal Distal (3/3 A165V)	5/11	3/11	0/11	Normal– 6-fold	8/11 2/11	Myopathic Myopathic-neurogenic Neurogenic
Griggs et al. (2007)	10	AD	p.A165V	44–49	6/6	Distal LL	n.r.	n.r.	n.r.	n.r.	1/11	n.r.
Desminopathy												
Horowitz et al. (1994) Sjöberg et al. (1999)	12	AD	p.L345P	24–46	10/10	Distal (7/10 LL>UL)	0/9	7/12	3/6	Normal– 4-fold	6/9	Myopathic
Dalakas et al. (2000)	12	AD (7/12) CH (3/12) De novo (2/12)	p.A337P (2/12), p.I451M (3/12), p.N342D (2/12), p.R406W (1/12), p.A360P/p.N393I (3/12)	20–43	6/12 6/12	Proximal-distal Distal (5/6 LL)	n.r.	7/12	2/12	Normal– 4-fold		n.r.
Walter et al. (2007)	15	AD	p.R350P	30–50	6/15 2/15 5/15 2/15	Proximal (LL>UL) Proximal-distal (LL>UL) Distal LL Scapuloperoneal	4/8	7/15	7/15	Normal– 16-fold	9/15 3/15	Myopathic Myopathic-neurogenic
Filaminopathy												
Present study	31	AD	p.W2710X	24–57	25/28 1/28 1/28 1/28	Proximal (19/21 LL>UL) proximal-distal LL distal LL neck flexors	(5/28)	9/24	14/30	Normal– 10-fold	14/15	Myopathic

CK = creatine kinase; EMG = electromyography; AD = autosomal dominant; CH = compound heterozygote; LL, lower limbs; UL, upper limbs; increased; n.r. = not reported.

with different mutations in *desmin* had cardiomyopathy (Olive *et al.*, 2007). Findings included cardiac arrhythmias and conduction blocks up to complete AV block requiring implantation of a permanent pacemaker, dilated cardiomyopathy, left ventricle diastolic dysfunction, restrictive cardiomyopathy and congestive heart failure. Some patients died of cardiac complications before age 30 (Goldfarb *et al.*, 2004; Olive *et al.*, 2007). In this series of filaminopathy patients, about one-third showed cardiac abnormalities comprising conduction blocks, tachycardia, diastolic dysfunction and left ventricular hypertrophy. Since filamin C is expressed in striated muscles including cardiac muscle (Maestrini *et al.*, 1993), these findings indicate that p.W2710X mutation may induce cardiac pathology. However, this hypothesis has to be verified in heart muscle specimens of filaminopathy patients or appropriate filamin C mouse models.

Peripheral nerve involvement assessed by clinical, neurophysiological or histological criteria has been described in a subset of MFM patients (Table 5) and postmortal studies in one desminopathy patient detected axonal spheroidal formations immunoreactive for neurofilaments in spinal cord and spinal roots (Ariza *et al.*, 1995). In our filaminopathy cohort, muscle specimen of half of the patients biopsied suggested neurogenic changes but nerve conduction velocities were normal in all individuals examined and only one person reported on distal hypaesthesia. These findings are not sufficient to verify an involvement of peripheral nervous system in filaminopathy and have to be complemented by further studies such as nerve biopsies.

Functional studies on the mutated filamin C polypeptide provided insights into the pathomechanisms of filaminopathy

The filamin molecule is composed of an amino-terminal actin-binding domain followed by 24 immunoglobulin-like modules. The most carboxy-terminally situated Ig-like domain is required and sufficient for dimerisation (Himmel *et al.*, 2003), which enables the filamins to link actin filaments into bundles or meshwork. The p.W2710X mutation in *FLNC* causes a deletion of the carboxyterminal 16 amino acids. *In vitro* biochemical studies revealed a less stable secondary structure of the Ig-like domain 24 and a reduced structural stability of the mutant protein (Vorgerd *et al.*, 2005). As a consequence, the mutant protein does not dimerize properly and has a strong tendency for uncontrolled aggregation (Vorgerd *et al.*, 2005; Löwe *et al.*, 2007). A variety of different proteins are subsequently recruited into these aggregates, thereby destabilizing tissue homoeostasis. Some of the proteins, e.g. myotilin and Xin, detected by immunofluorescence in aggregates or in ectopic distribution within muscle fibres of patients are binding partners of filamin C (van der Ven *et al.*, 2000*b*; van der Ven *et al.*, 2006). In adult skeletal

muscle the localization of Xin is normally restricted to the myotendinous junction and there is evidence that Xin is involved in developmental and adaptive remodelling of the actin cytoskeleton in cross-striated muscle cells (van der Ven *et al.*, 2006). Filamin C also interacts with γ- and δ-sarcoglycan, two members of the dystrophin-associated glycoprotein complex which are deficient in LGMD 2C and 2F, respectively (Thompson *et al.*, 2000). As a result of the ectopic accumulation of γ- and δ-sarcoglycan, the connection of the myofibrillar cytoskleleton to the extracellular matrix may be weakened and the dystrophin-associated signalling affected (Löwe *et al.*, 2007).

Symptomatic therapy is mandatory in filaminopathy

To date, no specific therapy is available for filaminopathy and the other subtypes of MFM. Symptomatic treatment should consider respiratory and cardiac complications and frequent respiratory and cardiological function tests are necessary during the disease course. Since respiratory insufficiency increases the risk of chest infections, patients may require intermittent or continuous positive pressure ventilation. Treatment of conduction blocks, arrhythmias and cardiac hypertrophy must follow cardiological guidelines. Regular physical therapy may help to maintain muscle strength but excessive training should be avoided because of potential damage of myofibres (Goldfarb *et al.*, 2004). The efficacy of creatine treatment, which has been shown to increase muscle strength in muscular dystrophies (Kley *et al.*, 2007), should be investigated by controlled trials.

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