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ABSTRACT

Introduction: Two families with autosomal dominant limb-girdle muscular dystrophy have been linked to a locus on chromosome 7q36 already 10 years ago. The locus has been denominated both LGMD1D and 1E, but in lack of further families to narrow down the linked region of interest this disease has remained elusive.

Methods: A large Finnish family was clinically and genetically investigated. Laboratory parameters were determined including CK value, neurographic and EMG studies, cardiac and respiratory function examinations, muscle biopsies and muscle imaging by CT or MRI.

Results: The patients had onset of muscle weakness in the pelvic girdle between the fourth and the sixth decade with an autosomal dominant pattern of inheritance. CK values were slightly elevated and EMG was myopathic only. Muscle biopsies showed myopathic and/or dystrophic features with very minor rimmed vacuolation and protein aggregation findings. Molecular genetic analysis indicates linkage of the disease to the locus on chromosome 7q36 completely overlapping with the previously reported locus LGMD1D/E.

Discussion: Advancement towards the causative gene defect in the 7q36-linked disease needs new additional families to narrow the region of interest. The phenotype in the previously linked families has not been reported in full detail, which may be one reason for the shortage of additional families. We now report the comprehensive clinical and morphological phenotype of chromosome 7q36 linked autosomal dominant LGMD with a restricted and updated 6.4 Mb sized haplotype.

Abbreviations: LGMD, Limb-girdle muscular dystrophy; OMIM, Online Mendelian Inheritance in Man database; ENMC, European Neuromuscular Council; EMG, Electromyography; ECG, Electrocardiogram; CK, Creatine kinase

INTRODUCTION

Limb-girdle muscular dystrophies (LGMD) are characterized by progressive weakness and wasting of shoulder and hip girdle muscles and they constitute a heterogeneous group of inherited muscle disorders without congenital symptoms.[1-3] The clinical course of the different LGMD variants range from severe forms with onset in early childhood, to milder forms with late adult onset and slow progression.[4, 5] Currently there are more than 12 genetically identified forms of autosomal recessive LGMD2 and seven autosomal dominant LGMD1 subtypes.[6]

Due to the clinical heterogeneity, the final diagnosis of LGMD relies on a combination of muscle biopsy immunohistochemical–immunoblotting analyses and molecular genetic methods. The phenotype-genotype correlation is weak and, in general, the different LGMD diseases are difficult or impossible to differentiate on clinical grounds only.[7] If no exact molecular diagnosis can be established, clinical diagnosis of LGMD relies on the familial occurrence, clinical examination, laboratory, electrophysiological, muscle imaging and muscle biopsy findings.[8]

To date seven autosomal dominant forms (LGMD 1A–G) are genetically determined, three of them having exact gene identification: 1A myotilinopathy,[9, 10] 1B laminopathy[11, 12] and 1C caveolinopathy.[13] Autosomal dominant LGMD1 forms are rare and cover approximately 10% of all LGMDs.[1] They are generally considered to be less severe than the autosomal recessive forms and many patients remain ambulatory in late adult life. However, the spectrum of phenotypes in LGMD1B is very large and includes cardiac and respiratory complications. There are very few published clinical descriptions of the forms 1A, D, E, F, G.[14-17] and regarding LGMD1D-1G, the range of phenotypic variation is still unknown.

In the OMIM database, 7q36-linked LGMD1 is determined as LGMD1D, whereas in the original ENMC report on classification [4] and in later reviews [2, 18] this locus is termed LGMD1E.

Here we describe the clinical and morphological phenotype of a dominant LGMD in a large Finnish family in which molecular genetic genome wide screening showed linkage on chromosome 7q36, overlapping perfectly with the LGMD1 locus previously mapped on chromosome 7q.[16]

MATERIALS AND METHODS

Patients

The family originates from central parts of Finland. All patients and members of the family indicated in the pedigree (Figure 1) were clinically examined including manual muscle testing using British Medical Research Council (MRC) scoring (Table 1). All examinations were performed with informed consent according to the Helsinki declaration. The oldest known affected family members were deceased and their history was obtained from the relatives.

Laboratory

Creatine kinase (CK) values were determined in six patients (III-6, III-8, III-12, IV-7, IV-9 and IV-10). Muscle biopsy was performed on six patients (III-6, III-8, III-12, IV-7, IV-9 and IV-10) and on one of them (III-12) at three occasions (Table 2). EMG studies were performed on multiple muscles on the upper and lower limbs, as well as neurographic nerve conduction and amplitude measurements in seven patients (III-2, III-6, III-8, III-12, IV-7, IV-9, IV-10) and in three of them (III-8, III-12, IV-9) it was repeated at least once. Cardiac examination by electrocardiogram (ECG) was performed in three patients (III-6, III-12, IV-9) and echocardiography in two of the oldest patients (III-6, III-12.). Spirometry was performed in one older patient (III-12).

Pathology

All muscle biopsies were of lower limb muscles: three vastus lateralis (III-6, III-12, IV-9), two medial gastrocnemius (IV-7, IV-10) and one soleus muscle (III-8). Biopsies were snap frozen in liquid-nitrogen-chilled isopentane, and 6–10 μm frozen sections were stained for Hematoxylin-eosin (H&E), Gomori trichrome, Reduced nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-TR), combined succinic dehydrogenase – cytochrome oxidase (SDH-COX), Congo red, Herovici, adenosine triphoshatase (ATPase) or myosin heavy chain isoform (slow/fast MyHC)

immunostaining according to established protocols.[19] In addition, a number of antibodies for immunohistochemistry was applied (Table 3).

Muscle imaging

Computed tomography (CT) transversal sections of lower leg, thigh, pelvic and scapular regions were performed on four patients (III-12, III-8, III-6, IV-7), whereas two patients (IV-9, IV-10) had magnetic resonance imaging (MRI) scans (T1-weighted and STIR sequences) on lower limb muscles.

Molecular genetics

Patients and family members indicated in the pedigree were genotyped for microsatellite markers for all LGMD1 loci known at the time (LGMD1A–G) (Table 4A and B). Fluorescently labeled PCR products were analyzed using Applied Biosystems 377 or 3730 equipment and Genotyper 2.0 or Gene Mapper 3.0 software (Applied Biosystems, Foster City, CA, USA). As another approach to identify and confirm the genomic region associated with the disease, a genome-wide linkage scan (GWS) using 400 microsatellite markers was performed at the Finnish Genome Center. Linkage analysis was carried out using the Genehunter program for multipoint analysis and the MLINK program for 2-point linkage analysis. The GWS data were supplemented by fine mapping with additional microsatellite markers in the potentially linked regions, as described above. Haplotypes were manually constructed, minimizing the number of crossing-overs. The GWS results were also used for separate assessment of the LGMD1 locus on chromosome 6q23, the 1F locus and the myofibrillar myopathy gene loci outside 7q.

RESULTS

Clinical findings (Table 1)

Onset of proximal lower limb weakness varied from 20 to 60. All except one 80 years old patient (III-2) had remained ambulatory. Typically, the first symptom was difficulty in walking and climbing stairs. All patients showed more pelvic girdle than shoulder girdle weakness. Three out of eight patients showed no shoulder girdle signs at ages: 69, 45 and 43 years. No contractures were observed. None of patients complained of dysphagia, respiratory difficulties, dysarthric problems or of muscle pain. Two patients had lower back pain diagnosed, associated with degenerative column changes. One patient (III-8) had a previous carpal tunnel syndrome surgically treated. One younger patient (IV-9) had been investigated because of non-specific arthritic pain at the time of LGMD diagnosis (Table 1).

The pattern of muscle weakness by MRC scoring is detailed in Table 1. Neck flexors and extensors were normal in all patients. Hip flexion and extension were weak in all patients as were knee flexion and extension. Ankle dorsiflexion was slightly weak in four patients and plantar flexion in four partly different patients. In the upper extremities, abduction weakness was present in four patients (III-2, III-10, III-12 and IV-9), biceps was weak in four of the patients (III-2, III-6, III-10 and IV-7) and triceps weakness was present in four of the patients (III-6, III-10, IV-7 and IV-10) but to a lesser extent than biceps. Wrist and finger muscles were normal in all patients.

The disease segregates in an autosomal dominant fashion in the family and occurs in both genders in all generations including male to male transmission (Figure 1).

Case reports

1. Index patient (III-12)

At the age of 42 years, the patient had noted weakness and stiffness in her legs and lower back. Climbing stairs was difficult and she could not get up from the squat without the help of her arms. Proximal muscles were weak, particularly on the lower extremities, with mild scapular atrophies and normal tendon reflexes. At the age of 49 years, muscle weakness had increased, with the maximal walking distance being 500 meters. She had mild calf hypertrophy and her gait was waddling and slow. Lower limb reflexes were absent and serum CK was 338 (upper normal limit 150). EMG showed myopathic changes including fibrillations and complex repetitive discharges. At age 68 she used a walker and could not get up from the chair without assistance (Figure 2).

2. Index patient's daughter (IV-9)

The patient noticed symptoms first at age 27 when climbing stairs became difficult. On examination at the age of 31, walking was slow and squatting was difficult, but muscle atrophy was not detected. Reflexes were normal and CK was 375. EMG showed myopathic changes including abundant fibrillation activity in all thigh and calf muscles examined. Upper limb proximal muscles were intact. At age 43 her walking was slow and slightly waddling, with a maximal walking distance of 500 meters. She complained of stiffness in her leg muscles, and her proximal leg muscles were weak on examination (Table 1). Upper limbs were normal and she had mild lateral calf hypertrophy.

Laboratory investigations

CK values were slightly or moderately elevated in the majority of patients. Five patients had CK values ranging from 187 to 820 (upper normal limit: in female 150 U/l, in male 220 U/l). One patient (IV-10) had normal CK value. EMG showed myopathic changes in all examined patients, particularly small and/or polyphasic motor unit potentials, early recruitment and spontaneous activity with fibrillations in many muscles and multiple repetitive discharges in some. The patient

with previous carpal tunnel syndrome had distal neurogenic findings in the affected limb. Motor and sensory nerve conduction velocities were normal in all patients.

All ECG recordings were normal. Echocardiographic and spirometry evaluations were also normal.

Muscle biopsy findings (Table 2): All biopsies showed myopathic changes including fiber size variation, some regenerating fibers, mild to moderate fibrosis and some excess of adipose tissue as in the gastrocnemius muscle biopsy of patient III-10 (Figure 3). In two biopsies (III-6 and III-8) the muscle morphology was more severely dystrophic. All biopsies contained low and variable numbers of fibers with rimmed vacuoles. In five biopsies (III-8, III-12: gluteus and vastus lateralis, IV-7 and IV-10) there were some fibers with dark or purple cytoplasmic inclusions on trichrome stain, which on immunohistochemistry contained minor accumulations of proteins including myotilin, alphaB-crystallin, desmin and ectopic dystrophin. In one biopsy (III-8), there was a mild neurogenic change with fiber type grouping. Increase of internal nuclei was found in four biopsies out of eight (III-6, III-8, III-12 and IV-7). Fiber necrosis and a few endomysial inflammatory infiltrates were found in half of the muscle biopsies (III-12: gluteus, vastus lateralis, IV-7, IV-9). Electron microscopy confirmed regions of myofibrillar disintegration and Z-disc streaming. In the rimmed vacuolar spaces, rare 15–18 nm filamentous inclusions were observed, besides myeloid bodies, small vesicles and debris material. Immunohistochemistry using the other antibodies listed in Table 3 did not show any abnormalities in the other biopsies.

Muscle imaging, CT and MRI scans, showed fatty degeneration and replacement in the affected muscles. The first muscles to be involved in the thigh were semimembranosus and adductor magnus muscles (IV-10, Figure 4C), and in the calf the medial gastrocnemius and soleus muscles (Figure 4D). At later stages (III-12), large replacement was observed in all thigh muscles except rectus

femoris and sartorius (Figure 4A), and in gastrocnemius, soleus and deep toe flexor muscles in the calf (Figure 4B).

Molecular genetics

The chromosomal loci of LGMD1A, B, C and E were excluded by genotyping the corresponding microsatellite markers. In a subsequent genome-wide screen, linkage to 7q was confirmed with a LOD score 3.76 for marker D7S1823 and a LOD score 3.07 for marker D7S2465. The linked region overlapped with the previously defined 7q locus[16] denoted as the LGMD1D locus in the OMIM database. The GWS results also excluded linkage to the previously defined loci of LGMD1F and 1G, as well as the known myofibrillar myopathy loci of the genes DES, MYOT, LDB3, CRYAB, SEPN1 and BAG3. Fine mapping defined a common linked haplotype on 7q36, spanning up to 6.4 Mb between the flanking marker D7S798 and 7qter. The linked haplotype completely segregated with all clinically affected individuals and was not present in unaffected members of the family (Table 4).

DISCUSSION

The phenotype of this chromosome 7q36-linked autosomal dominant muscle disease includes onset of symptoms between 20–60 years, slowly progressive lower limb weakness with late loss of ambulance and severe shoulder girdle and biceps involvement around the age of 80 years. Serum CK was mildly elevated and EMG was myopathic. Several muscle biopsies showed myopathic/dystrophic features, infrequent fibers with rimmed vacuoles, rare 15-18 nm filamentous inclusions on electron microscopy and fibers with minor cytoplasmic protein aggregates by immunohistochemistry . Since the myofibrillar myopathology was minor and not always present a classification of the disease as a myofibrillar myopathy may not be accurate.

On clinical grounds the disease can be distinguished from most of the other autosomal dominant LGMD-forms based on the later onset and the rimmed vacuolar pathology. However, myotilinopathy LGMD1A may show similar features. The reported two LGMD1A families had an earlier onset of symptoms, but considering that the more common myotilinopathy phenotype is late onset distal myopathy, also later onset proximal LGMD-type of myotilinopathy may exist. Anyway, LGMD1A is excluded in our family by molecular genetic results. Dominant LGMD1 entities are very heterogeneous. In the three forms with identified genetic defect, LGMD1A-1C, different phenotypes may result from different mutations even in the same gene, and in the forms identified by linkage studies, LGMD1D-1G, the range of phenotypical variation is still unknown.

The clinical features and muscle pathology findings in our family show some similarities with the family described by Chutkow et al.[20] regarding age of onset, slow progression and pelvifemoral weakness preceding scapulohumeral weakness. However, linkage in our family was firmly established on chromosome 7q36, which is not the case in the Chutkow family. The linked locus in our family overlaps with the 7q locus reported by Speer et al.[16] That locus was defined based on

significant linkage in two families, #1047 and #1701, whereas the other three families in the same report were excluded from 7q.[16] Neither the #1047 nor the #1701 family corresponds to the Chutkow family and we have confirmed that the Chutkow family was #383[16] and excluded from 7q-linkage (Speer, personal communication via Udd 2007). Family #1701 was described earlier in an article in 1969 by Schneiderman.[21] The clinical phenotype of the two 7q-linked families, #1047 and #1701[16] was reported briefly as being compatible with the LGMD definition, and by a few lines in a table.[22] Dysphagia, never observed in our patients, was part of the clinical findings in three of the 15 affected patients in the #1701 family.

The LGMD1 nomenclature related to the linkage data, i.e. linkage assignment of LGMD1D and 1E is controversial. OMIM and HUGO denominates LGMD1D as being 7q-linked and the ENMC report 2002[23] and the later EFNS guidelines[2] assigns LGMD1E to chromosome 7q. Another autosomal dominant LGMD disease LGMD1F,[24] OMIM #608423, is also linked on 7q. However, the clinical phenotype in that disorder is very dissimilar and its locus 7q32 is clearly outside the locus 7q36 linked to the familial disorder we report here. Filamin C is located on 7q and would have been an excellent candidate gene considering the rimmed vacuolar pathology with minor myofibrillar myopathy-type changes. This gene is, however, clearly outside the linked region of interest. There are more than 20 known genes mapped to the 6.4 Mb linked area in the 7q36 region, and some of these are known to be expressed also in muscle tissue. In addition there are numerous hypothetical genes or pseudogenes. Using bioinformatics no very obvious muscle disease candidate gene can be identified within the locus.

Linkage studies performed in our family excluded all other known dominant LGMD loci[12-17] except the locus on chromosome 7q.[16] Since the two families originally found to be linked to chromosome 7q (#1047 and #1701) still are the only ones so far reported to be 7q36-linked, the

phenotype associated with this locus is not well established. Since the linked chromosomal region in our family overlaps with the reported 7q36 locus[16], our family may thus provide the clinical phenotype corresponding to this locus. However, muscle biopsy findings of families #1047 and 1701 were not reported by Speer et al.[23] to contain rimmed vacuolar pathology. Whether this and the dysphagia symptoms in a few patients in family #1701 are indications of a different disease and genetic background in our family compared to the two previously 7q36-linked families, cannot be settled. Further gene identifications underlying the molecular defects in these families will define if there are one or two genes for LGMD1 on chromosome 7q36. Our family restricts and updates the linked region of interest to 6.4 Mb, and the reported detailed phenotype will help the identification of additional families suitable for molecular genetic studies.

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COMPETING INTERESTS

None of the authors has competing interests.

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FIGURE LEGENDS

Figure 1. Pedigree. Clinically affected individuals with filled symbols and clinically examined family members indicated with a star and genotyping for markers on a 7q36 locus. In addition to the examined individuals DNA samples were also available of the family members II-5, III-7, III-11, IV-1, IV-3, IV-5

Figure 2. Index patient III-12 at the age of 68 years showing scapular winging and mild atrophy of the rhomboidei, trapezius and deltoid muscles.

Figure 3. HE staining of gastrocnemius muscle biopsy of (patient III-10) showing variation in fiber size, atrophic fibers, increased amount of internal nuclei, one rimmed vacuolated fiber, two basophilic fibers, few fat cells and minor fibrosis.

Figure 4. A and B: Muscle CT imaging of patient III-12 showing the advanced stage with almost all thigh muscles and all posterior calf muscles replaced by fatty and connective tissue.
C and D: MRI muscle imaging of patient IV-10 showing the early stage of the disease with involvement of semimembranosus and adductor magnus muscles in thigh area and medial gastrocnemius and soleus muscles in the calf.

Table 1. Clinical findings.

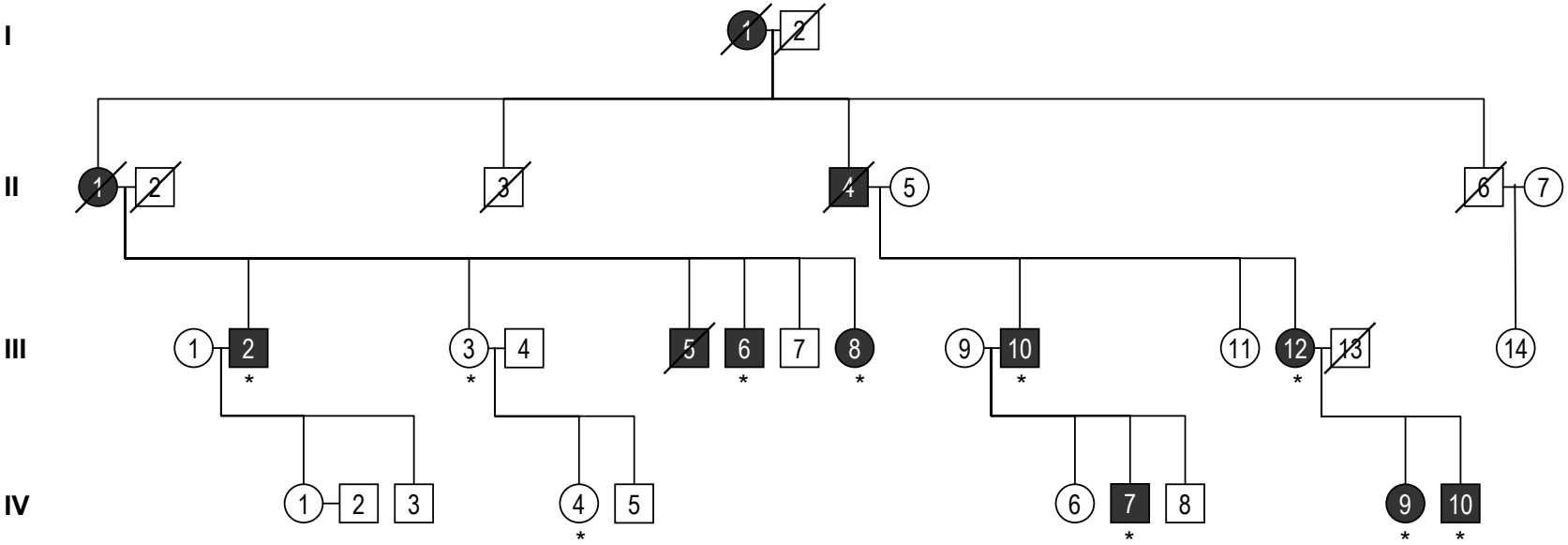
Patient		III-2	III-6	III-8	III-10	III-12	IV-7	IV-9	IV-10
Age at study		78	74	69	71	68	43	43	45
Gender		M	M	F	M	F	M	F	M
Age at onset		45	50	60	60	35	30	30	20
Symptom at onset		Difficulty in climbing stairs in all patients except IV-10							Slow running speed
Muscle weakness									
Arm	abduction	1	5	5	5–	4	5	4	5
Elbow	flexion	1	4	5	3	5	4	5	5
	extension	5	4	5	4	5	4	5	4
Hip	flexion	3	3	4	4	1	4	3	4
	extension	1	2	3	4	2	4	2	3
Knee	flexion	1	2	4	3	3	3	4	4
	extension	1	3	4	4	2	4	5–	5–
Ankle	dorsiflexion	4	4	5	5–	5	4	5	5
	plantarflexion	4	4	5	5–	5	5	5	3
EMG		myopathic	myopathic	myopathic CTS	–	myopathic	myopathic	myopathic	myopathic
CK (upper normal F 150 U/l, M 220 U/l)		–	400	187	–	338	820	375	175

CTS: Carpal tunnel syndrome

Table 2. Muscle biopsy pathology findings.

Patient	Muscle	Fiber type grouping	Fiber size variation	Fibrosis	Increase of internal nuclei	Necrosis	Lymphocyte infiltrates	Rimmed vacuoles	Eosinophilic incl. bodies	Desmin deposites	Myotilin deposites
III-6	VL	–	++	++	+	–	–	+	+	(+)	NA
III-8	S	+	++	++	++	–	–	+	+	(+)	++
III-12	QC/Gluteus/VL	–/–/–	(+)/(+)/(+)	(+)/(+)/(+)	–/–/(+)	–/–/+	–/(+)/+	(+)/+/+	–/++/+	NA/(+)/(+)	NA/NA/+
IV-7	G	–	+	+	+	+	(+)	+	++	+	+
IV-9	VL	–	+	+	–	+	(+)	+	+	(+)	NA
IV-10	QC	–	–	–	–	–	–	+	–	(+)	NA

VL, vastus lateralis; S, soleus; QC, quadriceps; G, gastrocnemius
–, finding absent; (+), minor presence; +, finding present; ++, marked finding; NA, not assessed



Marker	Pos (kb)	III-2D	IV-1	IV-3	III-3	IV-4	IV-5	III-5D	III-6D	III-7	II-5	III-10D	IV-7D	III-11	III-12D	IV-9D	IV-10D
D7S1815	152389	4 3	3 2	4 2	2 3	3 2	2 2	4 2	4 2	2 2	1 1	4 1	4 3	1 2	4 1	1 3	4 1
D7S798	152811	1 5	5 6	1 6	3 5	NA	NA	1 3	1 3	3 3	5 4	1 4	1 5	5 3	1 4	4 3	1 2
D7S2462	153561	1 1	1 5	1 5	2 1	3 2	2 2	1 2	1 2	2 2	2 4	1 4	1 3	2 2	1 4	1 1	1 3
D7S2546	154207	1 2	2 1	2 1	2 2	2 2	2 2	1 5	1 5	2 5	2 2	1 2	1 2	2 2	1 2	1 3	1 4
D7S2447	154461	1 2	2 1	2 1	1 2	1 2	1 1	1 2	1 2	1 2	1 2	1 2	1 2	1 1	1 2	1 3	1 3
D7S1823	154567	1 3	3 5	3 5	4 3	3 3	5 4	1 2	1 2	4 2	2 5	1 5	1 6	2 4	1 5	1 1	1 3
D7S550	155440	4 6	6 4	6 4	5 6	5 6	5 5	4 4	4 4	5 5	1 6	4 4	4 5	5 5	4 4	4 3	4 4
D7S2465	156114	2 3	3 1	3 1	1 3	NA	NA	2 1	2 1	1 1	4 1	2 1	2 1	4 5	2 1	2 6	2 1
D7S2423	157620	1 1	1 3	1 3	3 1	3 3	1 3	1 1	1 1	3 1	1 1	1 1	1 3	1 1	1 1	1 1	1 3
D7S427	158903	2 3	3 1	3 1	2 3	2 3	3 3	2 2	2 2	2 3	3 2	2 2	2 1	3 3	2 2	2 3	2 3
Chr7 tel	159100																

*, clinically examined family members
D, affected patients
NA, not assessed

Figure 2

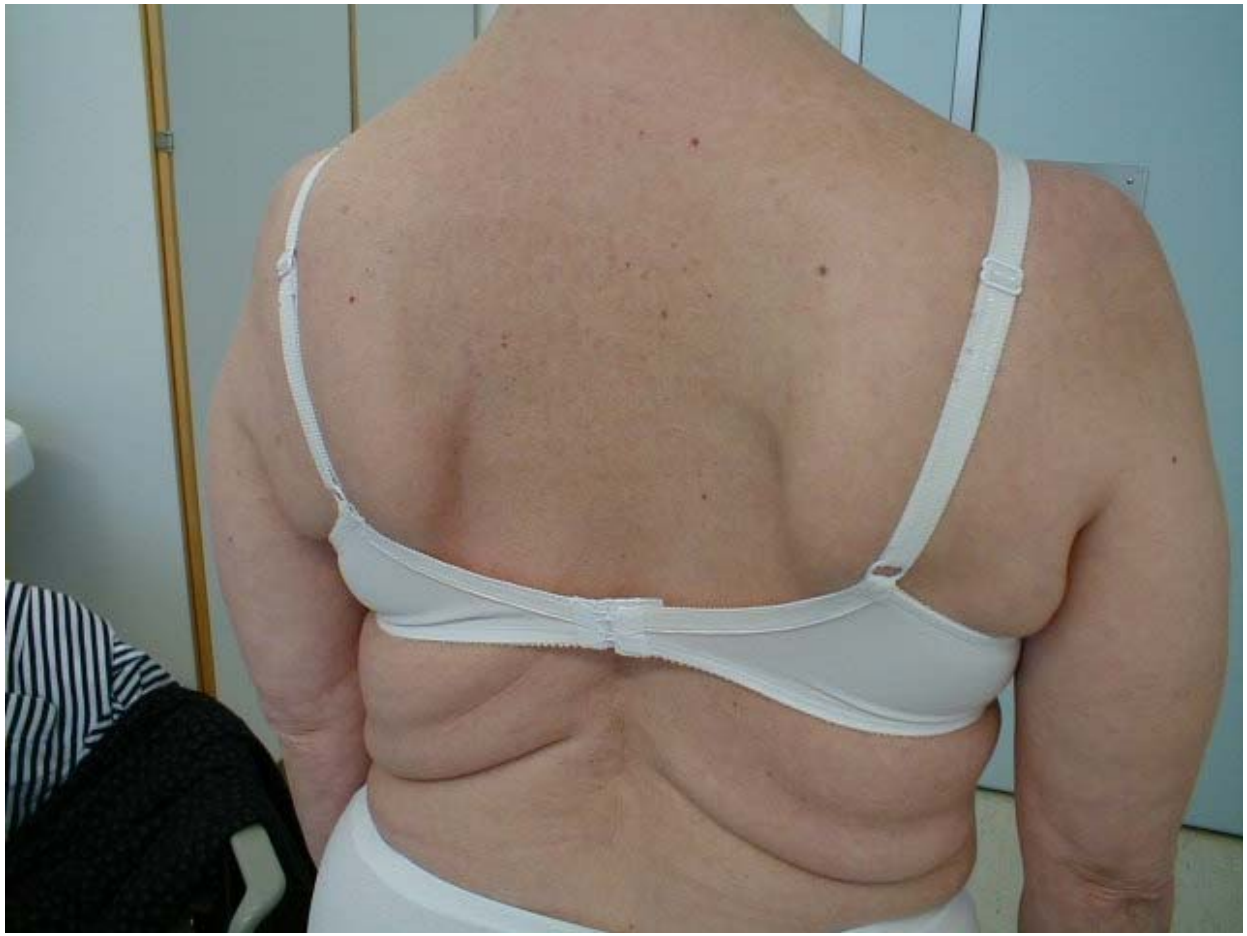


Figure 3

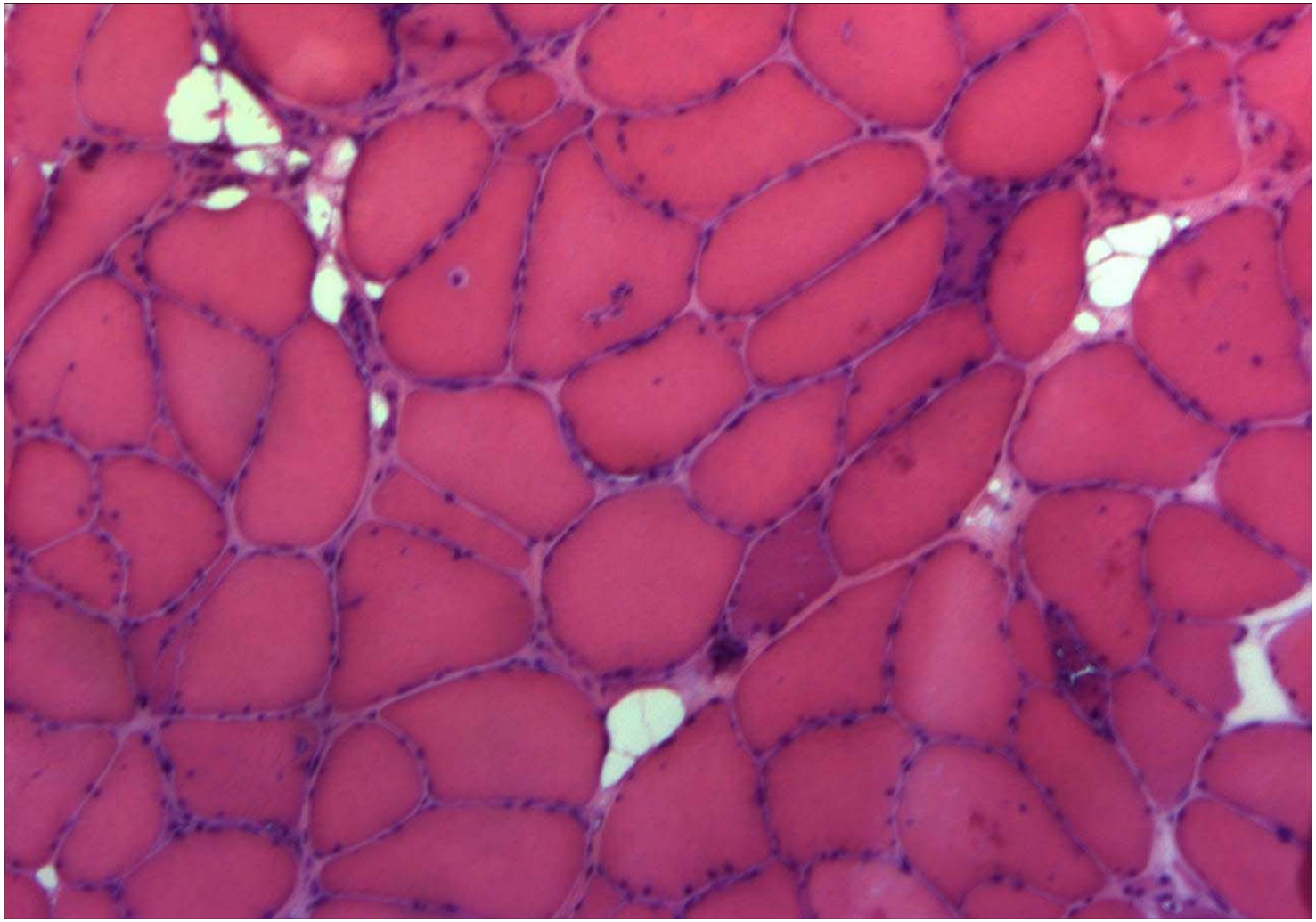


Figure 4 A–D