CYSTINOSIS DISTAL MYOPATHY, NOVEL CLINICAL, PATHOLOGICAL AND GENETIC FEATURES

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ABSTRACT

Nephropathic cystinosis is an autosomal recessive lysosomal disease in which cystine cannot exit the lysosome to complete its degradation in the cytoplasm, thus accumulating in tissues. Some patients develop a distal myopathy involving mainly hand muscles. Myopathology descriptions from only 5 patients are available in the literature. We present a comprehensive clinical, pathological and genetic description of 3 patients from 2 families with nephropathic cystinosis. Intrafamiliar variability was detected in one family in which one sibling developed a severe distal myopathy while the other sibling did not show any signs of skeletal muscle involvement. One of the patients was on treatment with Cysteamine for over 12 years but still developed the usual complications of nephropathic cystinosis in his twenties. Novel pathological findings consisting in sarcoplasmic deposits reactive for slow myosin were identified. Three previously known and one novel mutation are reported. Conclusion: Nephropathic cystinosis should be included in the differential diagnosis of distal myopathies in those with early renal failure. Novel clinical and pathological features are reported here contributing to the characterization of the muscle involvement in nephropathic cystinosis.

Key Words: Cystinosis, distal myopathy, slow myosin, *CTNS*

Abbreviations:

CK: Creatine kinase, EMG: Electromyography.

INTRODUCTION

Cystinosis is a lysosomal storage disease with an estimated prevalence of 0.5 to 1 per 100,000 live births[1]. It is a recessive disease with complete penetrance, caused by mutations in *CTNS* [2]. The product of *CTNS* is cystinosin, a ubiquitously expressed lysosomal transporter. Cystinosin is necessary for the transport of cystine (which is the oxidised dimer form of the amino-acid cysteine) from the lysosomal lumen to the cytoplasm, where it is reduced to cysteine. The defective cystinosin protein leads to accumulation and crystallization of cystine in the lysosome, but how this causes the tissue damage, and leads to the development of the typical clinical symptoms is not well understood[1]. Lysosomal overload, vesicular transport defects and endoplasmic reticulum stress have been suggested as possible mechanisms [3].

The infantile nephropathic form of cystinosis accounts for 95% of the cases presenting in the first year of life. Juvenile nephropathic cystinosis has a later onset during late childhood or adolescence[1]. Nephropathic cystinosis presents with symptoms related to Fanconi syndrome, with polyuria, growth retardation and photophobia. End-stage renal failure leads to dialysis or renal transplantation at about age 10. In 1960, renal transplant became available, prolonging the survival of these patients, however, the damage to non-renal organs continues, [4] leading to late complications[5]. The persistent intracellular accumulation of cystine in other tissues in patients not treated with Cysteamine leads to complications such as retinal blindness and ocular posterior synechiae, diabetes mellitus, infertility in males due to primary hypogonadism[6], encephalopathy with confusion, memory loss and cerebral atrophy[7] , or a distal myopathy[8, 9].

Involvement of skeletal muscle was first described in 1988 [8]. Limb muscle involvement has been reported to happen in 24 to 50% of patients [4, 9]. Hand muscles are the earliest and more severely involved with weakness and wasting resembling neurogenic atrophy. As the disease progresses, distal muscles of the lower extremities and proximal muscles can become affected. Swallowing difficulties have been reported in 60% of patients[4]. Restrictive respiratory insufficiency is present in 69% of patients, mainly related to muscle weakness. Morphological chest abnormalities from soft bones caused by phosphataemic rickets (a well-known effect of cystinosis) and delayed growth, may also contribute or be the only cause of respiratory insufficiency [10]. No pulmonary parenchymal dysfunction has been detected [10].

This distribution of muscle involvement brings cystinosis myopathy into the differential diagnosis of distal myopathies. This presentation, with early involvement of hand muscles, in the setting of a patient with early renal failure, should raise the suspicion of a cystinosis myopathy. However, due to it being uncommon, most neurologists are not familiar with the condition.

Little is known about the aetiology of cystinosis distal myopathy. Data on myopathology of the cystinosis distal myopathy is old and scarce, with only a handful of descriptions of biopsies available [8, 9, 11], in which autophagic vacuoles have been described. The concentration of cystine in the muscle fibres of patients with cystinosis myopathy is elevated, and it correlates with the degree of severity of the muscle involvement [9]. Unlike other tissues, no crystals are formed within the muscle fibres, although they can be seen ultrastructurally in the perimysium. However, the accumulation of cystine is still considered the most likely cause of the myopathy[9] .

Here we report 2 unrelated families with nephropathic cystinosis and distal muscle involvement in which additional clinical and pathological features are presented.

PATIENTS AND METHODS

Family 1

Patient 1 is a 55 years old male born to non-consanguineous parents of German origin. He has a younger sister affected with the same condition (Patient 2), and 3 unaffected siblings.

The patient developed renal failure at the age of 13 and was kept on dialysis until the age of 17, when he underwent renal transplant. He has photophobia from childhood. He later developed hoarse voice. He has restrictive respiratory insufficiency and needs non-invasive ventilation at night. At age 30 he developed weakness and atrophy of intrinsic muscles of the hands, which later progressed, involving proximal muscles and distal muscles of the lower limbs. At age 44 he was diagnosed with diabetes mellitus. He also developed high blood pressure. At age 54 he started experiencing dysphagia, having choking episodes.

On examination at age 55 he was found to be 165cm in height and having light hair and skin. He had hypophonic voice and stridor. Mild facial weakness was present. Extraocular movements were preserved. He had marked atrophy and weakness (2-3/5) of intrinsic muscles of the hands and forearms. Strength in biceps was graded 4+/5 bilaterally. He could not walk on his heels, and calf muscles were atrophic. Tibialis anterior and extensor digitorum brevis were normal in muscle bulk but weak. The strength in the remaining muscle groups was normal. Deep tendon reflexes were normal except for ankle reflexes that were absent bilaterally. Sensory examination was normal.

CK was 113 international units (IU) (normal range 30 to 190 IU). Slit lamp examination demonstrated the presence of corneal crystals.

Patient one had an EMG performed at the age of 41 years. Sensory nerve conduction studies were performed on ulnar, median and radial nerves showing normal responses. Motor conduction studies in ulnar and median nerves showed reduced amplitude of compound motor action potentials (CMAP) with normal nerve conduction velocity and distal latency in distal stimulation. Proximal stimulation was not done. An EMG was performed to study first dorsal interoseii, abductor pollicis brevis, biceps brachii, triceps brachii and deltoid. All examined muscles showed fibrillations, positive waves and reduced recruitment pattern, with myopathic motor units.

At the age of 55, after he was referred to neurology, a muscular CT scan was performed within the regular work-up of a myopathy. Extensive fat replacement of muscles in the posterior compartment of the legs (Figure 1B) and, to a lesser extent, posterior compartment of thighs (Figure 1A) was identified, revealing subclinical involvement of proximal muscles of the lower extremities. An MRI could not be performed due to the respiratory difficulties of the patients when lying flat.

Determination of 1/2 cystine content in leukocytes was 0.4nmol/mg protein, which was within normal range for the reference laboratory (Normal: < 0.5 nmol 1/2 cystine/mg protein). The control value was 0.03 nmol 1/2 cystine/mg of protein. However, viability of the patient sample received in the laboratory was 30%.

Patient 2 is the younger sister of patient 1. She also developed renal failure in her childhood and had a renal transplant at age 16. She is 162cm. She has photophobia, hoarse voice,

stridor and respiratory insufficiency requiring the use of non-invasive ventilation at night. However, she does not have any limb muscle weakness or atrophy and the neuromuscular examination at age 54, is normal. Her CK was normal.

Cystine/glutathione ratio in cultured fibroblasts from a skin biopsy was 102%, which was considered elevated compared to the reference range for normal controls (0-15%) and in the range seen in cystinosis patients (60-300%).

The diagnosis of cystinosis was confirmed in patients one and two at age 55 and 54 years respectively and they both declined starting treatment with cysteamine.

Family 2

Patient 3 is a 22 years old African American male diagnosed with Fanconi syndrome, rickets and nephropathic cystinosis at 18 months of age. He is an adopted child, so there are no records of family history. At age 4, hypothyroidism was detected. He started treatment with Cysteamine at age 10. He later developed renal failure and eventually underwent renal transplant at age 11.

From age 18 he noticed slowly progressive weakness affecting mainly his hands. He has difficulties straightening his hands and opening jars. He also has noticed difficulty swallowing with occasional choking episodes. He enjoys working out and has no proximal or facial weakness.

Neurological examination showed normal cranial nerves. He had atrophy of the intrinsic muscles of the hands with prominent weakness of his distal muscles affecting mainly wrist

extension and grip bilaterally. He has mild weakness of proximal muscles with normal facial, neck and lower limb strength.

Eye assessment with slit lamp showed full thickness crystals throughout the corneal stroma in both eyes. Modified barium swallow showed mild-to-moderate pharyngeal dysfunction.

Sensory nerve conduction studies performed on median, ulnar and sural nerves were normal. Motor nerve conduction studies of median, ulnar, peroneal and tibial nerves showed reduced CMAPs with normal nerve conduction velocity and distal latencies in proximal and distal stimulations, without signs of nerve conduction block. Electromyography was performed on deltoid, triceps brachii, biceps brachii, extensor carpi radialis longus, first dorsal interosseous and vastus medialis. It showed irritable myopathic changes with fibrillations and positive waves in all examined muscles except for vastus medialis, with short polyphasic motor unit potentials and a myopathic pattern of recruitment. Serum CK was 819 IU/l (normal range 30 to 200 IU).

All patients signed the appropriate consent forms. IRB of both centres involved in the study had approved it.

RESULTS

Muscle biopsies:

Patient 1 had two muscle biopsies. The first one was taken from a deltoid muscle at the age of 41 years and showed increased variation in fibre size without any other abnormalities. Vacuoles were not seen. A second biopsy was taken from biceps at the age of 55 years. Biceps was chosen as it was a moderately involved muscle. It showed mild variation in fibre size and shape with a few atrophic fibres. There was no myonecrosis and no regenerating

fibres were seen (Figure 2). Occasional fibres showed subsarcolemmal pale eosinophilic material resembling hyaline bodies (Figure 2) . There were several smaller fibres with peripheral triangular accumulation of mitochondria resembling lobulated fibres. There were no vacuolated fibres. The hyaline bodies stained light green on the modified Gomori trichrome stain (not shown). There was normal staining for ATPase in the muscle fibres (not shown).

Routine immunostains for dystrophin, utrophin, sarcoglicans, B-dystroglycan, merosin, caveolin-3, were performed on the deltoid biopsy. They were all normal. On the second biopsy (biceps), the immuno-histochemistry panel was completed with, desmin (Dako, DE-R-11) and alpha-B crystallin stains (Novocastra, G2JF). Slow myosin (Novocastra, NCL-MHCs), and fast myosin (Novocastra, NCL-MHCf) immunohistochemistry were also performed for fibre type distribution. The hyaline bodies were positive for slow myosin, negative for fast myosin and showed a peripheral rim of positive staining for desmin and alpha-B crystallin.

Electron microscopy performed on the sample obtained from the biceps biopsy showed subsarcolemmal accumulation of filamentous material corresponding to the hyaline bodies seen on light microscopy. The filaments were of similar diameter to myosin filaments. There were rectangular crystal-shaped electron lucent spaces in keeping with cystine crystals in a fibroblast (Figure 2).

Patient 3 had a right deltoid muscle biopsy which showed prominent variation of muscle fiber size with mildly increased endomysial connective tissue (figure 2F). There were scattered muscle fibers with rimmed vacuoles (Figure 2F), which content stained positive on congo red staining and it is birefringent under polarized light, representing amyloid

deposits. Acid phosphatase stain (Figure 2G) demonstrated they were autophagic vacuoles. There was no mononuclear cell inflammation and vessels were normal. PAS and Sudan staining showed no storage materials. Plastic embedded sections showed intracytoplasmic vacuoles, either large single or multiple small vacuoles. It also showed scattered macrophages with clear inclusions inside them as well as some muscle fibres with central dark inclusions (not shown). Immunostain for slow myosin was normal and did not show the presence of hyaline bodies (not shown).

Electron microscopy showed intracellular crystals consistent with cystine within the cytoplasm of macrophages. The crystals varied in size and shape and were mostly square, rectangular and shaft-like and may be seen to be confined within membrane bound spaces (Figure 2H).

Genetic studies

Whole exome sequencing in patient 1 identified an apparently homozygous 21bp deletion in *CTNS* exon 5 (ENST00000381870.3: c.198_218del (p.66_73del)). Microarray analysis however showed the presence of the common 57kb deletion, which includes the first 10 exons of CTNS, in a heterozygous state, indicating compound heterozygosity for the large and small deletions. The same results were confirmed in his affected sister.

As slow myosin aggregates in muscle fibres have been previously described in patients with mutations in *MYH7*, whole exome sequencing results from patient 1 were screened for variations in *MYH7*. The coverage of the entire gene was over 30 reads, except for a part of exon 28, which was read only 6 times and was therefore Sanger sequenced. No pathogenic variants were found in coding exons. Genes encoding other conventional and

unconventional myosins were also screened in the exome data without finding any variants other than known polymorphisms.

In patient 3 whole exome sequencing identified one known and one novel splice variant: ENST00000381870.3 c.225+5_225+6delGTinsCC [12]and c.226-3C>G. cDNA analysis failed to amplify any *CTNS* transcripts, suggesting nonsense mediated decay of both transcripts.
In both patients, mutations in other distal myopathy associated genes were also excluded through exome sequencing.

DISCUSSION

Cystinosis myopathy is another lysosomal disease to be included in the list of distal myopathies. Unlike other lysosomal diseases involving the skeletal muscle, such as Pompe's diseases or Danon's disease, little is known about cystinosis myopathy. Its pathological features have been described on only a few patients. Although the lysosomal involvement of the muscle tissue is clear as seen by the presence of autophagic, acid phosphatase positive vacuoles, it is unclear, why cystine crystals are not formed in the actual muscle fibres as they do in other tissues. Autophagic vacuoles are a pathological hallmark of cystinosis myopathy, however, as seen here as well as in previous reports, they are not invariably seen in every biopsy from cystinotic myopathy patients, and its absence should not rule out the diagnosis.

We have reported three different cystinosis patients at three different points of the phenotypic spectrum. In intermediate or late onset nephropathic cystinosis, symptoms are

usually restricted to kidneys and eyes. However, patient 1, with a juvenile onset, developed diabetes, hypothyroidism and a distal myopathy. However, his sister, with the same mutations did not have a distal myopathy, showing intrafamilial variability in the clinical presentation for a fully penetrant genetic disease, which, to our knowledge, has not been reported before in this condition.

In patient one, a determination of cysteine in white blood cells resulted unexpectedly in a normal result. Although this is a highly sensitive test, several circumstances can lead to false negative results[13]. In this case, the low viability of the sample received was probably the cause of the false negative result. In the right clinical settings, the diagnosis of cystinosis should still be pursued.

We have described here compound heterozygous splicing mutations including the novel CTNS c.226-3C>G and the previously described CTNS c.225+5_225+6delGTinsCC [12] showing how the combination of these mutations produce no detectable transcript of the gene.

Patients one and two were only diagnosed with cystinosis at age 55 and 54 and they never received Cysteamine treatment. However patient three developed renal failure, respiratory failure and muscle involvement although he had been on Cysteamine since age 10.

The presence of slow myosin aggregates in muscles from cystinosis patients has never been reported before, perhaps because immunostains for slow myosin are not routinely performed in every laboratory. This unexpected finding prompted us to look for mutation in *MYH7* with the idea that double trouble in this case may explain the phenotypical differences with his affected sister, especially considering that mutations in *MYH7* also

cause a distal myopathy, although with different distribution of symptoms[14]. But no *MYH7* mutations were identified. However, in patient 3 the same hyaline bodies were not identified. More pathological description in subsequent patients are necessary to stablish what features are specific to the disease.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare

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AUTHORS CONTRIBUTIONS

M Cabrera-Serrano: Study concept, acquisition of data, analysis and interpretation, draft of the manuscript.

A. Alisheri: Acquisition of data.

R. Juckenstorf: Adquisition of data.

A Pestronk: Adquisition of data

N. Laing: Study concept, critical revision of the manuscript for important intellectual content.

C. Weihl: Acquisition of data, analysis and interpretation.

P Lamont: Acquisition of data, critical revision of the manuscript for important intellectual content.

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FIGURE LEGENDS

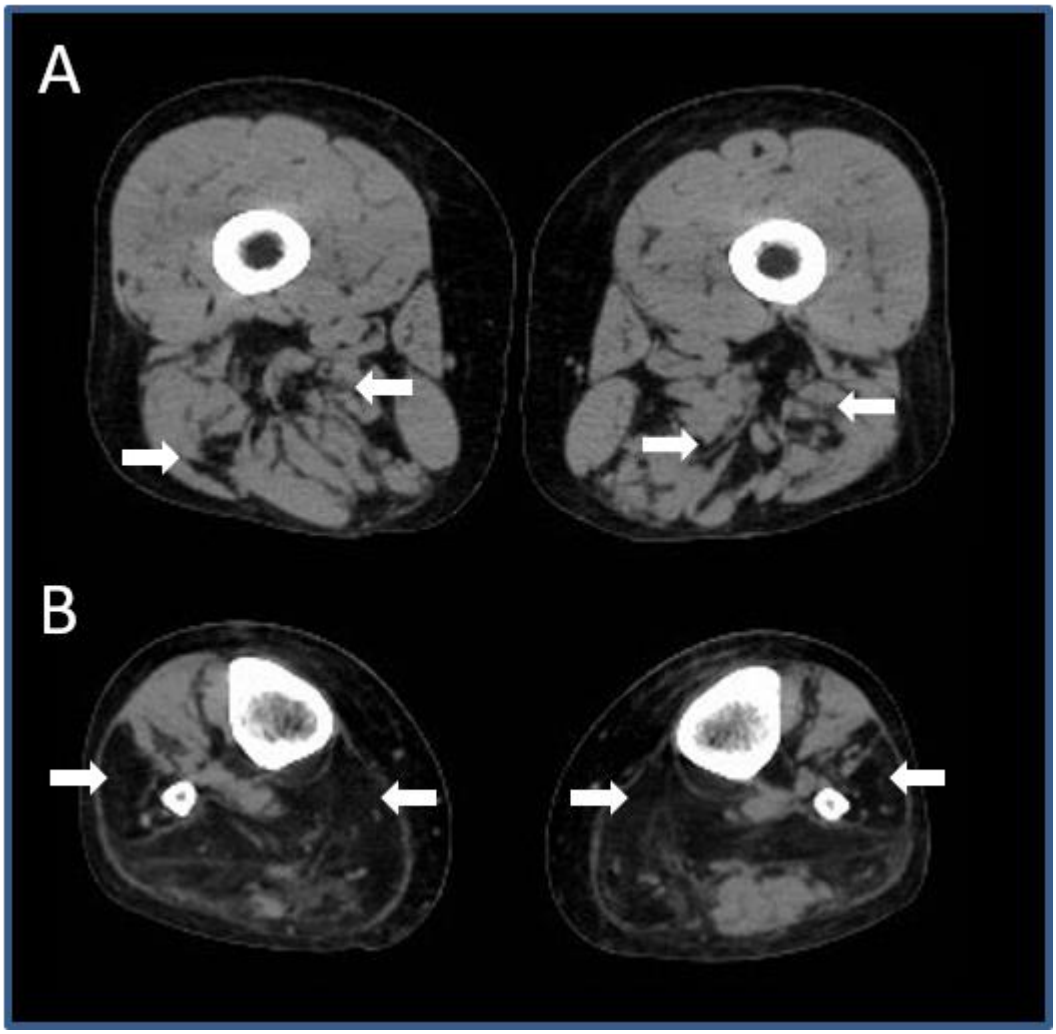

Figure 1: Muscle CT scan of patient 1 at age 55.

Fat infiltration involving posterior compartment of thighs (mainly biceps femoris, semitendinous and semimembranous), severe fat infiltration and atrophy of all muscles in posterior compartments of legs including soleus, gastrocnemious and also peroneal muscles, bilaterally. Patchy involvement of tibialis anterior. Arrows indicate the main areas of fat infiltration.

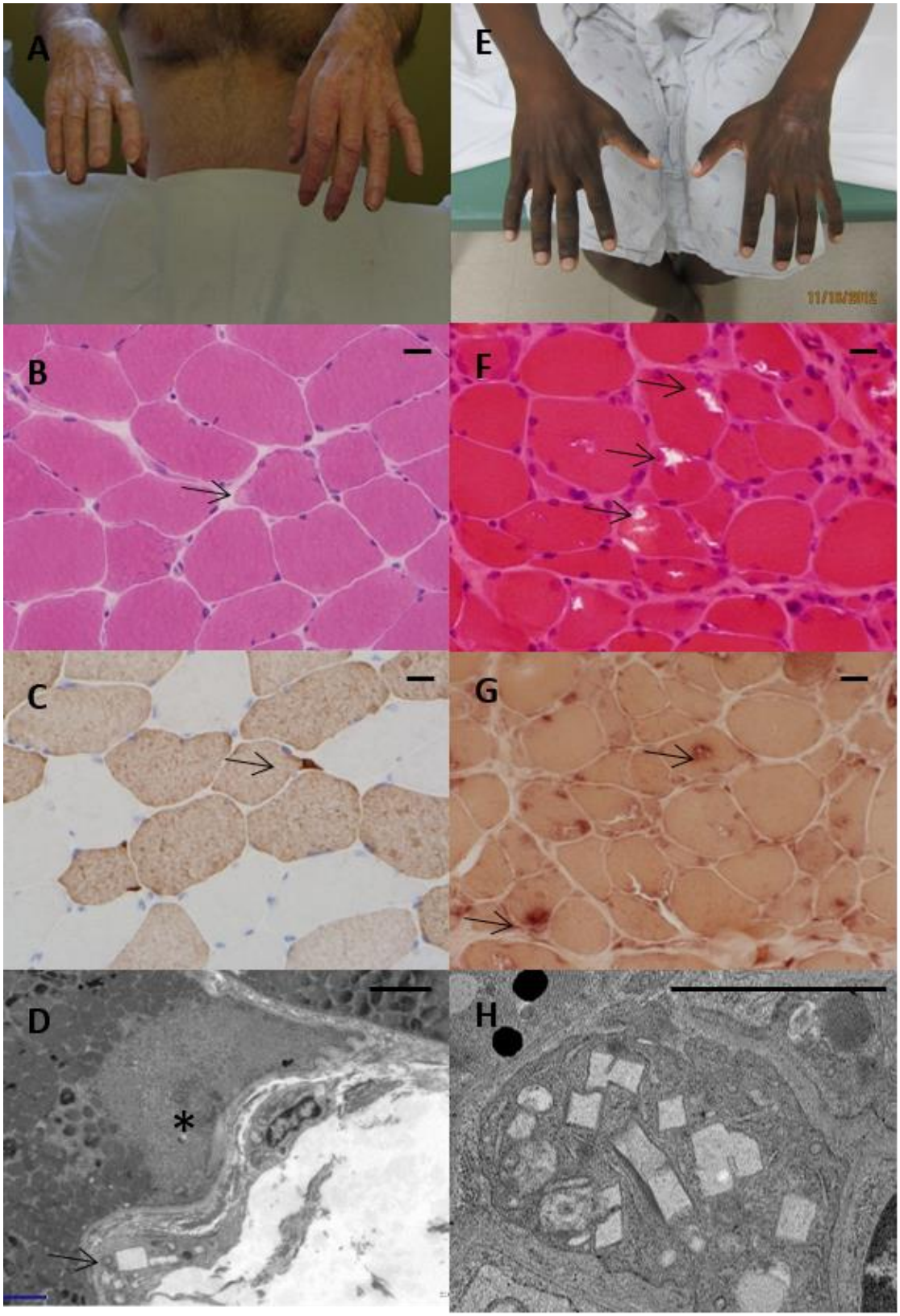

Figure 2 : Muscle biopsies of patients 1 and 3.

A to D: patient 1, B to D, biceps biopsy. E to H: patient 3, F to H: deltoid biopsy. A and E: Intrinsic hand muscle atrophy. B and F: Hematoxilin and Eosin stains showing a hyaline body in patient from

family 1 (B, arrow) and vacuoles in patient from family 2 (F, arrows). No myonecrosis or regenerating fibers are seen. C: Immunostain for slow myosin showing reactive aggregates (arrow) similar in localization and shape to the hyaline bodies observed in hematoxilin and eosin stain in the same patient. D and H: Electron microscopy showing filamentous material in the myofiber (D, asterisk), a cystine crystal in a nearby fibroblast (D, arrow) and cystine crystals in a macrophage (H). G: Acid phosphatase stain, showing positive reaction corresponding to the vacuoles (arrows). H:
Scale bars at the top left represent 20μm in panels B, C, F and G, and 2.5 μm in panels D and H.