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Case report

Calpainopathy with macrophage-rich, regional inflammatory infiltrates

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Abstract

Mutations in calpain-3 cause limb girdle muscular dystrophy 2A. Biopsy pathology is typically dystrophic, sometimes characterized by frequent lobulated fibres. More recently calpain mutations have been shown in association with eosinophilic myositis, suggesting that calpain mutations may render muscle susceptible to inflammatory change. We present the case of a 33-year old female with mild proximal muscle weakness and high CK levels (6698 IU/L at presentation). Muscle biopsy showed clusters of fibre necrosis associated with very dense macrophage infiltrates and small numbers of lymphocytes, raising the possibility of an inflammatory myopathy. No eosinophils were observed. Immunosuppressive treatment was started without clinical improvement. MRI demonstrated bilateral fatty replacement in posterior thigh and calf muscles. Western blot results prompted Sanger sequencing of the calpain-3 gene revealing compound heterozygous mutations c.643_663del and c.1746-20C>G. Our case widens the myopathological spectrum of calpainopathies to include focal macrophage rich inflammatory change.

Keywords: Limb girdle muscular dystrophy; inflammatory myopathy; inflammatory dystrophy; muscle MRI

1. Introduction

Mutations in the calpain-3 gene (*CAPN3*) cause Limb girdle muscular dystrophy 2A. Muscle biopsy often shows dystrophic changes, sometimes with prominent lobulated fibres. More recently calpain-3 mutations have been found in paediatric and adult patients with eosinophilic myositis, indicating that calpainopathy can also result in inflammatory changes in muscle. We diagnosed calpainopathy in a patient with regional macrophage rich myositis, suggesting an extension of the inflammatory phenotype.

2. Case report

A 33 year old female patient was referred to us with a one and a half year history of mildly progressive lower limb proximal weakness. She did not have previous neuromuscular complaints and had normal motor milestones in childhood, although mild scoliosis had been present since an early age. On examination she had mildly abnormal gait, and was unable to stand from squatting. Muscle strength in her lower limbs was reduced for hip flexion and adduction at 4/5 MRC bilaterally. Upper limb strength was normal but there was scapular winging. There was no facial weakness or dysphagia. Sensory examination was normal. Her CK level was 6698 IU/L. Family history was negative for neuromuscular conditions with the possible exception of her sister who had moderate scoliosis since age 12 and joint hypermobility.

Examination of a muscle biopsy from left quadriceps revealed increased variability in fibre size with scattered round smaller fibres, occasional internal nuclei, and mildly increased endomysial connective tissue in all fascicles (Fig. 1). In addition, several distinct regions within different fascicles demonstrated sheets of necrotic or small basophilic fibres with large internal nuclei, accompanied by a dense cellular infiltrate composed predominantly of macrophages with some lymphocytes. Eosinophils were not observed. Necrotic/regenerating regions appeared to respect fascicular boundaries with some fascicles quite unaffected. Immunohistochemistry confirmed a dense

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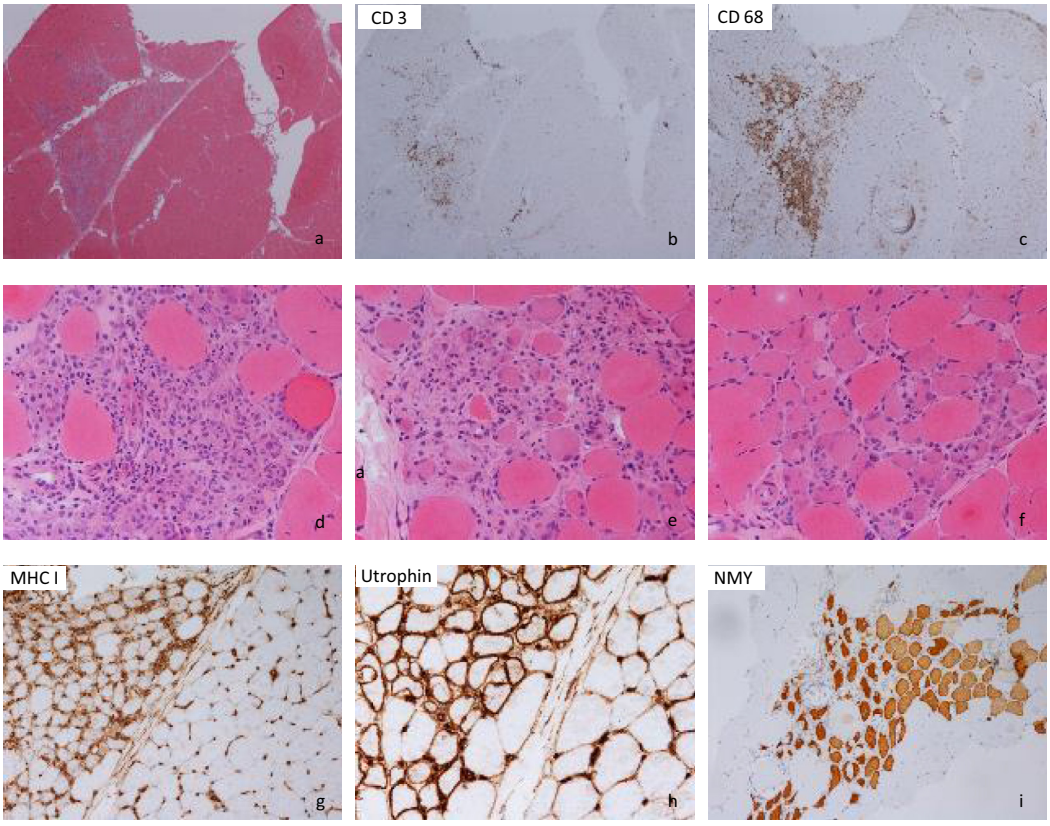

Fig. 1. Muscle biopsy from left quadriceps demonstrated regional clusters of necrotic and regenerating fibres surrounded by an inflammatory infiltrate (a). In some areas the process appeared to respect fascicular boundaries. The inflammatory infiltrate revealed moderate numbers of T-cells combined with numerous macrophages upon CD3 (b) and CD68 (c) immunohistochemical studies (same area as a). Clusters differed in regard to the presence of small basophilic fibres (d–f), suggesting various stages of regeneration. MHC Class I upregulation was restricted to regenerating fibres and was not present on neighboring normal appearing fibres (g). Regenerating fibres also expressed membranous utrophin (h) and neonatal myosin (i).

macrophage infiltrate (CD68) in these areas with moderate numbers of T-cells (CD3) of which a small proportion were CD8-positive. The disproportionate presence of macrophages accompanied by a moderate lymphocyte infiltrate supports a description as macrophage rich inflammatory process. Small basophilic fibres were generally positive for neonatal myosin with increased sarcolemmal expression of utrophin and MHC Class I. Normal appearing fibres in the remainder of the biopsy did not show this profile, nor was there a significant inflammatory infiltrate. Complement membrane attack complex (MAC) deposits were prominent in necrotic fibres and occasional scattered normal appearing fibres showed granular sarcolemmal deposits. There was no capillary MAC labelling. Several vessels had plump endothelial cells, but there was no evidence of vasculitis. An immunohistochemical dystrophy panel was normal, including dysferlin staining. Electron microscopy was not performed.

The areas of necrosis and marked inflammatory infiltration raised concern for an immune mediated process, possibly in the spectrum of immune myopathy with abundant macrophages, dermatomyositis with regional infarction, or vasculitis with microinfarction. After initial observation, immunosuppressive therapy with azathioprine was started. Although subjective improvement was reported, there was minimal clinical improvement and CK levels were only mildly reduced to around 2500 IU/L.

Muscle MRI while on immunosuppressive therapy revealed fatty replacement of adductor magnus and posterior thigh muscles, soleus, and medial gastrocnemius bilaterally (Fig. 2). There was no increased water content on STIR sequence. MRI examination of the patient's sister showed similar results. Suspicion of a dystrophic process increased and azathioprine treatment was discontinued without subsequent clinical change.

Following these investigations, Western blot analysis was initiated. There were no bands detected on probing with calpain-3 exon 1 antibody (Fig. 3). Exon 8 antibody revealed a barely detectable band for the full sized protein but no degradation bands. Sanger sequencing demonstrated a compound heterozygous state for a pathogenic in-frame deletion (c.643_663del [p.Ser215_Gly221del]) and a splice site mutation (c.1746-20C>G [1]) in *CAPN3*.

3. Discussion

The present case illustrates regional macrophage rich inflammation with fascicular distribution in a patient with late onset, mild proximal lower limb weakness, scapular winging, and mild scoliosis. Calpainopathy was diagnosed based on Western blot and Sanger sequencing. Symmetric fatty replacement of muscles in the posterior thigh compartment and superficial posterio-medial calf compartment were in keeping with a calpain-3 mutation.