Calpainopathy with macrophage-rich, regional inflammatory infiltrates.

Peter W. Schutz[a,#], Renata S. Scalco[a], Rita Barresi[b], Henry Houlden[c,d], Matthew Parton[d], Janice L. Holton[a,c,d,*]

[a]Division of Neuropathology, UCL Institute of Neurology, Queen Square, London, UK

[b]Muscle Immunoanalysis Unit, The Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK; The John Walton Muscular Dystrophy Research Centre and MRC Centre for Neuromuscular Diseases, Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne, UK.

[c]Department of Molecular Neuroscience, UCL Institute of Neurology, Queen Square, London, UK.

[d]MRC Centre for Neuromuscular Diseases, UCL Institute of Neurology and National Hospital for Neurology and Neurosurgery, Queen Square, London, UK

[#]Present address: Division of Neuropathology, Vancouver General Hospital, Vancouver, BC, Canada.

[*]Corresponding author: janice.holton@ucl.ac.uk; phone +44 (0)20 344 84239; fax +44 (0)20 344 84486

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.

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.

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 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 MRIwhile 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*.

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.

Histological findings prompted the initial suspicion of an inflammatory myopathy with subsequent azathioprine treatment. Macrophage-rich inflammation is not generally known to be associated with a hereditary condition, as opposed to, for instance, lymphocyte rich inflammation in dysferlinopathy or eosinophilic myositis in calpainopathy. The pattern of muscle involvement on MRI together with a similar pattern in the patient's sister provided a clue to pursue the possibility of a hereditary condition more vigorously, illustrating the potential importance of this diagnostic modality.

Although clearly inflammatory in nature, the muscle biopsy result was not indicative of a specific inflammatory myopathy. Regionality and selective fascicular involvement with numerous macrophages were striking histological features. These findings may raise a histological differential including immune myopathy with abundant macrophages (IMAM), dermatomyositis with microinfarcts, or vasculitis with microinfarcts. However, MHC class I expression was restricted to regenerating fibres arguing against a diffuse inflammatory process. There was no capillary membrane attack complex (MAC) deposition, which might be expected in dermatomyositis with regional infarcts. There was also no evidence of vasculitis. Although these findings did not support an immune mediated process, they were equally insufficient to exclude it.

Regional necrosis is not typically reported as a biopsy feature of calpainopathy, but has previously been noted in a single report of two patients homozygous for R110X in *CAPN3*. However, prominent inflammatory infiltrates were absent [2]. Larger series of eosinophilic myositis with calpainopathy mostly report focal inflammation in association with regional necrosis, with some micrographs demonstrating similar fascicular involvement as in our case [3, 4]. Nevertheless, the presence of eosinophils is thought to be a pointer in these instances. The present case indicates that the inflammatory infiltrate may also be predominantly composed of macrophages without eosinophils. Taken together, present evidence indicates that regional or fascicular necrosis and regeneration can occur in calpainopathy and may be associated with eosinophilic, macrophage rich, or scanty inflammatory infiltrates.

This case also illustrates our increasing understanding of the variability of biopsy findings in both inflammatory and hereditary conditions, and broadens the spectrum of inflammatory manifestations of limb girdle muscular dystrophies. It also illustrates the utility of MRI

investigations for the diagnosis of muscle conditions in adults. In the future, the routine use of panel sequencing approaches may further increase diagnostic efficiency. The present case would recommend a liberal use of panel approaches when biopsy reveals equivocal inflammatory findings.

Conclusion

The histological phenotype of calpain-3 mutations extends to macrophage-rich inflammatory infiltrates in a regional distribution and should be considered in its differential alongside microinfarcts and IMAM.

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