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LETTER TO THE EDITORS

Myopathy caused by anoctamin 5 mutations and necrotizing vasculitis

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Dear Sirs,

Recessive mutations in the *ANO5* gene, encoding anoctamin 5, cause either a proximal limb-girdle muscular dystrophy or a distal myopathy phenotype [2]. The latter has many similarities to Miyoshi myopathy, caused by mutations in the dysferlin gene. Both disorders usually manifest in young adults by initial calf muscle weakness, very high creatine kinase (CK) levels, and dystrophic features on muscle biopsy. Dysferlin immunostaining is normal in anoctaminopathy whereas it is absent in dysferlinopathy. This is the first report on muscle necrotizing vasculitis observed in a myopathy caused by *ANO5* gene mutations.

A 46-year-old man was admitted to a general hospital for malaise. He was diagnosed with *Chlamydia pneumoniae* infection and treated successfully with roxithromycine. A painless lower limb muscle weakness was also noticed. The first symptom was difficulty in running at age 34 years. Family history was negative for neuromuscular disease. Serum CK levels were 3,612 and 1,168 IU/l (normal value <190). Left vastus lateralis biopsy, performed 2 weeks after admission, disclosed variation in fiber size and adipose infiltration. Many vessels were surrounded or infiltrated by inflammatory cells (numerous T lymphocytes CD3+CD8− and a few histiocytes CD68+). The wall of several arterioles exhibited fibrinoid necrosis and nuclear debris, and some vessels were occluded (Fig. 1a–c). On the basis of these pathological findings, the patient was given corticosteroids at 1 mg/kg/day.

When referred to our center 2 months later, he was in a good general condition and denied improvement of muscle strength. His gait was slightly waddling, and walking on tip-toes was impossible. All the thigh and posterior leg muscles were wasted in accordance with muscle imaging (Fig. 2a–c). Manually tested muscle strength (MRC scale) was 3–/5 for foot and knee extensors, 3/5 for knee flexors, around 4/5 for pelvic muscles, and 5/5 for upper limb muscles. The rest of the muscular and general examination was normal. CK level was 1,951 IU/l. The following investigations were normal or negative: chest X-ray, respiratory function tests, electrocardiogram, echocardiography, erythrocyte sedimentation rate, C-reactive protein, eosinophil count, search for proteinuria and microscopic hematuria, hepatitis and HIV blood tests, serum protein electrophoresis, antinuclear and anti-neutrophil cytoplasmic antibodies (performed on two blood samples including one retrieved before corticosteroids introduction). A genetically determined muscular dystrophy was suspected and corticosteroids were stopped within 2 months. A second left vastus lateralis biopsy showed several necrotic or regenerative fibers, increased endomysial connective tissue and fat infiltration. No inflammatory lesions were present. Dystrophin, dystroglycans, sarcoglycans, caveolin, dysferlin and telethonin immunostaining were normal. Calpain3 was found to be reduced

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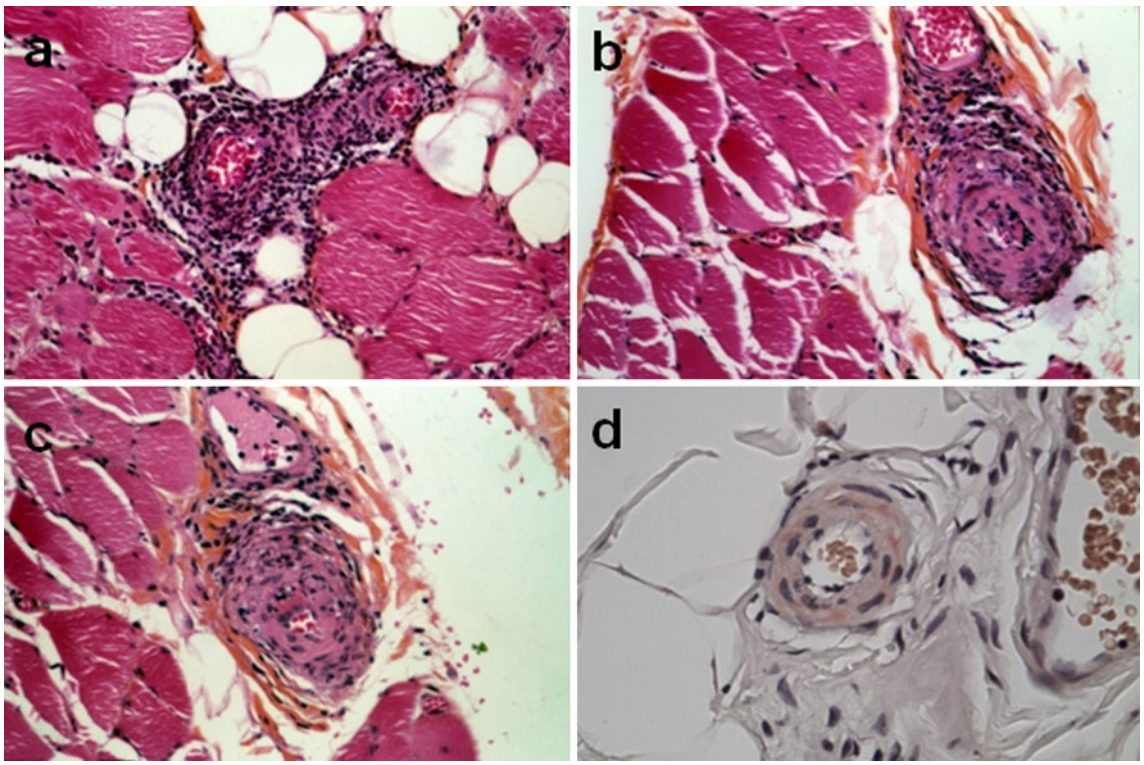

Fig. 1 Paraffin embedded sections of the first quadriceps muscle biopsy. Hematoxylin–eosin–safran staining. **a** A small vessel is surrounded and invaded by an extensive inflammatory infiltrate, **b, c** serial sections of an arteriole showing recent thrombosis and necrosis of the media which is vacuolated and contains pycnotic nuclei. **d** Congo red stained section showing congophilic deposits within the wall of an arteriole

by Western blot analysis but no mutation was detected in the *CAPN3* gene. Weakness remained confined to lower limb muscles at age 56 and neither thrombosis nor other systemic features occurred during follow-up. Diagnosis of Miyoshi-like myopathy with normal dysferlin expression was made by default until direct sequence analysis of *ANO5* identified two novel heterozygous mutations: exon 19 variant c. 2235+1G>A predicted to be pathogenic as it disrupts the consensus splice site and exon 7 variant c. 400C>T which results in the substitution of a conserved basic histidine residue with a neutral polar tyrosine residue (p. His134Tyr) and is predicted as pathogenic by in silico analysis. No mutation was detected in the two unaffected siblings and the mother was identified heterozygous for c. 400C>T. Father's DNA was unavailable. Congo red staining, performed after *ANO5* gene analysis, disclosed congophilic deposits, birefringent under polarized light, within the wall of several arterioles in both biopsies (Fig. 1d).

This is the first case showing that anoctaminopathy may cause a secondary reduction in calpain3 muscle expression. It also confirms that amyloid deposits are present in muscle blood vessel walls [12]. Both features are similar to that reported in primary dysferlinopathies [1, 14]. Two hypotheses may explain the necrotizing vasculitis of muscle which has not been observed in anoctaminopathy previously. (1) Unrelated to the muscular dystrophy, the preceding *Chlamydia pneumoniae* infection may have caused vasculitis; cutaneous vasculitis was reported in context similar case [16]. (2) The vasculitis may be related to anoctaminopathy, as similar inflammatory features are observed in other muscular dystrophies, especially dysferlinopathy, and often lead to misdiagnosis [3, 6, 11, 13, 15]. In dysferlinopathy, the infiltrates are endomysial or perivascular, and consist of T-cells and macrophages. Among the few muscle histology reports in anoctaminopathies [2, 4, 7, 9, 10, 12], inflammatory infiltrates were noticed in a single patient but their location was not reported [7]. The mutations found in our patient affect the cytoplasmic topological domain and the 7th transmembrane domain of the anoctamin 5 protein. Its precise function is not known [5]. Two studies suggested it may play a role in the muscle membrane repair pathway [2, 8]. In conclusion, muscle vasculitis could be a feature of anoctaminopathies, and *ANO5* gene analysis is warranted if compatible phenotype.

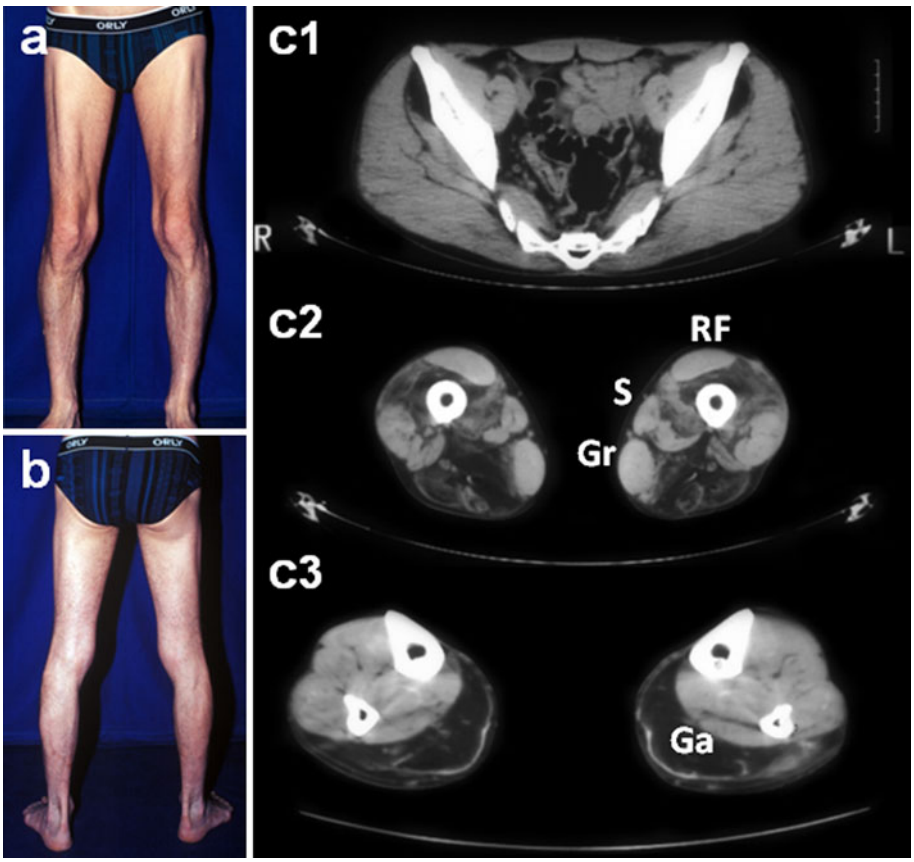

Fig. 2 **a, b** Frontal and posterior views of the patient's lower limbs showing severe atrophy of vasti, hamstrings and posterior leg muscles. **c** Muscle CT scan at (*c1*) pelvis, (*c2*) thigh, and (*c3*) leg levels showing symmetric fatty infiltration of vasti, harmstrings, and mostly gastrocnemius (*Ga*) muscles while rectus femoris (*RF*), sartorius (*S*), gracilis (*Gr*) and the anterior leg muscles are spared

Conflicts of interest None.

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