NEUROPATHOLOGY

Neuropathology 2011; **31**, 455–457 doi:10.1111/j.1440-1789.2011.01221.x

Neuropathology Education

Lobulated fibers in a patient with 46-year history of limb-girdle muscle weakness

Rie Tsuburaya,[1] Takateru Suzuki,[2] Ken Saiki,[3] Ikuya Nonaka,[1] Hideo Sugita,[1] Yukiko K. Hayashi[1] and Ichizo Nishino[1]

[1]Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP), Kodaira, Tokyo, [2]Suzuki Clinic, Morioka and [3]Saiki Hospital, Ichinoseki, Iwate, Japan

CLINICAL COURSE

A 78-year-old man presented with a 46-year history of slowly progressive muscle weakness. He had a past medical history of hypertension but had no familial history of neuromuscular disorders. He first noticed mild muscle weakness affecting his thigh at the age of 32 when he could not keep up with others during mountain climbing. The proximal muscle weakness worsened gradually to difficulty in climbing stairs after the age of 40, and progressed to involve his upper extremities when he could not raise his arms after the age of 50. When he first visited the clinic at the age of 57, physical examination revealed proximal-dominant and symmetric muscle weakness with atrophy in his four limbs, pelvic and shoulder girdle muscles. He had no facial muscle involvement or calf muscle hypertrophy. He neither had cardiac nor respiratory abnormality. Serum creatine kinase (CK) level was 1774 (normal <200) IU/L. Electromyography was reported to be myogenic. He was diagnosed by skeletal muscle biopsy and genetic analysis at the age of 58. It became increasingly difficult for him to stand up at the age of 63. He started using a wheelchair but still could walk with a hand-rail at the age of 66. He could drive a car until the age of 74. Since the age of 75, he has been wheelchair-bound.

PATHOLOGICAL FINDINGS

Muscle biopsy taken at the age of 58 from biceps brachii revealed dystrophic changes (Fig. 1). There was a marked variation in fiber size, moderate endomysial fibrosis and moderate adipose tissue infiltration. A few necrotic and regenerating fibers were seen. On nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-TR) stain, intermyofibrillar networks were disorganized, showing lobulated appearance in the numerous fibers. On cytochrome *c* oxidase (COX) and succinate dehydrogenase (SDH) stains, lobulated fibers were highlighted. On ATPase, type 1 fiber atrophy and scattered type 2C fibers were seen. Lobulated fibers were mostly type 1 fibers. Immunostaining with antibodies against dystrophin, dysferlin, caveolin-3, α/β/γ–sarcoglycans, α/β-dystroglycans, collagen VI, merosin and emerin all revealed normal findings.

GENETIC ANALYSIS

Genomic DNA sequencing of calpain 3 (*CAPN3*; OMIM 114240) gene revealed a compound heterozygous mutation of c.2102 A > G (p. D707G) and c.1976dupA (p.K659fsX6).

DIAGNOSIS

Limb-girdle muscular dystrophy type 2A (LGMD2A; OMIM 253600)

DISCUSSION

Several points can offer clues for diagnosis, including adult-onset, slowly-progressive and proximal dominant muscle weakness, high serum CK level, and dystrophic changes with lobulated fibers on muscle pathology. From the clinical point of view, differential diagnoses can include LGMD, Becker muscular dystrophy (BMD), metabolic myopathies such as adult-onset Pompe disease, and inflammatory myopathies, but these differentials can be narrowed by taking the findings in pathology into careful consideration.

Fiber size variation, endomysial fibrosis, and adipose tissue infiltration reflect chronic myopathic changes. A few

Correspondence: Ichizo Nishino, MD, PhD, Department of Neuromuscular Research, National Institute of Neuroscience, National Centerof Neurology and Psychiatry (NCNP), 4-1-1 Ogawahigashi-cho, Kodaira, Tokyo 187-8502, Japan. Email: nishino@ncnp.go.jp

Published online 26 April 2011.

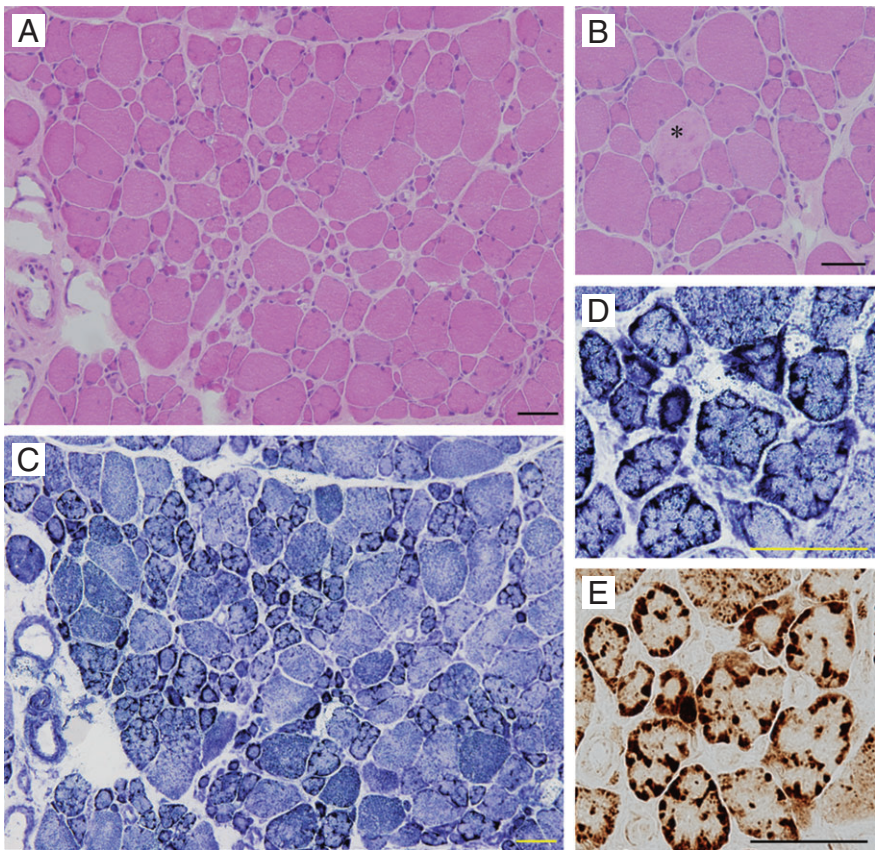

Fig. 1 Muscle pathology findings. Hematoxylin and eosin (a, b), NADH-tetrazolium reductase (c, d), and cytochrome *c* oxidase (e). (a) Marked variation in fiber size, moderate endomysial fibrosis and adipose tissue infiltration. (b) A necrotic fiber (asterisk). (c) Lobulated fibers, especially in small type 1 fibers. (d, e) Oxidative enzyme activity in lobulated fibers is high in the periphery and partially decreased or absent in the center. (Bar: 50 μm).

necrotic and regenerating fibers and scattered type 2C fibers indicate dystrophic features. The most distinct finding is "lobulated fiber" which is also called trabecular or lacy fiber on cross-section.[1] Lobulated fiber is usually type 1 fiber and appears small in size. On oxidative enzyme stains such as NADH-TR, COX and SDH, lobulated fibers show an irregular and coarse appearance due to maldistribution of mitochondria,[1] as opposed to the normal appearance of intermyofibrillar networks that show a fine and regular lattice pattern (Fig. 1). Oxidative enzyme activity in lobulated fibers is high in the periphery, giving the semblance of wedged septa, but it is partially decreased or absent in the center. Electron-micrographs reveal misaligned myofibrils and accumulation of morphologically normal mitochondria in the subsarcolemmal region (Fig. 2).

Attempts to characterize lobulated fibers in LGMD2A by gene expression profiling suggested the involvement of abnormal upregulation of actin binding protein,[2] but up to now the paucity of studies trying to understand how lobulated fibers are formed limits the elucidation of lobulated fiber formation. A previous report has alluded that the number of lobulated fibers is higher in the later stage of the disease but do not correlate with disease severity.[3] Lobulated fibers are often seen in slowly progressive myopathies, such as LGMD, especially in type 2A and type 2B, BMD and facioscapulohumeral muscular dystrophy.

BMD, caused by mutations in *DMD* gene, shows a wide variety of phenotypes from childhood-onset severe muscular dystrophy to mild adult-onset form with only high CKemia. Adult-onset patients often present with calf muscle hypertrophy and cardiac involvement. Diagnosis is partly supported by the faint and patchy pattern of immunostaining and decreased amounts of altered-sized dystrophin on Western blot.

LGMD2B is caused by mutations in *DYSF* gene encoding dysferlin, a transmembrane protein for membrane repairing. Proximal muscle weakness is usually noted in the late teens or later with slow progression. The serum CK level is markedly elevated. Diagnosis can be made by a deficient dysferlin on immunostaining and Western blot. Both BMD and LGMD2B were excluded by immunostainings in this patient.

Lobulated fibers are non-specific but are noted mostly in LGMD2A. In the repository of National Center of Neurology and Psychiatry, 19/58 (32.8%) muscles from genetically confirmed LGMD 2A patients had lobulated fibers. LGMD2A is caused by mutations in *CAPN3* gene encoding calpain 3, non-lysosomal calcium-dependent protease.

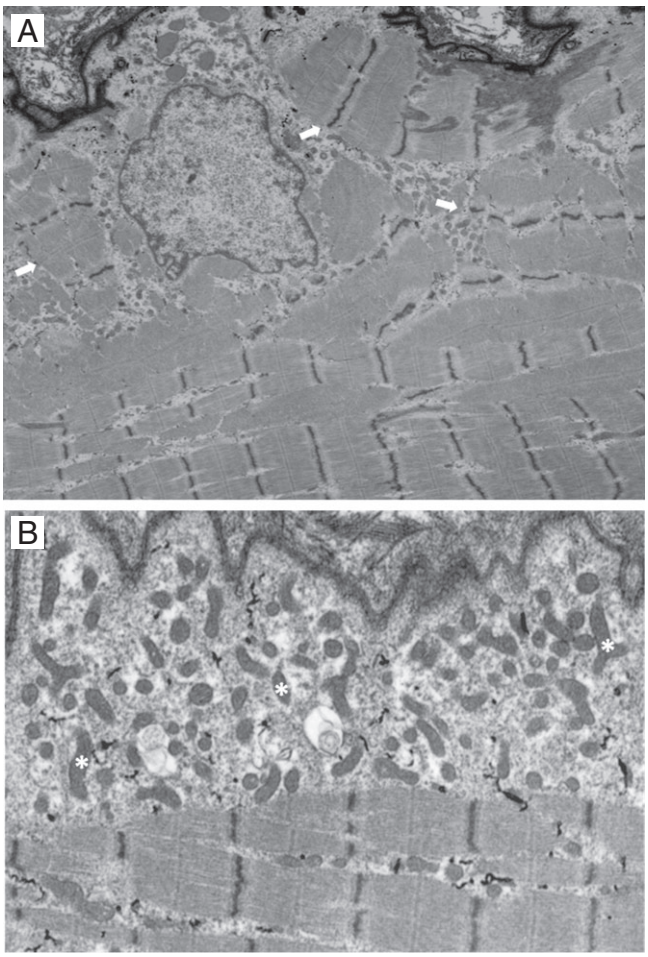

Fig. 2 Electron-microscopic findings. (a) Misaligned myofibrils (arrows) in lobulated fibers and (b) accumulation of mitochondria (asterisks).

Onset is variable, ranging from early childhood to the 40s. As shown in this report, patients usually present with slowly progressive proximal muscle weakness, invariably leading to loss of ambulation, usually 10–30 years from onset of disease. Cardiac or respiratory involvement is fairly uncommon. Serum CK level is moderately elevated. Diagnosis is suggested by deficiency of calpain 3 on Western blot analysis, although sometimes straightforward interpretation is complicated by the possibility of secondary deficiency. In addition, amounts of calpain 3 are sometimes preserved even in the genetically confirmed LGMD2A patients. Immunostaining is also not helpful, as most of the antibodies against calpain 3 are only suitable for Western blot. Definitive diagnosis is ultimately made by finding *CAPN3* gene mutation.

In conclusion, lobulated fibers can be seen in a variety of muscle diseases, but can arguably be a diagnostic clue for LGMD2A. *CAPN3* analysis should be considered in the patient with longstanding clinical course and dystrophic pathology with lobulated fibers.

ACKNOWLEDGMENTS

We thank Dr May Christine V. Malicdan (National Institute of Neuroscience, NCNP) for reviewing the manuscript.

REFERENCES

1. Weller B, Carpenter S, Lochmüller H, Karpati G. Myopathy with trabecular muscle fibers. *Neuromuscul Disord* 1999; **9**: 208–214.
2. Branger-Figarella D, El-Dassouki M, Saenz A *et al*. Myopathy with lobulated muscle fibers: evidence for heterogeneous etiology and clinical presentation. *Neuromuscul Disord* 2002; **12**: 4–12.
3. Keira Y, Noguchi S, Kurokawa R *et al*. Characterization of lobulated fibers in limb girdle muscular dystrophy type 2A by gene expression profiling. *Neurosci Res* 2007; **57**: 513–521.