A novel *CAPN3* mutation in late-onset limb-girdle muscular dystrophy with early respiratory insufficiency

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ABSTRACT

We describe a 70 year-old independently ambulatory man with a 10-year history of progressive axial and limb-girdle weakness, hyperCKemia, and a 5-year history of dyspnea requiring nocturnal ventilatory support due to a known c.1309C>T (p.Arg437Cys) variant and a novel in-frame deletion of exons 17–19 in the calpain-3 encoding gene (*CAPN3*). Pulmonary function tests revealed neuromuscular respiratory weakness. Biceps femoris biopsy showed chronic myopathic changes, numerous lobulated fibers, and reduced calpain-3 immunoreactivity. Muscle immunoblot showed markedly reduced calpain-3 expression. Respiratory insufficiency is uncommon in autosomal recessive calpainopathy, and generally develops in the advanced stages of the disease when individuals become wheelchair-dependent. Our patient broadens the phenotypic spectrum of recessive calpainopathy to include early respiratory insufficiency and also further expands its molecular spectrum.

1. Case report

A 70-year old man developed gait difficulty at age 60 with subsequent lower limb weakness and upper limb weakness in his late-60's. He described a 5-year history of exertional dyspnea requiring nocturnal oxygen for two years. He ambulated independently. He had no bulbar symptoms or rhabdomyolysis. His parents were asymptomatic (Fig. 1A).

Neurologic examination was remarkable for a waddling, severely hyperlordotic gait, trace orbicularis oculi weakness, and severe symmetric shoulder and pelvic girdle weakness, including the thigh adductors and hamstrings with sparing of thigh abductors and quadriceps. There was mild intrinsic hand and toe extensor weakness. He had scapular winging and periscapular muscular atrophy (Fig. 1B,C).

Creatine kinase (CK) was 1338 U/L (normal < 232 U/L) five years ago and 253 U/L (normal < 336 U/L) on our evaluation. Acid alpha-glucosidase level was normal. Needle electromyography showed small motor unit potentials in proximal and axial muscles with rare fibrillation potentials. His vital capacity was 62% of the predicted value and maximal respiratory pressures were reduced. Echocardiogram and Holter monitor were normal.

Biceps femoris biopsy showed numerous lobulated fibers and attenuated calpain-3 immunoreactivity (Fig. 2A–D). Muscle immunoblot showed markedly reduced calpain-3 expression (Fig. 2E).

2. Molecular genetic studies

Facioscapulohumeral dystrophy type 1 and 2 genetic testing was unremarkable (University of Iowa Diagnostic Laboratories). Next generation sequencing of 120 genes causative of myopathies and congenital myasthenic syndromes (Invitae; supplementary material) showed a known c.1309C>T variant in exon 10 (p.Arg437Cys) and a novel in-frame deletion of exons 17–19 in the calpain-3 encoding gene (*CAPN3*).

3. Discussion

Our patient had clinical and histopathologic features compatible with adult-onset calpainopathy, except for early respiratory insufficiency [1]. He carried compound heterozygous *CAPN3* mutations, a known pathogenic mutation (c.1309C>T) and a novel deletion of exons 17–19. Calpain-3 is a muscle specific non-lysosomal cysteine protease involved in muscle regeneration, sarcolemmal remodeling, cytoskeleton regulation, and calcium homeostasis [2]. The c.1309C>T variant has been reported in homozygotes and compound heterozygotes, but genotypic association with respiratory status has not been described [3]. The exon 17–19 deletion removes a calcium-binding EF hand domain believed important for protease function [4]. Functional studies have not been performed for this deletion, but a missense mutation within the deleted region was reported as pathogenic in mouse models [5]. Furthermore, the greatly reduced amount of calpain-3 detected by immunostaining and immunoblotting strongly supports the pathogenicity of this novel deletion of exons 17–19.

Recessive *CAPN3* mutations have been long known to cause a common limb-girdle muscular dystrophy, LGMD2A, featuring adolescent-onset, hip-girdle and axial weakness with wide inter- and intra-familial phenotypic variability. Scapular winging and hip extensor, thigh adductor and hamstring involvement is common [1]. Recently, a heterozygous 21-base pair in-frame deletion (c.643_663del21) in *CAPN3* was described in autosomal dominant LGMD of milder phenotype [6,7]. While we cannot determine if the patient's *CAPN3* variants are heteroallelic, asymptomatic parents support autosomal recessive inheritance.

While calpainopathy is typically associated with minimal respiratory involvement [8,9], *Mori-Yoshimura et al.* reported moderate-to-severe respiratory insufficiency in 20% of Japanese calpainopathy patients, whom were 65 and older, had longer disease duration, were non-ambulatory, and had lower CK levels than patients with normal respiratory function. There was no genotypic correlation with respiratory dysfunction in this cohort, and mutations reported in these cases did not overlap with our patient [10]. *Pollitt et al.* reported a non-ambulatory individual with respi-

Abbreviations: CAPN3, calpain-3; AR, autosomal recessive; CK, creatine kinase; LGMD, limb girdle muscular dystrophy; LGMD2A, limb-girdle muscular dystrophy type 2A; del, deletion; Arg, arginine; Cys, cysteine.

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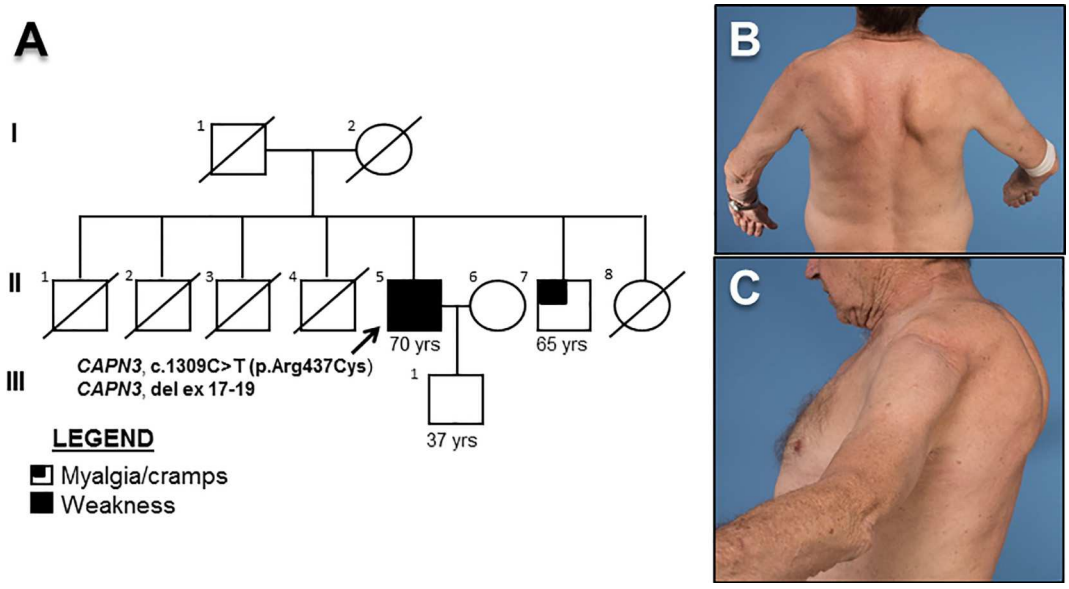

Fig. 1. Family pedigree and proband. (A) The arrow indicates the proband (II-5). A brother (II-7) developed myalgia and thigh cramping in his 60's. A sister (II-8) died in her 60's from unclear causes. Four brothers had coronary artery disease but none had a pacemaker/defibrillator. Photographs of the proband depicting (B) asymmetric scapular winging with arm flexion and (C) periscapular atrophy.

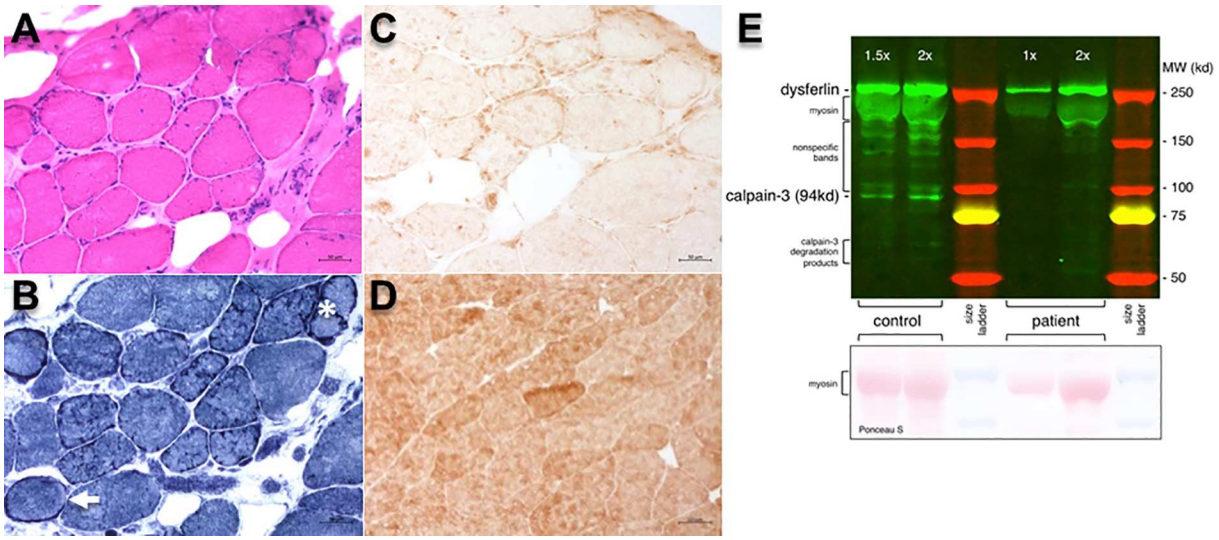

Fig. 2. Muscle histological and western blot findings. Biceps femoris muscle biopsy of the proband showing muscle fiber variation and an increase in fibrous and fatty connective tissue (A, hematoxylin and eosin). NADH dehydrogenase reacted sections (B) show numerous lobulated fibers (asterisk) or ring fibers (arrowhead). (C) Calpain-3 immunoreactivity (anti-calpain-3 2C4 antibodies) is reduced in the proband. (D) A normal muscle section with preserved calpain-3 immunoreactivity is shown for comparison. (E) Control muscle was compared to the muscle biopsy from the proband. While dysferlin appears normal in size and amount for the proband, full-length (94kd) calpain-3 and calpain-3 degradation products are greatly reduced. The antibodies used for Western blotting were Hamlet (anti-dysferlin) and 12A2 (anti–calpain-3), both purchased from Leica Biosystems.

ratory insufficiency requiring non-invasive ventilation, who carried a c.566 T > C variant in exon 4 and a splice site variant in exon 18 [11], the latter which overlaps with our patient's deletion. Respiratory involvement in our patient is unique as it occurred early in the course of the disease while he remained ambulatory.

Our findings further expand the spectrum of recessive calpainopathy to include early respiratory insufficiency and highlight another calpain-3 region necessary for protein function. Whether mutations affecting a calcium-binding EF hand domain are associated with respiratory insufficiency remains to be elucidated.

4. Disclosures

The authors have no conflicts of interest or disclosures to report.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jocn.2018.04.025.

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A novel homozygous NDRG1 mutation in a Chinese patient with Charcot-Marie-Tooth disease 4D

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ABSTRACT

Charcot-Marie-Tooth disease 4D (CMT4D) is characterized by severe peripheral neuropathy and deafness. It is caused by mutations in the N-myc downstream-regulated gene 1 (*NDRG1*). We report a Chinese man with a homozygous mutation c.675C > T of *NDRG1* that resulted in Q185X, representing the third known CMT4D patient of non-European ancestry. The patient presented with a 15-year-long history of progressive limb weakness accompanied by hearing loss and dysarthria. There was abnormal differentiation and increased interpeak latencies in brainstem auditory evoked potentials. Compound muscle action potentials (CMAP) of the peripheral nerves were not elicited in distal segments, while prolonged distal latencies and decreased CMAP were present in proximal nerves. A mild enlargement of the lateral ventricles showed in brain magnetic resonance imaging studies. Q185X of *NDRG1* is a novel mutation with CMT4D, which are demonstrated in Asian population. Q185X of the *NDRG1* expands the clinical and mutational spectrum of CMT4D.

1. Introduction

Charcot-Marie-Tooth disease 4D (CMT4D) is a severe, early-onset autosomal recessive polyneuropathy caused by mutations in the N-Myc downstream-regulated gene 1 (*NDRG1*) [1–3]. CMT4D was first identified in the Gypsy community of Lom in Bulgaria, who had a history of consanguineous marriages, and was subsequently reported in several other European countries [4–5].

Patients with CMT4D progress towards severe disability in adulthood and neural deafness develops during the second or third decade of life [3]. Electrophysiological studies of CMT4D patients revealed a severe reduction of motor nerve conduction velocity in the youngest patients, and unobtainable CMAP in distal segments after the age of 15 [3]. Pathological findings in the sural nerve of patients with CMT4D suggest a marked reduction of myelinated fibers, and that the remaining fibers have relatively thin myelin sheaths and onion bulb formations [2,3].

There are six known disease-causing *NDRG1* mutations [6–9]. The most common mutation in *NDRG1* is R148X in exon 7, which is associated with a founder effect [1,2]. Here we report the clinical, electrophysiological, neuroradiological, and genetic features of the Chinese patient with a novel *NDRG1* mutation.

2. Case report

The patient was a 21-year-old man who was admitted in 2014 to the Outpatient Department of Neurology at Beijing Tiantan Hospital of Capital Medical University. A detailed medical history was obtained from the patient and his older brother. A clinical evaluation of the patient was undertaken by two independent examiners. The patient underwent electrophysiological testing and brain magnetic resonance imaging (MRI) [10]. The study protocol was approved by the Medical Ethics Committee of the Beijing Tiantan Hospital.

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