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# Late-onset camptocormia caused by a heterozygous in-frame *CAPN3* deletion

Marco Spinazzi<sup>a,g,\*</sup>, Jerome Poupiot<sup>b,c</sup>, Julien Cassereau<sup>a</sup>, France Leturcq<sup>d</sup>, Laurent Brunereau<sup>e</sup>, Edoardo Malfatti<sup>f</sup>, Isabelle Richard<sup>b,c</sup>, Franck Letournel<sup>g</sup>

<sup>a</sup>Neuromuscular Reference Center, Department of Neurology, CHU d'Angers, France

<sup>b</sup>Genethon, 91000, Evry, France

<sup>c</sup> Université Paris-Saclay, Univ Evry, Inserm, Généthon, Integrare research unit UMR\_S951, 91000, Evry-Courcouronnes, France

<sup>d</sup>Laboratory of Genetics and Molecular Biology, Hôpital Cochin, Paris, France

<sup>e</sup>Radiology Department, CHU de Tours, France

<sup>f</sup>Neuromuscular Reference Center, Department of Neurology, Hôpital Henri-Mondor Paris, France <sup>g</sup>Institute of Neurobiology and Neuropathology CHU d'Angers, France

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#### Abstract

Camptocormia is defined by a pathological involuntary flexion of the thoracic and lumbar spine that is fully reducible in the supine position. Although originally described as a manifestation of conversion disorder, it is more commonly caused by a wide range of neurological diseases, in particular movement and neuromuscular disorders. We describe here a rare case of late onset camptocormia caused by autosomal dominant calpainopathy due to a heterozygous in-frame deletion in *CAPN3* leading to loss of a single lysin amino acid in the catalytic domain of calpain-3. Creatine kinase levels, electromyography, and thigh muscle MRI were normal. Muscle biopsy did not show lobulated fibers and calpain-3 protein expression was not decreased, but *in vitro* functional assays showed impaired proteolytic function of. Lys254del CAPN3. Autosomal dominant calpainopathy should be considered in the differential diagnosis of late onset camptocormia and unexplained paravertebral myopathies even in presence of normal creatine kinase levels, and in absence of lobulated fibers, of decreased calpain-3 protein expression, and of muscle limb involvement.

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Keywords: Calpainopathy; Calpain-3; Camptocormia; Bent-spine; Paravertebral myopathy.

#### 1. Introduction

Camptocormia (from the Greek camptos = bent and cormos = trunk) defines a pathological flexion of the thoracic and lumbar spine that is fully reversible in the supine position and worsens in the standing position or with walking. Since the original description by Brodie in 1818 [1], this term has been widely used during the world wars I and II to describe psychogenic postural manifestations of stress among soldiers, and until few decades ago it has been implicitly linked to conversion disorder. Only in 1992 camptocormia has been used to describe cases of myopathy

\* Corresponding author. *E-mail address:* marco.spinazzi@chu-angers.fr (M. Spinazzi). of paravertebral muscles [2]. In 1999, Djaletti reported camptocormia in eight patients with Parkinson's disease [3], and follow-up studies reported paraspinal myopathy as the structural base of Parkinson's disease camptocormia [4]. It is now recognized that camptocormia comprises a very heterogeneous group of diseases with different mechanisms that ultimately result into paraspinal muscles weakness. The most common etiologies include a wide range of movement and neuromuscular disorders, and degenerative joint diseases, whereas psychogenic camptocormia is nowadays exceedingly rare [5]. Within the neuromuscular etiologies, camptocormia can be caused by diseases affecting the motor unit at any anatomical level, from the motor neuron to the neuromuscular junction and the axial skeletal muscles [6]. It has been recently recognized that axial involvement is very common

in muscle diseases as part of a more widespread involvement [7]. More rarely, different myopathies can manifest with an exclusive or predominant axial involvement [7–9]. Calpainopathy is a muscular dystrophy affecting the limb girdles most commonly inherited with an autosomal recessive transmission [10], but rare autosomal dominant cases have been recently reported [11,12].

#### 2. CAPN3 proteolytic activity methods

L254del CAPN3 cDNA was first generated as a synthetic DNA (Geneart Gene synthesis, Thermo Scientific, Waltham, MA) then cloned into an expressing plasmid under the control of the CMV promoter. WT CAPN3 and the R572P CAPN3 mutant were previously cloned into an expression plasmid [13]. HEK-293 cells were co-transfected with these plasmids, alone or with a reporter plasmid expressing an inactive CAPN3 carrying a mutation in the cysteine of the catalytic site (C129A Substrate) or with the active form of calpain3 (WT). In this construct, the inactive human calpain 3 coding sequence is fused with the fluorescent proteins YFP at the N-Terminus and GFP at the C-terminus. For each transfection, the total quantity of DNA used was 1.8µg. Four days after transfection, cell extracts were prepared in RIPA buffer (Thermo Scientific, Waltham, MA) with Protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO). A BCA protein quantification (Thermo Scientific) was performed to normalize the quantity of protein loaded on the gel. After transfer on a nitrocellulose membrane, a western blot was performed using antibodies against GFP (Rabbit antibody Ab6556, Abcam, dilution 1:500) and Calpain 3 (Mouse antibody NCL-CALP-12A2, Leica Biosystems, dilution 1:200 and Goat antibody COP-080049, Operon Biotechnologies, dilution 1:1000).

## 3. Case report and CAPN3 proteolytic function analysis results

A 76-year-old female progressively developed since the age of 67 difficulties in gaining the upright position after bending which she compensated by pushing her hips forward with her hands. This difficulty was increased by bringing small weights or by walking, thus reducing her walking distance to a maximum of 200 m. She complained of frequent cervical and lumbar pain. Her medical history was uneventful and she did not take any drug. In her family, there was no consanguinity. Her father had a waddling gait for which he never underwent any clinical investigation. He died at 98 years following a cerebrovascular accident without significant disability. She had no children, and one brother still alive had no muscular symptoms. Clinical examination revealed a height of 167 cm for 55 Kg. There was a marked kyphosis enhanced by standing and walking, completely abolished in the supine position. Range of motion in spine extension and flexion was normal. Muscle strength was strictly normal except for spine extensors (2/5 MRC), neck flexors (4/5), and hip extensors muscles (3/5). There was no muscular atrophy. Deep tendon reflexes were normal at the upper extremities and reduced at the lower extremities. There was no extrapyramidal rigidity, no bradikynesia, nor tremors. Pulmonary function tests were normal.

Serum creatine kinase levels were normal (136U/L; nv < 145). Normal blood tests included: TSH, lactate, pyruvate, anti-AchR, anti-MUSK antibodies, blood acid maltase activity. EMG was normal. Whole body muscle MRI showed a selective severe symmetrical fatty infiltration of thoracic and lumbar paraspinal muscles, middle and small glutei, without pathological changes at the level of thigh muscles (Fig. 1A).

A left vastus lateralis muscle biopsy showed mildly increased fiber size variability, slight irregularities in the distribution of the oxidative stainings, but absence of necrosis, regenerating fibers, nor lobulated fibers (Fig. 1B). Western blot for dystrophin, dysferlin, sarcoglycans did not show any abnormality. The overall expression of calpain 3 was not reduced, but the ratio between full length 94 KDalton CAPN3, and the cleaved 60 KDa form, was increased in the patient muscle compared to a healthy control and two patients affected by other myopathies unrelated to CAPN3 (Fig. 1C), a finding compatible with altered proteolytic activity. Gene tests for myotonic dystrophy type II (PROMM) and facioscapulohumeral dystrophy type 1 resulted negative.

Next-generation sequencing of 38 genes involved in limb girdle muscular dystrophies (ANO5, CAPN3, CAV3. COL6A1, COL6A2, COL6A3, DAG1. DES. DMD, DNAJB6, DYSF, EMD, FHL1, FKRP, FKTN, GAA, GMPPB, GNE, HNRNPDL, ISPD, LAMA2, LISM2, LMNA, MYOT, PLEC, POMGNT1, POMT1, POMT2, SGCA, SGCB, SGCC, SGCG, TCAP, TNPO3, TRAPPC11, TRIM32, TTN, VCP) showed a heterozygous in-frame deletion in CAPN3 c.759\_761del (p.Lys254del). His-brother, who had no neuromuscular complaint and a normal neuromuscular examination, did not carry this CAPN3 variant.

In order to investigate the functional impact of L254del CAPN3 we tested both autolytic and proteolytic activity of L254del CAPN3 in HEK-293 cells, which do not have endogenous expression of CAPN3. WT CAPN3 is able to digest itself (autolysis), as well as other CAPN3 molecules and other substrates (hetero-proteolysis). The control conditions led to the expected results: WT CAPN3 underwent autolysis and R572P CAPN3, a variant known to have impaired proteolytic activity [13], had severely impaired autolysis (Fig. 2A). L254del CAPN3 showed a complete loss of autolytic activity (Fig. 2A). Cotransfection experiments with both the catalytically inactive calpain 3 substrate C129A and L254del CAPN3 or controls, showed that only WT, but not L254del CAPN3 or R572P CAPN3, were capable to proteolyze the C129 substrate, indicating a complete loss of hetero-proteolytic activity in L254del CAPN3 (Fig. 2B). Finally, cotransfection with both WT CAPN3 (WT) and L254del CAPN3 or controls, led to CAPN3 cleavage in all conditions, indicating lack of a dominant negative functional effect of L254del CAPN3 in this condition (Fig. 2C).



Fig. 1. (A) Whole body muscle MRI shows severe fatty replacement in thoracic and lumbar paravertebral muscles (first two images from the top, white arrows) and in small gluteus muscles (third image from the top, white arrows) in T1 images, but normal thigh muscles. (B) Muscle pathological analysis from quadriceps biopsy (scale bar:100  $\mu$ m). H&E staining (top panel) shows increased muscle fiber size variability with scattered atrophic fibers. Succinate dehydrogenase (SHD) histoenzymatic reaction (bottom panel) shows non-specific irregularities of the intermyofibrillar network without the evidence of lobulations. (C) Multiplex western blot of total skeletal muscles with anti-dystrophin (DYS), anti-dysferlin (DYSF), anti-calpain-3 (CAPN3), anti- $\gamma$ -sarcoglycan ( $\gamma$ SG) antibodies. Calpain-3 recognizes both full-length calpain-3 (94KDa) as well as its proteolytic product migrating at 60KDa. Myosin heavy chain (MYO) stained by Coomassie is the loading control. *P* = patient, *M* = disease controls (patients with myopathies unrelated to *CAPN3*), *C* = healthy control).

#### 4. Discussion

Calpainopathy is among the most common forms of autosomal recessive limb girdle muscular dystrophy worldwide (LGMD2A/LGMDR1). It is caused by mutations in the *CAPN3* gene, encoding for a skeletal muscle-specific non lysosomal Ca<sup>2+</sup>-dependent cysteine protease expressed in the cytosol. Its physiological role is poorly understood. Clinically, it is typically characterized by limb girdle or scapulo-peroneal progressive muscle weakness with variable age at onset between two to forty years [14], with constantly

elevated serum creatine kinase. Biochemically, the majority of the mutations are associated with reduced muscle calpain-3 levels or impaired autocatalytic activity [15].

More recently, calpainopathies with autosomal dominant forms have been reported in carriers of a single mutated allele, now reclassified as LGMD D4 [16]. The first study of dominant calpainopathy described 37 patients within 10 families carrying a distinct heterozygous 21 base pairs in-frame deletion in the *CAPN3* gene (c.643\_663del21) [12].

Although this new nosological entity has been initially questioned [17], similar findings have been reported by

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Fig. 2. (A) Autolytic CAPN3 assay. HEK-293 cells were either non transfected or transfected with a control plasmid (GFP), or WT CAPN3 (WT), L254del CAPN3 or the previously described c.1715G>*C CAPN3* mutation, leading to R572P CAPN3, as positive control [13]. Cleavage of CAPN3 is revealed by anti CAPN3 antibody (red). The expression of the control plasmid is detected with anti GFP antibody (in green). Only WT CAPN3, but not L254del or R572P are cleaved, indicating the complete loss of autolytic activity of the L254del *CAPN3* mutant. (B) Proteolytic CAPN3 assay with catalytic inactive substrate. HEK 293 cells were cotransfected with a control plasmid (GFP), WT *CAPN3* (WT) or the two *CAPN3* mutants (L254del or the positive control R572P) plus the C129A catalytically inactive CAPN3 substrate fused with YFP at the N terminus and GFP at the C terminus (C129A, not recognized by anti CAPN3 antibody). Cleavage of the full-length catalytic inactive substrate is revealed by anti GFP (green, at about 150KDa). Cleavage of cotransfected WT and the two *CAPN3* mutants (L254del or R572P) is revealed by anti CAPN3 antibody (red). Only WT, but not L254del or R572P CAPN3, cleaves the catalytic inactive substrate, indicating loss of proteolytic activity of the L254del *CAPN3* deletion. The asterisk indicates nonspecific bands recognized by anti GFP, WT *CAPN3* (WT) or the two *CAPN3* mutants (L254del or R572P) plus WT *CAPN3*. Cleavage of CAPN3 antibody (red). Cotransfection of WT and any of the two *CAPN3* mutants was not associated with impaired CAPN3 proteolysis indicating absence of dominant functional effect of the L254del *CAPN3*.

independent groups reporting additional families carrying the same in-frame deletion [18], and more recently, during the revision of the current article, also missense mutations [13,19,20]. These reported cases of dominant calpainopathy are usually characterized by a much milder and later onset phenotype compared to LGMDR1. Patients commonly presented an axial muscular involvement associated with limb weakness, in particular at the level of scapular, gluteal, hamstring and medial gastrocnemius muscles [12,18–20] and CPK levels ranging from normal to markedly elevated.

Late onset camptocormia has been previously reported in a single patient carrying the same in-frame c.759–761delGAA *CAPN3* deletion that we report here [11]. Muscle biopsy showed an identical marked fatty infiltration of paravertebral and gluteal muscles as in our case, but with an additional iliopsoas and thigh muscles involvement which was absent in our case.

Unlike our patient, muscle biopsy showed a marked presence of lobulated fibers, a common but incompletely sensitive and specific histological finding in calpainopathy [11]. The authors strongly suspected but could not conclude a diagnosis of a dominant form of calpainopathy based on a single observation. This deletion, already described as pathogenic in some autosomal recessive cases, seems to be relatively rare since it has been reported in about 3.7/100'000 exomes in the European population in the gnomAD database in dbSNP (rs794727697). Our independent observation supports the c.759–761delGAA *CAPN3* heterozygous deletion as the cause of the reported late-onset camptocormia.

The molecular mechanism underlying autosomal dominant calpainopathy is unclear. Severe reduction in CAPN3 protein expression has been observed in most [12,20] but not all cases with dominant calpainopathy [19] leading to hypothesize a possible dominant negative molecular mechanism on protein stability, possibly via polymerization of WT and deleted calpain-3 in dimers [12] or homo-trimers [21]. In our case the overall expression of CAPN3 was not reduced, arguing against a dominant negative effect on CAPN3 protein stability. Instead, we noticed an increased ratio of full length uncleaved CAPN3 over the cleaved form compared to normal or disease controls, suggesting partially impaired catalytic activity [22], which we expected since L254del leads to a loss of a lysine in catalytic domain of CAPN3. This hypothesis was further confirmed in our cell-based proteolytic assays that indicate a loss of autolytic and hetero-proteolytic function of L254del CAPN3, but no dominant negative functional effect. These data suggest that in our case the molecular mechanism is likely due to haploinsufficiency. Impaired proteolytic activity has been recently reported in two other CAPN3 variants associated with dominant calpainopathy in which functional experiments have been conducted [13,19]. Although we cannot rule out that non-enzymatic functions

of CAPN3 are involved we believe that decreased CAPN3 proteolytic activity, likely play an important role in the pathogenesis of dominant calpainopathy.

Dominant calpainopathy seems to be associated with variable age of onset comprised between 15 and 84 years of age [12,18,19], and severity ranging from asymptomatic to severely affected patients. As in other genetic diseases the underlying reason for this phenotypic variability and incomplete penetrance is unknown. The D4Z4 repeat contraction associated with facio-scapulo-humeral dystrophy has been found to be present in as many as 3% healthy individuals while the prevalence of this disease occurs only in about 1/20'000 [23]. Therefore, in dominant calpainopathy, as well as in facio-scapulo-humeral muscular dystrophy and other genetic diseases, additional genetic, epigenetic, and likely also non-genetic factors are involved and deserve to be investigated further.

The pattern of muscle involvement is also variable in dominant calpainopathy, and is typically characterized by limb girdle and axial muscles [12,18-20]. Camptormia has been reported in few other cases of dominant calpainopathy, but, unlike our case, associated with thigh muscles involvement [11,19]. The reasons for the frequent paravertebral muscle involvement is not known, but paravertebral muscles are more susceptible than leg muscles to age-related degeneration [24], perhaps due to their prolonged activation with significant anti-gravity workload. We believe that heterozygous CAPN3 mutations may be an under-recognized cause of camptocormia and age-related axial muscle weakness. We speculate whether single allele mutations in CAPN3 may constitute a risk factor to develop paravertebral myopathy during aging. Conceptually, a similar genetic mechanism has been observed in carriers of mutations in GBA, encoding for the enzyme galactocerebrosidase. Homozygous or compound heterozygous mutations cause the lysosomal-storage disease Gaucher disease, while heterozygous mutations confer a 20fold increased risk to develop Parkinson's disease by the age of 80 [25]. Future studies are warranted to investigate the prevalence of CAPN3 mutations in patients with unexplained camptocormia and of paraspinal myopathy in heterozygous carriers of CAPN3 mutations.

In conclusion, our observations support a pathogenic role of the c.759\_761del *CAPN3* deletion as one possible cause of camptocormia by a haploinsufficiency mechanism. *CAPN3* gene mutations should be considered in the differential diagnosis of unexplained late-onset camptocormia and axial myopathies even in absence of lobulated fibers, normal creatine kinase levels and absence of decreased CAPN3 expression and thigh muscles involvement. Increased ratio between full length and cleaved CAPN3 can be a hint for impaired CAPN3 proteolytic activity warranting further investigation.

#### **Declaration of Competing Interest**

None.

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