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AUTOSOMAL DOMINANT CALPAINOPATHY DUE TO HETEROZYGOUS *CAPN3* C.643_663DEL21

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Abstract

Introduction—A calpain-3 (*CAPN3*) gene heterozygous deletion (c.643_663del21) was recently linked to autosomal dominant (AD) limb-girdle muscular dystrophy. However, the possibility of digenic disease was raised. We describe 3 families with AD calpainopathy carrying this isolated mutation.

Methods—Probands heterozygous for *CAPN3* c.643_663del21 were identified by targeted next generation or whole exome sequencing. Clinical findings were collected for probands and families. Calpain-3 muscle Western blots were performed in 3 unrelated individuals.

Results—Probands reported variable weakness in their 40s or 50s, with myalgia, back pain, or hyperlordosis. Pelvic girdle muscles were affected with adductor and hamstring sparing. Creatine kinase was normal to 1,800 U/L, independent of weakness severity. Imaging demonstrated lumbar paraspinal muscle atrophy. Electromyographic findings and muscle biopsies were normal to mildly myopathic. Muscle calpain-3 expression was reduced.

Discussion—This study provides further evidence for AD calpainopathy associated with *CAPN3* c.643_663del21. No pathogenic variants in other genes known to cause myopathy were detected.

Keywords

autosomal dominant myopathy; axial myopathy; CAPN3; limb-girdle muscular dystrophy

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Calpain-3 (CAPN3) is a muscle-specific non-lysosomal cysteine protease essential for normal muscle function.[1] It interacts with several proteins, including dysferlin, titin, filamin C, and sarcoplasmic/ endoplasmic calcium–ATPase (SERCA).[2–4] Calpain-3 plays a role in multiple physiologic and pathophysiologic functions, including muscle regeneration, sarcolemmal repair, sarcomere remodeling, cytoskeleton regulation, and calcium homeostasis.[2–5]

In 1995, *CAPN3* recessive mutations were recognized as causative for one of the most common type 2 limb-girdle muscular dystrophies: LGMD2A.[1,6] The majority of patients manifested in the first 2 decades of life with proximal lower limb and axial weakness. There was inter- and intrafamilial variability, but patients with 2 null mutations demonstrated a more severe phenotype than those carrying at least 1 mis-sense mutation.[6] Creatine kinase (CK) levels were elevated but normalized in severely weak patients. The mechanism by which *CAPN3* mutations lead to weakness remains undefined but seems related to the perturbation of various homeostatic mechanisms, including calcium dysregulation.[7]

Recently, Vissing and colleagues reported 10 European families with an autosomal dominant (AD) LGMD co-segregating with an in-frame 21-bp deletion (c.643_663del21, p.Ser215_Gly221del) in *CAPN3*.[8] Affected individuals had a milder phenotype than those affected by LGMD2A, normal expression of the mutated mRNA, and [CAPN3] loss by Western blot analysis, suggesting a dominant negative effect of the *CAPN3* deletion. A critique of the article suggested the alternative possibility of a digenic disease.[9] Although the original authors rebutted the critique,[10] the concept of AD calpainopathy remains controversial. To our knowledge, none of the published subjects with AD calpainopathy underwent extensive molecular testing to search for coexisting mutations in other genes causative of myopathies.

We report the clinical and laboratory findings of 3 unrelated multigenerational families with AD calpainopathy-3 as further support for dominant as well as recessive inheritance of this myopathy.

METHODS

We identified 2 unrelated probands with AD myopathy and another proband with a history suggestive of AD myopathy, all heterozygous for the in-frame 21-bp deletion in *CAPN3*, c. 643_663del21, p.Ser215_Gly221del. Clinical history and findings, CK values, electromyographic (EMG), imaging, and muscle biopsy findings were reviewed. Family history was obtained from the probands and review of available familial medical records. Scrutiny of affected and asymptomatic individual's surnames within the 3 families, who come from different areas of the United States, revealed no overlap or similarity. The study was approved by the institutional review board of the Mayo Clinic, Rochester, Minnesota.

Genetic testing was performed via targeted next generation sequencing (NGS) of 120 genes causative of myopathies or congenital defects of neuromuscular transmission, Sanger sequencing (Invitae, San Francisco, California; PreventionGenetics, Marshfield, Wisconsin; or EGL Genetics, Tucker, Georgia), or whole exome sequencing (WES; Mayo Clinic). WES

findings were verified by targeted Sanger sequencing. Briefly, WES was performed on genomic DNA using capture reagent (Agilent SureSelect All Exon V5 Exome; Agilent Technologies, Santa Clara, California) and >95% of the target region was sequenced >20×. The coding region of the *CAPN3* gene was completely sequenced and the heterozygous c. 643_663del21 mutation was detected by 32× at an average read depth of 72×.

Three unrelated individuals had Western blot analysis on their muscle biopsies (University of Iowa). Cryosections were cut and pooled from each muscle biopsy, then homogenized before separating proteins on a 3%–13% gradient gel.[11] The antibodies used for Western blotting were Hamlet (anti-dysferlin) and 12A2 (anti–calpain-3), both purchased from Leica Biosystems (Wetzlar, Germany).

RESULTS

Family Descriptions

Family 1 (Norwegian Ancestry)—The proband (IV-16) manifested at age 51 after years of back pain and myalgias. In her mid-60s, she developed a waddling gait and subsequently mild symmetric weakness of the iliopsoas and gluteus medius. Multiple maternal family members were variably affected (Fig. 1A and Table 1).

Family 2 (German Ancestry on Maternal Side and Scandinavian Ancestry on Paternal Side)—The proband (V-6) had low back pain, hyperlordosis, and elevated CK in his 20s. He developed proximal limb and abdominal wall muscle weakness in his 40s (Fig. 2A–C). Lumbar spine MRI showed paraspinal muscle atrophy (Fig. 2D). Gluteus medius biopsy revealed mild, chronic myopathic changes (fiber splitting, rare regenerating fibers, mild-to-moderate increase in internalized nuclei, and type 1 fiber predominance). Multiple maternal family members were variably symptomatic (Fig. 1B and Table 1).

Family 3 (Mixed Northern European Ancestry)—The proband (II-2) developed myalgias in his mid-30s and weakness by his 50s. He had mild proximal weakness and waddling gait at age 57. Lumbar spine MRI revealed fatty replacement of paraspinal muscles. Several family members not available for examination are symptomatic (Fig. 1C).

Probands in family 1 and 2 had no decrement on 2-Hz repetitive nerve stimulation of the ulnar nerve. With the exception of subject III-10 in family 1, who developed atrial fibrillation in her 80s, affected individuals had no cardiac disease via history and when screened with a 12-lead electrocardiogram and echocardiogram.

Molecular Data and Muscle Western Blot

All 3 probands carried the *CAPN3* (c.643_663del21) deletion. Family 1's proband had no additional pathogenic or presumed pathogenic variants (point mutations, deletions, or duplications) on NGS of 120 genes causative of myopathies or congenital defects of neuromuscular transmission that can sometimes be associated with myopathy. Previous genetic testing detected no pathogenic variants in *LMNA* (laminin A) and *CNBP* (CCHC-type zinc finger nucleic acid binding protein) in a cousin (IV-13), and there were no pathogenic variants in *FKRP* (fukutin-related protein), *LMNA*, *CAV3* (caveolin-3), *DYSF*

(dysferlin), *SGCA*, *SGCB*, *SGCD*, *SGCG* (sarcoglycans alpha, beta, gamma, and delta, respectively), or *MYOT* (myotilin) in subject III-3.

Family 2's proband had no additional pathogenic or suspected pathogenic variants by WES in *CAPN3* or in any other gene currently known to cause myopathy or hyperCKemia. Because Starling *et al.*[12] reported subjects with LGMD carrying a single *CAPN3* in-frame deletion associated with a *KX* gene (X-linked Kx blood group protein) mutation (McLeod syndrome), we also searched for mutations in *KX*, but found no variants. In addition, the family 1 and family 2 probands had no acanthocytosis, and no subject within the 3 families has chorea. Family 3's proband had no pathogenic variants in genes causative of other LGMD.

Muscle Western blot analysis in the 3 unrelated individuals showed greatly reduced [CAPN3] and normal dysferlin expression (Fig. 2F).

DISCUSSION

We have described 3 unrelated families of northern European ancestry with AD LGMD due to the c.643_663del21 deletion in *CAPN3*. These individuals presented with a spectrum of disease severity and weakness affecting predominantly pelvic girdle and axial muscles in mid-adulthood. Back pain and hyperlordosis preceded the reported onset of weakness by years, suggesting early axial involvement. As previously reported by Vissing *et al.*,[9] our patients had a milder phenotype than that commonly observed in recessive calpainopathy, but with phenotypic intrafamilial variability. The abdominal wall muscle weakness, previously recognized as a feature of LGMD2A, was a prominent early clinical finding in family 2's pro-band, suggesting that this feature also occurs in dominant calpainopathy. Conversely, the early involvement of thigh adductors and hamstrings of LGMD2A[13] was not observed in our patients, who had normal strength in these muscles. Of interest, some patients showed no myopathic EMG changes in clinically weak muscles and minimal muscle histopathologic findings. No patients had lobulated fibers, often observed in LGMD2A.[13] The CK values did not correlate with disease severity, as some affected individuals had normal CK during their clinical course, including family 2's proband.

No additional pathogenic or presumed pathogenic variants were detected in other genes so far known to cause myopathy. In particular, no relevant variants were detected in *TTN* (titin) *DYSF* (dysferlin), *FLNC* (filamin C), and *SERCA1*, which encode for proteins that interact with [CAPN3]. Therefore, it is unlikely that the loss in [CAPN3] expression detected by Western blot is secondary to another gene so far known to cause myopathy. In addition, muscle biopsies in 3 affected individuals showed no structural abnormalities typical of a specific genetic defect.

[CAPN3] has 4 structural domains and the p.Ser215_Gly221del is in the first domain within a region making many contacts with a loop from the third domain. Although this specific deletion has not been studied *in silico*, a deletion in this region could theoretically disrupt the interaction between the first and third domains and affect the assembly and activation of the protein. *In-silico* studies on known point mutations in the first domain have suggested a

reduction in the rigidity of this domain and decreased interaction with the third domain, facilitating calpain-3 inactivation.[14] As Vissing *et al.*[8] postulated, it is possible that the mutated calpain-3 could exert a dominant negative effect.

Our study is limited by the lack of objective data for some individuals within each family and a lack of genetic testing of asymptomatic subjects with normal CK levels. We were also unable to test first-order relatives within a family to follow the segregation of the *CAPN3* mutation. Despite this, there were enough affected individuals and findings in family 1 and family 2 with complete information to confirm the AD pattern of inheritance of this mutation.

This study has provided further evidence of the pathogenic dominant effect of the *CAPN3* c.643_663 in-frame deletion and identified no other potentially pathogenic variants in genes so far known to be causative of myopathies, decreasing the likelihood of digenic disease in individuals harboring this specific mutation.

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Abbreviations

AD	autosomal dominant
CAPN3	calpain-3
CAV3	caveolin-3
CK	creatine kinase
CNBP	CCHC-type zinc finger nucleic acid binding protein
del	deletion
DYSF	dysferlin
EMG	electromyography
FKRP	fukutin-related protein
FLNC	filamin C
Gly	glycine
KX	X-linked Kx blood group protein
LGMD	limb-girdle muscular dystrophy
LGMD2A	limb-girdle muscular dystrophy type 2A

LMNA	laminin A
mRNA	messenger RNA
MYOT	myotilin
NGS	next generation sequencing
Ser	serine
SERCA	sarcoplasmic/endoplasmic calcium–ATPase
SGC (A,B, D, G)	sarcoglycan (alpha, beta, delta, gamma)
TTN	titin
WES	whole exome sequencing

References

1. Richard I, Broux O, Allamand V, Fougerousse F, Chiannilkulchai N, Bourg N, et al. Mutations in the proteolytic enzyme calpain 3 cause limb-girdle muscular dystrophy type 2A. Cell. 1995; 81:27–40. [PubMed: 7720071]
2. Huang Y, de Morree A, van Remoortere A, Bushby K, Frants RR, Dunnen JT, et al. Calpain 3 is a modulator of the dysferlin protein complex in skeletal muscle. Hum Mol Genet. 2008; 17:1855–1866. [PubMed: 18334579]
3. Taveau M, Bourg N, Sillon G, Roudaut C, Bartoli M, Richard I. Calpain 3 is activated through autolysis within the active site and lyses sarcomeric and sarcolemmal components. Mol Cell Biol. 2003; 23:9127–9135. [PubMed: 14645524]
4. Toral-Ojeda I, Aldanondo G, Lasa-Elgarresta J, Lasa-Fernandez H, Fernandez-Torron R, Lopez de Munain A, et al. Calpain 3 deficiency affects SERCA expression and function in the skeletal muscle. Exp Rev Mol Med. 2016; 18:1–14.
5. Hauerslev S, Sveen ML, Duno M, Angelini C, Vissing J, Krag TO, et al. Calpain 3 is important for muscle regeneration: evidence from patients with limb girdle muscular dystrophies. BMC Musc Dis. 2012; 13:1–14.
6. Richard I, Hogrel JY, Stockholm G, Payan CA, Fougerousse F. the Calpainopathy Study Group. Natural history of LGMD2A for delineating outcome measures in clinical trials. Ann Clin Transl Neurol. 2016; 3:248–265. [PubMed: 27081656]
7. Vallejo-Illarramendi A, Toral-Ojeda I, Aldanondo G, Lopez de Munain A. Dysregulation of calcium homeostasis in muscular dystrophies. Exp Rev Mol Med. 2014; 16:1–23.
8. Vissing J, Barresi R, Witting N, Van Ghelue M, Gammelgaard L, Bindoff LA, et al. A heterozygous 21-bp deletion in CAPN3 causes dominantly inherited limb girdle muscular dystrophy. Brain. 2016; 139:2154–2163. [PubMed: 27259757]
9. Saenz A, Lopez de Munain A. Dominant LGMD2A: alternative diagnosis of hidden digenism? Brain. 2016; 140:1–3.
10. Vissing J, Duno M. Reply: Dominant LGMD2A: alternative diagnosis or hidden digenism? Brain. 2017; 140:e8. [PubMed: 28137959]
11. Quinn C, Moore SA, Bardakjian TM, Karam C. Clinical reasoning: a 30-year-old man with progressive weakness and atrophy. Neurology. 2016; 87:e227–230. [PubMed: 27821570]
12. Starling A, Schlesinger D, Kok F, Passos-Bueno MR, Vainzof M, Zatz M. A family with McLeod syndrome and calpainopathy with clinically overlapping diseases. Neurology. 2005; 65:1832–1833. [PubMed: 16344536]
13. Gallardo E, Saenz A, Illa I. Limb-girdle muscular dystrophy 2A. Handb Clin Neurol. 2011; 101:97–110. [PubMed: 21496626]

14. Jia Z, Petrounevitch V, Wong A, Moldoveanu T, Davies PL, Elce JS, et al. Mutations in calpain 3 associated with limb girdle muscular dystrophy: analysis by molecular modeling and by mutation in m-calpain. Biophys J. 2001; 80:2590–2596. [PubMed: 11371436]

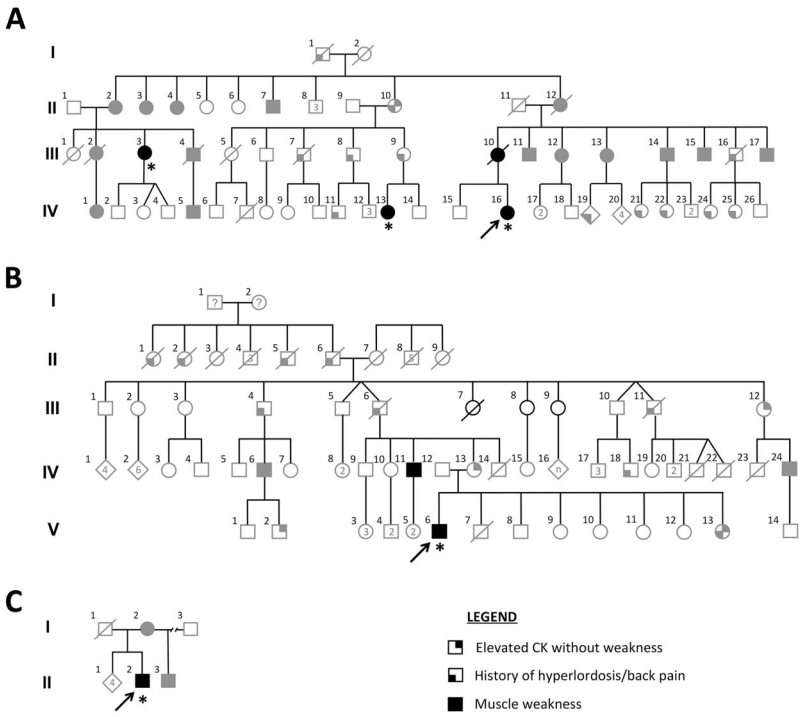

FIGURE 1.

Pedigrees of family 1 **(A)**, family 2 **(B)**, and family 3 **(C)**. Asterisks denote individuals carrying the *CAPN3* mutation. Gray color indicates symptomatic individuals or subjects with elevated CK for whom a neurological evaluation was not available.

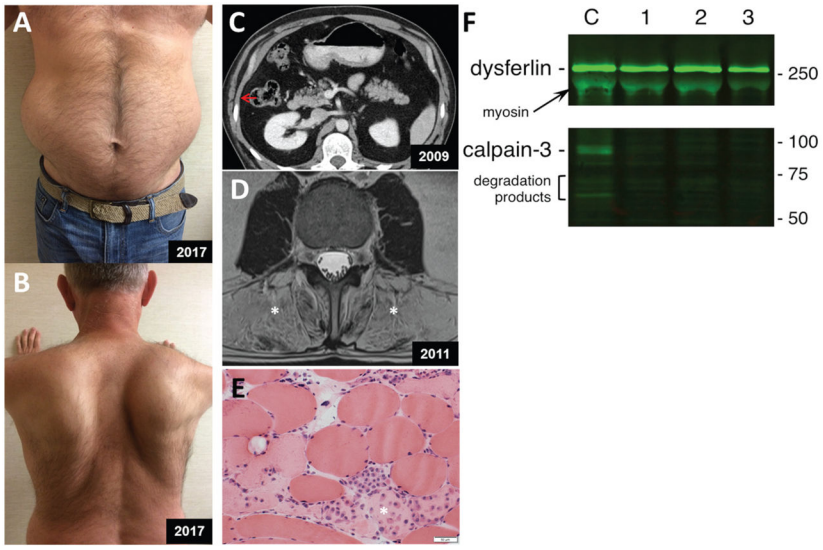

FIGURE 2.

Clinical, radiological, muscle histological, and Western blot findings. Photographs depicting **(A)** abdominal wall muscle weakness and **(B)** asymmetric scapular winging for proband (V-6) in family 2. **(C)** Axial computed tomography image of the abdomen showing oblique muscle atrophy (red arrow) for this proband. **(D)** Axial T2 lumbar spine MRI highlights marked atrophy and fatty replacement of the posterior paraspinal musculature (asterisks) for this proband. **(E)** Triceps muscle biopsy shows small clusters of necrotic fibers (asterisk indicates a necrotic fiber) in subject IV-13 of family 1 (hematoxylin–eosin stain). **(F)** Control muscle (lane C) was compared with the muscle biopsy from subject IV-13 of family 1 (lane 1) and proband muscle biopsies from families 2 and 3 (lanes 2, and 3). Although dysferlin appears normal in size and amount for each affected subject, full-length (94-kd) calpain-3 and calpain-3 degradation products are greatly reduced in each subject.

Table 1

Features of individuals with objective weakness and/or documented creatine kinase elevation.

Individual (age/gender)	Weakness onset (years)	Distribution of weakness*	Myalgia/ LBP†	CK	EMG‡	Muscle biopsy
Family 1						
III-3 (70/F)	60s	Proximal LL	No	2.3–4.7 × Nl	Axial myopathy	ND
III-10 (74/F)	60s	Proximal LL > UL, axial	LBP	Nl–1.9 × Nl	Axial myopathy	ND
IV-13 (57/F)	Mid-50s	Proximal LL > UL	Myalgia	2.4 × Nl	Axial/proximal myopathy	Necrotic/ regenerating fibers; type 1 fiber grouping
IV-16 (71/F)	Mid-60s	Proximal LL	Myalgia, LBP	1.3–8.7 × Nl	Normal	ND
Family 2						
III-12 (adult/F)	—	No	LBP	5.7 × Nl	ND	ND
IV-11 (55/M)	50s	Proximal UL	Myalgia	5.1 × Nl	Normal	Rare atrophic fibers; type 1 fiber grouping
IV-13 (66/F)	—	No	No	2.9–4.2 × Nl	ND	ND
V-2 (adult/M)	—	No	LBP	5.7 × Nl	ND	ND
V-6 (47/M)	Early 40s	Proximal LL > UL, axial	LBP	Nl§ (previously 1.2–6.1 × Nl)	Axial myopathy	Fiber type splitting; rare regenerating fibers; type 1 fiber predominance
Family 3						
II-2 (57/M)	50s	Proximal LL, UL	Myalgia	3.3–6.6 × Nl	Normal	Mild fiber size variation

CK, creatine kinase; EMG, electromyography; F, female; LBP, low back pain; M, male; LL, lower limb; Nl, normal (upper limit); ND, not done; UL, upper limb.

*In lower limbs weakness affected iliopsoas and often gluteus medius and maximus, but thigh adductors and hamstrings were clinically spared.

†Myalgia and low back pain preceded onset of weakness by years.

‡No fibrillation potentials, positive sharp waves or myotonic discharges were recorded in any patient.

§CK values have been persistently normal for the past 7 years. The first CK elevation was documented at age 24 years. At that time he had only low back pain.