Received: 25 May 2023 | Revised: 9 January 2024 | Accepted: 14 January 2024

DOI: 10.1002/mus.28045

CLINICAL RESEARCH SHORT REPORT

MUSCLE&NERVE WILEY

The CAPN3 p.Lys 254del variant is not always associated with dominant CAPN3-related muscular dystrophy

Andrea Valls MSc[1,2] | Gerardo Gutiérrez-Gutiérrez MD[2,3,4] | Agustín Martínez MD[5] | Cristina Ruiz-Roldán MSc[1,2] | Pilar Camaño PhD[1,2,6] | Adolfo López de Munain MD, PhD[1,2,7,8,9] | Amets Sáenz PhD[1,2]

[1]Neurosciences Area, Biodonostia Health Research Institute, San Sebastian, Spain

[2]CIBERNED, CIBER, Spanish Ministry of Science & Innovation, Carlos III Health Institute, Madrid, Spain

[3]Department of Neurology, Hospital Universitario Infanta Sofía, Madrid, Spain

[4]Neuromuscular Diseases Unit, Universidad Europea de Madrid, Madrid, Spain

[5]Hospital Público da Mariña–Burela, Burela, Spain

[6]Molecular Diagnostics Platform, Biodonostia Health Research Institute, San Sebastian, Spain

[7]Department of Neurology, Donostialdea Integrated Health Organisation, Osakidetza, San Sebastian, Spain

[8]Department of Neurosciences, University of the Basque Country UPV-EHU, San Sebastian, Spain

[9]Faculty of Medicine, University of Deusto, Bilbao, Spain

Correspondence
Amets Sáenz, Neurosciences Area, Biodonostia Health Research Institute, P° Dr Begiristain s/n, 20014 San Sebastian, Spain.
Email: amets.saenzpena@osakidetza.eus

Funding information
Instituto de Salud Carlos III (ISCIII), co-funded by the European Union, Grant/Award Number: PI21/00047; Department of Health of the Government of the Basque Country, Grant/Award Number: 2021111022; Center for Networked Biomedical Research on Neurodegenerative Diseases (CIBERNED), Grant/Award Number: CB06/05/1126; GENE (Association of Neuromuscular diseases of Gipuzkoa)

Abstract

Introduction/Aims: Limb-girdle muscular dystrophy R1 (LGMDR1) calpain 3-related usually presents as a recessively transmitted weakness of proximal limb-girdle muscles due to pathogenic variants in the *CAPN3* gene. Pathogenic variants in this gene have also been found in patients with an autosomal dominantly inherited transmission pattern (LGMDD4). The mechanism underlying this difference in transmission patterns has not yet been elucidated. Camptocormia, progressive limb weakness, myalgia, back pain, and increased CK levels are common clinical features associated with dominant forms. The p.Lys254del pathogenic variant was associated with camptocormia in two LGMDD4 families. This study aimed to present carriers found in recessively transmitted LGMDR1 families bearing the p.Lys254del variant that do not show muscle weakness.

Methods: DNA sequencing was performed on exon 5 of *CAPN3* in family members to establish the carrier status of the pathogenic variant. They were evaluated clinically and MRI was performed when available.

Results: Two families presented with the p.Lys254del pathogenic variant in a homozygous or compound heterozygous state. Family members carrying only the pathogenic variant in the heterozygous state did not demonstrate the myopathic

Abbreviations: LGMDD4, limb-girdle muscular dystrophy dominant 4-calpain 3 related; LGMDR1, limb girdle muscular dystrophy recessive 1-calpain 3 related; MRI, magnetic resonance imaging; OSAHS, obstructive sleep apnea hypopnea syndrome.

characteristics described in dominant patients. Camptocormia and other severe clinical symptoms were not observed.

Discussion: We conclude that the p.Lys254del pathogenic variant per se cannot be solely responsible for camptocormia in dominant patients. Other undisclosed factors may regulate the phenotype associated with the dominant inheritance pattern in *CAPN3* pathogenic variant carriers.

KEYWORDS
calpain 3, dominant inheritance pattern, camptocormia, *CANP3*, LGMDD4, LGMDR1

1 | INTRODUCTION

Recessively inherited limb-girdle muscular dystrophy R1 (LGMDRI) calpain 3-related, one of the most common forms of LGMD, is typically associated with progressive weakness of proximal limb-girdle muscles.[1,2] LGMDR1 is secondary to pathogenic variants in *CAPN3*,[3] the gene that encodes calpain-3, which is predominantly expressed in skeletal muscle.[4]

In 2012, Liewluck et al.[5] reported a patient with axial myopathy and camptocormia bearing a pathogenic variant in *CAPN3*. Vissing et al.[6] were the first to identify families with an autosomal dominant inheritance pattern with a single pathogenic variant in *CAPN3* (LGMDD4); however, since then, several studies have described families showing this transmission pattern.[7–13]

Although the reason for the different transmission patterns is still unknown, some authors propose that the only pathogenic variant found in the *CAPN3* gene may have a dominant negative effect, not allowing normal proteins to perform their functions because of the presence of mutated versions of the protein in the muscle fiber.[6,10]

Two studies reporting this inheritance pattern described two patients bearing the p.Lys254del pathogenic variant (c.759_761del) without a second identified pathogenic variant in *CAPN3* who showed camptocormia.[5,13]

In this study, we report two LGMDR1 families, including six carriers of the p.Lys254del pathogenic variant. None of the carriers of this pathogenic variant in the families presented with clinical or radiological characteristics that were previously associated with dominant patients.

2 | METHODS

LGMDR1 patients carrying the p.Lys254del pathogenic variant and their families (Figure 1) were recruited from Hospital Universitario Infanta Sofía (Madrid) and Hospital Público da Mariña–Burela (Lugo). The study was approved by the Ethics Committee of the hospital of origin, and all participants provided written informed consent.

DNA/RNA were extracted from peripheral blood samples according to standard procedures. Amplification of *CAPN3* coding regions was performed as described by Richard et al.[3] We used a previously described protocol for the cDNA analysis.[14] All the LGMDR1 patients from Families A and B underwent clinical and genetic testing several years ago. For this study, serum creatine kinase (CK) analysis of all family members was performed in the hospitals' laboratories, clinical information was obtained from consultations, and for Family A, 1.5 Tesla MRI images of three non-affected relatives were also obtained. MRIs were evaluated by experienced radiologists using the modified Fischer scale.[15]

In Family A, seven healthy family members were genetically analyzed for research purposes when the mutation was implicated in the dominant forms. In contrast, four healthy family members in Family B were analyzed to characterize their carrier status prior to this study for clinical and genetic counseling purposes.

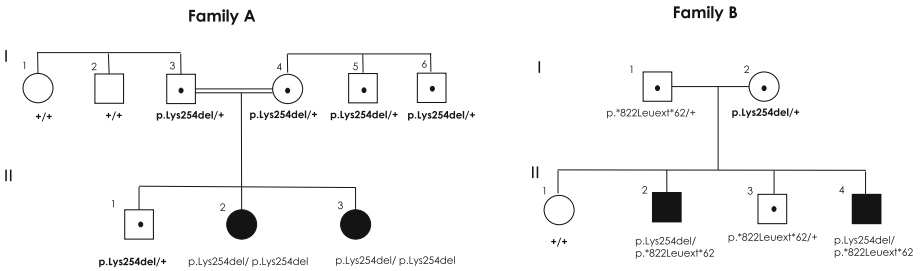

FIGURE 1 Pedigrees of the two families carrying the p.Lys254del pathogenic variant. Circles: Females. Squares: Males. Filled symbols designate affected LGMDR1 patients. Open symbols designate unaffected individuals. Open symbols with a black dot designate p.Lys254del pathological variant carriers. + indicates non-mutated allele and +/+ non-carriers. Double line: consanguineous marriage. Roman numerals indicate generations. Arabic numbers indicate individuals in a given generation. p.Lys254del pathogenic variant carriers are indicated in bold.

3 | RESULTS

In our series of patients with LGMDR1, nine patients from six families carried the p.Lys254del pathogenic variant in a homozygous or compound heterozygous state. However, only two of the six families were available for the study of pathogenic variant carriers.

Family A: The probands were two sisters homozygous for the p.Lys254del pathogenic variant, who were born to consanguineous parents. They showed typical symptoms of LGMDR1: age at onset of 13 years, both nonambulatory (currently 39 and 41 years old), and affected MRI available for one of them (Figure 2). The carrier status was confirmed in the DNA of the consanguineous parents. Five other family members were also analyzed, three of whom carried the pathogenic variant (Figure 1). Age, CK levels, clinical symptoms, and MRI findings are detailed in Table 1.

Family B: In this family, the two affected patients were compound heterozygous for p.Lys254del/p.*822Leuext*62 pathogenic variants. The patients presented with a classic calpainopathy phenotype. Their ages at onset were 7 and 12 years. At the time of the study, they had severe weakness of the pelvic and scapular girdle muscles and were nonambulatory. Healthy family members were evaluated; the patients' mother carried the p.Lys254del pathogenic variant. The oldest sibling did not carry any pathogenic variants, but the father and other sibling were carriers of the p.*822Leuext*62 pathogenic variant (Figure 1 and Table 1).

Finally, in our calpainopathy patients series, we identified two carriers of different pathogenic variants: a patient's mother (p.Gly222Arg/+) and the brother of another patient (p.Arg788Serfs*14/+), both with increased CK levels (244 and 510 U/L, respectively) but without muscle symptoms.

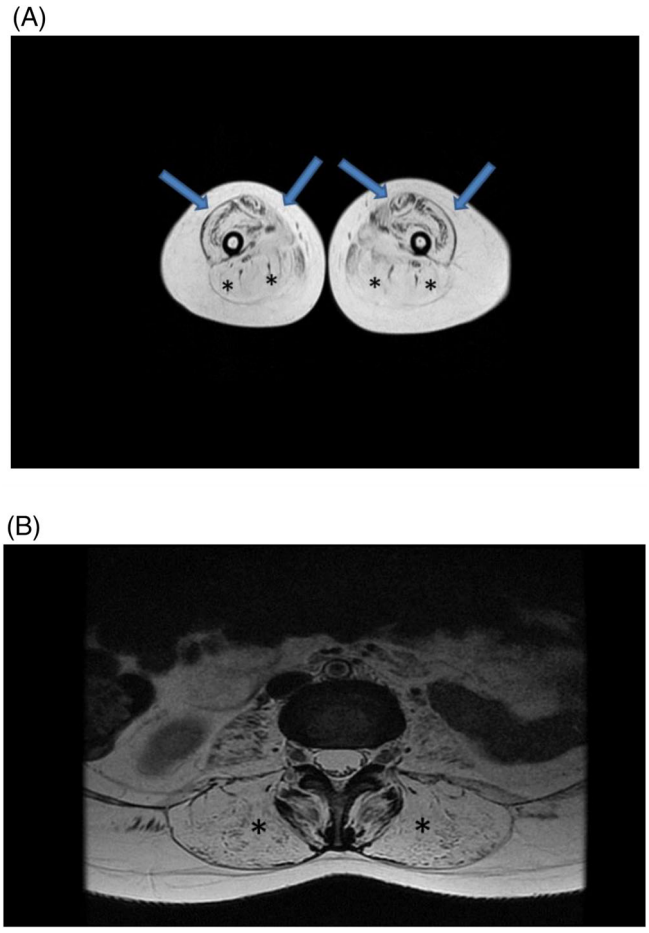

FIGURE 2 MRI of Family A II-2 patient, homozigous for the p.Lys254del pathogenic variant. (A) Axial T1 weighted MRI of the thighs with severe involvement (Fischer grade 3–4) of all anterior thigh muscles (arrows) with a predominance (Fischer grade 4) in posterior muscles (asterisks). (B) Axial T1 weighted MRI of the lumbar spine of the patient. Severe involvement of paraspinal muscles (Fischer grade 4) (asterisks).

4 | DISCUSSION

A large number of families with *CAPN3* variants and an autosomal dominant inheritance pattern have been described,[5–13] confirming the existence of this new calpain 3-related muscular dystrophy form. In other muscular dystrophies, the possibility of a dominant or recessive inheritance pattern associated with pathogenic variants in the same gene has already been described, as in titinopathies or RYR1-related myopathies.[16–20] However, the mechanisms underlying the different inheritance patterns remain unclear for many of them.

Most carrier members of the two families described here were beyond the age of onset of the common symptomatology described for the dominant forms. Thus, the observed lack of muscle symptoms and the normal MRI of the paraspinal muscles ruled out camptocormia as a direct consequence of the p.Lys254del pathogenic variant. Muscle MRI was performed in only two out of five carriers with CK elevation. After clinical evaluation, the neurologist found no muscular weakness in carriers I-5 and I-6; carrier I-2 from Family B moved to a different community, and therefore we could not perform a follow-up MRI.

Moreover, two previously reported p.Lys254del pathogenic variant carriers exhibited phenotypic differences.[5,13] These patients presented with a paravertebral involvement. Although these patients showed similar ages and duration of symptoms, they showed differences in thigh muscle impairment, emphasizing the differences in clinical presentation between the dominant forms due to the same pathogenic variant.

In contrast, *CAPN3* pathogenic variant carriers (healthy relatives of recessive forms bearing only one pathogenic variant) who show no muscle atrophy or severe clinical signs demonstrate that muscle function is not compromised. Therefore, the requirement of a regulatory or modifying factor to induce impairment is supported by this observation. In studies carried out on LGMDD4, genes that cause known muscular dystrophies were ruled out.[7–12] Thus, other genes without clear involvement in muscular dystrophies but encoding proteins with relevant cellular functions, such as metabolism, signal transduction, ion transport, and methylation, should be considered.

Previous studies have suggested that the nature of the pathogenic variant is the most plausible reason for the dominant transmission of

TABLE 1 LGMDR1 patient's and p.Lys254Δ mutation carriers' clinical information.

	Mutation in *CAPN3* gene	Age (years)	CK[a] U/L	Status	Clinical symptoms	MRI
Family A						
I-3	p.Lys254Δ/+	76	981	Carrier	Moderate OSAHS	Normal thighs and paraspinal muscles
I-4[b]	p.Lys254Δ/+	70	85	Carrier	No muscular dystrophy	Normal thighs
I-5	p.Lys254Δ/+	80	233	Carrier	No muscular dystrophy	N.A.
I-6	p.Lys254Δ/+	70	275	Carrier	No muscular dystrophy	N.A.
II-1	p.Lys254Δ/+	44	254	Carrier	No muscular dystrophy	Normal thighs
II-2	p.Lys254Δ/ p.Lys254Δ	41	N.A.	LGMDR1	Proximal muscle weakness	Affected thighs and paraspinal muscles
II-3	p.Lys254Δ/ p.Lys254Δ	39	N.A.	LGMDR1	Proximal muscle weakness	N.A.
Family B						
I-2	p.Lys254Δ/+	66	344	Carrier	No muscular dystrophy	N.A.
II-2	p.Lys254del/p.*822Leuext*62	51	N.A.	LGMDR1	Proximal muscle weakness	N.A.
II-4	p.Lys254del/p.*822Leuext*62	42	N.A.	LGMDR1	Proximal muscle weakness	N.A.

Abbreviations: CK, creatine kinase; N.A., not available; OSAHS, obstructive sleep apnea/hypopnea síndrome.
[a]CK: <171 U/L is considered normal.
[b]Pseudofibromialgia.

calpain 3-associated dystrophies.[6,10] However, proteolytic assays did not identify a dominant-negative functional effect in p.Lys254del mutants[13] and it was considered that the molecular mechanism underlying dominant forms was likely related to haploinsufficiency, which may constitute a risk factor for paravertebral myopathy with aging.

Finally, in recessive muscular dystrophies, such as α-sarcoglycanopathies and dysferlinopathies, carriers can have subclinical findings (elevated CK levels and abnormal MRI findings).[21–23] All p.Lys254del carrier members in the present study had elevated CK levels, except for case I-4 in Family A. This suggests that the increased CK levels are not only due to the p.Lys254del pathogenic variant but also caused by additional factors. In contrast, in our LGMDR1 patient series, we found two carriers with increased CK levels but without muscle symptoms. One of the carriers presented with the p.Arg788Serfs*14 mutation. The consequence of this mutation, at the protein level, is the total absence of calpain 3; therefore, it could not be considered as the cause of a dominant negative effect.

In conclusion, these findings strongly suggest that the p.Lys254del pathogenic variant alone does not generate a clinical phenotype (camptocormia) but may present with asymptomatic hyperCKemia in some individuals, thus requiring additional unknown factors that modulate the phenotype. Further in-depth studies are required to unveil the underlying mechanisms related to the dominant pattern of inheritance and the phenotype of carriers of this *CAPN3* pathogenic variant.

AUTHOR CONTRIBUTIONS

Andrea Valls: Formal analysis; investigation; methodology; writing – review and editing. **Gerardo Gutiérrez-Gutiérrez:** Formal analysis; resources; investigation; methodology; writing – review and editing; supervision. **Agustín Martínez:** Formal analysis; resources; investigation; methodology; writing – review and editing; supervision. **Cristina Ruiz-Roldán:** Investigation; methodology; writing – review and editing; supervision. **Pilar Camaño:** Investigation; methodology; writing – review and editing; supervision. **Adolfo López de Munain:** Resources; funding acquisition; writing – review and editing; supervision. **Amets Sáenz:** Formal analysis; resources; conceptualization; investigation; methodology; funding acquisition; writing – original draft; writing – review and editing; supervision.

ACKNOWLEDGMENTS

The authors would like to thank the patients and their families.

FUNDING INFORMATION

This study has been funded by Instituto de Salud Carlos III (ISCIII) through the project "PI21/00047" and co-funded by the European Union. It has been funded also by Department of Health of the Government of the Basque Country (project ref 2021111022). This work was, in part, supported by the Center for Networked Biomedical Research on Neurodegenerative Diseases (CIBERNED: CB06/05/1126 to Andrea Valls, Pilar Camaño, Adolfo López de Munain, and Amets Sáenz), GENE (Association of Neuromuscular diseases of Gipuzkoa), and Fundación "la Caixa" (Acción Social).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The study was approved by the Ethics Committee of the hospital of origin, and all participants provided written informed consent.

ORCID

Amets Sáenz https://orcid.org/0000-0002-0704-1150

REFERENCES

1. Urtasun M, Sáenz A, Roudaut C, et al. Limb-girdle muscular dystrophy in Guipuzcoa (Basque Country, Spain). *Brain*. 1998;121:1735-1747. doi:10.1093/brain/121.9.1735
2. Fardeau M, Hillaire D, Mignard C, et al. Juvenile limb-girdle muscular dystrophy clinical, histopathological and genetic data from a small community living in the Reunion Island. *Brain*. 1996;119:295-308. doi:10.1093/brain/119.1.295
3. Richard I, Broux O, Allamand V, et al. Mutations in the proteolytic enzyme calpain 3 cause limb-girdle muscular dystrophy type 2A. *Cell*. 1995;81:27-40. doi:10.1016/0092-8674(95)90368-2
4. Sorimachi H, Imajoh-Ohmi S, Emori Y, et al. Molecular cloning of a novel mammalian calcium-dependent protease distinct from both m- and μ-types. Specific expression of the mRNA in skeletal muscle. *J Biol Chem*. 1989;264:20106-20111. doi:10.1016/S0021-9258(19)47225-6
5. Liewluck T, Goodman BP. Late-onset axial myopathy and camptocormia in a calpainopathy carrier. *J Clin Neuromuscul Dis*. 2012;13:209-213. doi:10.1097/CND.0b013e3182461a9c
6. Vissing J, Barresi R, Witting N, et al. A heterozygous 21-bp deletion in CAPN3 causes dominantly inherited limb girdle muscular dystrophy. *Brain*. 2016;139:2154-2163. doi:10.1093/brain/aww133
7. Martinez-Thompson JM, Niu Z, Tracy JA, et al. Autosomal dominant calpainopathy due to heterozygous CAPN3 C.643_663del21. *Muscle Nerve*. 2018;57:679-683. doi:10.1002/mus.25970
8. Nallamilli BRR, Chakravorty S, Kesari A, et al. Genetic landscape and novel disease mechanisms from a large LGMD cohort of 4656 patients. *Ann Clin Transl Neurol*. 2018;5:1574-1587. doi:10.1002/acn3.649
9. Cerino M, Bartoli M, Riccardi F, et al. Autosomal dominant segregation of CAPN3 c.598_612del15 associated with a mild form of calpainopathy. *Ann Clin Transl Neurol*. 2020;7:2538-2540. doi:10.1002/acn3.51193
10. Cerino M, Campana-Salort E, Salvi A, et al. Novel CAPN3 variant associated with an autosomal dominant calpainopathy. *Neuropathol Appl Neurobiol*. 2020;46:564-578. doi:10.1111/nan.12624
11. González-Mera L, Ravenscroft G, Cabrera-Serrano M, et al. Heterozygous CAPN3 missense variants causing autosomal-dominant calpainopathy in seven unrelated families. *Neuropathol Appl Neurobiol*. 2021;47:283-296. doi:10.1111/nan.12663
12. Vissing J, Dahlqvist JR, Roudaut C, et al. A single c.1715G>C calpain 3 gene variant causes dominant calpainopathy with loss of calpain 3 expression and activity. *Hum Mutat*. 2020;41:1507-1513. doi:10.1002/humu.24066
13. Spinazzi M, Poupiot J, Cassereau J, et al. Late-onset camptocormia caused by a heterozygous in-frame CAPN3 deletion. *Neuromuscul Disord*. 2021;31:450-455. doi:10.1016/j.nmd.2021.02.012
14. Blázquez L, Azpitarte M, Sáenz A, et al. Characterization of novel CAPN3 isoforms in white blood cells: an alternative approach for limb-girdle muscular dystrophy 2A diagnosis. *Neurogenetics*. 2008;9:173-182. doi:10.1007/s10048-008-0129-1
15. Fischer D, Kley RA, Strach K, et al. Distinct muscle imaging patterns in myofibrillar myopathies. *Neurology*. 2008;71:758-765. doi:10.1212/01.wnl.0000324927.28817.9b
16. Hackman P, Vihola A, Haravuori H, et al. Tibial muscular dystrophy is a titinopathy caused by mutations in TTN, the gene encoding the giant skeletal-muscle protein titin. *Am J Hum Genet*. 2002;71:240-248. doi:10.1086/342380
17. Hackman JPV, Vihola AK, Udd AB. The role of titin in muscular disorders. *Ann Med*. 2003;35:434-441. doi:10.1080/07853890310012797
18. Udd B, Vihola A, Sarparanta J, Richard I, Hackman P. Titinopathies and extension of the M-line mutation phenotype beyond distal myopathy and LGMD2J. *Neurology*. 2005;64:636-642. doi:10.1212/01.WNL.0000151853.50144.82
19. Klein A, Lillis S, Munteanu I, et al. Clinical and genetic findings in a large cohort of patients with ryanodine receptor 1 gene-associated myopathies. *Hum Mutat*. 2012;33:981-988. doi:10.1002/humu.22056
20. Snoeck M, van Engelen BGM, Küsters B, et al. RYR1-related myopathies: a wide spectrum of phenotypes throughout life. *Eur J Neurol*. 2015;22:1094-1112. doi:10.1111/ene.12713
21. Ben Hamida M, Fardeau M, Attia N. Severe childhood muscular dystrophy affecting both sexes and frequent in Tunisia. *Muscle Nerve*. 1983;6:469-480. doi:10.1002/mus.880060702
22. Fischer D, Aurino S, Nigro V, Schröder R. On symptomatic heterozygous alpha-sarcoglycan gene mutation carriers. *Ann Neurol*. 2003;54:674-678. doi:10.1002/ana.10738
23. Illa I, De Luna N, Domínguez-Perles R, et al. Symptomatic dysferlin gene mutation carriers: characterization of two cases. *Neurology*. 2007;68:1284-1289. doi:10.1212/01.wnl.0000256768.79353.60

How to cite this article: Valls A, Gutiérrez-Gutiérrez G, Martínez A, et al. The CAPN3 p.Lys 254del variant is not always associated with dominant CAPN3-related muscular dystrophy. *Muscle & Nerve*. 2024;69(4):472-476. doi:10.1002/mus.28045