A Roma founder *BIN1* mutation causes a novel phenotype of centronuclear myopathy with rigid spine

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Abstract

Objective

To describe a large series of *BIN1* patients, in which a novel founder mutation in the Roma population of southern Spain has been identified.

Methods

Patients diagnosed with centronuclear myopathy (CNM) at 5 major reference centers for neuromuscular disease in Spain (n = 53) were screened for *BIN1* mutations. Clinical, histologic, radiologic, and genetic features were analyzed.

Results

Eighteen patients from 13 families carried the p.Arg234Cys variant; 16 of them were homozygous for it and 2 had compound heterozygous p.Arg234Cys/p.Arg145Cys mutations. Both *BIN1* variants have only been identified in Roma, causing 100% of CNM in this ethnic group in our cohort. The haplotype analysis confirmed all families are related. In addition to clinical features typical of CNM, such as proximal limb weakness and ophthalmoplegia, most patients in our cohort presented with prominent axial weakness, often associated with rigid spine. Severe fat replacement of paravertebral muscles was demonstrated by muscle imaging. This phenotype seems to be specific to the p.Arg234Cys mutation, not reported in other *BIN1* mutations. Extreme clinical variability was observed in the 2 compound heterozygous patients for the p.Arg234Cys/p.Arg145Cys mutations, from a congenital onset with catastrophic outcome to a late-onset disease. Screening of European Roma controls (n = 758) for the p.Arg234Cys variant identified a carrier frequency of 3.5% among the Spanish Roma.

Conclusion

We have identified a *BIN1* founder Roma mutation associated with a highly specific phenotype, which is, from the present cohort, the main cause of CNM in Spain.

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Glossary

cDNA = complementary DNA; CNM = centronuclear myopathy; DM1 = myotonic dystrophy type I; NGS = next-generation sequencing; SDH = succinate dehydrogenase.

BIN1 (bridging integrator-1) recessive mutations are a rare cause of centronuclear myopathy (CNM), first described in 2007, with 6 reported mutations in 8 families.^{1–5} These patients have a wide range at onset from neonatal to adulthood with usually mild, slowly progressive proximal limb weakness and ophthalmoparesis.³ An abundance of central nuclei and predominance of type I fibers are the typical histopathologic findings and normally establish the diagnosis in conjunction with an appropriate phenotype.^{6,7} Amphiphysin-2, encoded by *BIN1*, is involved in T-tubule formation,⁸ membrane recycling,⁹ and nuclear positioning and shape.¹⁰ The BAR domain of amphiphysin-2 participates in membrane remodeling, curvature sensing, and tubulation.^{11,12}

The Roma population (also known as Romani, Gypsies, or “Gitanos” as they call themselves in Spain) is the most numerous ethnic minority in Europe. The Council of Europe estimates a European population of 11 million (coe.int/en/web/portal/roma/). The Roma originated in northwest India 1,500 years ago. Nomadic, they followed different routes through Persia and into Europe. Social and economic pressures led to gradual fragmentation, generating divergent groups separated by strict rules of endogamy. Common origins resulted in the sharing of ancient disease-causing mutations across geographically dispersed Roma subisolates,^{13,14} with younger disease-causing mutations being restricted to specific Roma groups. A small number of ancestors, subsequent endogamy, and population fissions causing secondary founder effects explain the increased frequency of mendelian disorders in this population. Here, we present a novel Roma founder *BIN1* mutation that represents a major cause of CNM in Spain. Novel clinical, radiologic, and pathologic findings are described.

Methods

Identification of CNM cases

We prospectively and retrospectively selected all cases diagnosed with CNM at 5 major centers in Spain: Hospital Universitario Virgen del Rocío, Sevilla (Andalusia), Hospital 12 de Octubre, Madrid (Madrid), Hospital Bellvitge, Barcelona (Catalonia), Hospital La Fe, Valencia (Valencian Community), and Hospital de Sant Pau, Barcelona (Catalonia). These centers are national reference centers for diagnosis of neuromuscular disease, receiving most muscle biopsies from their reference area in Spain. Clinical and pathology databases were screened. Cases with muscle biopsies showing abundance of central nuclei in the absence of features typical of other muscle disease, in patients with the appropriate phenotype, were given the histopathologic diagnosis of CNM and selected for the study.

Cases were divided in 2 groups according to their clinical presentation. Severe, congenital-onset, myotubular-myopathy-like cases are referred to as “early-onset CNM” and cases with childhood or adult onset are referred to as “late-onset CNM.”

Genetic workup

DNA was extracted from blood or muscle using standard procedures. Patients 1.1 and 8.1 (figure 1) were studied using next-generation sequencing (NGS) (whole-exome sequencing in patient 1.1 and a panel of 256 neuromuscular disease genes including all known CNM genes in patient 8.1) on HiSeq and NextSeq Illumina (San Diego, CA) platforms, respectively. Patients 2.1 and 3.1 were screened by Sanger sequencing of *BIN1* coding exons and intron-exon boundaries. DNA samples from the remaining probands with molecularly unresolved CNM were screened by Sanger sequencing of exons 6 and 8 of *BIN1*. Mutations detected by NGS and the *BIN1* c.700C>T, p.Arg234Cys or c.433C>T, p.Arg145Cys mutations in affected and unaffected relatives of probands were confirmed by Sanger sequencing.

To exclude the presence of variants in other disease-causing genes, a sample from an early-onset patient (6.1) was sent for NGS using the same neuromuscular disease genes panel. Myotonic dystrophy type I (DM1) was excluded in the early-onset case.

Relatedness of the different families was studied by haplotype analysis. Six microsatellite markers spanning 10 Mb (10.1 cM) around *BIN1* (figure 1) were amplified by PCR using fluorescently labeled forward primers and unlabeled reverse primers (primers available on request). The PCR products were analyzed in an Applied Biosystems 3500 Genetic Analyzer (Foster City, CA), and their sizes were estimated by the GeneMapper software version 4.1 using a size standard loaded with each sample.

Screening for the *BIN1* c.700C>T, p.Arg234Cys mutation in a panel of Roma population controls was performed with a custom-designed TaqMan assay with primers ACCTGG CCCCATCCTT (forward) and GATGCTCTGGAACGT GTTGAC (reverse), and reporters ACCTACGCGGCTACA G and ACCTACGCAGCTACAG. Heterozygotes identified by the screening assay were confirmed by Sanger sequencing.

Transcript analysis

To investigate whether the common *BIN1* c.700C>T, p.Arg234Cys variant affects splicing, we sequenced complementary DNA (cDNA) obtained from muscle RNA from 2 patients homozygous for the variant and 2 control muscles. RNA was extracted from frozen muscle using a tissue RNA

Figure 1 *BIN1* families: Pedigrees and haplotype analysis

A conserved haplotype (red) is shared by all families identified among normal controls, which segregates with the p.Arg234Cys mutations. A second haplotype, shared by families 1 and 6 segregates with the p.Arg145Cys mutation (green). Arrow: recombination in family 4. Arrowhead: position of *BIN1* in relation to the markers used for the haplotype analysis.

purification kit (ref. 17200; Norgen, Thorold, ON, Canada). Immediately after, a PrimeScript RT Master Mix kit (ref. RR036A; Takara Bio, Shiga, Japan) was used to obtain cDNA. Primers CAAGCTGGTGGACTACGACA and GATCACC AGCACCACATCAC, annealing at exons 6 and 18, present in all transcripts, were used for sequencing. The amplified transcripts were identified according to the unique expected length of the amplicons and confirmed by Sanger sequencing.

Clinical assessment

Patients and first-degree relatives were assessed by one of the authors. For patients lost to follow-up, clinical information was obtained from medical records.

Muscle histology

Muscle samples were obtained by open biopsy and processed following the standard procedures of the different centers taking part in the study. Hematoxylin & eosin, Gomori trichrome, periodic acid–Schiff, NADH (nicotinamide adenine dinucleotide), succinate dehydrogenase (SDH), cytochrome *c* oxidase (COX)-SDH, and ATPase (adenosine triphosphatase) stains were performed for all samples. Immunohistochemistry for emerin and dystrophin were performed in 4 cases and spectrin in one.

The study was approved by the institutional review board of every center, and all patients signed an informed consent.

Data availability

Anonymized data from this study will be shared by request from qualified investigators.

Results

Fifty-two cases of CNM were identified including 8 cases of early-onset CNM (table e-1, links.lww.com/WNL/A589). Forty-two were molecularly unresolved at the time the study was started and were included in it. (However, the diagnoses shown in table e-1 have been updated at the time of completion of the study.) Fifteen patients with CNM in this series were of known Roma background (Spanish Gypsies),¹⁵ all of them molecularly unresolved at the time of inclusion.

Identification of novel *BIN1* mutations

A novel *BIN1* c.700C>T (NM_139343), p.Arg234Cys variant was identified in 18 patients from 13 families (figure 1). Twelve of these 18 patients were from southern Spain. Sixteen patients were homozygous and 2 were compound heterozygous for the *BIN1* p.Arg234Cys variant and a second novel *BIN1* variant: c.433C>T, p. Arg145Cys. All of the Roma patients in this series had the p.Arg234Cys variant. The remaining 3 patients with the mutations were of unknown ethnic origin and were lost to follow-up.

Evidence of pathogenicity of the *BIN1* p.Arg234Cys and p.Arg145Cys mutations

Both p.Arg234Cys and p.Arg145Cys involve conserved residues across species. Both variants are within the BAR domain of amphiphysin-2, known to be relevant for the function of the protein.^{11,12} GnomAD shows a carrier frequency of 0.0008% (2/244,264) for the p.Arg234Cys variant and 0.0004% (1/246,074) for the p.Arg145Cys variant. Both variants are predicted to be damaging by all in silico tools applied (PROVEAN, PolyPhen-2, SIFT, and MutationTaster).

Cosegregation with the disease of the homozygous state for the *BIN1* p.Arg234Cys variant or compound heterozygous state for the *BIN1* p.Arg234Cys and p.Arg145Cys variants was confirmed in all families.

Application of the American College of Medical Genetics and Genomics criteria for interpretation of genetic variants¹⁶ shows the *BIN1* p.Arg234Cys and p.Arg145Cys variants are likely pathogenic (class 4).

Alternative molecular diagnoses were reasonably excluded by NGS in patients 1.1, 6.1, and 8.1 (see methods), finding no other candidate mutations.

The c.700C>T p.Arg234Cys involves the second to last nucleotide in exon 8. Splicing changes were excluded by muscle cDNA sequencing. Two different previously reported transcripts,¹⁷ corresponding to isoforms 8 and 12, were identified, with isoform 8 being the predominant transcript. No differences were found between patients and controls.

In our series, the *BIN1* p.Arg234Cys mutation causes 34% of all CNM cases (18/52 cases). It is also responsible for 39% (17/44) of late-onset CNM and 12.5% (1/8) of early-onset CNM. These 2 mutations explained 100% of the CNM cases identified in the Roma population in this cohort. *BIN1* mutations are therefore the most frequently identified molecular cause of CNM in our cohort. *MTM1* and *DNM2* mutations follow, being identified in 8 patients (15%) each (table e-1, links.lww.com/WNL/A589).

The *BIN1* p.Arg234Cys mutation is a Roma founder mutation

The analysis of microsatellite markers around the position of the variants shows a common haplotype shared by all affected individuals and 4 of the 5 heterozygous carriers identified among Roma controls from whom DNA was available. This haplotype segregates with the p.Arg234Cys variant, proving a common ancestry. A second haplotype is shared by families 1 and 7 segregating with the *BIN1* p.Arg145Cys variant (figure 1).

Control screening revealed high carrier rates in a Spanish Roma community

For a general Roma population screen, DNA samples from 758 healthy European Roma controls (including 141 samples from Spanish Gypsies) were genotyped for the p.Arg234Cys variant. Five heterozygous carriers were identified, all from Spain, giving a carrier rate of 3.55% among Spanish Gypsies. No homozygotes were identified in the population controls.

A novel clinical phenotype with prominent axial involvement is associated with the p.Arg234Cys *BIN1* mutation

Of the 18 patients carrying the p.Arg234Cys *BIN1* mutation, 17 had a late onset. One early-onset patient presented in the neonatal period and had deceased in the first month of life.

Age at onset in the 17 late-onset patients was variable, from early childhood to 55 years, and severity was mild to moderate. All late-onset patients were ambulant at last examination (age range 10–58 years). The presentation and rate of progression were similar within the late-onset group: patients with childhood and adulthood onset. The most common clinical signs were proximal limb weakness and axial weakness frequently accompanied by prominent and severe rigid spine, involving cervical, dorsal, and lumbar regions (figure 2). Ophthalmoplegia and mild ptosis were present in most patients, upward gaze being more prominently involved than lateral or downward gaze. Neck flexors weakness was the only abnormal sign in patient 7.3 and limitation of upward gaze was the only abnormal sign in patient 2.2 at last examination at ages 22 and 30 years, respectively. These 2 patients are in the mildest end of the severity spectrum, while their siblings are more severely affected, demonstrating intrafamilial variability. None of the patients had mental retardation (table 1).

Six patients had an EMG; 3 of them had abundant myotonic discharges and 2 had clinical myotonia. Four patients had respiratory insufficiency with a restrictive pattern in pulmonary function tests, one of whom required nocturnal non-invasive ventilation. Four patients had an echocardiogram: one patient had mild septal hypertrophy and the other 3 patients were normal.

Muscle imaging reveals a specific pattern of muscle involvement

Limb and/or axial MRI or CT scans from 10 patients were available for review. A consistent pattern was found in all patients homozygous for the p.Arg234Cys mutation. Axial scans show almost complete fat replacement of paravertebral cervical (figure 3, B and C) and dorsal and lumbar (figure 3, E–J) muscles. This finding is in keeping with the prominent axial muscle involvement found on clinical examination. Lower limb muscle imaging demonstrated predominant involvement of the posterior compartment of the thighs and soleus and medial gastrocnemius at the leg level (figure 3, K–N). The described pattern was present in all available muscle scans from patients who were homozygous for the p.Arg234Cys variant. Only patient 1.1, with the compound heterozygous p.Arg234Cys/p.Arg145Cys mutations, diverted from this pattern showing diffuse and severe involvement of all muscles in the thighs and lower legs (figure 3O).

Histopathologic findings: Central nuclei, central oxidative clusters, and central empty spaces are the most common features

Muscle biopsies from 13 patients were available for review (table 2). Central nuclei were present in 6% to 98% of the fibers. In 5 cases (38%), central nuclei were seen infrequently (less than 10% of the fibers). Ten patients (77%) had central areas that were devoid of staining (figure 4, I–P) or filled with mitochondria as demonstrated by increased SDH activity (figure 4L). In 5 cases (38%), these central empty spaces were identified in more than 10% of the fibers. Negativity of the

Figure 2 Rigid spine

(A–C) Cervical rigid spine. (D and E) Dorsal and lumbar rigid spine.

central areas for spectrin and emerin immunostains (figure 4, N and O) demonstrates they are not membrane bound and they do not correspond to nuclei, in keeping with previously reported ultrastructural data.¹⁷ The strong reactivity for oxidative enzymes in the central areas of the fibers is referred to as “central oxidative clusters” in table e-1 (links.lww.com/WNL/A589). Significant fat replacement and endomysial fibrosis was present in most cases (77%).

The histopathologic pattern in the early-onset case (patient 6.1) was indistinguishable from myotubular myopathies caused by *MTM1* mutations, with rounded hypotrophic fibers displaying nuclear chains at the center of the fibers and a characteristic peripheral subsarcolemmal halo devoid of oxidative activity (figure 4, E–H).

Discussion

Herein we report 2 novel pathogenic *BIN1* variants, both confined to Roma, and in particular, to Spanish Gypsies. We demonstrate the novel *BIN1* p.Arg234Cys variant is a Roma founder mutation with a high carrier rate in Spain. Our failure to detect p.Arg234Cys carriers in other European Roma

groups suggests that the mutation is of relatively recent, local origin possibly from the south of Spain (Andalusia) from where it spread north to other areas of the country. The second novel *BIN1* mutation, p.Arg145Cys, was identified in 2 related affected Spanish Gypsy families in the compound heterozygous state with p.Arg234Cys. Its role as another founder mutation, potentially important for European Roma, remains to be established. In our series, *BIN1* mutations are thus the most frequently identified molecular cause of CNM in Spain and account for 100% of Spanish Gypsy patients. Moreover, the *BIN1* p.Arg234Cys, present in 18 patients in this cohort, is by far the most common *BIN1* mutation reported to date.

Although it is not known which function of amphiphysin-2 is affected by the described novel mutations, both are located within the BAR domain of the *BIN1*-encoded protein. The BAR domain is known to be important for the function of the protein, and pathogenic mutations in it have previously been identified and functionally characterized.¹⁷ Our cohort therefore represents a large series of patients with *BIN1* mutations involving a specific functionally important domain of the protein, providing clinical, radiologic, and histopathologic descriptions that contribute to the

Table 1 *BIN1* patients' clinical data

Family	Patient	Age at onset	Proximal limb weakness	Distal limb weakness	Facial weakness	Bulbar weakness	Axial weakness	Rigid spine, dorsolumbar	Rigid spine, cervical	Scoliosis	Ophthalmoparesis	Ptosis	Ankle contractures	Muscle scan	CK, IU	Pulmonary function test
1	1.1	Childhood	Yes	Yes	Yes	No	Yes	No	No	Yes	Yes	Yes	Yes	Yes	70	Restrictive
2	2.1	20 y	Yes	Yes	No	No	Yes	Yes	Yes	No	Yes	Yes	—	Yes	1,900	NP
2	2.2	22 y	No	No	No	No	No	No	No	No	Yes	No	No	No	—	NP
3	3.1	Childhood	Yes	Yes	No	No	Yes	Yes	Yes	No	Yes	Yes	—	No	2,615	NP
4	4.1	29 y	Yes	Yes	Yes	No	Yes	No	Yes	No	Yes	Yes	No	Yes	167	NP
5	5.1	40 y	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	90	Restrictive
5	5.2	Childhood	Yes	—	Yes	—	Yes	—	—	—	No	Yes	Yes	No	—	NP
6	6.1	Congenital	Deceased in the first month of life											No	—	NP
7	7.1	Childhood	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No	No	—	NP
7	7.2	Childhood	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	1,609	NP
7	7.3	Adulthood	No	No	No	No	Yes	No	No	No	No	No	No	No	—	NP
8	8.1	39 y	Yes	No	No	No	Yes	No	No	Yes	Yes	Yes	Yes	Yes	520	Restrictive
8	8.2	30 y	Yes	No	No	No	Yes	No	No	No	No	Yes	Yes	Yes	491	Restrictive
9	9.1	55 y	Yes	—	—	—	Yes	—	—	No	—	—	—	Yes	—	NP
10	10.1	10 y	Yes	Yes	No	No	—	—	—	No	Yes	No	No	No	323	NP
11	11.1	Childhood	Yes	No	No	No	—	—	—	No	No	No	No	No	239	NP
12	12.1	Adulthood	Yes	No	No	No	Yes	Yes	Yes	No	Yes	No	No	No	889	NP
13	13.1	Childhood	Yes	No	No	No	Yes	No	No	No	Yes	No	No	Yes	240	NP

Abbreviations: CK = Creatine kinase; IU = international units; NP = not performed.

Figure 3 *BIN1* patients' muscle imaging

(A) Cervical T1 MRI of a normal control in sagittal view showing normal signal intensity in paravertebral muscles (stars). (B, C, and E–J) Axial muscle scans showing prominent to complete fat replacement in paravertebral muscles at the various levels examined (stars). (B) Cervical T1 MRI, sagittal view, of patient 9.1. (C) Cervical T1 MRI, sagittal view, of patient 9.1. (D) Lumbar T1 MRI of a normal control, sagittal view, showing normal signal intensity of paravertebral muscles (stars). (E) Lumbar T1 MRI, sagittal view, in patient 10.1. (F) Lumbar T1 MRI, sagittal view, of patient 13.1. (G) CT scan at the thoracic level in patient 10.1. (H) CT scan at the thoracic level of patient 13.1. (I) CT scan at the dorsal level, axial view, of patient 5.1. (J) Lumbar MRI, sagittal view, patient 2.1. (K–N) Lower limbs muscle scans showing fat replacement predominantly involving the posterior compartment of the thighs (top pictures) and soleus and medial gastrocnemius in the lower legs (bottom pictures). (K) T1 MRI, patient 5.1; (L) T1 MRI, patient 7.2; (M) CT scan, patient 8.1; (N) CT scan, patient 2.1; (O) muscle T1 MRI of patient 1.1 showing diffuse involvement of thighs and lower legs.

knowledge of the condition and of genotype-phenotype correlations.

Both novel mutations here described involve CpG sites that are susceptible to C>T transition. In addition, positive electrostatic charges at the BAR domain are important for the protein to bind membranes and shape them; therefore, variants removing positive charges would likely be deleterious.⁸ These 2 mechanisms could explain why mutations in the BAR domain preferentially involve arginine residues.

Novel clinical features seen in our patients, such as rigid spine with prominent fat infiltration of axial muscles, may be specific to the mutation, as they do not seem to appear in patients with other *BIN1* mutations. However, it is also possible that they are common to *BIN1* mutations but unnoticed because of a shortage of previous descriptions. Early-onset CNM is most frequently caused by mutations in *MTM1*. Congenital DM1 can have an identical clinical and histopathologic presentation

and is the main differential diagnosis of myotubular myopathy. Only one family with severe early-onset CNM due to *BIN1* mutations has previously been communicated,⁴ and here we report a second severe early-onset case in which compound heterozygosity for *BIN1* p.Arg234Cys/p.Arg145Cys mutations has been identified. Of note, a second patient in our series who had the same compound heterozygous p.Arg234Cys/p.Arg145Cys mutations presented during late childhood with a mild phenotype. This reveals wide clinical variability of these mutations that includes congenital onset with catastrophic outcome and late-onset disease in related patients. Although NGS in both patients and testing for DM1 in the early-onset case have reasonably excluded mutations in other known genes as an alternative cause of this clinical variability, the possibility of a second muscle disease to explain this severe phenotype in patient 6.1 may still be considered.

Myotonia has previously been reported in patients with CNM¹⁸ including *DNM2* mutations,¹⁹ and in a single patient

Table 2 Pathologic data of *BIN1* cases

Patient	Age at biopsy, y	Muscle sample	Fibrosis/ATR	CN, %	IN, %	CA, %	COC	CN clustering	Membrane invagination	RSS	Type I, %	Type I hypotrophy
1.1	22	Biceps	+++/>+++	97	1	2	+++	+++	–	+	90	+
2.1	36	Biceps	++/>+	7	16	15	–+	–	+++	–+	46	–+
3.1	21	Gastrocnemius	++/>–	6	21	<1	–	–	++	+	60	–
4.1	29	Deltoid	+++/>+	98	1	0	+++	++	+	+	99	++
5.1	52	Deltoid	+/>++	75	5	10	+++	+	–	+	95	++
6.1	0	Quadriceps	+/>–	45	0	2	+++	–	–	–	45	+++
7.2	23	Deltoid	++/>–	6	9	10	+++	–+	+	+	86	++
8.1	42	Biceps	+/>–	8	12	0	+	+	–	–	60	++
9.1	65	Deltoid	++/>+++	90	1	3	++	+++	–	–	90	+
10.1	42	—	++/>+++	54	6	23	+++	++	++	+	64	+++
11.1	56	—	++/>++	10	14	13	+	+	+++	+	53	+++
12.1	54	Quadriceps	+/>–	23	5	2	–+	+/>++	–	–	58	+++
13.1	52	Biceps	+++/>+++	45	15	0	+	+	+	–	80	+++

Abbreviations: ATR = adipose tissue replacement; CA = central areas devoid of hematoxylin & eosin staining; CN = central nuclei; COC = central oxidative cluster; IN = internal nuclei (noncentral); RSS = radial sarcoplasmic strands.

with *BIN1* mutations.³ Our observation of clinical and/or electrical myotonia in 3 patients confirms this association, bringing CNM, and *BIN1* mutations in particular, into the differential diagnosis for myotonic disorders.

We have identified a specific pattern of radiologic muscle involvement that may help in the differential diagnosis, with prominent fat infiltration of paravertebral muscles, posterior compartment of the thighs, soleus, and medial gastrocnemius. Limited data are available on the muscle imaging pattern of patients with other *BIN1* mutations to establish whether the pattern described here applies to all *BIN1* mutations or is specific to the p.Arg234Cys mutation.^{2,3,20} Patients with CNM due to *DNM2* mutations present with predominant radiologic involvement of distal muscles, including soleus and gastrocnemius, but at a late stage, there may be some similarities with the pattern we described for *BIN1* with involvement of the posterior compartment of the thighs, soleus, and medial gastrocnemius. However, fat infiltration of paravertebral muscles has not been reported in *DNM2* mutations.²¹

Central nucleation is the histopathologic hallmark of CNM. We have observed great variability in the percentage of fibers with central nuclei, being as low as 6% to 10% in 38% of patients, displaying in some cases an atypical histopathologic pattern that may not be easy to recognize as a CNM (figure 4D). However, central oxidative clusters were, in some patients, more numerous than central nuclei. These central areas represent the space between 2 consecutive nuclei, which is devoid of myofibrils and often occupied with mitochondria. Central empty spaces and central oxidative clusters have been previously reported in

MTM1 mutations.⁷ Ultrastructural images have also demonstrated accumulation of glycogen and mitochondria in the space between 2 nuclei in samples from patients with dominant²⁰ and recessive¹⁷ CNM due to *BIN1* mutations. Aggregates of desmin in central areas of the fibers have previously been observed⁷ and here we show deposition of dystrophin in the same localization, probably in relation to the disorganization of myofibrils that is known to occur around the nuclei.^{2,3} Our findings suggest that central empty spaces and central oxidative clusters are characteristic of CNM and should be included as histopathologic diagnostic criteria, along with the presence of central nuclei. In cases with atypical patterns and scarce central nuclei, they can provide valuable diagnostic clues. Although the different biopsy sites may account for part of the variability observed in the histologic patterns, this variability is still observed among samples from the same muscle (i.e., samples from biceps brachii show central nuclei in 7%–97% of the fibers). Larger numbers of samples from each muscle would be necessary to conclude to what extent variability is the result of different biopsy sites.

The histopathologic variability observed here supports the idea previously discussed by other authors who consider the condition as a continuum with other congenital myopathies, such as central core disease and congenital fiber-type disproportion, from the observation of type 1 fiber atrophy, or cores in the *RYR1* or *TTN* mutation.²² Moreover, the abundance of fibrosis and fat replacement observed in our cohort overlaps with features typical of a muscular dystrophy.

Geographically dispersed and socially and linguistically divergent Roma groups share unique mendelian disorders and

Figure 4 Histopathologic findings in *BIN1* patients

(A–C) Typical centronuclear myopathy histopathologic pattern with frequent central nuclei, some forming clusters and endomysial fibrosis with fat replacement. (D) Atypical case with infrequent central nuclei, few internal nuclei, and occasional central vacuoles. (E–H) Congenital-onset case with myotubular features showing central nuclei (E), central accumulations of glycogen (F), a peripheral rim devoid of oxidative activity (G), and type I hypotrophy (H). (I–P) Serial sections of the same areas stained with different techniques to demonstrate frequent central areas devoid of H&E staining (I), not containing glycogen (J), or myofibrils (K). These areas show a strong reactivity to SHD (L) and NADH (M), and they are negative to spectrin (N) and emerin (O) immunostains. Dystrophin immunoreactive material is present in central areas. (A) Patient 1.1, biceps (H&E). (B) Patient 9.1, deltoid (H&E). (C) Patient 13.1, biceps (H&E). (D) Patient 2.1, biceps (H&E). (E–H) Patient 6.1, quadriceps (E: H&E; F: PAS; G: NADH; H: ATPase 4.6). (I–P) Patient 7.2, deltoid (I: H&E; J: PAS; K: ATPase 9.4; L: SDH; M: NADH; N: spectrin; O: emerin; P: dystrophin C-terminal domain). ATPase = adenosine triphosphatase; H&E = hematoxylin & eosin; NADH = nicotinamide adenine dinucleotide; PAS = periodic acid–Schiff; SDH = succinate dehydrogenase.

founder mutations: *CHRNE* 1267delG and *NDRG1* R148X (causing congenital myasthenia and Lom neuropathy, respectively) are common to Roma groups throughout Europe, demonstrating a common origin and founder effect.²³ Other mutations, such as *CTDP1* IVS6+389C→T, causing congenital cataracts, facial dysmorphism, and neuropathy are restricted to a few Balkan Roma groups, with haplotype studies suggesting a younger age of the mutation.¹⁴ The restricted presence of the *BIN1* mutation p.Arg234Cys in Spanish Gypsies in context with their migrational history, suggests that the mutation might have originated during the settlement of the Roma in Spain, dating to the 15th century, although alternative scenarios of losing the mutation by drift in other Roma groups may not be discarded. Identifying common founder mutations greatly facilitates the molecular diagnosis of rare diseases. This has implications for

establishing carrier screening, prenatal diagnosis, and counseling. The high carrier rate detected among the Spanish Gypsies, and the fact that the p.Arg234Cys mutation causes 100% of CNM cases in this population, suggests genetic counseling, prenatal diagnosis, and carrier screening programs would be feasible, increasing the number of diagnoses and allowing prevention strategies.

The identification of a common founder *BIN1* mutation in the Roma population allowed us to describe a large series of *BIN1* patients, providing detailed clinical characterization of the condition and identifying of a novel CNM phenotype.

Author contributions

M.C.-S.: study concept, acquisition of data, analysis and interpretation, draft of the manuscript. F.M.: acquisition of data,

analysis and interpretation, composition of tables and figures. V.B.: acquisition of data, analysis and interpretation. E.R.: acquisition of data, analysis and interpretation. B.M.: acquisition of data, analysis and interpretation. A.H.-L.: acquisition of data, analysis and interpretation. M.O.: acquisition of data, analysis and interpretation. N.M.: acquisition of data, analysis and interpretation. E.K.: acquisition of data, analysis and interpretation. A.C.: acquisition of data, analysis and interpretation. P.Q.: acquisition of data, analysis and interpretation. J.D.-M.: acquisition of data, analysis and interpretation. M.D.: analysis and interpretation. R.Á.: analysis and interpretation. C.D.: analysis and interpretation. N.B.R.: analysis and interpretation. J.J.V.: analysis and interpretation. D.C.: acquisition of data, critical revision of the manuscript for important intellectual content. N.G.L.: analysis and interpretation, critical revision of the manuscript for important intellectual content. J.L.: critical revision of the manuscript for important intellectual content. L.K.: analysis and interpretation, critical revision of the manuscript for important intellectual content. C.P.: study concept, acquisition of data, critical revision of the manuscript for important intellectual content and study supervision.

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Disclosure

The authors report no disclosures relevant to the manuscript. Go to Neurology.org/N for full disclosures.

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References

- Bohm J, Vasli N, Maurer M, et al. Altered splicing of the BIN1 muscle-specific exon in humans and dogs with highly progressive centronuclear myopathy. *PLoS Genet* 2013; 9:e1003430.
- Bohm J, Yis U, Ortac R, et al. Case report of intrafamilial variability in autosomal recessive centronuclear myopathy associated to a novel BIN1 stop mutation. *Orphanet J Rare Dis* 2010;5:35.
- Claeys KG, Maissonobe T, Bohm J, et al. Phenotype of a patient with recessive centronuclear myopathy and a novel BIN1 mutation. *Neurology* 2010;74:519–521.
- Nicot AS, Toussaint A, Tosch V, et al. Mutations in amphiphysin 2 (BIN1) disrupt interaction with dynamin 2 and cause autosomal recessive centronuclear myopathy. *Nat Genet* 2007;39:1134–1139.
- Mejaddam AY, Nennesmo I, Sejersen T. Severe phenotype of a patient with autosomal recessive centronuclear myopathy due to a BIN1 mutation. *Acta Myol* 2009;28: 91–93.
- Fattori F, Maggi L, Bruno C, et al. Centronuclear myopathies: genotype-phenotype correlation and frequency of defined genetic forms in an Italian cohort. *J Neurol* 2015; 262:1728–1740.
- Romero NB, Bitoun M. Centronuclear myopathies. *Semin Pediatr Neurol* 2011;18: 250–256.
- Wu T, Shi Z, Baumgart T. Mutations in BIN1 associated with centronuclear myopathy disrupt membrane remodeling by affecting protein density and oligomerization. *PLoS One* 2014;9:e93060.
- Prokic I, Cowling BS, Laporte J. Amphiphysin 2 (BIN1) in physiology and diseases. *J Mol Med* 2014;92:453–463.
- D'Alessandro M, Hnia K, Gache V, et al. Amphiphysin 2 orchestrates nucleus positioning and shape by linking the nuclear envelope to the actin and microtubule cytoskeleton. *Dev Cell* 2015;35:186–198.
- Frost A, Unger VM, De Camilli P. The BAR domain superfamily: membrane-molding macromolecules. *Cell* 2009;137:191–196.
- Ren G, Vajjhala P, Lee JS, Winsor B, Munn AL. The BAR domain proteins: molding membranes in fission, fusion, and phagy. *Microbiol Mol Biol Rev* 2006;70:37–120.
- Kalaydjieva L, Morar B, Chaix R, Tang H. A newly discovered founder population: the Roma/Gypsies. *Bioessays* 2005;27:1084–1094.
- Morar B, Gresham D, Angelicheva D, et al. Mutation history of the Roma/Gypsies. *Am J Hum Genet* 2004;75:596–609.
- Gresham D, Morar B, Underhill PA, et al. Origins and divergence of the Roma (Gypsies). *Am J Hum Genet* 2001;69:1314–1331.
- Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;17:405–424.
- Toussaint A, Cowling BS, Hnia K, et al. Defects in amphiphysin 2 (BIN1) and triads in several forms of centronuclear myopathies. *Acta Neuropathol* 2011;121:253–266.
- Gil-Peralta A, Rafel E, Bautista J, Alberca R. Myotonia in centronuclear myopathy. *J Neurol Neurosurg Psychiatry* 1978;41:1102–1108.
- Dabby R, Sadeh M, Gilad R, Jurkat-Rott K, Lehmann-Horn F, Leshinsky-Silver E. Myotonia in DNM2-related centronuclear myopathy. *J Neural Transm* 2014;121: 549–553.
- Bohm J, Biancalana V, Malfatti E, et al. Adult-onset autosomal dominant centronuclear myopathy due to BIN1 mutations. *Brain* 2014;137:3160–3170.
- Fischer D, Herasse M, Bitoun M, et al. Characterization of the muscle involvement in dynamin 2-related centronuclear myopathy. *Brain* 2006;129:1463–1469.
- Jungbluth H, Gautel M. Pathogenic mechanisms in centronuclear myopathies. *Front Aging Neurosci* 2014;6:339.
- Mendizabal I, Lao O, Marigorta UM, Kayser M, Comas D. Implications of population history of European Romani on genetic susceptibility to disease. *Hum Hered* 2013;76: 194–200.