




ORIGINAL ARTICLE

A founder variant in the *RYR1* gene is associated with hyperCKemia, myalgia and muscle cramps

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Abstract

Background and purpose: Pathogenic variants in the *RYR1* gene have been associated with a variety of conditions, ranging from congenital myopathy to adult manifestations. Our aim was to characterize the p.Leu2286Val variant in 17 Basque patients, to accurately determine its correlation with clinical features and to explore the possible founder effect of the variant.

Methods: Families harbouring the p.Leu2286Val *RYR1* variant underwent a detailed clinical evaluation, including muscle magnetic resonance imaging, electromyography and muscle biopsy. Haplotypes were analysed in available patients and their relatives.

Results: Individuals carrying the p.Leu2286Val shared a common haplotype, suggesting a founder event in the Basque Country population. The most prevalent features were exertional myalgia, high creatine kinase (CK) levels, cramps and muscle hypertrophy. None of the patients carrying only the p.Leu2286Val showed progression to severe muscle weakness and muscle magnetic resonance imaging showed a heterogeneous muscle involvement. Muscle biopsy revealed non-specific findings in two patients and features associated with central core disease in one patient carrying only the p.Leu2286Val and two patients harbouring an additional *RYR1* variant. Three individuals carrying an in *trans* *RYR1* variant presented with an earlier onset and more severe phenotype.

Conclusion: Here, it is shown that the dominantly inherited p.Leu2286Val *RYR1* founder variant is associated with a milder phenotype of exercise intolerance, myalgia and hyperCKemia.

KEYWORDS

exercise intolerance, hyperCKemia, myalgia, *RYR1*-related myopathies

For affiliations refer to page 7.

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INTRODUCTION

Pathogenic variants in the *RYR1* gene have been associated with a variety of conditions and represent the most common cause of congenital myopathies [1–3]. The *RYR1* gene encodes the skeletal muscle type 1 ryanodine receptor (RyR1), a homotetrameric calcium channel located in the sarcoplasmic reticulum that plays an essential role in excitation–contraction coupling [4].

Pathogenic variants in the *RYR1* gene can result in dysregulation of channel function, leading to alterations in calcium homeostasis and abnormalities in skeletal muscle contraction. Both autosomal dominant and recessive modes of inheritance have been described in *RYR1*-related myopathies (*RYR1*-RM). Dominant pathogenic variants are usually clustered in three hotspot regions of *RYR1* (hotspot 1, exons 2–19; hotspot 2, exons 39–47; and hotspot 3, exons 85–104) [5, 6] and are mostly associated with central core disease (CCD), characterized by the presence of central cores on muscle biopsy and malignant hyperthermia susceptibility (MHS) [7], a genetic predisposition syndrome triggered by exposure to volatile halogenated anaesthetics. In contrast, recessive variants appear to be distributed throughout the gene and are most commonly associated with more severe clinical presentations, such as multiminicore disease [8], centronuclear myopathy [9] and congenital fibre-type disproportion [10], as well as phenotypes involving ophthalmoplegia and facial weakness.

In recent decades, the phenotypic spectrum of *RYR1*-RM has broadened considerably, ranging from severe congenital myopathies to late-onset manifestations [11–13]. Until 2013, the occurrence of MHS and exercise-induced rhabdomyolysis episodes in patients with heterozygous pathogenic *RYR1* variants was sporadically reported [14–17]. In 2013, Dlamini et al. identified a pathogenic *RYR1* variant in 35% of families with exertional rhabdomyolysis (ERM) or myalgia without MHS and muscle weakness, thus highlighting the association of *RYR1* variants with myalgia and ERM [11]. In a genetic diagnostic setting, the large size of the *RYR1* gene, the high proportion of variants of uncertain significance (VUS) and the broad clinical spectrum of *RYR1*-RM make the interpretation of rare *RYR1* variants challenging.

In the present study, a novel *RYR1* founder variant (p.Leu2286Val) is described in 17 Basque individuals who presented with myalgia, muscle cramps, muscle hypertrophy and elevated serum creatine kinase (CK) levels with minimal associated muscle weakness. The variant was found in a heterozygous state in all patients; however, three of them carried another *trans* *RYR1* variant. The individuals with p.Leu2286Val shared the same haplotype in the *RYR1* region, suggesting a single founder event in the Basque Country population.

MATERIALS AND METHODS

Clinical assessment

A total of 17 patients, 11 males and six females, from seven unrelated families with the p.Leu2286Val variant were included in the study. None of the families reported consanguinity. Since

Basque surnames are based on the Basque language, they are unequivocally recognizable; moreover, knowledge about the surnames of grandparents and often great-grandparents is common. Individuals with Basque origin were defined as those with at least one Basque surname, which was the case for all individuals carrying the p.Leu2286Val variant. This study was approved by the ethics committee of Hospital de la Santa Creu i Sant Pau (Barcelona, Spain) (REF: IIBSP-MIO-2022-120). Written informed consent was obtained from the participants. Clinical assessment was performed on unaffected relatives when possible.

Clinical evaluation was performed in three university hospitals located in San Sebastian (families 1–5), Pamplona (family 6) and Bilbao (family 7) (Spain). The clinical assessment included a detailed neurological examination by an expert neurologist. Serum CK levels were measured in all index cases and available relatives. Muscle magnetic resonance imaging (MRI) and electromyography (EMG) were performed in all index cases. Six patients (1.II.4, 3.II.1, 4.II.1, 5.III.1, 6.II.1 and 7.I.2) underwent muscle biopsy and routine histochemical studies as part of the diagnostic process.

Genetic studies

DNA samples extracted from peripheral blood of patients 1.II.4, 2.II.1, 3.II.1 and 4.II.1 were sequenced using a neuromuscular disease gene panel (including 138 genes, Table S1) (Nonacus, Birmingham, UK) at the Hospital de la Santa Creu i Sant Pau (Barcelona, Spain). Patient 4.II.1 also underwent custom clinical exome sequencing, with custom designed probes against genes associated with human disease (Roche, MA, USA). Patients 5.II.2, 6.II.1 and 7.I.2 were enrolled in the international MYO-SEQ project, and whole-exome sequencing was performed at the Broad Institute Genomics Platform, as previously described [18]. Variants in coding regions and splice-site regions were selected when the read depth was greater than 30x and the minor allele frequency was less than 0.5% in population allele frequency databases. Data were examined on the *datagenomics* (<https://datagenomics.es/>) and *seqr* (<https://seqr.broadinstitute.org/>) platforms, respectively. Variants were classified according to the guidelines of the American College of Medical Genetics (ACMG) [19]. *RYR1* variants were annotated in the canonical transcript NM_000540.3 and the human reference genome hg19. The c.6856C>G *RYR1* (p.Leu2286Val) variant was validated by Sanger sequencing in all index cases, and segregation analysis was performed in five families (families 1 and 4–7) (primers detailed in Table S2).

The haplotype background of the p.Leu2286Val variant was explored by genotyping the rs2228072, rs2071088, rs111227660, rs143752962, rs141905132 and rs11541756 single nucleotide polymorphisms (SNPs), which are all within the *RYR1* gene except for rs11541756 and span 147.8kb (Figure 1b) and are thus expected to be associated with p.Leu2286Val. These SNPs were selected because most patients were heterozygous according to their next-generation sequencing data. SNP genotypes were determined by Sanger sequencing in all patients and available relatives (primers

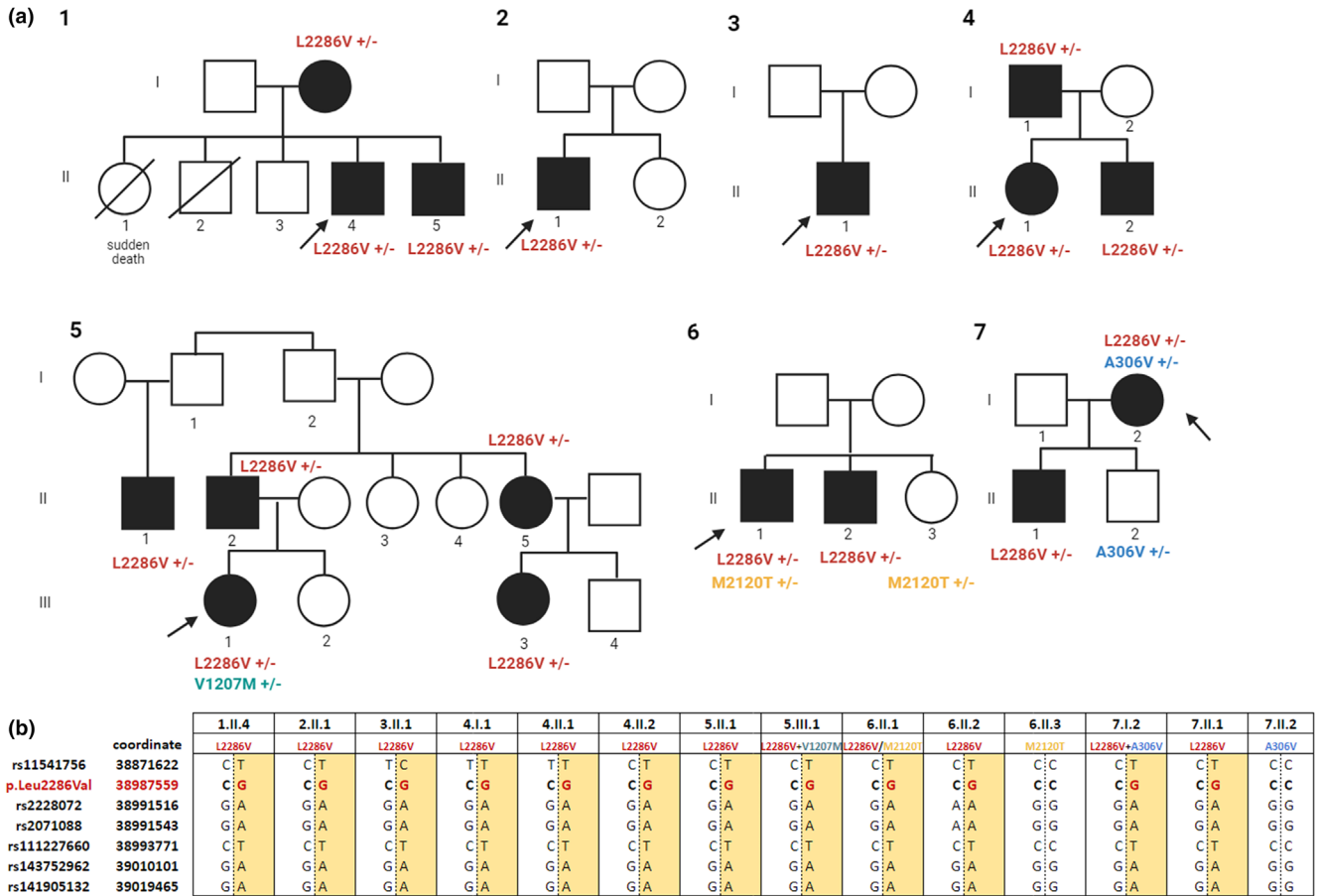


FIGURE 1 Seventeen individuals with the RYR1 p.Leu2286Val sharing a common haplotype. (a) Pedigrees of the seven families with p.Leu2286Val RYR1 (NM_000540.3:c.6856C>G). Arrows indicate index cases in each family. In families 5, 6 and 7, an *in trans* RYR1 variant with the p.Leu2286Val was detected (c.3619G>A, p.Val1207Met in 5.II.1, c.6359T>C, p.Met2120Thr in 6.II.1 and c.917C>T, p.Ala306Val in 7.I.2). (b) The genotype of index cases and available relatives of six selected SNPs spanning 147.8 kb. p.Leu2286Val-associated haplotype is highlighted in yellow.

detailed in Table S2). These SNPs were also analysed from the next-generation sequencing data of 62 Basque and 200 non-Basque control individuals to assess the prevalence of the p.Leu2286Val-associated haplotype in the general population.

RESULTS

The p.Leu2286Val variant is shared by 17 individuals of Basque origin

Through a neuromuscular gene panel, the RYR1 chr19:38987559C>G, c.6856C>G (p.Leu2286Val) variant was detected in heterozygosity in four index cases (1.II.4, 2.II.1, 3.II.1 and 4.II.1), one of which was previously reported (4.II.1) [20]. As these individuals were all from the same geographical area in the Basque Country and the variant was not present in any other individual from an in-house database (Hospital de la Santa Creu i Sant Pau, n=3,179), a founder event was suspected. The p.Leu2286Val variant has also been reported in three index cases by the MYO-SEQ consortium [18], all of them

with Basque origin (5.II.2, 6.II.1 and 7.I.2) who were subsequently included in the present study. In gnomAD v4.1.0, only one individual was found with the p.Leu2286Val in the heterozygous state (allele frequency 0.000001592), and the variant was absent from the Broad Institute in-house database (n=26,164 whole-exome sequencing samples). In total, the p.Leu2286Val variant was detected in 17 individuals from seven Basque families, including seven index cases and ten relatives (Figure 1a).

According to ACMG guidelines, the p.Leu2286Val variant was initially classified as a VUS (PP3, PM1 and PM3). This variant was absent in general population databases, and *in silico* algorithms supported its pathogenicity (SIFT, deleterious; PolyPhen2, deleterious; MutationTaster, deleterious; CADD score, 24.2; Alpha Missense, pathogenic). The L2286 residue is evolutionarily conserved in up to 10 species, and it is located in the MHS/CCD hotspot 2 of the RYR1 gene. Segregation analysis in families 1, 4, 5, 6 and 7 revealed an association between the p.Leu2286Val variant and elevated CK levels, suggesting an autosomal dominant inheritance pattern (Figure 1a, Table 1). Thus, the p.Leu2286Val variant was reclassified as likely pathogenic. However, regarding MHS,

TABLE 1 Clinical presentation of the patients carrying only the RYR1 p.Leu2286Val founder variant.

Family	Patient	Sex	Age of onset	Present age	High CK	Range CK levels	Myalgia	ERM	Cramps	Muscle hypertrophy	Muscle weakness	Observations
1	I.2	F	-	79	No	215–242	No	No	No	No	No	
1	II.4*	M	32	46	Yes	500–1000	Yes	No	Yes	Yes	No	
1	II.5	M	-	47	Yes	539	No	No	No	No	No	
2	II.1*	M	53	58	Yes	14,000	Yes	No	No	Yes	No	
3	II.1*	M	42	45	Yes	500–11,000	Yes	Yes	Yes	No	No	
4	I.1	M	-	78	Yes	800	Yes	No	Yes	No	Asymmetrical distal weakness	
4	II.1*	F	43	50	Yes	700	Yes	No	No	No	Severe, proximal and distal	Concurrent diagnosis of ALS
4	II.2	M	-	48	Yes	375–500	No	No	No	No	No	
5	II.1	M	-	48	Yes	1,300–1,500	No	No	No	No	No	
5	II.2	M	-	71	No	270	No	No	No	No	No	
5	II.5	F	-	64	Yes	400–500	No	No	No	No	No	
5	III.3	F	-	24	No	117	No	No	No	No	No	
6	II.2	M	-	62	Yes	600–900	Yes	No	No	No	No	
7	II.1	M	-	50	No	180	Yes	No	No	Yes	No	

Note: Index cases are indicated with an asterisk. Index cases of families 5–7 harbouring an in trans RYR1 variant are detailed in [Table S5](#). Patient 4.II.1 is highlighted in italics because it was excluded from the analysis due to a concomitant diagnosis of ALS.

Abbreviations: ALS, amyotrophic lateral sclerosis; CK, creatine kinase; ERM, exertional rhabdomyolysis.

the p.Leu2286Val variant is classified as a VUS according to the European Malignant Hyperthermia Group guidelines and ClinGen criteria (PSb and PPb) [21].

Patients 5.III.1, 6.II.1 and 7.I.2 harboured a second RYR1 variant classified as a VUS (p.Val1207Met, p.Met2120Tyr and p.Ala306Val, respectively) in *trans* with the p.Leu2286Val variant (Figure 1a). The p.Val1207Met (5.III.1) and p.Met2120Thr (6.II.1) variants have been reported in patients with RYR1-recessive myopathy [22], whereas p.Ala306Val (7.I.2) has not been previously described in RYR1-RM. Pathogenic and likely pathogenic variants in metabolic myopathy genes were excluded through exome sequencing in families 4–7 (Table S3).

Dominant RYR1 variants associated with ERM and myalgia were collected from the literature to study their localization in the RYR1 gene (Table S4, Figure S1).

Haplotype analysis supporting a founder event in the Basque Country

To investigate the ancestral origin of the p.Leu2286Val allele in Basque families, the genotype of six SNPs in the RYR1 and PSMD8 loci (both located in 19q13.2) were analysed in all available patients and family members ($n=14$) (Figure 1b). All individuals with p.Leu2286Val shared the same haplotype, spanning 147.8 kb, whereas individuals 6.II.3 and 7.II.2 without p.Leu2286Val had a different haplotype (Figure 1b).

The p.Leu2286Val-associated haplotype was analysed in control individuals with Basque origin ($n=62$) and Spanish individuals with non-Basque origin ($n=200$). The disease-associated haplotype without p.Leu2286Val was present in 1/124 Basque chromosomes and 6/400 non-Basque chromosomes. Thus, the p.Leu2286Val-associated haplotype represents an uncommon haplotype in general Basque and non-Basque populations, suggesting a common ancestry of p.Leu2286Val patients.

Clinical manifestations

Seventeen individuals from seven unrelated families were identified to carry the p.Leu2286Val variant in RYR1. Segregation analysis in families 1, 4, 5, 6 and 7 allowed the identification of relatives with variable symptoms ranging from asymptomatic (1.I.2, 5.II.2 and 5.III.3), isolated hyperCKemia with or without myalgia (1.II.5, 4.II.2, 5.II.1, 5.II.5 and 6.II.2) to mild muscle weakness (4.I.1, 5.III.1), highlighting intrafamilial clinical heterogeneity. To explore the clinical presentation associated with p.Leu2286Val, patients carrying an additional in *trans* RYR1 variant (5.III.1, 6.II.1 and 7.I.2) and patient 4.II.1, who has a concurrent diagnosis of amyotrophic lateral sclerosis (ALS), were excluded from further analysis.

The presence of myalgia with disabling pain over time (6/13), muscle cramps (3/13) and gastrocnemius hypertrophy (3/13) were

the most prominent clinical features of individuals only carrying the p.Leu2286Val RYR1. High serum CK levels were present in most patients (9/13), although they were variable ranging from normal levels to 700–14,000 IU/L (Table 1). Only patient 3.II.1 experienced a single episode of rhabdomyolysis after exercise and also presented elevated aspartate transaminase and alanine transaminase levels. The first symptoms in patient 1.II.4 were triggered by statin treatment and persisted after discontinuation. None of the patients showed progression to severe weakness or disability (defined by the use of walking aids), except for patient 4.II.1 because of the concomitant presence of ALS. A metabolic myopathy was suspected in the majority of the index cases. Individuals carrying only the p.Leu2286Val variant exhibited normal strength except patient 4.I.1 who presented mild asymmetrical distal weakness (Table 1). Nonetheless, patients 5.III.1, 6.II.1 and 7.I.2 with an additional RYR1 variant in compound heterozygosity with p.Leu2285Val exhibited mild-moderate muscle weakness and an earlier disease onset (Table S5).

The p.Leu2286Val variant is located in one of the MHS/CCD hotspot regions. However, none of the patients was previously exposed to halogenated anaesthetics or reported a family history of malignant hyperthermia except for the sudden death of 1.II.1, although the presence of the variant was not genetically confirmed. No fresh muscle biopsies were available to perform the in vitro contracture testing to assess MHS induced by p.Leu2286Val.

Muscle imaging was performed in eight individuals only carrying the p.Leu2286Val RYR1 (Figure 2). Four individuals showed no muscle fat infiltration (4.II.2, 5.II.1, 6.II.2 and 7.II.1). The remaining patients had a heterogeneous involvement, although some common patterns were identified with involvement of the medial gastrocnemius (3/8), soleus (1/8), tibialis anterior (1/8), posterior compartment of the thigh (2/8) and gluteus minimus (3/8) (Figure 2). Furthermore, a hypertrophic pattern, including iliacus and thigh hypertrophy, was identified in most patients (6/8) (Figure 2b,c). Cases with an additional in *trans* RYR1 variant (5.III.1, 6.II.1 and 7.I.2) were more severely affected on muscle imaging (Figure S2). EMG showed a myopathic pattern in two patients (3.II.1 and 7.I.2) and a normal pattern in six patients (1.II.4, 2.II.1, 5.II.1, 5.III.1, 6.II.1 and 7.II.1).

Muscle biopsies were performed on five individuals, three of them with compound heterozygous RYR1 variants (5.III.1, 6.II.1 and 7.I.2, Figure S3) and two in patients carrying only the p.Leu2286Val (1.II.4 and 3.II.1, Figure 3). None of them had abnormalities suggesting a glycogen, lipid or mitochondrial disorder. In p.Leu2286Val patients, muscle biopsy showed non-specific findings (1.II.4, Figure 3a), with predominance of type I fibres, increased central nuclei and ring fibres, or was normal (3.II.1, Figure 3b). Individuals carrying biallelic variants showed features associated with CCD or minicore disease (Figure S3), except for patient 6.II.1 whose biopsy was normal (data not shown). The ultrastructural study in patient 7.I.2 revealed areas of myofibril disorganization, Z-line streaming and a scarcity or absence of mitochondria (Figure S3c).

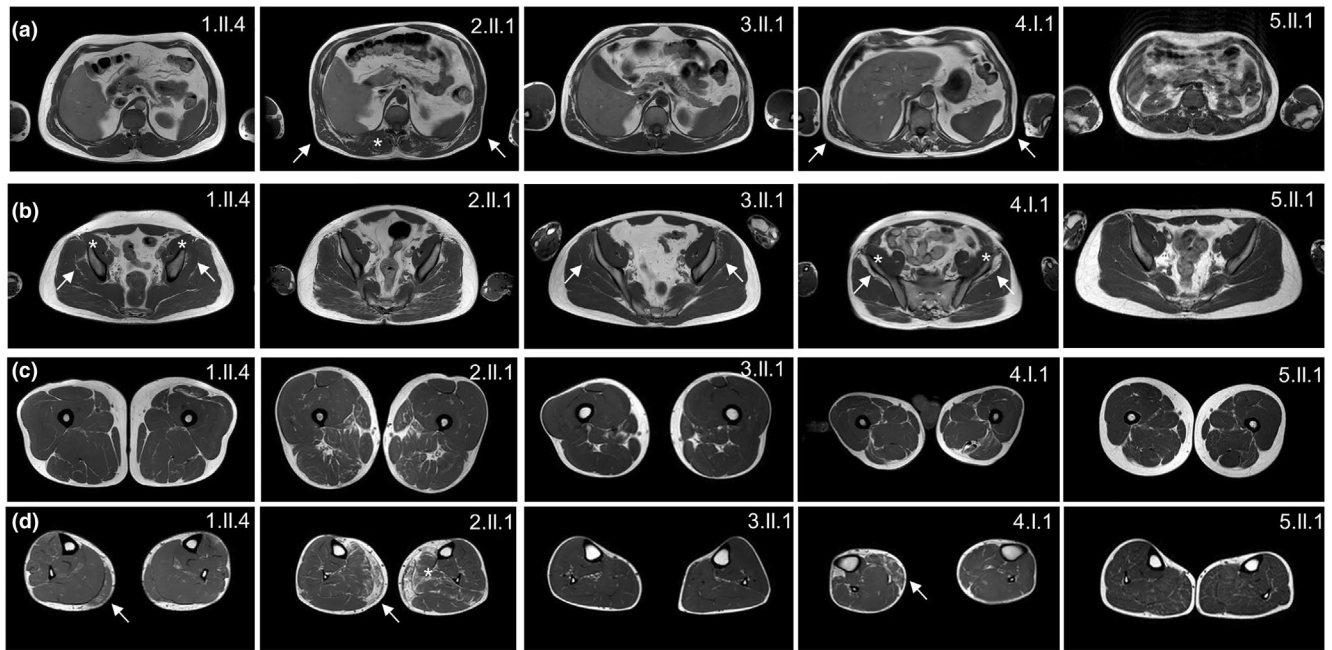


FIGURE 2 MRI patterns in *RYR1* p.Leu2286Val patients. (a) Latissimus dorsi was mildly affected in patients 2.II.1 and 4.I.1 (white arrow). Paravertebral muscles were also involved in patient 2.II.1 (asterisk). (b) Glutei minimum involvement (white arrow), as well as iliopsoas hypertrophy (asterisk), were present in patients 1.II.4, 3.II.1 and 4.I.1. (c) In the thighs, the posterior compartment was more affected than the anterior, especially sartorius, adductor magnus and biceps femoris. Thigh hypertrophy was present in 1.II.4, 3.II.1 and 2.II.1. (d) In the legs, medial gastrocnemius (white arrows) was affected in patients 1.II.4, 2.II.1 and 4.I.1; soleus in patient 2.II.1 (asterisk) and tibialis anterior in patient 4.I.1.

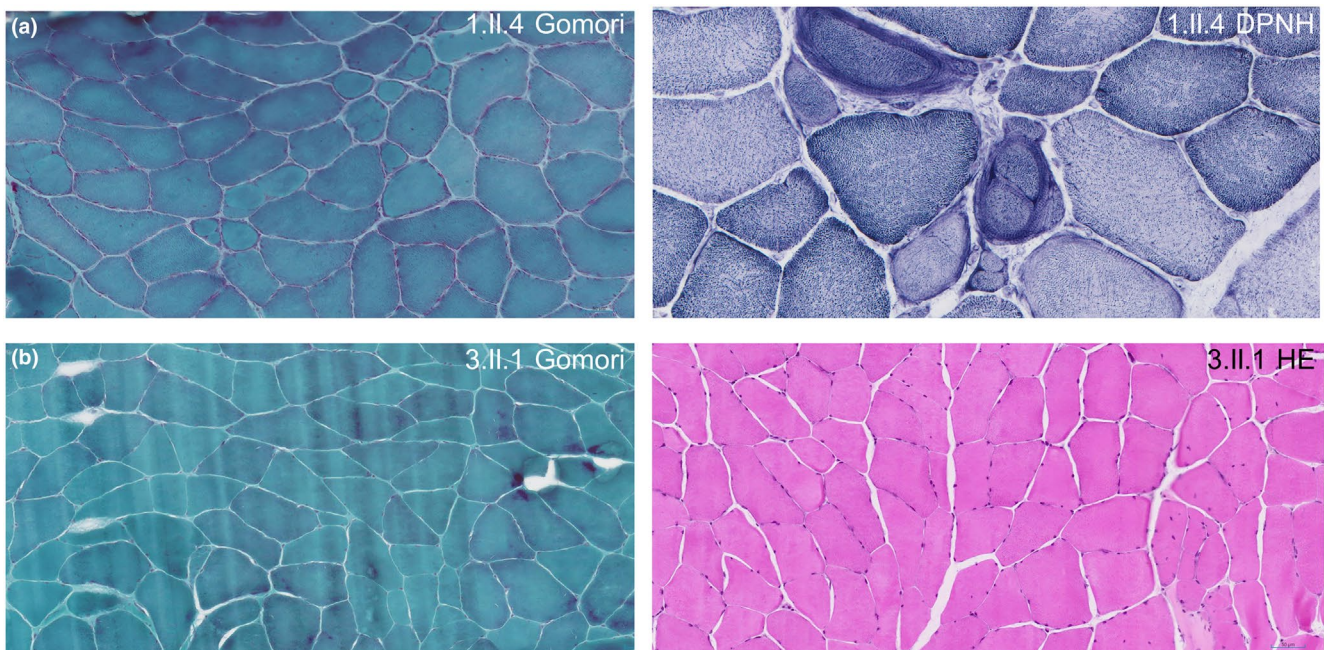


FIGURE 3 Histological studies from muscle biopsies of patients 1.II.4 and 3.II.1. (a) Muscle biopsy from patient 1.II.4 showed variable-size fibres with central nuclei and ring fibres (hematoxylin and eosin not shown). (b) Muscle biopsy of patient 3.II.1 showed central nuclei in a few fibres.

DISCUSSION

Pathogenic variants in *RYR1* have been associated with distinct phenotypes ranging from congenital myopathy to MHS, adult-onset myopathy and ERM. *RYR1* variants previously associated with MHS are

being progressively identified in apparently healthy individuals with episodes of ERM [11, 23, 24]. Here, the pathogenic p.Leu2286Val *RYR1* founder variant in patients with Basque origin showing high CK levels, exertional myalgia, cramps and muscle hypertrophy with minimal progression is reported.

The p.Leu2286Val variant was identified in 17 individuals from seven Basque families (Figure 1, Table 1) and is located in MHS/CCD hotspot 2 of RyR1. Individuals with the p.Leu2286Val share a disease-associated haplotype in the RYR1 region, suggesting a founder effect in the Basque population. To date, a nonnegligible number of founder variants in neurological diseases have been described in the Basque population [25, 26]. The most prevalent features in this RYR1-RM cohort were hyperCKemia, myalgia and muscle cramps, which is consistent with the findings of a recent prospective study of patients with RYR1-associated MHS and/or ERM [27]. Most of the reported patients with autosomal dominant RYR1-associated myopathy presented with ERM [11, 24]. However, in our cohort, only one patient had an episode of rhabdomyolysis after strenuous exercise (patient 3.II.1). Relatives of families 1 and 4–7 presented high CK levels and harboured the p.Leu2286Val variant (Figure 1a, Table 1). Nonetheless, four carriers remained asymptomatic and had normal CK levels. These findings highlight the high phenotypic variability amongst individuals within the same family, which has also been described in other RYR1-associated ERM families [11, 24, 28]. Such clinical heterogeneity can be explained by exposure to external factors (such as physical exercise, heat, fever, ageing or polymorphisms modulating calcium homeostasis). In our cohort, patients with an additional in *trans* RYR1 variant in combination with p.Leu2286Val (5.III.1, 6.II.1 and 7.I.2) (Table S5, Figure 1a) exhibited a more severe phenotype with earlier onset of symptoms, progressive muscle weakness and extensive fat infiltration. This suggests a possible synergetic dose effect between p.Leu2286Val and other RYR1 variants, which should be considered in the genetic counselling of these families.

Regarding muscle MRI, half of the cases carrying the p.Leu2286Val variant had a normal MRI, whilst the remaining showed common patterns involving the medial gastrocnemius, soleus, posterior thigh compartment and gluteus minimus (Figure 2). Muscle hypertrophy was also a common feature in these patients, consistent with previously described patients with RYR1-related rhabdomyolysis and myalgia [24]. Globally, muscle MRI and biopsy correlated with disease severity. Thus, patients carrying two RYR1 variants had more severe clinical symptoms, MRI muscle involvement and pathological features on muscle biopsy (except for patient 6.II.1, whose muscle biopsy was normal) (Figures S2, S3). In individuals carrying only the p.Leu2286Val variant, muscle MRI involvement was less severe or even normal and, in the two patients who had a muscle biopsy, correlated with the MRI findings (1.II.4 had mild involvement in MRI and muscle biopsy, whilst 3.II.1 had only mild involvement of glutei minimus in MRI and normal biopsy) (Figures 2 and 3).

Fifty likely pathogenic variants previously reported to be associated with exercise-induced rhabdomyolysis, cramps and myalgia were extracted from the literature (Figure S1a, Table S4). Of these, two variants are splicing-altering variants, one is an in-frame duplication, and the remaining are missense variants (47/50; 94%). Amongst them, 29 variants have been previously associated with MHS (either with positive in vitro contracture testing in patients or present in patients with a malignant hyperthermia episode) (29/50; 58%). Most of the variants were localized in hotspot regions of RyR1 (36/50; 72%).

Taking into account each hotspot length (number of reported variants in each hotspot/residues present in the hotspot), pathogenic variants associated with the phenotype described here are more frequently located in hotspot 2 than in the other hotspots (Figure S1).

The cohort presented here shows that the presence of asymptomatic and pauci-symptomatic individuals with RYR1 variants makes genetic interpretation difficult. The familial segregation of families 1 and 4–7 confirmed the autosomal dominant inheritance, although functional characterization would further confirm the underlying pathological mechanism of the variant in the RyR1 channel. Early detection of dominantly inherited RYR1 variants will avoid unnecessary testing, inappropriate treatments and possible malignant hyperthermia complications, although these complications were not observed in our cohort of patients. Based on RYR1 variants reported in the literature, those causing RYR1-myalgia/rhabdomyolysis syndrome are preferentially clustered in CCD/MH hotspot 2 (Figures S1b,c). Therefore, the presence of rare variants in hotspot 2 of RYR1 should be considered and further investigated in patients presenting with exercise intolerance, myalgia, hyperCKemia and absence or minimal muscle weakness and an excluded metabolic disorder.

AUTHOR CONTRIBUTIONS

Alba Segarra-Casas: Conceptualization; investigation; writing – original draft; formal analysis; data curation. **Pablo Iruzubieta:** Investigation; writing – original draft; formal analysis; data curation. **Solange Kapetanovic:** Investigation; writing – original draft; formal analysis; data curation. **Aurelio Hernández-Láin:** Investigation; writing – review and editing; formal analysis. **Ivonne Jericó:** Investigation; writing – original draft; formal analysis; data curation. **Roberto Fernández-Torrón:** Investigation; writing – review and editing. **Miren Maneiro:** Investigation; writing – review and editing. **Pablo Marco-Moreno:** Investigation; writing – review and editing. **M. Victoria Zelaya-Huerta:** Investigation; writing – review and editing. **Benjamín Rodríguez-Santiago:** Investigation; writing – review and editing. **Francesc Calafell:** Investigation; writing – review and editing. **Ana Töpf:** Investigation; writing – review and editing; funding acquisition. **Volker Straub:** Funding acquisition; writing – review and editing. **Ainara Vallejo-Illarramendi:** Investigation; writing – review and editing. **Adolfo López de Munain:** Investigation; writing – review and editing. **Pia Gallano:** Investigation; funding acquisition; conceptualization; writing – original draft. **Lidia Gonzalez-Quereda:** Conceptualization; investigation; funding acquisition; writing – original draft.

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CONFLICT OF INTEREST STATEMENT

AVI and ALdM are co-funders of Miramoon Pharma S.L. The remaining authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

Data supporting this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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