Update on RYR1-related myopathies

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Purpose of review
RYR1-related myopathy (RYR1-RM) is a group of myopathies caused by mutations in the *RYR1* gene, which encodes the ryanodine receptor 1 (RYR1). This review discusses recent advances in the clinical features, pathology, pathogenesis, and therapeutics of RYR1-RM.

Recent findings
Although treatments such as salbutamol, pyridostigmine, and N-acetylcysteine have been explored as potential therapies for RYR1-RM, none have been conclusively proven to be effective. However, recent clinical trials of Rycal ARM210 in patients with RYR1-RM have shown promising results, including reduced fatigue and improved proximal muscle strength.
Recent advances in three-dimensional structural analysis of RYR1 channels, facilitated by cryo-electron microscopy (cryo-EM), have elucidated the distinct molecular mechanisms underlying RYR1 functionality. Additionally, high-throughput screening methods, including FRET-based and endoplasmic reticulum Ca^{2+}-based assays, have been successful in identifying potential candidates for the treatment of RYR1-RM.

Summary
Recent advances in clinical and pathological understanding have provided new insights into RYR1-RM. Novel pathomechanisms elucidated by cryo-EM and rapid screening methods have led to the identification of several promising drug candidates. We are hopeful about the potential of Rycal, other new drugs, and gene therapy, offering a promising outlook for the future.

Keywords
central core disease, congenital myopathy, Rycal, RYR1, therapy

INTRODUCTION

RYR1-related myopathy (RYR1-RM) is one of the most common forms of congenital myopathy and is characterized by mutations in the *RYR1* gene, which encodes the ryanodine receptor 1 (RYR1). The RYR1 is a membrane protein that spans the sarcoplasmic reticulum membrane, forming a channel that allows the release of calcium ions from the sarcoplasmic reticulum into the cytoplasm and plays a crucial role in muscle contraction. Although both dominant and recessive mutations can cause RYR1-RM, the latter form presents a more severe phenotype, such as fetal akinesia [1▪▪]. The frequency of RYR1-RM is estimated to be greater than 1 in 90 000 individuals [2]. Given this lower frequency, long-term natural history data have been scarce. However, recently, substantial big data on the natural long-term history of RYR1-RM has been published, offering critical insights for developing future therapies [3▪▪]. Furthermore, the pathological findings of RYR1-RM are very unique and varied, encompassing central core disease (CCD), multiminicore disease (MmD), centronuclear myopathy (CNM), congenital fiber type disproportion (CFTD), congenital neuromuscular disease with uniform type 1 fiber (CNMDU1), core-rod myopathy, and dusty core disease (DuCD) [4,5]. Several studies have reported novel pathological findings in RYR1-RM [6▪]. Notably, individuals with malignant hyperthermia susceptibility associated with dominant *RYR1* mutations may or may not show cores, but if present, they often do not exhibit typical central cores [7].

Currently, there are no established treatments for RYR1-RM, but recent advancements have introduced some potential therapeutic options [8▪▪].

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KEY POINTS

- Large natural history study for RYR1-related myopathy has been conducted.
- The clinical trial of Rycal ARM210 has concluded with a small number of positive results, showing reduced fatigue and improved proximal muscle strength.
- High-throughput screening methods have identified several plausible medicines for RYR1-related disorders.

Additionally, the structural details of the RYR1 protein have been elucidated using cryo-electron microscopy (cryo-EM), providing insights into its molecular mechanisms [9]. Furthermore, novel methods such as high-throughput screening (HTS) techniques, including FRET-based and endoplasmic reticulum Ca^{2+}-based assays, have facilitated the discovery of new drugs more efficiently [10].

Here, we present recent advances in the clinicopathological findings, pathogenesis, and potential therapeutic approaches of RYR1-RM.

CLINICOPATHOLOGICAL FEATURES OF RYR1-RELATED MYOPATHY

Clinically, autosomal dominant RYR1-RM typically presents a mild phenotype. In contrast, autosomal recessive RYR1-RM is characterized by a more severe phenotype, including neonatal hypotonia, ptosis, and ophthalmoplegia [1▪▪].

A recent social media survey conducted by the RYR-1 Foundation, which received 226 responses (63.3% women and 36.7% men), revealed that the most common RYR1-related disorder type was CCD (53.2%), followed by malignant hyperthermia susceptibility (MHS) (16.2%) and CNM (5.2%). Inheritance patterns included autosomal dominant (27.0%), autosomal recessive (26.1%), de-novo (9.3%), and unknown 32.7%. Most participants (64.2%) walked unassisted. Physical symptoms were self-reported as either progressive (47.1%), stable (33.9%), or uncertain (7.5%). A significant 86% were willing to participate in clinical trials, with many maintaining strength through regular exercise [1▪▪].

A long-term natural history study of pediatric RYR1-RM was recently published [3]. The study included 69 patients with RYR1-RM, with onset ranging from birth to 7 years, among whom 29 had autosomal dominant, 31 autosomal recessive, six de-novo dominant, and three uncertain inheritances. At the initial assessment of the 50 patients older than 2 years, 45 were able to walk with or without support (age range 2.3–15.7 years; median age 5.2 years). Scoliosis was found in 30% and spinal rigidity in 17% of patients. Respiratory issues were present in 22%, with 12% requiring ventilatory support from a median age of 7 years. Muscle MRI from 19 patients predominantly showed abnormalities in the anterior thigh muscles, including the vastus lateralis, sartorius, and adductor longus. Key findings revealed significant motor, respiratory, and feeding difficulties, with recessive patients exhibiting more severe symptoms. No malignant hyperthermia episodes were reported in this cohort [3▪▪]; however, there is a considerable overlap in clinical and histopathological features between patients with RYR1-RM and those predisposed to malignant hyperthermia and/or exertional rhabdomyolysis. This overlap highlights the need for vigilant clinical management and the consideration of malignant hyperthermia risk in patients with *RYR1* mutations [11].

From the report of an Italian study, researchers investigated *RYR1* mutations in 153 patients with core myopathy (cores and minicores) [12]. Of these, they found 68 cases with at least one mutation in the *RYR1* gene. Additionally, they examined the genotype-phenotype correlation of core myopathies and discovered that mutations in the pore domain are associated with fetal hypokinesia, contractures, and foot deformities. The study also highlighted that patients with mutations in other regions of the *RYR1* gene exhibited varied clinical presentations, including differences in muscle strength, respiratory function, and the presence of scoliosis, indicating the complexity and diversity of RYR1-RM [12].

Recent findings have expanded the pathological spectrum of RYR1-RM. Researchers identified *RYR1* mutations (p.Thr2206Met and p.Gly2434Arg) in two patients with tubular aggregate myopathy (TAM), characterized by increased CK levels and episodic stiffness triggered by repetitive muscle contraction or exposure to cold. Notably, these two mutations have also been primarily detected in individuals with malignant hyperthermia susceptibility (MHS), highlighting the overlapping clinical manifestations associated with *RYR1* mutations [6▪]. Furthermore, three cases of periodic paralysis, both with and without associated myopathy, have been reported in autosomal dominant and recessive RYR1-RM [13]. Considering that periodic paralysis caused by mutations in *SCN4A*, *CACNA1S*, and *KCNJ2* is sometimes associated with tubular aggregates [14], it is plausible that *RYR1* mutations could be related to both conditions.

PATHOGENESIS OF RYR1-RELATED MYOPATHY

In skeletal muscle, excitation-contraction (E-C) coupling is initiated when the action potential of

transverse tubule membranes triggers Ca^{2+} release from the sarcoplasmic reticulum. The RYR1 mediates two key Ca^{2+} release mechanisms in skeletal muscle: depolarization-induced Ca^{2+} release (DICR) and Ca^{2+}-induced Ca^{2+} release (CICR). DICR is triggered by depolarization, which activates RYR1 through physical interaction with the dihydropyridine receptor (DHPR), and is crucial for muscle contraction. CICR, which is triggered by direct Ca^{2+} binding to RYR1, contributes minimally to Ca^{2+} release in skeletal muscle [10].

RYR1 mutations are associated with various diseases, including malignant hyperthermia, central core disease (CCD), multiminicore disease (MmD), and dusty core disease (DuCD) [5]. Consequently, the pathomechanisms of *RYR1* mutations are diverse and can be categorized into three mechanism: gain-of-function, loss-of-function, and decreased expression of RYR1 [10]. Gain-of-function *RYR1* mutations associated with malignant hyperthermia cause hyperactive CICR, leading to massive Ca^{2+} release from the sarcoplasmic reticulum. Conversely, loss-of-function *RYR1* mutations (mostly located in the pore domain) associated with CCD inhibit Ca^{2+} release via depolarization. In RYR1-RM linked to autosomal recessive mutations such as MmD or DuCD, a severe reduction in RYR1 protein expression is observed, likely due to epigenetic allele silencing. This also results in muscle weakness due to the loss-of-function of *RYR1* [10].

Recently, other downstream pathogenic events in RYR1-RM have been studied [15▪]. Sonne *et al.* [15▪] investigated posttranslational modifications (PTMs) and ATP turnover time in myosin in RYR1-RM. They discovered abnormal acetylation and phosphorylation on myosin molecules in RYR1-RM, which led to a significant decrease in the ATP turnover time of myosin molecules in the disordered-relaxed state. These mechanisms could be related to muscle weakness in RYR1-RM [15▪,16].

The association between endoplasmic reticulum (ER) stress and RYR1 protein levels was evaluated in various diseases, including RYR1-RM. A negative association was found between ER stress markers (GRP78-Bip and CHOP-DDIT3 protein levels) and muscle RYR1 content in various myopathies. Additionally, the accumulation of sphingolipids was observed in myotubes derived from RyR1-depleted myoblasts, which may be associated with ER stress [17▪].

HIGH-RESOLUTION Cryo-ELECTRON MICROSCOPY ANALYSIS OF RYR1-RELATED MYOPATHY

Cryo-electron microscopy (Cryo-EM) has significantly enhanced our understanding of the molecular mechanisms of RYR1. By providing high-resolution 3D structures, Cryo-EM allows for the identification of functional domains and the structural impacts of pathogenic mutations. Additionally, Cryo-EM allows us to pinpoint ligand-binding sites for molecules such as ATP, Ca^{2+}, and caffeine [18]. Through Cryo-EM, researchers have uncovered the structural and functional effects of the severe RyR1 Y523S mutation (equivalent to human Y522S) in its open and closed states. The study revealed widespread conformational changes across multiple domains, leading to preactivation of the channel and altered interactions with the DHPR [19▪]. This precise visualization is crucial for unraveling the molecular basis of RYR1-RM and informing the development of targeted treatments [9]. Melville *et al.* [20] elucidated the structure of the Rycal compound (ARM210)-bound RYR1. This compound cooperatively binds with ATP at a second ATP-binding site, stabilizing the closed state of the RYR1 channel [20].

DRUG SCREENING OF RYR1-RELATED MYOPATHY

Recently, two methods for discovering new drugs have been invented. These include HTS techniques, such as FRET-based and endoplasmic reticulum Ca^{2+}-based assays, which have significantly improved the efficiency of drug discovery [10].

Cornea *et al.* [21] developed a rapid HTS assay using FRET, employing FK506 binding protein and calmodulin, regulatory molecules for RYR1. The assay measures FRET signals to identify RYR1 activity changes. By testing 727 compounds, six were found to alter FRET signals, with four increasing RYR1 activity and two inhibiting it. Further improvements identified chloroxine and myricetin as novel RYR1 inhibitors, showing potential as therapeutic candidates for RYR1-RM [22].

Murayama *et al.* [10] developed an HTS platform using ER Ca^{2+} measurements to identify RYR1 inhibitors. They expressed RYR1 in HEK293 cells, using R-CEPIA1er to monitor ER Ca^{2+} levels. Through the screening of 1535 compounds, they found three potential inhibitors (oxolinic acid, 9-aminoacridine, alexidine) and over 50 activators. Oxolinic acid derivatives, particularly Cpd1, showed significant potency and selectivity for RYR1, effectively preventing and treating malignant hyperthermia crises in mouse models carrying *RYR1* mutations (R163C, G2434R, and R 2509C). Additionally, Cpd1 has advantages over dantrolene in water solubility and rapid clearance [10,23]. Further analysis is necessary to evaluate the effectiveness of these drugs for RYR1-RM.

CLINICAL TRIALS OF RYR1-RELATED MYOPATHY

A phase 1, open-label, dose-escalation trial of Rycal S48168 (ARM210), an RYR1 calcium release channel stabilizer, for RYR1-RM was recently conducted [8▪▪]. The Rycal compound was well tolerated and did not cause any serious adverse events, exhibiting a dose-dependent pharmacokinetic profile. Among the four participants who received a 200 mg/day dose, three showed improvements in fatigue and proximal muscle weakness during physical examination [8▪▪]. However, randomized double-blind trials are necessary to further evaluate the long-term safety, tolerability, and potential therapeutic benefits of Rycal in RYR1-RM. Dantrolene, a drug used for malignant hyperthermia caused by *RYR1* mutations, has been tried in malignant hyperthermia susceptible (MHS) patients with myopathic symptoms [24▪]. In this study, among 476 MHS patients who tested positive for the caffeine-halothane contracture test, 193 had muscle symptoms and were older than 25 years. Of these 193 cases, 164 patients used oral dantrolene, and none of them experienced severe side effects. Additionally, 142 patients adhered to the therapy and showed improvements in myalgia (55%), fatigue (22%), and rhabdomyolysis/hyperCKemia (22%) [24▪]. Additionally, a 5-year-old patient with RYR1-related exertional myalgia/rhabdomyolysis was treated with dantrolene and showed improvements in CK levels, enhanced performance on the 6-min walk test, and a reduction in the frequency and intensity of myalgia episodes [25▪]. Considering the drug mechanism of dantrolene, which suppresses CICR in the sarcoplasmic reticulum, and the pathogenesis of MHS and RYR1-RM, dantrolene may not be beneficial for all RYR1-RM patients. Clinicians should use it with caution and carefully monitor patients.

Interestingly, some patients have been reported to have pyridostigmine-responsive myasthenia-like symptoms [26,27]. However, more cases and further evaluations are necessary to delineate this subcategory and understand its pathophysiology.

GENE THERAPY OF RYR1-RELATED MYOPATHY

In recent years, gene therapy has been successfully developed for several neuromuscular disorders, demonstrating remarkable efficacy, particularly for spinal muscular atrophy. However, for RYR1-RM, the situation is more challenging due to the large size of the RYR1 gene. To address this issue, researchers are exploring several innovative strategies. One potential approach is to split the *RYR1* sequence into multiple parts, each fitting into an adeno-associated virus (AAV) vector, and then induce fusion between the different segments using a trans-splicing mechanism [28]. Another promising approach is exon skipping, which modulates splice sites to restore partial functionality of the protein. Additionally, CRISPR/Cas9-based gene editing is being explored to directly correct mutations – such as deletions, modifications, insertions, or replacements of DNA segments – within the *RYR1* gene, though this technology is still in the experimental stage for this application [28]. Recently, the first successful correction of an *RYR1* gene mutation using prime editing was reported, marking a significant advancement toward gene therapies for RYR1-RM. In human myoblasts, researchers achieved a 59% correction rate of the T4709 M mutation in the *RYR1* gene through RNA delivery of prime editing components [29▪]. However, as this research is still *in vitro*, further investigation is necessary to determine its efficacy and safety in clinical settings.

CONCLUSION

Knowledge of genotype-phenotype correlations in RYR1-RM has been gradually accumulating. Thanks to advancements in cryo-EM, novel pathomechanisms have been elucidated, paving the way for the discovery of new drug efficacies. Additionally, two rapid screening methods – FRET-based and ER Ca^{2+}-based assays – have facilitated the testing of numerous drugs, resulting in the identification of several promising candidates. Randomized double-blind clinical trials are necessary to evaluate the long-term safety and efficacy of the RYR1 calcium channel stabilizer, Rycal. Continued research and clinical trials will be crucial in developing effective treatments for RYR1-RM, ultimately improving patient outcomes and quality of life.

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Conflicts of interest

Competing interests: The authors report no competing interests.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

1. O'Connor TN, van den Bersselaar LR, Chen YS, *et al.* RYR-1-related diseases
■■ International Research Workshop: from mechanisms to treatments Pittsburgh, PA, U.S.A, 21-22 July 2022. J Neuromuscul Dis 2023; 10:135–154.

This workshop united experts and patients to advance research and develop therapies for RYR1-related diseases.

2. Amburgey K, McNamara N, Bennett LR, *et al.* Prevalence of congenital myopathies in a representative pediatric United States population. Ann Neurol 2011; 70:662–665.
3. Sarkozy A, Sa M, Ridout D, *et al.* Long-term natural history of pediatric
■■ dominant and recessive RYR1-related myopathy. Neurology 2023; 101: e1495–e1508.

This study introduces the long-term natural history of pediatric patients with RYR1-RM.

4. Ogasawara M, Nishino I. A review of major causative genes in congenital myopathies. J Hum Genet 2023; 68:215–225.
5. Ogasawara M, Nishino I. A review of core myopathy: central core disease, multiminicore disease, dusty core disease, and core-rod myopathy. Neuromuscul Disord 2021; 31:968–977.
6. Vattemi GNA, Rossi D, Galli L, *et al.* Ryanodine receptor 1 (RYR1) mutations
■ in two patients with tubular aggregate myopathy. Eur J Neurosci 2022; 56:4214–4223.

This study expands on the pathological findings of RYR1-RM, showing the presence of tubular aggregates.

7. Ibarra MC, Wu S, Murayama K, *et al.* Malignant hyperthermia in Japan: mutation screening of the entire ryanodine receptor type 1 gene coding region by direct sequencing. Anesthesiology 2006; 104:1146–1154.
8. Todd JJ, Lawal TA, Chrismer IC, *et al.* Rycal S48168 (ARM210) for RYR1-
■■ related myopathies: a phase one, open-label, dose-escalation trial. EClinicalMedicine 2024; 68:102433.

This study explains that S48168 (ARM210) showed promising initial results in safety and efficacy for RYR1-RM patients.

9. Iyer KA, Barnakov V, Samsó M. Three-dimensional perspective on ryanodine receptor mutations causing skeletal and cardiac muscle-related diseases. Curr Opin Pharmacol 2023; 68:102327.
10. Murayama T, Kurebayashi N, Ishida R, Kagechika H. Drug development for the treatment of RyR1-related skeletal muscle diseases. Curr Opin Pharmacol 2023; 69:102356.
11. van den Bersselaar LR, Jungbluth H, Kruijt N, *et al.* Neuromuscular symptoms in patients with RYR1-related malignant hyperthermia and rhabdomyolysis. Brain Commun 2022; 4:fcac292.
12. Fusto A, Cassandrini D, Fiorillo C, *et al.* Expanding the clinical-pathological and genetic spectrum of RYR1-related congenital myopathies with cores and minicores: an Italian population study. Acta Neuropathol Commun 2022; 10:54.
13. Matthews E, Neuwirth C, Jaffer F, *et al.* Atypical periodic paralysis and myalgia: a novel RYR1 phenotype. Neurology 2018; 90:e412–e418.
14. Vivekanandam V, Männikkö R, Skorupinska I, *et al.* Andersen-Tawil syndrome: deep phenotyping reveals significant cardiac and neuromuscular morbidity. Brain 2022; 145:2108–2120.
15. Sonne A, Antonovic AK, Melhedegaard E, *et al.* Abnormal myosin posttranslational modifications and ATP turnover time associated with human congenital myopathy-related RYR1 mutations. Acta Physiol (Oxf) 2023; 239:e14035.

This study shows RYR1 mutations alter myosin structure and function, contributing to congenital myopathy through secondary pathogenic mechanisms.

16. Chase PB, Coons AN. Ryanodine receptor-associated myopathies: what's myosin got to do with it? Acta Physiol (Oxf) 2023; 239:e14058.
17. Vidal J, Fernandez EA, Wohlwend M, *et al.* Ryanodine receptor type 1 content
■ decrease-induced endoplasmic reticulum stress is a hallmark of myopathies. J Cachexia Sarcopenia Muscle 2023; 14:2882–2897.

This study reveals that decreased RyR1 protein levels are common in various myopathies and contribute to muscular disorders through ER stress and mitochondrial dysfunction.

18. Melville Z, Kim K, Clarke OB, Marks AR. High-resolution structure of the membrane-embedded skeletal muscle ryanodine receptor. Structure 2022; 30:172–180; e3.
19. Iyer KA, Hu Y, Klose T, *et al.* Molecular mechanism of the severe MH/CCD
■ mutation Y522S in skeletal ryanodine receptor (RyR1) by cryo-EM. Proc Natl Acad Sci U S A 2022; 119:e2122140119.

This study underscores the importance of utilizing advancements in cryo-EM technology for structural studies of single-point mutations.

20. Melville Z, Dridi H, Yuan Q, *et al.* A drug and ATP binding site in type 1 ryanodine receptor. Structure 2022; 30:1025–1034; e4.
21. Rebbeck RT, Essawy MM, Nitu FR, *et al.* High-throughput screens to discover small-molecule modulators of ryanodine receptor calcium release channels. SLAS Discov 2017; 22:176–186.
22. Rebbeck RT, Singh DP, Janicek KA, *et al.* RyR1-targeted drug discovery pipeline integrating FRET-based high-throughput screening and human myofiber dynamic Ca(2+) assays. Sci Rep 2020; 10:1791.
23. Yamazawa T, Kobayashi T, Kurebayashi N, *et al.* A novel RyR1-selective inhibitor prevents and rescues sudden death in mouse models of malignant hyperthermia and heat stroke. Nat Commun 2021; 12:4293.
24. Ibarra Moreno CA, Kraeva N, Zvaritch E, *et al.* Oral dantrolene for myopathic
■ symptoms in malignant hyperthermia-susceptible patients: a 25-year retrospective cohort study of adverse effects and tolerability. Anesth Analg 2023; 136:569–577.

This study reports that oral dantrolene is effective in treating fatigue, rhabdomyolysis, and pain in patients susceptible to malignant hyperthermia.

25. de Lima Silva EV, Donis KC, Machado FRC, *et al.* Oral dantrolene reduces
■ myalgia and hyperckemia in a child with RYR1-related exertional myalgia/Rhabdomyolysis. J Neuromuscul Dis 2023; 10:1145–1149.

This case report describes the first successful use of oral dantrolene in a pediatric patient with RYR1-related exertional myalgia/rhabdomyolysis, showing notable symptom improvement and no adverse effects.

26. Lester EB, Larsen MJ, Laulund LW, *et al.* Ryanodine receptor 1 related myasthenia like myopathy responsive to pyridostigmine. Eur J Med Genet 2023; 66:104706.
27. Illingworth MA, Main M, Pitt M, *et al.* RYR1-related congenital myopathy with fatigable weakness, responding to pyridostigimine. Neuromuscul Disord 2014; 24:707–712.
28. Marty I, Beaufils M, Fauré J, Rendu J. Gene therapies for RyR1-related myopathies. Curr Opin Pharmacol 2023; 68:102330.
29. Godbout K, Rousseau J, Tremblay JP. Successful correction by prime editing
■ of a mutation in the RYR1 gene responsible for a myopathy. Cells 2023; 13:1–16.

This study demonstrates the first successful prime editing correction of the *RYR1* gene mutation, showing a 59% correction rate in human myoblasts.