

REVIEW

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Preclinical model systems of ryanodine receptor 1-related myopathies and malignant hyperthermia: a comprehensive scoping review of works published 1990–2019

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Abstract

Background: Pathogenic variations in the gene encoding the skeletal muscle ryanodine receptor (RyR1) are associated with malignant hyperthermia (MH) susceptibility, a life-threatening hypermetabolic condition and *RYR1*-related myopathies (*RYR1*-RM), a spectrum of rare neuromuscular disorders. In *RYR1*-RM, intracellular calcium dysregulation, post-translational modifications, and decreased protein expression lead to a heterogenous clinical presentation including proximal muscle weakness, contractures, scoliosis, respiratory insufficiency, and ophthalmoplegia. Preclinical model systems of *RYR1*-RM and MH have been developed to better understand underlying pathomechanisms and test potential therapeutics.

Methods: We conducted a comprehensive scoping review of scientific literature pertaining to *RYR1*-RM and MH preclinical model systems in accordance with the PRISMA Scoping Reviews Checklist and the framework proposed by Arksey and O'Malley. Two major electronic databases (PubMed and EMBASE) were searched without language restriction for articles and abstracts published between January 1, 1990 and July 3, 2019.

Results: Our search yielded 5049 publications from which 262 were included in this review. A majority of variants tested in *RYR1* preclinical models were localized to established MH/central core disease (MH/CCD) hot spots. A total of 250 unique *RYR1* variations were reported in human/rodent/porcine models with 95% being missense substitutions. The most frequently reported *RYR1* variant was R614C/R615C (human/porcine total $n = 39$), followed by Y523S/Y524S (rabbit/mouse total $n = 30$), I4898T/I4897T/I4895T (human/rabbit/mouse total $n = 20$), and R163C/R165C (human/mouse total $n = 18$). The dyspedic mouse was utilized by 47% of publications in the rodent category and its RyR1-null (1B5) myotubes were transfected in 23% of publications in the cellular model category. In studies of transfected HEK-293 cells, 57% of *RYR1* variations affected the RyR1 channel and activation core domain. A total of 15 *RYR1* mutant mouse strains were identified of which ten were heterozygous, three were compound heterozygous, and a further two were knockout. Porcine, avian, zebrafish, *C. elegans*, canine, equine, and drosophila model systems were also reported.

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Conclusions: Over the past 30 years, there were 262 publications on MH and *RYR1*-RM preclinical model systems featuring more than 200 unique *RYR1* variations tested in a broad range of species. Findings from these studies have set the foundation for therapeutic development for MH and *RYR1*-RM.

Keywords: Ryanodine receptor, *RYR1*, Congenital myopathy, Central core disease, Preclinical, Mouse, Zebrafish, HEK-293, Porcine, Malignant hyperthermia

Introduction

Ryanodine receptor 1-related myopathies (*RYR1*-RM) are a diverse spectrum of rare monogenic neuromuscular disorders that manifest from variations in the *RYR1* gene [1, 2]. In total, > 700 *RYR1* variations have been identified; many of which are private to an individual case or family [3]. *RYR1* exhibits little functional variation (per a recently developed bioinformatic residual variance intolerance [RVIS] scoring system: -8.29 [0.01%]) [4] and encodes a 2.2 megadalton homotetrameric calcium ion channel (RyR1) that is localized to the sarcoplasmic reticulum (SR) membrane in skeletal muscle [5]. The physical connection between the RyR1 cytosolic shell and dihydropyridine receptor (DHPR) enables a coordinated release of SR calcium to the muscle cell cytosol, a process that facilitates excitation-contraction coupling in response to depolarization of the transverse-tubule membrane [6, 7]. ER/SR calcium concentration is an estimated 1000–10,000 times greater than cytosolic calcium concentration, and maintenance of this steep gradient is imperative to the health of the cell [8, 9]. Preclinical studies have identified intracellular calcium dysregulation as the central pathomechanism resulting from *RYR1* variations characterized by SR calcium leak or excitation-contraction uncoupling [10]. In addition, the presence of truncation variations often reported in compound heterozygous cases can lead to decreased RyR1 expression [11, 12]. Owing to > 100 cysteine residues per subunit, RyR1 are susceptible to post-translational modifications, which in the case of mutant channels, further exacerbate intracellular calcium dysregulation through a previously reported feed-forward mechanism [13, 14]. For example, an elevated level of S-nitrosylated cysteines greatly increases channel activity, thus perpetuating calcium release. *RYR1*-RM pathomechanisms have been reviewed in detail elsewhere [10].

RYR1-RM can be inherited in a dominant or recessive manner and are slowly progressive with clinical manifestations including proximal muscle and facial weakness, joint contractures, scoliosis, ophthalmoplegia, and respiratory muscle weakness [15]. Although presentation often occurs at birth or in early childhood, adult-onset cases have also been reported [16, 17]. Affected individuals are considered at risk of malignant hyperthermia (MH) susceptibility. Genetic predisposition to MH can result in a potentially fatal hypermetabolic response and skeletal muscle rigidity

upon exposure to triggers such as volatile anesthetics, exercise in the heat, and influenza [18, 19]. In addition to myopathy, other clinical phenotypes attributed to *RYR1* variations include rhabdomyolysis-myalgia syndrome and intermittent periodic paralysis [20, 21]. Historically, *RYR1*-RM were sub-categorized based on skeletal muscle histopathology. This yielded subtypes such as central core disease, multiminicore disease, centronuclear myopathy, and congenital fiber-type disproportion [22]. Despite being the most frequently reported non-dystrophic neuromuscular disorder [23], there is currently no approved treatment for *RYR1*-RM.

A decade after the first report of central core disease in humans [24], Hall and colleagues observed a fatal hypermetabolic response to suxamethonium in pigs [25]. This was the first MH animal model system whose phenotype, also referred to as porcine stress syndrome, was later attributed to the R615C variation in *RYR1* [26, 27]. Since this landmark discovery, technological and scientific advances have led to the development of preclinical model systems that can be grouped into cell culture and animal categories, each with their own advantages and limitations [28–32].

Objective

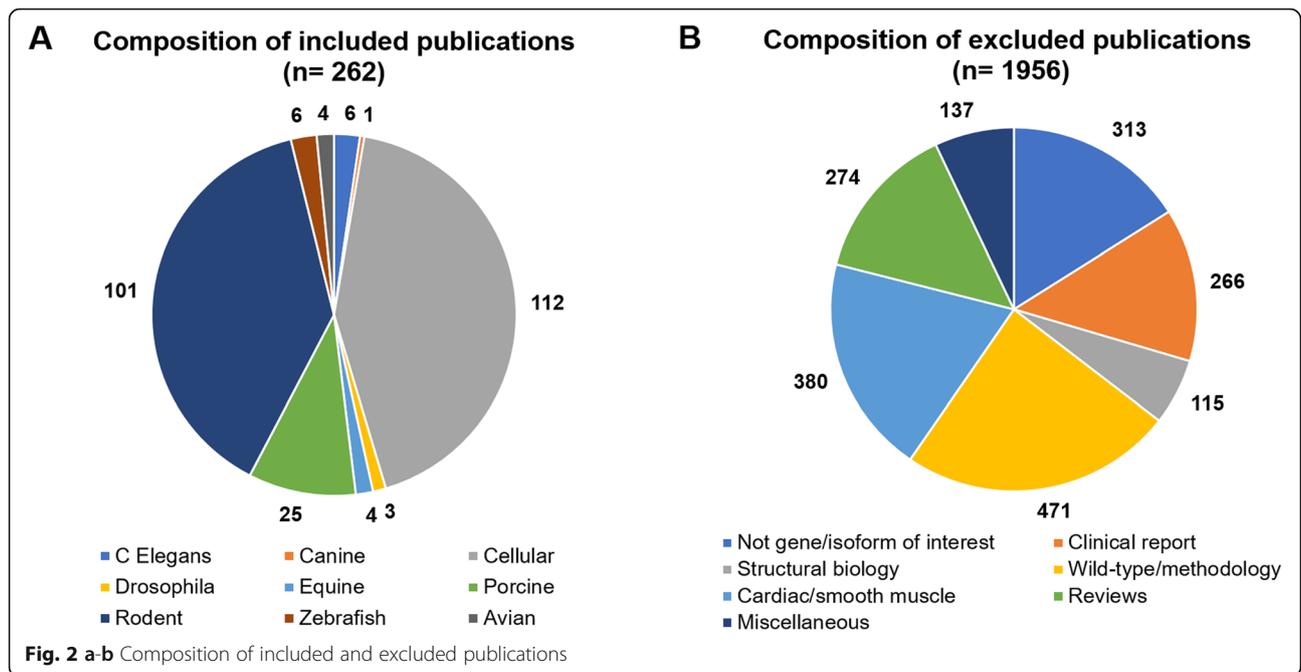
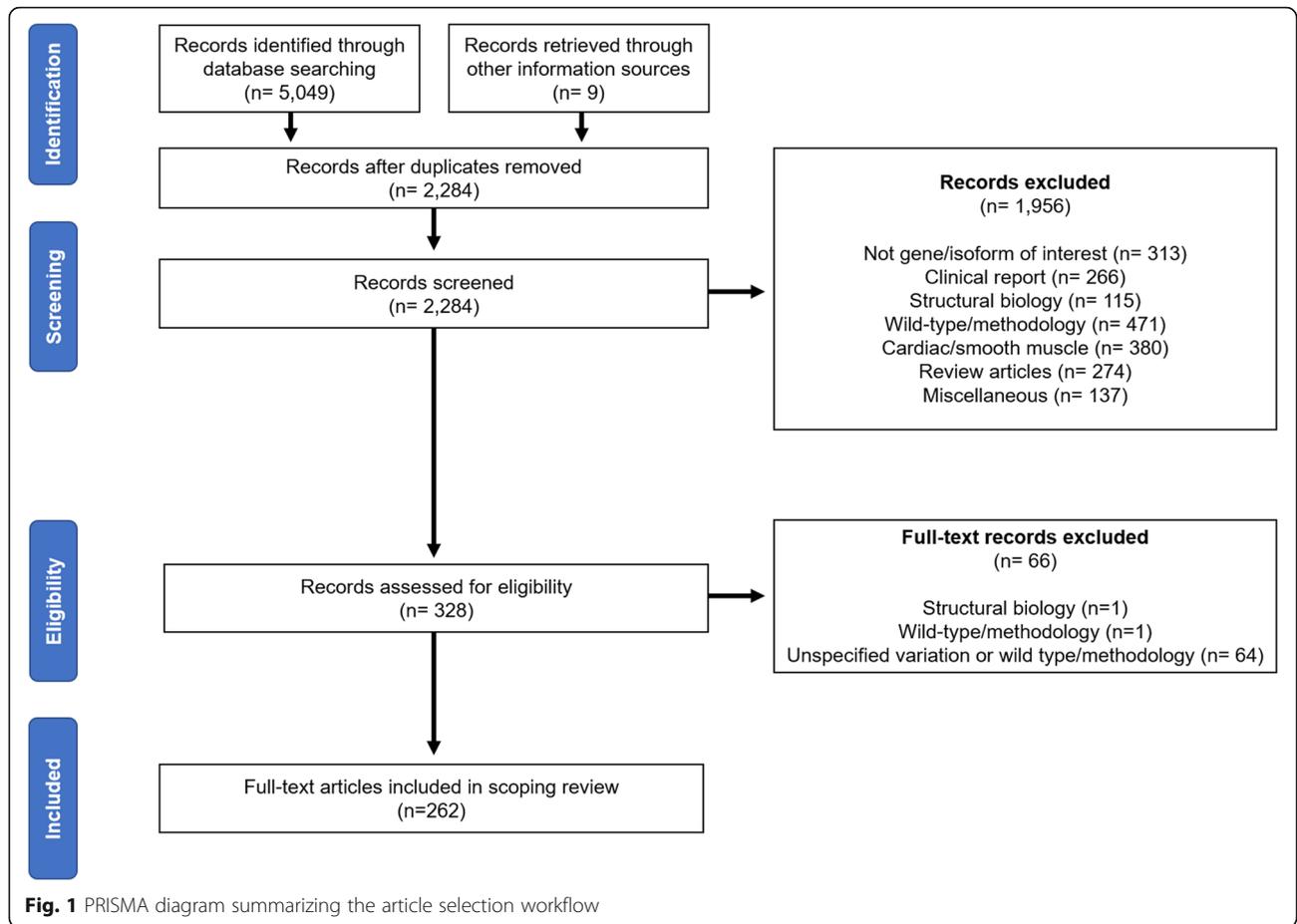
The objective of this scoping review was to comprehensively review the scientific literature for MH and *RYR1*-RM preclinical model systems, thus generating a resource to guide future research.

Methods

The PRISMA extension for Scoping Reviews (PRISMA-ScR) Checklist and the framework proposed by Arksey and O'Malley [33] were used to guide this scoping review. The overarching research question was: what preclinical model systems have been reported for MH and *RYR1*-RM?

Identifying relevant studies

Two major electronic databases (PubMed and EMBASE) were searched without language restriction for articles and abstracts published between January 1, 1990 and July 3, 2019. The search strategy comprised the following a priori search terms present in the title or abstract



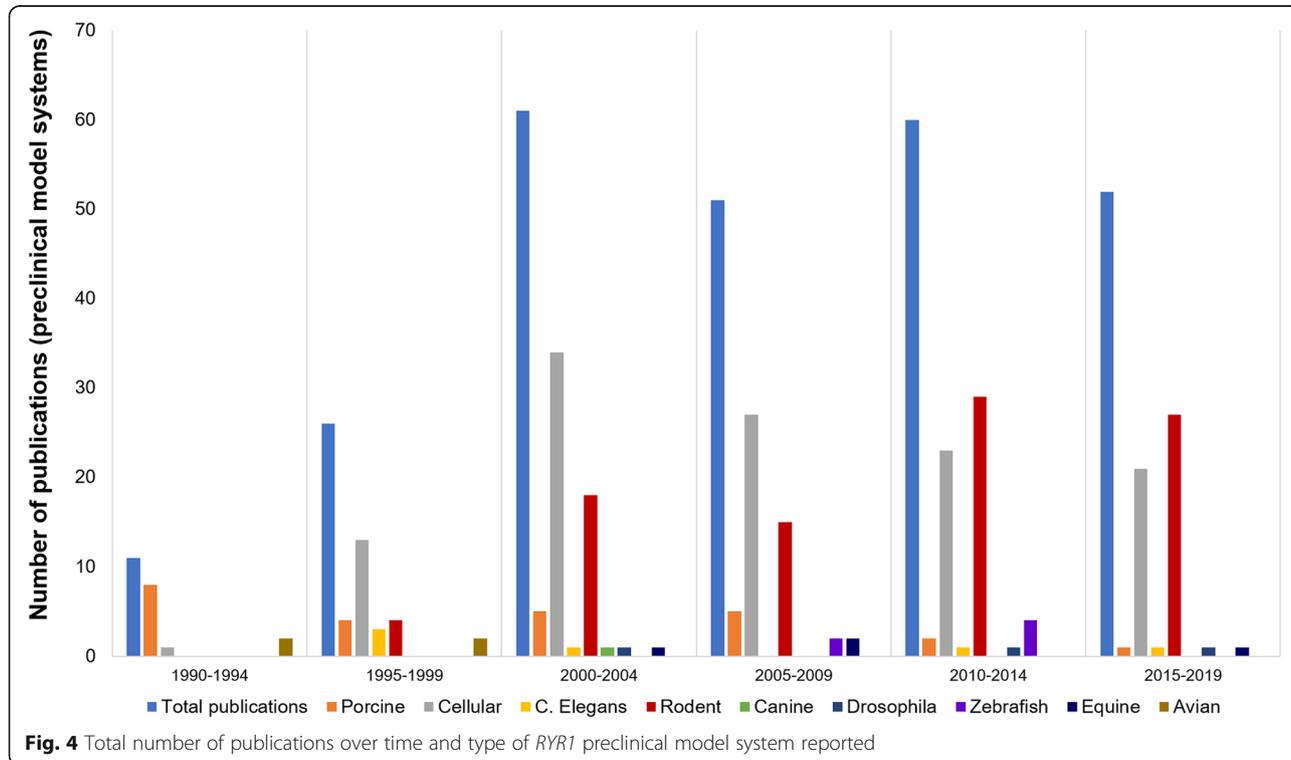
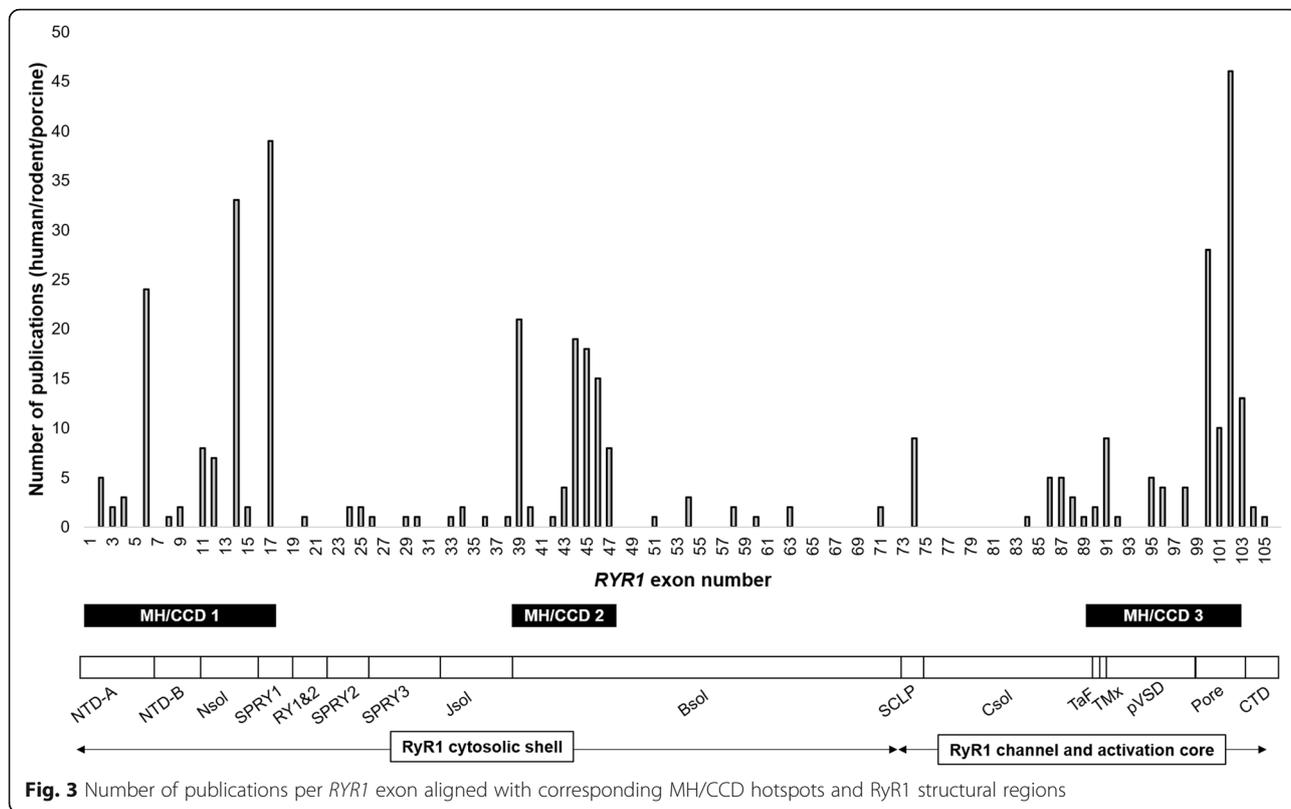


Table 1 Cellular *RYR1* model systems: Human embryonic kidney (HEK-293) cells

| Author/Year | <i>RYR1</i> variant(s) | Title | Conclusions |
|--------------------------------|--|--|---|
| HEK-293 | | | |
| Chirasani VR, et al. [34] 2019 | Q3970K, Q3970E | A central core disease mutation in the Ca ²⁺ binding site of skeletal muscle ryanodine receptor impairs single channel regulation | RyR1-Q3970K is likely a CCD-associated loss-of-function channel that conducts Ca ²⁺ |
| Xu L, et al. [35] 2018 | G4934D, G4934K, G4941D, G4941K, G4941M, D4938N, D4945N | G4941K substitution in the pore-lining S6 helix of the skeletal muscle ryanodine receptor increases RyR1 sensitivity to cytosolic and luminal Ca ²⁺ | Luminal Ca ²⁺ accesses Ca ²⁺ activation sites as they pass through the pore rather than traveling to openings that lie outside the pore |
| Xu L, et al. [36] 2018 | E3893Q, E3893V, E3967Q, E3967V, T5001A | Ca ²⁺ – mediated activation of the skeletal-muscle ryanodine receptor ion channel | Removal of negative charges in a RyR1 Ca ²⁺ binding site impairs activation of RyR1 by physiological concentrations of Ca ²⁺ , and suggests loss of binding to or reduced Ca ²⁺ affinity of the site |
| Xu L, et al. [37] 2008 | G4898E, G4898R, ΔV4926, ΔI4927, R110W, L486V | Single channel properties of heterotetrameric mutant RyR1 ion channels linked to core myopathies | Homozygous RyR1 mutations associated with core myopathies abolish or greatly reduce sarcoplasmic reticulum Ca ²⁺ release during excitation-contraction coupling |
| Schiemann AH, et al. [38] 2018 | D2431Y | A genetic mystery in malignant hyperthermia 'solved'? | The D2431Y variant is pathogenic for MH and should be added to the European Malignant Hyperthermia Group (EMHG) list of diagnostic mutations |
| Murayama T, et al. [39] 2018 | G342R, R2435H, L4824P | Efficient High-Throughput Screening by Endoplasmic Reticulum Ca ²⁺ Measurement to Identify Inhibitors of Ryanodine Receptor Ca ²⁺ – Release Channels | In the current high throughput screening of 1535 compounds, we identified four RyR1 inhibitors |
| Kondo T, et al. [40] 2018 | T84M | Genetic and functional analysis of the <i>RYR1</i> mutation pThr84Met revealed a susceptibility to malignant hyperthermia | Functional analysis of T84M demonstrated higher responsiveness to caffeine and 4CmC |
| Parker R, et al. [41] 2017 | M4640I, V4849I, F4857S, D4918N | Functional Characterization of C-terminal Ryanodine Receptor 1 Variants Associated with Central Core Disease or Malignant Hyperthermia | The V4849I variant should be considered a risk factor for malignant hyperthermia, while the F4857S and D4918N variants should be classified as pathogenic for CCD |
| Merritt A, et al. [42] 2017 | R2336H, R2355W, E3104K, G3990V, V4849I, D3986E | Assessing the pathogenicity of <i>RYR1</i> variants in malignant hyperthermia | Functional analyses in HEK293 cells provided evidence to support the use of R2336H, R2355W, E3104K, pG3990V and V4849I for diagnostic purposes but not D3986E |
| Chen W, et al. [43] 2017 | R164C, Y523S, R2136H, R2435H, Y4796C | Reduced threshold for store overload-induced Ca ²⁺ release is a common defect of RyR1 mutations associated with malignant hyperthermia and central core disease | All mutations reduced the threshold for SOICR |
| Stephens J et al. [44] 2016 | ΔE2348, T214M | Functional analysis of <i>RYR1</i> variants linked to malignant hyperthermia | ΔE2348 could be added to the list of diagnostic mutations for susceptibility to malignant hyperthermia T214M, does not appear to significantly alter sensitivity to agonist in the same system |
| Murayama T, et al. [45] 2016 | R2163C, R2163H, V2168M, T2206M, A2350T, G2375A, G2434R, R2435H, R2454C, R2454H, R2458C, R2458H, R2508C, R2508H | Genotype–Phenotype Correlations of Malignant Hyperthermia and Central Core Disease Mutations in the Central Region of the <i>RYR1</i> Channel | In live-cell Ca ²⁺ imaging, the mutant channels exhibited an enhanced sensitivity to caffeine, a reduced endoplasmic reticulum Ca ²⁺ content, and an increased resting cytoplasmic Ca ²⁺ level |
| Gomez AC, et al. [46] 2016 | F4732D, G4733E, R4736W, R4736Q, T4825I, H4832Y, T4082M, S4113L, N4120Y | Malignant hyperthermia-associated mutations in the S2-S3 cytoplasmic loop of type 1 ryanodine receptor calcium channel impair calcium-dependent inactivation | Nine RyR1 mutants associated with skeletal muscle diseases were differently regulated by Ca ²⁺ and Mg ²⁺ |
| Murayama T, et al. [47] 2015 | C36R, R164C, R164L, G249R, G342R, R402C, R402H, Y523C, Y523S, R615C, R615L | Divergent Activity Profiles of Type 1 Ryanodine Receptor Channels Carrying Malignant Hyperthermia and Central Core Disease Mutations in the Amino-Terminal Region | The mutations increased the gain and the sensitivity to activating Ca ²⁺ in a site-specific manner. Gain was consistently higher in both MH and MH/CCD mutations |

Table 1 Cellular *RYR1* model systems: Human embryonic kidney (HEK-293) cells (*Continued*)

| Author/Year | <i>RYR1</i> variant(s) | Title | Conclusions |
|----------------------------------|--|--|--|
| Miyoshi H, et al. [48] 2015 | R2508H, R2508G, R2508S, R2508K | Several Ryanodine Receptor Type 1 Gene Mutations of pArg2508 Are Potential Sources of Malignant Hyperthermia | Cells transfected with each of the 4 mutants, R2508H, R2508G, R2508S, or R2508K, were more sensitive to caffeine and 4CmC than wild-type cells |
| Mei Y, et al. [49] 2015 | G4934A, G4934V, G4941V, G4941A, G4941I | Channel Gating Dependence on Pore Lining Helix Glycine Residues in Skeletal Muscle Ryanodine Receptor | Both glycines are important for RyR1 channel function by providing flexibility and minimizing amino acid clashes |
| Shirvanyants D, et al. [50] 2014 | M4887G, M4887A, M4887V, V4891A, I4897Y | Pore dynamics and conductance of RyR1 transmembrane domain | Loss of these interactions in the case of polar substitution I4897T results in destabilization of the selectivity filter, a possible cause of the CCD-specific reduced Ca ²⁺ conductance |
| Roesl C, et al. [51] 2014 | R2452W | Functional characterisation of the R2452W ryanodine receptor variant associated with malignant hyperthermia susceptibility | R2452W results in a hypersensitive ryanodine receptor 1 and is likely to be causative of MH |
| Miyoshi H, et al. [52] 2014 | R2508C, R2508H, R2508K, R2508S | Two different variants of p.2508 in Japanese malignant hyperthermia patients causing hypersensitivity of ryanodine receptor 1 | All alterations in the p.2508 portion of RyR1 play important roles in the pathogenesis of MH |
| Sato K, et al. [53] 2013 | R44C, R163C, R401C, R533C, R533H, H4833Y | Skeletal muscle ryanodine receptor mutations associated with malignant hyperthermia showed enhanced intensity and sensitivity to triggering drugs when expressed in human embryonic kidney cells | These six mutations cause functional abnormality of the calcium channel, leading to higher sensitivity to a specific agonist |
| Kraeva N, et al. [54] 2013 | M4640R, L4647P, F4808L, D4918N, F4941C | Novel excitation-contraction uncoupled <i>RYR1</i> mutations in patients with central core disease | Homotetrameric RyR1 mutants harbouring L4646P, F4807P, D4917N and R4892Q mutations abolished caffeine-induced Ca ²⁺ release |
| Merritt A, et al. [55] 2012 | D1056H | Functional analysis of the pD1056H <i>RYR1</i> variant associated with malignant hyperthermia and exertional heat stroke | Cells expressing D1056H exhibited a trend for greater calcium release and increased sensitivity than wild-type at low doses of caffeine |
| Murayama T, et al. [56] 2011 | T4825A, T4825I, I4826A, L4827A, S4828A, S4829A | Role of amino-terminal half of the S4-S5 linker in type 1 ryanodine receptor (RyR1) channel gating | Four mutants had reduced CICR activity without changing Ca ²⁺ sensitivity, whereas the L4827A mutant formed a constitutive active channel T4825I, a disease-associated mutation for malignant hyperthermia, exhibited enhanced CICR activity |
| Haraki T, et al. [57] 2011 | A4894T, A4894P, A4894S, A4894G | Mutated p.4894 RyR1 function related to malignant hyperthermia and congenital neuromuscular disease with uniform type 1 fiber (CNMDU1) | The hypersensitive A4894T-RyR1 is associated with MH and the poorly functional A4894P-RyR1 with CNMDU1 |
| Zhou H, et al. [58] 2010 | R2939K | Multi-minicore disease and atypical periodic paralysis associated with novel mutations in the skeletal muscle ryanodine receptor (<i>RYR1</i>) gene | The R2435K mutation did not affect two characteristic functional properties of RyR1, as both Ca ²⁺ dependence and activation by caffeine were not altered |
| Sato K, et al. [59] 2010 | R163C, G248R, T4826I, H4833Y, I4898T, G4899R | Functional studies of <i>RYR1</i> mutations in the skeletal muscle ryanodine receptor using human <i>RYR1</i> complementary DNA | MH mutations showed a higher response, whereas CCD mutants (I4898T and G4899R) did not respond to 4-Cm C |
| Merritt A, et al. [60] 2010 | G3990V | Functional analysis of the pGly3990Val <i>RYR1</i> variant using a human cDNA clone in HEK293 cells | A statistically significant increase in Ca ²⁺ release was observed in G3990V mutants at each caffeine concentration that elicited a response |
| Migita T, et al. [61] 2009 | R2508C | Functional analysis of ryanodine receptor type 1 pR2508C mutation in exon 47 | The transfected <i>RYR1</i> mutant was more sensitive to caffeine and 4CmC than wildtype <i>RYR1</i> |
| Migita T, et al. [62] 2009 | R2508C, A4894T | Do Ca ²⁺ channel blockers improve malignant hyperthermia crisis? | The dantrolene-induced decline effect of Ca ²⁺ of skeletal muscle was not disappeared in the presence of Ca ²⁺ blockers. In MH crisis, we do not recommend to administer Ca ²⁺ blockers because of its potent effect to increase Ca ²⁺ |

Table 1 Cellular *RYR1* model systems: Human embryonic kidney (HEK-293) cells (Continued)

| Author/Year | <i>RYR1</i> variant(s) | Title | Conclusions |
|------------------------------|--|---|--|
| Ghassemi F, et al. [63] 2009 | R2435L | A recessive ryanodine receptor 1 mutation in a CCD patient increases channel activity | R2435L does not affect resting Ca^{2+} , or sensitivity of RyR1 to pharmacological activators. Instead it reduces the release of Ca^{2+} from intracellular stores induced by pharmacological activators as well as by KCl via the voltage sensing dihydropyridine receptor |
| Jiang D, et al. [64] 2008 | R615C | Reduced threshold for luminal Ca^{2+} activation of RyR1 underlies a causal mechanism of porcine malignant hyperthermia | R615C confers MH susceptibility by reducing the threshold for luminal Ca^{2+} activation and SOICR |
| Rossi D, et al. [65] 2007 | R4836fsX4838 | A truncation in the <i>RYR1</i> gene associated with central core lesions in skeletal muscle fibres | Subtle changes in Ca^{2+} release of human heteromeric RyR1/RyR1R4837fsX4839 channels, probably due to the reduced stability/assembly of these channels, may predispose individuals to MHS |
| Lyfenko AD, et al. [66] 2007 | R4214_F4216del, V4927_I4928del | Two central core disease (CCD) deletions in the C-terminal region of <i>RYR1</i> alter muscle excitation-contraction (EC) coupling by distinct mechanisms | Single channel data indicate that the Δ RQF mutation increases Ca^{2+} responsiveness without altering K^+ conductance and ion selectivity for Ca^{2+} compared to K^+ . In contrast, the Δ VI deletion abolished Ca^{2+} responsiveness, Ca^{2+} permeation, and significantly reduced K^+ conductance demonstrating that the Δ VI mutation introduced major alterations to the channel pore |
| Zhou H, et al. [67] 2006 | S71Y, R110W, L486V, A1578T, S2060C, N2283H | Characterization of recessive <i>RYR1</i> mutations in core myopathies | Recombinant channels with N2283H substitution showed an increased activity, whereas recombinant channels with S71Y + N2283H substitution lost activity upon isolation |
| Xu L, et al. [68] 2006 | D4938N, D4945N, D4953N, E4942Q, E4948Q, E4952Q, E4955Q | Two rings of negative charges in the cytosolic vestibule of type-1 ryanodine receptor modulate ion fluxes | D4938N and D4945N exhibited an attenuated block by neomycin to a greater extent from the cytosolic than luminal side. By comparison, charge neutralization of luminal loop residues (D4899Q, E4900N) eliminated the block from the luminal but not the cytosolic side |
| Wang Y, et al. [69] 2005 | D4899Q, E4900N | Probing the role of negatively charged amino acid residues in ion permeation of skeletal muscle ryanodine receptor | the negatively charged carboxyl oxygens of D4899 and E4900 side chains are major determinants of RyR ion conductance and selectivity |
| Brini M, et al. 2005 [70] | R615C, Y523S, I4898T | Ca^{2+} signaling in HEK-293 and skeletal muscle cells expressing recombinant ryanodine receptors harboring malignant hyperthermia and central core disease mutations | I4898T RyR1 channels produced cytosolic Ca^{2+} values which were similar to those observed for WT RyR1 channels. R615C augmented the amplitude of the cytosolic and mitochondrial Ca^{2+} transients following cell stimulation. By contrast, the mitochondrial Ca^{2+} transients were reduced in cells expressing Y523S |
| Du GG, et al. [71] 2004 | R4892W, I4897T, G4898E | Central core disease mutations R4892W, I4897T and G4898E in the ryanodine receptor isoform 1 reduce the Ca^{2+} sensitivity and amplitude of Ca^{2+} -dependent Ca^{2+} release | Ca^{2+} sensitivity is one of the serious defects in these three excitation-contraction uncoupling CCD mutations |
| Zozato F, et al. [72] 2003 | F4863_D4869delinsT | Clinical and functional effects of a deletion in a COOH-terminal luminal loop of the skeletal muscle ryanodine receptor | Channels carrying the deletion were less stable than the wild-type channels and disappeared rapidly when recorded at membrane potentials greater than ± 20 mV |
| Stange M, et al. [73] 2003 | S2843D, S2843A | Characterization of recombinant skeletal muscle (Ser-2843) and cardiac muscle (Ser-2809) ryanodine receptor phosphorylation mutants | Results did not support the view that phosphorylation of a single site (RyR1-Ser-2843 and RyR2-Ser-2809) substantially changes RyR1 and RyR2 channel function |

Table 1 Cellular *RYR1* model systems: Human embryonic kidney (HEK-293) cells (Continued)

| Author/Year | <i>RYR1</i> variant(s) | Title | Conclusions |
|---------------------------------|--|--|---|
| Loke JC, et al. [74] 2003 | R328W | Detection of a novel ryanodine receptor subtype 1 mutation (R328W) in a malignant hyperthermia family by sequencing of a leukocyte transcript | The mutant channel has increased sensitivity to both caffeine and halothane |
| Yamaguchi N, et al. [75] 2001 | V3619A, W3620A, L3624D, Δ 4274–4535 | Identification of apocalmodulin and Ca^{2+} – calmodulin regulatory domain in skeletal muscle Ca^{2+} release channel, ryanodine receptor | Two single amino acid substitutions distinctly change the regulation of the skeletal muscle Ca^{2+} release channel by CaM; one of which (L3624D) results in a loss of activation by apoCaM and an inhibition by CaCaM, whereas the other (W3620A) specifically abolishes CaCaM inhibition RyR1 Δ 4274–4535, showed an ~10-fold increased sensitivity to activating Ca^{2+} |
| Sun J, et al. [76] 2001 | C3635A | Cysteine-3635 is responsible for skeletal muscle ryanodine receptor modulation by NO | C3635A resulted in the loss of CaM-dependent NO modulation of channel activity and reduced S-nitrosylation by NO to background levels but did not affect NO-independent channel modulation by CaM or the redox sensitivity of the channel to O(2) and glutathione |
| Gaburjakova M, et al. [77] 2001 | V2461H, V2461E, V2461G, V2461I | FKBP12 binding modulates ryanodine receptor channel gating | Val2461 is a critical residue required for FKBP12 binding to RyR1 FKBP12 has a functional role in the RyR1 channel complex |
| Du GG, et al. [78] 2001 | G2370A, G2372A, G2373A, G2375A, Y3937A, S3938A, G3939A, K3940A | Mutations to Gly2370, Gly2373 or Gly2375 in malignant hyperthermia domain 2 decrease caffeine and cresol sensitivity of the rabbit skeletal-muscle Ca^{2+} – release channel (ryanodine receptor isoform 1) | Amino acids 2370–2375 lie within a sequence (amino acids 2163–2458) in which 8 RyR1 mutations associated with MH have been shown to be hypersensitive to caffeine and 4-chloro-m-cresol activation. By contrast, G2370A, G2373A and G2375A are hyposensitive to caffeine and 4-chloro-m-cresol. Amino acids 2163–2458 form a regulatory domain (MH regulatory domain 2) that regulates caffeine and 4-chloro-m-cresol sensitivity of RyR1 |
| Monnier N, et al. [79] 2000 | Y4796C | An autosomal dominant congenital myopathy with cores and rods is associated with a neomutation in the <i>RYR1</i> gene encoding the skeletal muscle ryanodine receptor | Expression of the mutant RYR1 cDNA produced channels with increased caffeine sensitivity and a significantly reduced maximal level of Ca^{2+} release. Single-cell Ca^{2+} analysis showed that the resting cytoplasmic level was increased by 60% in cells expressing the mutant channel |
| Gao L, et al. [80] 2000 | I4897A, I4897L, I4897V, D4917A, D4899A, D4899R, R4913E, G4894A, D4899N | Evidence for a role of the luminal M3-M4 loop in skeletal muscle Ca^{2+} release channel (ryanodine receptor) activity and conductance | Amino acid residues in the luminal loop region between the two most C-terminal membrane segments constitute a part of the ion-conducting pore of RyR1 |
| Tong J, et al. [81] 1999 | C36R, G249R, G342R, R553W, R615R, R615C, R2163C, G2435R, R2458C, R2458H, R164C, I404M, Y523S, R2163H, R2436H | Measurement of resting cytosolic Ca^{2+} concentrations and Ca^{2+} store size in HEK-293 cells transfected with malignant hyperthermia or central core disease mutant Ca^{2+} release channels | MH/CCD mutants were more sensitive to caffeine than WT RyR1, indicating that caffeine hypersensitivity observed with a variety of MH/CCD mutant RyR1 proteins is not dependent on extracellular Ca^{2+} concentration |
| Lynch PJ, et al. [82] 1999 | I4898T | A mutation in the transmembrane/luminal domain of the ryanodine receptor is associated with abnormal Ca^{2+} release channel function and severe central core disease | Single-cell analysis of co-transfected cells showed a significantly increased resting cytoplasmic Ca^{2+} level and a significantly reduced luminal Ca^{2+} level. These data are indicative of a leaky channel, possibly caused by a reduction in the Ca^{2+} concentration required for channel activation |
| Tong J, et al. [83] 1997 | R164C, G249R, G342R, I404M, Y523S, R615C, G2435R, R2436H, C36R, R553W, R615L, R2163C, R2163H, R2458C, R2458H | Caffeine and halothane sensitivity of intracellular Ca^{2+} release is altered by 15 calcium release channel (ryanodine receptor) mutations associated with malignant hyperthermia and/or central core disease | Abnormal sensitivity in the Ca^{2+} photometry assay provides supporting evidence for a causal role in MH for each of 15 single amino acid mutations in the ryanodine receptor |

Table 2 Cellular *RYR1* model systems: Transfected *RYR1*-null (dyspedic) myotubes

| Author/Year | <i>RYR1</i> variant(s) | Title | Conclusions |
|-----------------------------------|--------------------------------|--|--|
| Dyspedic myotubes | | | |
| Lefebvre R et al. [86] 2013 | R4892W, G4896V | Ca ²⁺ release in muscle fibers expressing R4892W and G4896V type 1 ryanodine receptor disease mutants | The dominant-negative effect of the R4892W mutant on voltage-gated Ca ²⁺ release in myotubes and adult muscle fibers was considerably less than that observed for G4896V |
| Groom L, et al. [87] 2011 | R3983C, D4505H | Identical de novo mutation in the type 1 ryanodine receptor gene associated with fatal, stress-induced malignant hyperthermia in two unrelated families | The functional impact of the two variants expressed in RyR1-null myotubes depends on whether the two variants are located on common or separate subunits |
| Booms P, et al. [88] 2009 | E2347del | Concentration dependence of caffeine-induced Ca ²⁺ release in dyspedic skeletal myotubes transfected with ryanodine receptor isoform-1 (RYR1) cDNAs | E2347del increases the sensitivity of RyR1 to caffeine |
| Yang T, et al. [89] 2007 | R164C, R165C, R2163C, T4825I | Elevated resting [Ca ²⁺] _i in myotubes expressing malignant hyperthermia RyR1 cDNAs is partially restored by modulation of passive calcium leak from the SR | Myotubes expressing any of the four MH RyR1s (at least 1 from all 3 mutation hot spots) had a higher resting [Ca ²⁺] _i than those expressing WTRyR1. The elevated resting [Ca ²⁺] _i observed in myotubes expressing the four MHRyR1s varied among the individual mutations. Treatment of myotubes expressing WT/MHRyR1s with ryanodine or FLA 365 had no effect on resting [Ca ²⁺] _i . Incubation of myotubes with bastadin 5 or ryanodine and bastadin 5 in combination significantly lowered resting [Ca ²⁺] _i in myotubes expressing either WTRyR1 and MHRyR1s. The percent decrease in resting [Ca ²⁺] _i after treatment with bastadin 5 or the combination of ryanodine and bastadin 5 in myotubes expressing MHRyR1s was significantly greater than in myotubes expressing WTRyR1s. |
| Yang T, et al. [90] 2007 | R615C, R2163C, T4825I | Enhanced excitation-coupled calcium entry in myotubes is associated with expression of RyR1 malignant hyperthermia mutations | RyR1 MH mutations are associated with an enhanced Ca ²⁺ entry through the sarcolemma during depolarization. Ca ²⁺ entry may contribute to maintaining Ca ²⁺ homeostasis in mammalian skeletal EC coupling and may play an important role in the pathophysiology of malignant hyperthermia. |
| Goonasekera SA, et al. [91] 2007 | D4878A, D4907A, E4908A | Triadin binding to the C-terminal luminal loop of the ryanodine receptor is important for skeletal muscle excitation contraction coupling | triadin binding to RyR1 enhances release channel activity during both voltage and ligand activation and that this critical regulation of release channel activity ensures robust and rapid calcium release during skeletal muscle EC coupling. |
| Lyfenko AD, et al. [66] 2007 | R4214_F4216del, V4927_I4928del | Two central core disease (CCD) deletions in the C-terminal region of RYR1 alter muscle excitation-contraction (EC) coupling by distinct mechanisms | R4214_F4216del promotes Ca ²⁺ depletion from intracellular stores by exhibiting a classic "leaky channel" behavior. V4927_I4928del deletion reduces Ca ²⁺ release by disrupting Ca ²⁺ gating and eliminating Ca ²⁺ permeation through the open channel. |
| Lee EH, et al. [92] 2006 | D4878A, D4907A, E4908A | Occurrence of atypical Ca ²⁺ transients in triadin-binding deficient-RYR1 mutants | There was similarly atypical Ca ²⁺ transients in response to caffeine in myotubes expressing all 3 mutations and the single mutant (D4907A). Differences in triadin-binding and SR Ca ²⁺ release observed in this study can be attributed to an alteration in a single amino acid (D4907). |
| Aracena-Parks P, et al. [93] 2006 | C3635A | Identification of cysteines involved in S-nitrosylation, S-glutathionylation, and oxidation to disulfides in ryanodine receptor type 1 | 12 of the 100 cysteines on RyR1 can be redox-modified and that 9 of these cysteines appear to be endogenously modified to some extent. We also show that the different redox agents target some of the same cysteines, but Cys-1040 and Cys-1303 are exclusively S-nitrosylated, whereas Cys-1591 and Cys-3193 are exclusively S-glutathionylated. On the other hand, Cys-3635 can be S-nitrosylated, S-glutathionylated, or oxidized to form a disulfide and also influences Ca ²⁺ release during EC coupling. |

Table 2 Cellular *RYR1* model systems: Transfected *RYR1*-null (dyspedic) myotubes (Continued)

| Author/Year | <i>RYR1</i> variant(s) | Title | Conclusions |
|---------------------------------|---|---|--|
| Hurne AM, et al. [94] 2005 | C4958S, C4961S | Ryanodine receptor type 1 (RyR1) mutations C4958S and C4961S reveal excitation-coupled calcium entry (ECCE) is independent of sarcoplasmic reticulum store depletion | There is an essential role of Cys(4958) and Cys(4961) within an invariant CXXC motif for stabilizing conformations of RyR1 that influence both its function as a release channel and its interaction with ECCE channels |
| Cheng W, et al. [95] 2005 | D3490_N3523del | Interaction between the dihydropyridine receptor Ca ²⁺ channel β -subunit and ryanodine receptor type 1 strengthens excitation-contraction coupling | EC coupling in skeletal muscle involves the interplay of at least two subunits of the DHPR, namely α 1S and β 1a, interacting with possibly different domains of RyR1 |
| Du GG, et al. [96] 2004 | 4274_4535del | Role of the sequence surrounding predicted transmembrane helix M4 in membrane association and function of the Ca(2+) release channel of skeletal muscle sarcoplasmic reticulum (ryanodine receptor isoform 1) | Maximal amplitudes of L-currents and Ca ²⁺ transients with Delta4274–4535 were larger than with wild-type RyR1, and voltage-gated sarcoplasmic reticulum Ca ²⁺ release was more sensitive to activation by sarcolemmal voltage sensors Thus, this region may act as a negative regulatory module that increases the energy barrier for Ca ²⁺ release channel opening |
| Dirksen RT, et al. [97] 2004 | Y4795C, R2435L, R2163H | Distinct effects on Ca ²⁺ handling caused by malignant hyperthermia and central core disease mutations in RyR1 | MH-only mutations modestly increase basal release-channel activity in a manner insufficient to alter net SR Ca ²⁺ content ("compensated leak"), whereas the mixed MH + CCD phenotype arises from mutations that enhance basal activity to a level sufficient to promote SR Ca ²⁺ depletion, elevate [Ca ²⁺], and reduce maximal VGCR ("decompensated leak") |
| Zhu X, et al. [98] 2004 | 3614_3643del | The calmodulin binding region of the skeletal ryanodine receptor acts as a self-modulatory domain | Depolarization-, caffeine- and 4-chloro-m-cresol (4-CmC)-induced Ca ²⁺ transients in these cells were dramatically reduced compared with cells expressing WT RyR1. Deletion of the 3614–3643 region resulted in profound changes in unitary conductance and channel gating RyR1 3614–3643 region acts not only as the CaM binding site, but also as an important modulatory domain for RyR1 function |
| Yang T, et al. [99] 2003 | R163C, G341R, R614C, R2163C, V2168M, R2458H, T4826I | Functional defects in six ryanodine receptor isoform-1 (RyR1) mutations associated with malignant hyperthermia and their impact on skeletal excitation-contraction coupling | These 7 MH mutations are all both necessary and sufficient to induce MH-related phenotypes Decreased sensitivity to Ca ²⁺ and Mg ²⁺ inhibition and inability of MHRyR1s to be fully inactivated at [Ca ²⁺] typical of normal myotubes at rest are key defects that contribute to the initiation of MH episodes |
| Avila G, et al. [100] 2003 | Y523S, Y4795C, I4897T, G4890R, R4892W, G4898E, G4898R, A4905V, R4913G | The pore region of the skeletal muscle ryanodine receptor is a primary locus for excitation-contraction uncoupling in central core disease | CCD mutations in exon 102 disrupt release channel permeation to Ca ²⁺ during EC coupling and that this region represents a primary molecular locus for EC uncoupling in CCD |
| Avila G, et al. [101] 2003 | V2461G, V2461I | FKBP12 binding to RyR1 modulates excitation-contraction coupling in mouse skeletal myotubes | None of the mutations that disrupted FKBP binding to RyR1 significantly affected RyR1-mediated enhancement of L-type Ca ²⁺ channel activity (retrograde coupling) FKBP12 binding to RyR1 enhances the gain of skeletal muscle EC coupling |
| O'Connell KM, et al. [102] 2002 | L3624D, W3620A | Calmodulin binding to the 3614–3643 region of RyR1 is not essential for excitation-contraction coupling in skeletal myotubes | Expression of either L3624D or W3620A in dyspedic myotubes restored both L-type Ca ²⁺ currents (retrograde coupling) and voltage-gated SR Ca ²⁺ release (orthograde coupling) to a similar degree as that observed for wild-type RyR1, although L-current density was somewhat larger and activated at more hyperpolarized potentials in W3620A-expressing myotubes CaM binding to the 3614–3643 region of RyR1 is not essential for voltage sensor activation of RyR1 |
| O'Brien JJ, et al. [103] 2002 | E4032A | Ca ²⁺ activation of RyR1 is not necessary for the initiation of skeletal-type excitation-contraction coupling | Depolarization of E4032A-RyR1-expressing myotubes elicited L-type Ca ²⁺ currents of approximately normal size and myoplasmic Ca ²⁺ transients that were skeletal-type, but about fivefold smaller than |

Table 2 Cellular *RYR1* model systems: Transfected *RYR1*-null (dyspedic) myotubes (Continued)

| Author/Year | <i>RYR1</i> variant(s) | Title | Conclusions |
|---------------------------------|-------------------------------------|---|---|
| | | | those for wild-type RyR1 The reduced amplitude of the Ca ²⁺ transient is consistent either with the possibility that Ca ²⁺ activation amplifies Ca ²⁺ release during EC coupling, or that the E4032A mutation generally inhibits activation of RyR1 Ca ²⁺ activation of RyR1 does not appear to be necessary for the initiation of Ca ²⁺ release during EC coupling in skeletal muscle |
| Feng W, et al. [104] 2002 | F1777R, F1782R | Homer regulates gain of ryanodine receptor type 1 channel complex | 1B5 dyspedic myotubes expressing RyR1 with a point mutation of a putative Homer-binding domain exhibit significantly reduced (approximately 33%) amplitude in their responses to K ⁺ depolarization compared with cells expressing wild type protein These results reveal that in addition to its known role as an adapter protein, Homer is a direct modulator of Ca ²⁺ release gain |
| Fessenden JD, et al. [105] 2001 | E4032A | Ryanodine receptor point mutant E4032A reveals an allosteric interaction with ryanodine | Results with the E4032A mutant channel suggest that ryanodine does not act as a pore blocker but instead, that ryanodine binding sites reside outside of the permeation pore, and that ryanodine binding to these sites has allosteric effects on calcium permeability |
| Avila G, et al. [106] 2001 | E4032A | Ca ²⁺ release through ryanodine receptors regulates skeletal muscle L-type Ca ²⁺ channel expression | Long-term expression of E4032A, a mutant RyR-1 that preferentially affects the orthograde signal of E-C coupling (i.e., fully restores L-channel activity but not SR Ca ²⁺ release) failed to increase functional DHPR expression |
| Avila G, et al. [107] 2001 | I4897T | Excitation - Contraction uncoupling by a human central core disease mutation in the ryanodine receptor | Muscle weakness suffered by individuals possessing the I4898T mutation involves a functional uncoupling of sarcolemmal excitation from SR Ca ²⁺ release, rather than the expression of overactive or leaky SR Ca ²⁺ release channel |
| Avila G, et al. [108] 2001 | R164C, I404M, Y523S, R2163H, R2435H | Functional effects of central core disease mutations in the cytoplasmic region of the skeletal muscle ryanodine receptor | Resting Ca ²⁺ levels were elevated in dyspedic myotubes expressing four of these mutants (Y523S > R2163H > R2435H R164C > I404M RyR1) |

using Boolean operators and MeSH terms: RYR-1 OR RYR1 OR RyR1s OR ryanodine receptor calcium release channel OR “ryanodine receptor 1” AND malignant hyperthermia OR “malignant hyperthermia” OR malignant hyperpyrexia OR anesthesia hyperthermia OR Muscular diseases OR muscular diseases OR myopathies OR myopathy OR muscle OR muscular OR muscle contraction OR muscle contraction OR smooth muscle OR cardiac muscle OR skeletal muscle OR muscle fiber OR myofibril. The full search strategy is provided in Additional file 1.

Study selection

Following removal of duplicates, titles and abstracts of all publications were reviewed independently by two of the authors and marked for inclusion if they discussed a MH or *RYR1*-RM preclinical model system. Publications were marked for exclusion if they were (1) not gene or isoform of interest (e.g. *CACNA1S*-related MH), (2) clinical report, (3) structural biology, (4) wild-type models and methods, (5) cardiac or smooth muscle, (6) review

articles, or (7) categorized as miscellaneous. All publications were discussed with a third author who adjudicated when there was discordance between the first two authors over whether publication should be included or excluded.

Charting data and reporting the results

The following data were extracted from full text publications selected for inclusion in the review: first author, year of publication, title of the publication, variation(s) of the preclinical model system(s), and conclusions of the publication on the disease model system(s). Data were tabulated according to type of preclinical model system. Categories included transfected human embryonic kidney cell (HEK)-293 cells, transfected *RYR1*-null (dyspedic) myotubes, immortalized B-lymphocytes, primary cell culture, porcine model systems, and rodent model systems. Data on all other preclinical model systems, including zebrafish, avian, *C. elegans*, and drosophila, were combined and tabulated separately. Two authors reviewed data extracted for each article. To

Table 3 Cellular *RYR1* model systems: Immortalized B-lymphocytes

| Author/Year | <i>RYR1</i> variant(s) | Title | Conclusions |
|---------------------------------|---|--|---|
| Zullo A, et al. [110] 2019 | R1335C, S2345R, S3098I, F4924_V4925ins | <i>RYR1</i> sequence variants in myopathies: Expression and functional studies in two families | Ca ²⁺ release in response to the RyR1 agonist 4-chloro-m-cresol and to thapsigargin showed that S2345R causes depletion of S/ER Ca ²⁺ stores and that the compound heterozygosity for variant S3098I and the 30-nucleotide insertion increases RyR1-dependent Ca ²⁺ release without affecting ER Ca ²⁺ stores |
| Johannsen S, et al. [111] 2016 | R4737W | Functional characterization of the RYR1 mutation pArg4737Trp associated with susceptibility to malignant hyperthermia | Intracellular resting calcium was slightly but significantly elevated in mutation positive cells. Calcium release following stimulation with 4-chloro-m-cresol was significantly increased in B lymphocytes carrying the R4737W mutation compared to mutation negative controls |
| Schiemann AH, et al. [112] 2014 | R2355W, V2354M | Functional characterization of 2 known ryanodine receptor mutations causing malignant hyperthermia | We propose that R2355W is confirmed as being an MH-causative mutation and suggest that V2354M is a <i>RYR1</i> mutation likely to cause MH |
| Lyfenko AD, et al. [66] 2007 | R4214_F4216del | Two central core disease (CCD) deletions in the C-terminal region of RYR1 alter muscle excitation-contraction (EC) coupling by distinct mechanisms | ΔRQF <i>RYR1</i> deletion did not significantly affect the sensitivity of lymphoblastoid cells to activation by 4-CmC. However, Ca ²⁺ release activated by a maximal concentration of 4-CmC (1 mM) was significantly reduced in ΔRQF-carrying cells |
| Schiemann AH, et al. 2013 | R1583C, V2102L | Sequence capture and massively parallel sequencing to detect mutations associated with malignant hyperthermia | The amount of Ca ²⁺ released after stimulation with 4-chloro-m-cresol from B lymphocytes of the MH-susceptible patients in the family was significantly greater compared with that of Ca ²⁺ released from cells of an MH-negative family member |
| Attali R, et al. [113] 2013 | Y3016C | Variable myopathic presentation in a single family with novel skeletal RYR1 mutation | Functional analysis on EBV immortalized cell lines showed no effect of the mutation on RyR1 pharmacological activation or the content of intracellular Ca ²⁺ stores |
| Vukcevic M, et al. [114] 2010 | R1679H, K1393R, E1058K, H382N, R2508G | Functional properties of RYR1 mutations identified in Swedish patients with malignant hyperthermia and central core disease | All B lymphoblastoid cell lines carrying RYR1 candidate mutations showed significantly increased resting cytoplasmic Ca ²⁺ levels as well as a shift to lower concentrations of 4-CmC inducing calcium release compared with controls |
| Grievink H, et al. [115] 2010 | H4833Y | Allele-specific differences in ryanodine receptor 1 mRNA expression levels may contribute to phenotypic variability in malignant hyperthermia | Allele-specific differences in RYR1 mRNA expression levels in heterozygous MHS samples, and can at least in part contribute to the observed variable penetrance and variations in MH clinical phenotypes |
| Zozato F, et al. [72] 2003 | F4863_D4869delinsT | Clinical and functional effects of a deletion in a COOH-terminal luminal loop of the skeletal muscle ryanodine receptor | Cells have depleted thapsigargin-sensitive intracellular Ca ²⁺ stores, exhibit release of Ca ²⁺ from intracellular stores in the absence of the addition of a pharmacological activator of the RYR1; and the unelicited Ca ²⁺ transient from the thapsigargin-sensitive stores could be blocked by dantrolene, a specific inhibitor of the skeletal muscle RYR |
| Zullo A, et al. [116] 2009 | R530H, R2163P, N2342S, E2371G, R2454H, C4664R | Functional characterization of ryanodine receptor (<i>RYR1</i>) sequence variants using a metabolic assay in immortalized B-lymphocytes | Increased acidification rate of lymphoblastoid cells in response to 4-CmC is mainly due to RYR1 activation. Cells expressing RYR1 variants in the N-terminal and in the central region of the protein (R530H, R2163P, N2342S, E2371G and R2454H) displayed higher activity compared with controls. Cell lines harboring RYR1(C4664R) were significantly less activated by 4-CmC |
| Levano S, et al. [117] 2009 | D554Y, R2336H, E2404K, D2730G | Increasing the number of diagnostic mutations in malignant hyperthermia | All <i>RYR1</i> mutations significantly increased resting calcium concentration and significantly affect either 4-CmC or caffeine dose-response curve to pharmacological activation Only one mutation (D2730G) showed a significant reduction in EC50 of both caffeine and 4-CmC |
| Anderson AA, et al. [118] 2008 | H4833Y | Identification and biochemical characterization of a novel ryanodine receptor gene mutation associated with malignant hyperthermia | B lymphocytes from patients with this mutation were approximately twofold more sensitive than MH-negative cells to activation with 4-CmC. The amount of Ca ²⁺ released from B lymphocytes of MH-susceptible patients was significantly greater than that released from cells of |

Table 3 Cellular *RYR1* model systems: Immortalized B-lymphocytes (Continued)

| Author/Year | <i>RYR1</i> variant(s) | Title | Conclusions |
|------------------------------|------------------------|--|---|
| Ducreux D, et al. [119] 2006 | P3527S, V4849I, R999H | Functional properties of ryanodine receptors carrying three amino acid substitutions identified in patients affected by multi-minicore disease and central core disease, expressed in immortalized lymphocytes | family members without this mutation P3527S in the homozygous state affected the amount of Ca ²⁺ released after pharmacological activation with 4-CmC and caffeine but did not affect the size of the thapsigargin-sensitive Ca ²⁺ stores. The other substitutions had no effect on the size of the intracellular Ca ²⁺ stores, or the amount of Ca ²⁺ released after ryanodine receptor activation P3527S and V4849I substitutions had a small but significant effect on the resting Ca ²⁺ concentration |
| Tilgen N, et al. [120] 2001 | R4861H, I4898T, G4899A | Identification of four novel mutations in the C-terminal membrane spanning domain of the ryanodine receptor 1: Association with central core disease and alteration of calcium homeostasis | Cell showed release of Ca ²⁺ from intracellular stores in the absence of any pharmacological activators of RYR, significantly smaller thapsigargin-sensitive intracellular calcium stores, compared to lymphoblasts from control individuals, and a normal sensitivity of the calcium release to the RYR inhibitor dantrolene |
| Girard T, et al. [121] 2001 | V2168M | B-lymphocytes from malignant hyperthermia-susceptible patients have an increased sensitivity to skeletal muscle ryanodine receptor activators | EBV immortalized cells harboring the V2168M <i>RYR1</i> gene mutation were more sensitive to the RYR activator 4-CmC and their peripheral blood leukocytes produce more interleukin-1beta after treatment with the RYR activators caffeine and 4-CmC, compared with cells from healthy controls |
| Hoppe K, et al. [122] 2016 | R530H, C4664R, R2163P | Hypermetabolism in B-lymphocytes from malignant hyperthermia susceptible individuals | Native B-lymphocytes from MHS individuals are more sensitive to 4-CmC than those from MHN, reflecting a greater Ca ²⁺ turnover. The acidification response, however, was less pronounced than in muscle cells, presumably reflecting the lower expression of RyR1 in B-lymphocytes. |

identify gaps in the literature where no preclinical model system had been reported for a specific *RYR1* protein-coding region, the number of publications per *RYR1* exon was mapped against established MH/CCD hotspot regions and sequence of the RyR1 protein structure. The composition of included and excluded publications was also summarized.

Results

Study characteristics

The search strategy utilized in this study yielded 5049 research publications between January 1, 1990 and July 3, 2019. Nine additional publications were retrieved through other information sources. Following removal of 2814 duplicates, 2284 abstracts were screened for inclusion. A total of 1956 publications were excluded at this point, leaving 328 for full text review. During full text review, 66 additional publications were excluded leaving 262 publications for inclusion in this review. An overview of this process is provided in Fig. 1.

The majority of publications that met inclusion criteria for this review focused on *RYR1* cellular and rodent model systems (43 and 39%, and respectively), Fig. 2a. Wild-type/methods publications formed the largest group of those excluded (24%), followed by those focused on cardiac/smooth muscle (19%), not isoform/gene of interest (16%), and clinical reports (13%), Fig. 2b.

The highest frequencies of variations reported in *RYR1* preclinical model systems were localized to established MH/central core disease (MH/CCD) hot spots 1, 2, and 3 located between exons 1–17, 39–46, and 90–103, respectively, Fig. 3. At least one *RYR1* preclinical model system was reported for every RyR1 structural region.

A total of 250 unique *RYR1* variations were reported in human/mouse/porcine model systems with 95% being missense substitutions. The most frequently reported *RYR1* variations reported across species were R614C/R615C (human/porcine total $n = 39$), Y523S/Y524S (rabbit/mouse total $n = 30$), I4898T/I4897T/I4895T (human/rabbit/mouse total $n = 20$), and R163C/R165C (human/mouse total $n = 18$). The dyspedic mouse was the most frequently reported mouse model system comprising 47% of publications in this category. The predominant type of *RYR1* preclinical model system used has varied over time. From 1990 to 1994, the R615C porcine model system was most frequently reported. Cellular model systems were then most frequently reported until 2010, after which this transitioned to rodent model systems including RyR1-null (dyspedic) and Y524S, R163C, and I4895T mutant mice, Fig. 4.

Cellular model systems

Expression of recombinant *RYR1* in heterologous cells

A total of 49 publications reported transfecting mutant *RYR1* cDNA into HEK-293 cells, which lack native RyR1

Table 4 Primary cell culture model systems

| Author/ Year | Species/ RyR1 variant(s) | Title | Conclusions |
|----------------------------------|---|--|---|
| Suman M, et al. [123] 2018 | N4575T, I1571V, L3136Rfs, R163C, I4898T, Q4837RfsX3 | Inositol triphosphate receptor-mediated Ca ²⁺ signaling stimulates mitochondrial function and gene expression in core myopathy patients | Remodeling of skeletal muscle Ca ²⁺ signaling following loss of functional RyR1 mediates bioenergetic adaptation |
| Choi RH, et al. [124] 2017 | R1976C | Dantrolene requires Mg ²⁺ to arrest malignant hyperthermia | Accumulation of the metabolite Mg ²⁺ from MgATP hydrolysis is required to make dantrolene administration effective in arresting an MH episode |
| Hoppe K, et al. [122] 2016 | G2434R, R614C | Hypermetabolism in B-lymphocytes from malignant hyperthermia susceptible individuals | Native B-lymphocytes from MHS individuals are more sensitive to 4-CmC than those from MHN, reflecting a greater Ca ²⁺ turnover. The acidification response, however, was less pronounced than in muscle cells, presumably reflecting the lower expression of RyR1 in B-lymphocytes |
| Kaufmann A, et al. [125] 2012 | A612P, R2458H, R3348C | Novel double and single ryanodine receptor 1 variants in two Austrian malignant hyperthermia families | Results suggest that these variants are new causative MH variants |
| Treves S, et al. [126] 2010 | V2168M, R2336H, R614C, D2730G, R44C, R789L | Enhanced excitation-coupled Ca ²⁺ entry induces nuclear translocation of NFAT and contributes to IL-6 release from myotubes from patients with central core disease | Excitation-coupled calcium entry is strongly enhanced in cells from patients with CCD compared with individuals with MH and controls. Excitation-coupled calcium entry induces generation of reactive nitrogen species and enhances nuclear localization of NFATc1, which in turn may be responsible for the increased IL-6 released by myotubes from patients with CCD |
| Kobayashi M, et al. [127] 2011 | L4838V, R2508C | Analysis of human cultured myotubes responses mediated by ryanodine receptor 1 | Among samples from CICR-accelerated patients, there was an increased sensitivity to RyR1 activators compared to non-accelerated patients. The EC50 values for these different compounds correlated with results of CICR testing. Using this approach may be a sensitive and specific method of identifying patients predispose to MH |
| Migita T, et al. [62] 2009 | R2508C, A4894T | Do Ca ²⁺ channel blockers improve malignant hyperthermia crisis? | The dantrolene-induced decline effect of Ca ²⁺ of skeletal muscle was not disappeared in the presence of Ca ²⁺ blockers. In MH crisis, we do not recommend to administer Ca ²⁺ blockers because of its potent effect to increase Ca ²⁺ |
| Migita T, et al. [128, 129] 2007 | R2508C, L4838V | Propofol-Induced Changes in Myoplasmic Calcium Concentrations in Cultured Human Skeletal Muscles from RyR1 Mutation Carriers | Increases in calcium concentrations in response to propofol dosage were limited to doses at least 100-fold greater than those used in clinical settings. These observations correlate well with clinical observations that propofol does not trigger malignant hyperthermia in susceptible humans |
| Zhou, et al. [130] 2006 | R109W, M402T, M2423K, R2939K, A4329D, T4709M | Epigenetic allele silencing unveils recessive RyR1 mutations in core myopathies | RyR1 undergoes polymorphic, tissue-specific, and developmentally regulated allele silencing and that this unveils recessive mutations in patients with core myopathies |
| Weigl LG, et al. [131] 2004 | G2434R | 4-Chloro-m-cresol cannot detect malignant hyperthermia equivocal cells in an alternative minimally invasive diagnostic test of malignant hyperthermia susceptibility | Cells of MHEH individuals showed low sensitivities against both caffeine and 4-CmC, comparable to those of the MHN group. Therefore, with myotubes, caffeine was able to discriminate between MHS and MHN cells, but both caffeine and 4-CmC failed to detect MHEH cells |
| Wehner M, et al. [132] 2004 | A2350T, R2355W, G2375A | Functional characterization of malignant hyperthermia-associated RyR1 mutations in exon 44, using the human myotube model | Investigation of calcium homeostasis with the calcium sensitive probe Fura 2 showed a higher sensitivity to the ryanodine receptor |

Table 4 Primary cell culture model systems (Continued)

| Author/ Year | Species/ RyR1 variant(s) | Title | Conclusions |
|---------------------------------|--|---|---|
| | | | agonists 4-chloro-m-cresol, caffeine and halothane for the myotubes derived from the mutation carriers as compared to those of the control group |
| Ducreux S, et al. [133] 2004 | V2168M, I4898T, R4893W | Effect of ryanodine receptor mutations on interleukin-6 release and intracellular calcium homeostasis in human myotubes from malignant hyperthermia-susceptible individuals and patients affected by central core disease | Abnormal release of calcium via mutated RYR enhances the production of the inflammatory cytokine IL-6, which may in turn affect signaling pathways responsible for the trophic status of muscle fibers |
| Wehner M, et al. [134] 2003 | I2182F, G2375A | Calcium release from sarcoplasmic reticulum is facilitated in human myotubes derived from carriers of the ryanodine receptor type 1 mutations Ile2182Phe and Gly2375Ala | In myotubes of individuals carrying the RyR1 Ile2182Phe or the RyR1 Gly2375Ala mutation, the EC(50) for caffeine and halothane was reduced; in the Ile2182Phe myotubes, the EC(50) for 4CmC was also reduced, all consistent with facilitated calcium release from the sarcoplasmic reticulum. From these data we conclude that both mutations are pathogenic for MH |
| Wehner M, et al. [135] 2003 | I2453T | The Ile2453Thr mutation in the ryanodine receptor gene 1 is associated with facilitated calcium release from sarcoplasmic reticulum by 4-chloro-m-cresol in human myotubes | The reduction of EC(50) indicates a facilitated calcium release from sarcoplasmic reticulum in the myotubes of the index patient suggesting that the RyR1 Ile2453Thr mutation is pathogenic for the malignant hyperthermia susceptibility and CCD of the two affected individuals |
| Wehner M, et al. [136] 2002 | T2206M | Increased sensitivity to 4-chloro-m-cresol and caffeine in primary myotubes from malignant hyperthermia susceptible individuals carrying the ryanodine receptor 1 Thr2206Met (C6617T) mutation | In myotubes the half-maximal activation concentration (EC(50)) for 4-chloro-m-cresol was reduced from 203 micro m (wild type) to 98 micro m (Thr2206Met), and for caffeine from 3.8 mm to 1.8 mm. From the reduction of EC(50) we conclude that the RyR1 Thr2206Met mutation is pathogenic for MH |
| Sei Y, et al. [137] 2002 | C35R, R163C, G248R, G341R, I403M, R552W, R614C, R614L, R2163C, R2163H, V2168, V2214I, A2367T, D2431N, G2434R, R2435H, R2454C, R2454H, R2458C, R2458H, I4898T | Patients with malignant hyperthermia demonstrate an altered calcium control mechanism in B lymphocytes | The Ca ²⁺ responses to caffeine or 4-chloro-m-cresol in B lymphocytes showed significant differences between MHS and MHN (or control) individuals. Although the molecular mechanisms of these alterations are currently undetermined, the results suggest that the enhanced Ca ²⁺ responses are associated with mutations in the RYR1 gene in some MHS individuals |
| Girard T, et al. [138] 2002 | R614C, G2434R, V2168M, R2458C | Phenotyping malignant hyperthermia susceptibility by measuring halothane-induced changes in myoplasmic calcium concentration in cultured human skeletal muscle cells | Measurements of Ca ²⁺ in human skeletal muscle cells can be used to phenotype MH susceptibility; however, we did not observe a specific effect of any mutation in the RYR1 gene on the halothane-induced increase in Ca ²⁺ |
| Brinkmeier H, et al. [139] 1999 | G2435R | Malignant hyperthermia causing Gly2435Arg mutation of the ryanodine receptor facilitates ryanodine-induced calcium release in myotubes | The phenotype of MH can be characterized using cultured human muscle and a culture-based test for MH susceptibility may eventually be developed. |
| Censier K, et al. [140] 1998 | R163C | Intracellular calcium homeostasis in human primary muscle cells from malignant hyperthermia-susceptible and normal individuals. Effect Of overexpression of recombinant wild-type and Arg163Cys mutated ryanodine receptors | Cultured human skeletal muscle cells derived from MH-susceptible individuals exhibit a half-maximal halothane concentration causing an increase in intracellular Ca ²⁺ concentration which is twofold lower than that of cells derived from MH-negative individuals. The resting Ca ²⁺ concentration of cultured skeletal muscle cells from MH-negative and MH-susceptible individuals is not significantly different |

Table 5 Rodent *RyR1* model systems

| Author/Year | <i>Ryr1</i> variant(s) | Title | Conclusions |
|--|------------------------|--|--|
| YS mouse (equivalent to Y522S in humans) | | | |
| Zullo A, et al. [141] 2018 | Y524S | Voltage modulates halothane-triggered Ca ²⁺ release in malignant hyperthermia-susceptible muscle | Binding of halothane to RyR1 alters the voltage dependence of Ca ²⁺ release in MH-susceptible muscle fibers such that the resting membrane potential becomes a decisive factor for the efficiency of the drug to trigger Ca ²⁺ release |
| O-Uchi J, et al. [142] 2017 | Y524S | Malignant hyperthermia-associated mutation of leaky RyR1 induces mitochondrial Ca ²⁺ overload in the heart | Chronic mitochondrial Ca ²⁺ overload via leaky mutant mRyR1 damages cardiac mitochondrial functions/structures, which may alter cytosolic Ca ²⁺ handling, induce cellular oxidation, and increase the arrhythmogenic events in MH |
| Abeele FV, et al. [143] 2019 | Y524S | TRPV1 variants impair intracellular Ca ²⁺ signaling and may confer susceptibility to malignant hyperthermia | Trpv1 may be contributing to the mechanism underlying the hyperthermia response of this Y524S RyR1 model TRPV1 and related mutants could be a new therapeutic target for treating muscle diseases due to altered regulation of Ca ²⁺ release |
| Michelucci A, et al. [144] 2017 | Y524S | Strenuous exercise triggers a life-threatening response in mice susceptible to malignant hyperthermia | Strenuous physical exertion triggers lethal episodes in MH-susceptible mice and these episodes share common features with MH episodes triggered by anesthetics and heat (ie, hyperthermia and rhabdomyolysis) |
| Michelucci A, et al. [145] 2017 | Y524S | Antioxidant Treatment Reduces Formation of Structural Cores and Improves Muscle Function in RYR1(Y522S/WT) Mice | NAC administration is beneficial to prevent mitochondrial damage and formation of cores and improve muscle function in RYR1Y522S/WT mice |
| Lopez RJ, et al. [146] 2016 | Y524S | An RYR1 mutation associated with malignant hyperthermia is also associated with bleeding abnormalities | Y522S mice had longer bleeding times than their WT littermates. Primary vascular smooth muscle cells from Y524S mice exhibited a higher frequency of subplasmalemmal Ca ²⁺ sparks, leading to a more negative resting membrane potential. The bleeding defect of Y524S mice and of one patient was reversed by treatment with the RYR1 antagonist dantrolene, and Ca ²⁺ sparks in primary vascular smooth muscle cells from Y524S mice were blocked by ryanodine or dantrolene |
| O-Uchi J, et al. [147] 2016 | Y524S | Malignant hyperthermia-associated mutation of RyR1 induces mitochondrial damages and cellular oxidation in the heart | Chronic mitochondrial Ca ²⁺ overload via leaky mutant mRyR1 damages cardiac mitochondrial functions/structures, reduces cytosolic Ca ²⁺ buffering capacity and induces cellular oxidation, which may increase arrhythmogenic events in MH |
| O-Uchi J, et al. [148] 2014 | Y524S | RyR1 mutation associated with malignant hyperthermia facilitates catecholaminergic stress-induced arrhythmia via mitochondrial injury and oxidative stress | Chronic mitochondrial damage by Ca ²⁺ overload via leaky mutant RyR1 induces cellular oxidation, which facilitates catecholaminergic stress-triggered arrhythmia |
| Yarotsky V, et al. [149] 2013 | Y524S | Accelerated activation of SOCE current in myotubes from two mouse models of anesthetic- and heat-induced sudden death | While an increased rate of SOCE current activation is a common characteristic of myotubes derived from Y524S/+ and dCasq-null mice and that the protective effects of azumolene are not due to a direct inhibition of SOCE channels |
| Vukcevic M, et al. [150] 2013 | Y524S | Gain of function in the immune system caused by a ryanodine receptor 1 mutation | Y524S mice have a gain in immune functions. Gain-of-function MH-linked RYR1 mutations might offer selective immune advantages to their carriers |
| Manno C, et al. [151] 2013 | Y524S | Altered Ca ²⁺ concentration, permeability and buffering in the myofibre Ca ²⁺ store of a mouse model of malignant hyperthermia | Y524S mutation causes greater openness of the RyR1, lowers resting SR Ca ²⁺ and alters SR Ca ²⁺ buffering in a way that copies the functional instability observed upon reduction of calsequestrin content |
| Knoblauch M, et al. [152] 2013 | Y524S | Mice with RyR1 mutation (Y524S) undergo hypermetabolic response to simvastatin | An acute dose of simvastatin triggers a hypermetabolic response in YS mice. In isolated YS muscle fibers, simvastatin triggers an increase in cytosolic Ca ²⁺ levels by increasing Ca ²⁺ leak from the sarcoplasmic reticulum (SR). With higher simvastatin doses, a similar cytosolic Ca ²⁺ increase occurs in wild type (WT) muscle fibers. Pre-treatment of YS and WT |

Table 5 Rodent *RyR1* model systems (Continued)

| Author/Year | <i>Ryr1</i> variant(s) | Title | Conclusions |
|---|------------------------|--|---|
| Lanner JT, et al. [153] 2012 | Y524S | AICAR prevents heat-induced sudden death in RyR1 mutant mice independent of AMPK activation | mice with AICAR prevents the response to simvastatin AICAR is probably effective in prophylactic treatment of humans with enhanced susceptibility to exercise- and/or heat-induced sudden death associated with RYR1 mutations |
| O-Uchi J, et al. [154] 2012 | Y524S | Malignant hyperthermia mutation of RYR1 (Y522S) increases catecholamine-induced cardiac arrhythmia through mitochondrial injury | Chronic mitochondrial damage by Ca ²⁺ overload through leaky mutant RyR1 induces mitochondrial structural and functional disruption, which facilitates arrhythmogenic outbursts under acute catecholaminergic stress |
| Loy RE, et al. [155] 2012 | Y524S | Allele-specific gene silencing in two mouse models of autosomal dominant skeletal myopathy | The temperature-dependent increase in resting Ca ²⁺ observed in FDB fibers from Y5/+ mice was normalized to WT levels after 2 weeks of treatment with Y5 allele-specific siRNA |
| Wei L, et al. [156] 2011 | Y524S | Mitochondrial superoxide flashes: metabolic biomarkers of skeletal muscle activity and disease | Uncontrolled mitochondrial superoxide production likely contributes to the pathogenic temperature-dependent increase in oxidative stress of RYR1Y524S/WT MH mice |
| Corona BT, et al. [157] 2010 | Y524S | Effect of prior exercise on thermal sensitivity of malignant hyperthermia-susceptible muscle | Eccentric, but not concentric, exercise attenuated the thermal sensitivity of MH-susceptible muscle |
| Boncompagni S, et al. [158] 2009 | Y524S | Characterization and temporal development of cores in a mouse model of malignant hyperthermia | Initial mitochondrial/SR disruption in confined areas causes significant loss of local Ca ²⁺ sequestration that eventually results in the formation of contractures and progressive degradation of the contractile elements |
| Andronache Z, et al. [159] 2009 | Y524S | A retrograde signal from RyR1 alters DHP receptor inactivation and limits window Ca ²⁺ release in muscle fibers of Y522S RyR1 knock-in mice | The increase in uncompensated SR Ca ²⁺ leak observed at rest following transient overexpression of the Y524S RyR1 mutant in myotubes is effectively suppressed after long-term expression of a normal complement of wild-type and mutant RyR1s in adult muscle fibers of WT/Y524S mice |
| Durham WJ, et al. [160] 2008 | Y524S | RyR1 S-nitrosylation underlies environmental heat stroke and sudden death in Y522S RyR1 knock-in mice | Ca ²⁺ release channels in RyR1Y524S/wt mice are leaky, producing elevations in resting Ca ²⁺ , ROS, RNS and basal stress at physiologically relevant temperatures. Ca ²⁺ leak enhances RNS production, and subsequent S-nitrosylation of RyR1 further increases Ca ²⁺ leak, resulting in regenerative Ca ²⁺ release that underlies uncontrolled contractions during heat stress |
| Corona BT, et al. [161] 2008 | Y524S | Eccentric contractions do not induce rhabdomyolysis in malignant hyperthermia susceptible mice | RYR1Y524S/wt protects skeletal muscle from exercise-induced muscle injury. Findings do not support a direct association between MH susceptibility and contraction-induced rhabdomyolysis when core temperature is maintained at lower physiological temperatures during exercise |
| Chelu MG, et al. [162] 2006 | Y524S | Heat- and anesthesia-induced malignant hyperthermia in an RyR1 knock-in mouse | Heterozygous expression of the Y524S mutation confers susceptibility to both heat- and anesthetic-induced MH responses |
| IT mouse (equivalent to I4898T in humans) | | | |
| Lee CS, et al. [163] 2017 | I4895T | A chemical chaperone improves muscle function in mice with a RyR1 mutation | Persistent ER stress/UPR, decreased protein synthesis, mitochondrial ROS production/damage and elevation of proapoptotic markers are defining features of RyR1 myopathy associated with the I4895T mutation in mice, making this myopathy distinct from that of the RyR1 myopathies that arise from Ca ²⁺ leak. Chemical chaperones and ER stress inhibitors may be better suited for mutations in RyR1 that produce ER stress/UPR |
| Zvartich E, et al. [164] 2015 | I4895T | Muscle spindles exhibit core lesions and extensive degeneration of intrafusal fibers in the RyR1(I4895T/ | Muscle spindles undergo severe deterioration that may precede structural changes in extrafusal |

Table 5 Rodent *RYR1* model systems (Continued)

| Author/Year | <i>Ryr1</i> variant(s) | Title | Conclusions |
|-----------------------------------|------------------------|--|---|
| | | wt) mouse model of core myopathy | myofibers Muscle spindles represent an important early target in RyR1-related disease pathology |
| De Crescenzo V, et al. [165] 2012 | I4895T | Type 1 ryanodine receptor knock-in mutation causing central core disease of skeletal muscle also displays a neuronal phenotype | RyR1 plays a role in voltage-induced Ca ²⁺ release in hypothalamic nerve terminals and a neuronal alteration accompanies the myopathy in IT/+ mice |
| Loy RE, et al. [155] 2012 | I4895T | Allele-specific gene silencing in two mouse models of autosomal dominant skeletal myopathy | Altered RyR1 function in FDB fibers of YS/+ and IT/+ knock-in mice can be normalized only two weeks after local in vivo delivery of ASGS siRNAs |
| Loy RE, et al. [166] 2011 | I4895T | Muscle weakness in RyR1I4895T/WT knock-in mice as a result of reduced ryanodine receptor Ca ²⁺ ion permeation and release from the sarcoplasmic reticulum | In vivo muscle weakness observed in IT/+ knock-in mice arises from a reduction in the magnitude and rate of RYR1 Ca ²⁺ release during EC coupling that results from the mutation producing a dominant-negative suppression of RYR1 channel Ca ²⁺ ion permeation |
| Boncompagni S, et al. [167] 2010 | I4895T | The I4895T mutation in the type 1 ryanodine receptor induces fiber-type specific alterations in skeletal muscle that mimic premature aging | Muscle fibers from IT/+ mice in a mixed 129S6/SvEvTac and 129S2/SvPasCrl background exhibit structural alterations of the type seen in CCD patients as well as in WT mice at older ages |
| Zvaritch E, et al. [168] 2009 | I4895T | Ca ²⁺ dysregulation in RyR1(I4895T/wt) mice causes congenital myopathy with progressive formation of minicores, cores, and nemaline rods | The IT/+ mouse line represents a unique and phenotypically valid model of RyR1-related congenital myopathy with minicores, cores, and rods |
| Zvaritch E, et al. [30] 2007 | I4895T | An RyR1I4895T mutation abolishes Ca ²⁺ release channel function and delays development in homozygous offspring of a mutant mouse line | IT/IT mice, in which RyR1-mediated Ca ²⁺ release is abolished without altering the formation of the junctional DHPR-RyR1 macromolecular complex, provide a valuable model for elucidation of the role of RyR1-mediated Ca ²⁺ signaling in mammalian embryogenesis |
| RC mouse (equivalent in humans) | | | |
| Truong KM, et al. [169] 2019 | R163C | Comparison of Chlorantraniliprole and Flubendiamide Activity Toward Wild-Type and Malignant Hyperthermia-Susceptible Ryanodine Receptors and Heat Stress Intolerance | Although nM-μM of either diamide is capable of differentially altering WT and MHS RyR1 conformation in vitro, human RyR1 mutations within putative diamide N- and C-terminal interaction domains do not alter heat stress intolerance in vivo |
| Eltit JM, et al. [170] 2013 | R163C | Nonspecific sarcolemmal cation channels are critical for the pathogenesis of malignant hyperthermia | nonselective sarcolemmal cation permeability, separate from the classic STIM/Orai pathway, is activated by SR depletion and plays a critical role in the causing cytosolic Ca ²⁺ and Na ⁺ overload both at rest and during the MH crisis |
| Estève E, et al. [171] 2012 | R163C | Malignant hyperthermia mutation alters excitation-coupled Ca ²⁺ entry in MH RyR1-R163C knock-in myotubes | Conformational changes induced by the R163C MH mutation alter the retrograde signal that is sent from RYR1 to the DHPR, delaying the inactivation of the DHPR voltage sensor |
| Giulivi C, et al. [172] 2011 | R163C | Basal bioenergetic abnormalities in skeletal muscle from ryanodine receptor malignant hyperthermia-susceptible R163C knock-in mice | Chronically elevated resting Ca ²⁺ in R163C skeletal muscle elicited the maintenance of a fast-twitch fiber program and the development of insulin resistance-like phenotype as part of a metabolic adaptation to the R163C RyR1 mutation |
| Feng W, et al. [173] 2011 | R163C | Functional and biochemical properties of ryanodine receptor type 1 channels from heterozygous R163C malignant hyperthermia-susceptible mice | R163C channels are inherently more active than WT channels, a functional impairment that cannot be reversed by dephosphorylation with protein phosphatase. Dysregulated R163C channels produce a more overt phenotype in myotubes than in adult fibers in the absence of triggering agents, suggesting tighter negative regulation of R163C-RyR1 within the Ca ²⁺ release unit of adult fibers |
| Estève E, et al. [174] 2010 | R163C | A malignant hyperthermia-inducing mutation in RYR1 (R163C): alterations in Ca ²⁺ entry, release, and retrograde signaling to the DHPR | Conformational changes induced by the R163C MH mutation alter the retrograde signal that is sent from RYR1 to the DHPR, delaying the inactivation of the DHPR voltage sensor and enhancing sarcolemmal Ca ²⁺ entry during depolarization |

Table 5 Rodent *RyR1* model systems (Continued)

| Author/Year | <i>Ryr1</i> variant(s) | Title | Conclusions |
|---------------------------------------|---|--|--|
| Bannister RA, et al. [175] 2010 | R163C | A malignant hyperthermia-inducing mutation in RYR1 (R163C): consequent alterations in the functional properties of DHPR channels | Mutations in RYR1 can alter DHPR activity and raise the possibility that this altered DHPR function may contribute to MH episodes |
| Cherednichenko G, et al. [176] 2008 | R163C | Enhanced excitation-coupled calcium entry in myotubes expressing malignant hyperthermia mutation R163C is attenuated by dantrolene | Myotubes isolated from mice heterozygous and homozygous for the ryanodine receptor type 1 R163C MH susceptibility mutation show significantly enhanced ECCE rates that could be restored to those measured in wild-type cells after exposure to clinical concentrations of dantrolene |
| Yang T, et al. [177] 2006 | R163C | Pharmacologic and functional characterization of malignant hyperthermia in the R163C RyR1 knock-in mouse | The newly developed R163C Het mouse line is a valid animal model for studying the largely unknown pathophysiology of MH |
| Other rodent models | | | |
| Brennan S, et al. [178] 2019 | T4706M/Indel (equivalent to T4709M in humans) | Mouse model of severe recessive RYR1-related myopathy | The first mouse model of severe, early-onset recessive <i>RYR1</i> -RM Mice exhibit clearly observable, early-onset phenotypes, premature mortality and a consistent pattern of myofibre hypotrophy |
| Elbaz M, et al. [179] 2019 | Q1970fsX16/A4329D (equivalent in humans) | Quantitative RyR1 reduction and loss of calcium sensitivity of RyR1Q1970fsX16+ A4329D cause cores and loss of muscle strength | The phenotype of the RyR1Q1970fsX16 + A4329D compound heterozygous mice recapitulates the clinical picture of multimimicore patients and provide evidence of the molecular mechanisms responsible for skeletal muscle defects |
| Elbaz M, et al. [180] 2019 | Q1970fsX16 (equivalent in humans) | Quantitative reduction of RyR1 protein caused by a single-allele frameshift mutation in RYR1 ex36 impairs the strength of adult skeletal muscle fibres | The RyR1Q1970fsX16 mouse model provides mechanistic insight concerning the phenotype of the parent carrying the RYR1 exon 36 mutation and suggests that in skeletal muscle fibres there is a functional reserve of RyR1 |
| RYR-1 Foundation [181] 2019 | T4706M/S1669C + L1716 del | Unpublished - https://wwwryr1.org/mice | Phenotype includes kyphosis and malocclusion. The model is still being fully characterized |
| Dulhunty AF, et al. [182] 2019 | P3528S | Unpublished - https://wwwryr1.org/edamame | Phenotype includes mild scoliosis and decreased mobility (heterozygous) and scoliosis, decreased mobility, hang time, and increased calcium sensitivity. The model is still being fully characterized |
| Lopez JR, et al. [183] 2018 | G2435R | Malignant hyperthermia, environmental heat stress, and intracellular calcium dysregulation in a mouse model expressing the pG2435R variant of RYR1 | RYR1 G2435R mice demonstrated gene dose-dependent in vitro and in vivo responses to pharmacological and environmental stressors that parallel those seen in patients with the human RYR1 variant G2434R |
| Hernandez-Ochoa EO, et al. [184] 2018 | L3625D | Loss of S100A1 expression leads to Ca ²⁺ release potentiation in mutant mice with disrupted CaM and S100A1 binding to CaMBD2 of RyR1 | RyR1D-S100A1KO muscle fibers exhibit a modest but significant increase in myoplasmic Ca ²⁺ transients and enhanced Ca ²⁺ release flux following field stimulation when compared to fibers from RyR1D mice |
| Bannister RA, et al. [185] 2016 | E4242G | Distinct Components of Retrograde Ca(V)11-RyR1 Coupling Revealed by a Lethal Mutation in RyR1 | E4242G markedly reduces L-type current density, CaV11 Po, and CaV11 expression, where this last effect is most likely a consequence of the absence of EC coupling. The effects of E4242G on current density, relative Po, and channel expression are similar to those occurring in dyspedic myotubes |
| Hanson MG, et al. [186] 2016 | E4242G | Potassium dependent rescue of a myopathy with core-like structures in mouse | Amelioration of potassium leaks through potassium homeostasis mechanisms may minimize muscle damage of myopathies due to certain <i>RYR1</i> mutations |
| Hanson MG, et al. [187] 2015 | E4242G | Rectification of muscle and nerve deficits in paralyzed ryanodine receptor type 1 mutant embryos | Contractility can be resumed through the masking of a potassium leak, and evoked vesicular release can be resumed via bypassing the defect in RyR1 induced calcium release |
| Yuen B, et al. [188] 2012 | T4826I | Mice expressing T4826I-RYR1 are viable but exhibit sex- and genotype-dependent susceptibility to | T4826I mice underscore the importance of gene × environment interactions in expression of clinical and |

Table 5 Rodent *RYR1* model systems (Continued)

| Author/Year | <i>Ryr1</i> variant(s) | Title | Conclusions |
|----------------------------------|------------------------|---|--|
| | | malignant hyperthermia and muscle damage | subclinical phenotype, and suggest that individuals with RyR1 mutations may represent particularly vulnerable populations to environmental stressors |
| Barrientos GC, et al. [189] 2012 | T4826I | Gene dose influences cellular and calcium channel dysregulation in heterozygous and homozygous T4826I-RYR1 malignant hyperthermia-susceptible muscle | Pronounced abnormalities inherent in T4826I-RYR1 channels confer MHS and promote basal disturbances of excitation-contraction coupling, $[Ca^{2+}]_{rest}$, and oxygen consumption rates. Considering that both Het and Hom T4826I-RYR1 mice are viable, the remarkable isolated single channel dysfunction mediated through this mutation in S4-S5 cytoplasmic linker must be highly regulated in vivo |
| Andersson DC, et al. [190] 2012 | S2844A | Stress-induced increase in skeletal muscle force requires protein kinase A phosphorylation of the ryanodine receptor | The molecular mechanism underlying skeletal muscle inotropy requires enhanced SR Ca^{2+} release due to PKA phosphorylation of S2844 in RyR1 |
| Andersson DC, et al. [191] 2011 | S2844D | Ryanodine receptor oxidation causes intracellular calcium leak and muscle weakness in aging | 6-month-old mice harboring leaky S2844D mutant channels exhibited skeletal muscle defects comparable to 24-month-old WT mice |
| Yamaguchi N, et al. [192] 2011 | L3625D | Modulation of sarcoplasmic reticulum Ca^{2+} release in skeletal muscle expressing ryanodine receptor impaired in regulation by calmodulin and S100A1 | L3625D removes both an early activating effect of S100A1 and CaM and delayed suppressing effect of CaCaM on RyR1 Ca^{2+} release |
| Felder E, et al. [193] 2002 | RYR1/DHPR double KO | Morphology and molecular composition of sarcoplasmic reticulum surface junctions in the absence of DHPR and RyR in mouse skeletal muscle | RyR nor DHPR, alone or separately, are necessary for T-SR docking and for the targeting and/or association of calsequestrin and triadin in the junctional SR. Both proteins are needed for appropriate muscle development |

channels, making this the most frequently utilized cellular model, Table 1. These 49 publications reported on 161 unique *RYR1* variations of which 153 were missense substitutions, six were deletions, one was a frameshift variant resulting in a truncation, and one was a deletion-insertion resulting in a truncation. Of these unique variations, 57% affected the RyR1 channel and activation core domain. In the 49 publications reporting on mutant HEK-293 cells, 13 variations were evaluated and/or functionally characterized (at least three times) (C36R [47, 81, 83], R164C [43, 47, 81, 83], G249R [47, 81, 83], G342R [39, 47, 81, 83], Y523S [43, 47, 70, 81, 83], R615C [47, 70, 81, 83], R2163C [45, 81, 83], R2163H [45, 81, 83], R2435H [39, 43, 45], R2458C [45, 81, 83], R2458H [45, 81, 83], R2508C [45, 52, 61], R2508H [45, 48, 52]). A majority (13/14) of these well-characterized missense substitutions affected the RyR1 cytosolic shell domain. In four publications, multiple variants were introduced to HEK-293 cells to evaluate their impact alone and in combination on RyR1 structural conformation and calcium homeostasis [35–37, 67]. Two additional publications reported on monkey-derived CV-1 in Origin with SV40 (COS)-7 cells that were transfected with mutant *RYR1* cDNA [84, 85].

Expression of recombinant *RYR1* in dyspedic myotubes

Transfection of dyspedic myotubes with mutant *RYR1* cDNA was reported in 25 publications, Table 2, in which a total of 49 unique variations were tested. This includes

studies that used the 1B5 cell line, derived by transduction of dyspedic mouse fibroblasts with *MyoD*, to evaluate mutant RyR1 channel function [109]. Of these 49 variations, 44 were missense substitutions and 5 were deletions with a majority (27/49) affecting the RyR1 channel and activation core domain. One missense substitution, E4032A [103, 105, 106], was evaluated and/or functionally characterized at least three times in transfected dyspedic myotubes.

Expression of endogenous mutant *RYR1*

Additionally, 16 publications reported immortalization of patient primary B-lymphocytes for downstream functional characterization, Table 3. These 16 publications included 32 unique missense substitutions, one deletion, and two deletion-insertions. A total of 50 unique *RYR1* variants, all missense substitutions, were tested in 19 publications utilizing primary cell culture model systems, Table 4.

Animal model systems

Mice

A total of 15 *RYR1* rodent model systems were identified of which ten were heterozygous, three were compound heterozygous, and a further two were knockout, Table 5. Variations discussed in this section are numbered according to the mouse sequence. Core formation was reported in three of the rodent model systems, excluding knockout (Y524S [158], Q1970fsX16 + A4329D [179],

Table 6 Porcine *RYR1* model system of malignant hyperthermia

| Author/Year | Genotype(s) | Title | Conclusions |
|---------------------------------|-------------|---|--|
| Popovski ZT, et al. [239] 2016 | R615C | Associations of Biochemical Changes and Maternal Traits with Mutation 1843 (C > T) in the <i>RYR1</i> Gene as a Common Cause for Porcine Stress Syndrome | Stress susceptible animals have an increased number of stillborn piglets and a reduced number of newborn piglets compared with heterozygous and normal animals |
| Scheffler TL, et al. [240] 2014 | R615C | Fiber hypertrophy and increased oxidative capacity can occur simultaneously in pig glycolytic skeletal muscle | RyR1 R615C increased mitochondrial proteins and DNA, but this was not associated with improved oxidative capacity, suggesting that altered energy metabolism in RyR1 R615C muscle influences mitochondrial proliferation and protein turnover |
| Bina S, et al. [241] 2010 | R615C | Lymphocyte-based determination of susceptibility to malignant hyperthermia: a pilot study in swine | 4CmC stimulation of porcine lymphocytes induces increased adenosine formation in MHS cells relative to those from normal swine |
| Liang X, et al. [242] 2009 | R615C | Impaired interaction between skeletal ryanodine receptors in malignant hyperthermia | Purified RyR1(R615C) from MH susceptible porcine skeletal muscle shows significantly reduced oligomerization when compared to RyR1(WT), indicating a potential loss of intrinsic intermolecular control |
| Ta TA, et al. [243] 2007 | R615C | Ryanodine receptor type 1 (RyR1) possessing malignant hyperthermia mutation R615C exhibits heightened sensitivity to dysregulation by non-coplanar 2,2',3,5',6-pentachlorobiphenyl (PCB 95) | A genetic mutation known to confer susceptibility to pharmacological agents also enhances sensitivity to an environmental contaminant |
| Stinckens A, et al. [244] 2007 | R615C | The RYR1 g.1843C > T mutation is associated with the effect of the IGF2 intron3-g.3072G > A mutation on muscle hypertrophy | The effect of IGF2 on muscle growth might partially be mediated by the calpain/calpastatin system and that this is dependent on RYR1-mediated Ca ²⁺ transport |
| Murayama T, et al. [245] 2007 | R615C | Postulated role of interdomain interaction between regions 1 and 2 within type 1 ryanodine receptor in the pathogenesis of porcine malignant hyperthermia | Stimulation of the RyR1MHS channel caused by affected inter-domain interaction between regions 1 and 2 is an underlying mechanism for dysfunction of Ca ²⁺ homeostasis seen in the MH phenotype |
| McKinney LC, et al. [262] 2006 | R615C | Characterization of Ryanodine Receptor-mediated Calcium Release in Human B Cells | Lymphocytes from MH pigs displayed an increased sensitivity to 4-CmC (EC50 decreased from 0.81 mM to 0.47 mM). The twofold magnitude of the shift was similar to that observed for 4-CmC-sensitive H-ryanodine binding in MH porcine skeletal muscle |
| Gallant EM, et al. [246] 2004 | R615C | Caffeine sensitivity of native RyR channels from normal and malignant hyperthermic pigs: effects of a DHPR II-III loop peptide | In MH-susceptible pig muscles the caffeine sensitivity of native RyRs was enhanced, the sensitivity of RyRs to a skeletal II-III loop peptide was depressed, and an interaction between the caffeine and peptide CS activation mechanisms seen in normal RyRs was lost |
| Zhao F, et al. [247] 2001 | R615C | Dantrolene inhibition of ryanodine receptor Ca ²⁺ release channels. Molecular mechanism and isoform selectivity | Both the RyR1 and the RyR3, but not the RyR2, may be targets for dantrolene inhibition in vivo |
| Gallant EM, et al. [248] 2001 | R615C | Arg(615) Cys substitution in pig skeletal ryanodine receptors increases activation of single channels by a segment of the skeletal DHPR II-III loop | Enhanced DHPR activation of RyRs may contribute to increased Ca ²⁺ release from SR in MH-susceptible muscle |
| Balog EM, et al. [249] 2001 | R615C | Divergent effects of the malignant hyperthermia-susceptible Arg(615)--> Cys mutation on the Ca ²⁺ and Mg ²⁺ dependence of the RyR1 | Reduced Mg ²⁺ inhibition of the MHS RyR1 compared with the normal RyR1 is due to both an enhanced selectivity of the MHS RyR1 A-site for Ca ²⁺ over Mg ²⁺ and a reduced Mg ²⁺ affinity of the I-site |
| Dietze B, et al. [250] 2000 | R615C | Malignant hyperthermia mutation Arg615Cys in the porcine ryanodine receptor alters voltage dependence of Ca ²⁺ release | Arg615Cys does not only promote ligand-induced Ca ²⁺ release but also the depolarization-induced release controlled by the DHP receptor voltage sensor |
| Laver DR, et al. [251] 1997 | R615C | Reduced inhibitory effect of Mg ²⁺ on ryanodine receptor-Ca ²⁺ release channels in malignant hyperthermia | The cytoplasmic Mg ²⁺ in vivo (approximately 1 mM), this Ca ²⁺ Mg ²⁺ inhibitory site will be close to fully saturated with Mg ²⁺ in normal RyRs, but less fully saturated in MHS RyRs. Therefore, MHS RyRs should be more sensitive to any activating stimulus, which would readily account for the development of an MH episode |
| Fruen BR, et al. [252] 1997 | R615C | Dantrolene inhibition of sarcoplasmic reticulum Ca ²⁺ release by direct and specific action at skeletal muscle ryanodine receptors | Results demonstrate selective effects of dantrolene on skeletal muscle ryanodine receptors that are consistent with the actions of dantrolene in vivo and suggest a mechanism of action in which dantrolene may act directly at the skeletal muscle ryanodine receptor complex to limit its activation by calmodulin and C ²⁺ |

Table 6 Porcine *RYR1* model system of malignant hyperthermia (Continued)

| Author/Year | Genotype(s) | Title | Conclusions |
|-------------------------------------|-------------|--|---|
| Bašić I, et al. [253] 1997 | R615C | Stress syndrome: Ryanodine receptor (RYR1) gene in malignant hyperthermia in humans and pigs | This study confirmed application of a method for large-scale, rapid, accurate, DNA-based laboratory diagnosis of the mutation associated with susceptibility to porcine stress syndrome |
| O'Driscoll S, et al. [254] 1996 | R615C | Calmodulin sensitivity of the sarcoplasmic reticulum ryanodine receptor from normal and malignant-hyperthermia-susceptible muscle | The central region of RYR1 is a potential binding domain for CaM in the absence of Ca ²⁺ . It is suggested that in vivo an enhanced CaM sensitivity of RYR1 might contribute to the abnormal high release of Ca ²⁺ from the SR of MHS muscle |
| Herrmann-Frank A, et al. [255] 1994 | R615C | 4-Chloro-m-cresol: a specific tool to distinguish between malignant hyperthermia-susceptible and normal muscle | 4-CmC is suggested to be a potent tool to distinguish between Ca ²⁺ release from MHS and normal muscle |
| Vogeli P, et al. [256] 1994 | R615C | Co-segregation of the malignant hyperthermia and the Arg615-Cys615 mutation in the skeletal muscle calcium release channel protein in five European Landrace and Pietrain pig breeds | DNA-based detection of the MH status in 238 MH-susceptible heterozygous (N/n) and homozygous (n/n) pigs was shown to be accurate, eliminating the 2% diagnostic error that is associated with the halothane challenge test |
| Ledbetter MW, et al. [257] 1994 | R615C | Tissue distribution of ryanodine receptor isoforms and alleles determined by reverse transcription polymerase chain reaction | The normal (Arg615) and mutant (Cys615) <i>ryr1</i> alleles were expressed in the brains of normal and malignant hyperthermia susceptible pigs, respectively. These results thus demonstrate expression of two <i>ryr</i> isoforms in each type of striated muscle, and all <i>ryr</i> isoforms in a number of regions of the nervous system. The wide distribution of <i>ryr1</i> in the brain provides a possible neurogenic etiology of malignant hyperthermia |
| Fagerlund T, et al. [258] 1994 | R615C | Search for three known mutations in the <i>RYR1</i> gene in 48 Danish families with malignant hyperthermia | Other mutations must underlie the disorder in most Danish malignant hyperthermia-susceptible families, and the "pig mutation" is not a frequent cause of malignant hyperthermia susceptibility in Denmark |
| Otsu K, et al. [259] 1992 | R615C | Refinement of diagnostic assays for a probable causal mutation for porcine and human malignant hyperthermia | PCR-amplified sequences contain constant internal controls for the reliable differentiation by restriction endonuclease digestion of normal, heterozygous, and MH genotypes |
| Hogan K, et al. [260] 1992 | R615C | A cysteine-for-arginine substitution (R614C) in the human skeletal muscle calcium release channel cosegregates with malignant hyperthermia | The cysteine-for-arginine mutation represents a shared calcium release channel pathogenesis between porcine malignant hyperthermia and a subset of mutations responsible for the human malignant hyperthermia syndrome |
| Otsu K, et al. [261] 1991 | R615C | Cosegregation of porcine malignant hyperthermia and a probable causal mutation in the skeletal muscle ryanodine receptor gene in backcross families | Substitution of T for C at nucleotide 1843 is the causative mutation in porcine MH |
| Fujii J, et al. [27] 1991 | R615C | Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia | A single point mutation in the porcine gene for the skeletal muscle ryanodine receptor (<i>ryr1</i>) was found to be correlated with MH in five major breeds of lean, heavily muscled swine |

I4895T [168]). Overall, six of the ten heterozygous rodent model systems had missense substitutions affecting the RyR1 cytosolic shell domain. Two compound heterozygous model systems had a single missense substitution engineered into one allele with a frameshift leading to a deletion or truncation on the opposite allele [178, 179]. In these model systems, one variation affected the RyR1 cytosolic shell and the other affected the RyR1 channel and activation core. An additional compound heterozygous model system had a single missense substitution affecting the RyR1 channel and activation core with a second missense substitution and deletion, on the opposite allele, affecting the RyR1 cytosolic shell [181]. Various forms of aberrant intracellular calcium dynamics

were reported in all rodent systems (except knockout). This included evidence of increased resting cytosolic calcium and RyR1-open probability under resting conditions (SR calcium leak) [173] as well as decreased calcium permeation (excitation-contraction uncoupling) [30]. The two most frequently reported *RYR1* rodent model systems were the dyspedic mouse, accounting for 47% of rodent publications [54, 109, 194–238], and the Y524S knock-in mouse, which accounted for 22% of rodent publications, Table 5. Studies utilizing dyspedic mice/1B5 myotubes not transfected with mutant *RYR1* cDNA, were primarily focused on elucidating the following: (a) relative importance and functional role of wild-type RyR isoforms [213, 227, 234], (b) fundamental

Table 7 Other *RYR1* preclinical model systems

| Author/Year | Species/ <i>RYR1</i> variant(s) | Title | Conclusions |
|-------------------------------|--|--|---|
| Gupta VA, et al. [263] 2013 | Zebrafish (<i>Danio rerio</i>)/ ryr1b mi340 | Developing therapies for congenital myopathies by high throughput chemical screening in ryanodine receptor 1 mutant zebrafish | A secondary screen using individual chemicals from positive pools is in progress to identify the best combination of chemical/s that improve muscle function and survival of ryr1b mutant fish |
| Dowling JJ, et al. [28] 2012 | Zebrafish (<i>Danio rerio</i>)/ ryr1b mi340 | Oxidative stress and successful antioxidant treatment in models of RYR1-related myopathy | Oxidative stress is an important pathophysiological mechanism in <i>RYR1</i> -related myopathies and that <i>N</i> -acetylcysteine is a successful treatment modality <i>ex vivo</i> and in a vertebrate disease model |
| Dowling JJ, et al. [264] 2011 | Zebrafish (<i>Danio rerio</i>)/ ryr1b mi340 | Increased oxidative stress and successful antioxidant treatment in a vertebrate model of RYR1 related myopathy | Increased oxidative stress is an important aspect of the pathogenesis of RYR1-related myopathies, and antioxidant treatment is a viable potential treatment strategy for patients |
| Dowling JJ, et al. [265] 2010 | Zebrafish (<i>Danio rerio</i>)/ ryr1b mi340 | Oxidative stress and RYR1-related myopathies | Loss of <i>RYR1</i> function in the zebrafish results in increased levels of basal oxidative stress and increased susceptibility to pro-oxidants. <i>RYR1</i> deficient zebrafish treated with anti-oxidants had significant improvements in motor function |
| Dowling JJ, et al. [266] 2009 | Zebrafish (<i>Danio rerio</i>)/ ryr1b mi340 | Oxidative stress and antioxidant therapy in a zebrafish model of multi minicore myopathy | Loss of <i>RYR1</i> function results in increased oxidative stress in a vertebrate model of <i>RYR1</i> related myopathy. Antioxidant therapy can improve motor function |
| Hirata H, et al. [267] 2007 | Zebrafish (<i>Danio rerio</i>)/ ryr1b mi340 | Zebrafish relatively relaxed mutants have a ryanodine receptor defect, show slow swimming and provide a model of multi-minicore disease | Zebrafish relatively relaxed mutants may be useful for understanding the development and physiology of MmD |
| Oyamada H, et al. [268] 2002 | Hamster (CHO cells)/ P4667S, L4837V, R615C | Novel mutations in C-terminal channel region of the ryanodine receptor in malignant hyperthermia patients | L4838V was responsible for increased sensitivity of RyR1 to caffeine P4667S had very little effect on the caffeine-induced Ca ²⁺ increase |
| Treves S, et al. [84] 1994 | Monkey (COS-7 cells)/ R615C | Alteration of intracellular Ca ²⁺ transients in COS-7 cells transfected with the cDNA encoding skeletal-muscle ryanodine receptor carrying a mutation associated with malignant hyperthermia. | Presence of the Arg-to-Cys point mutation in the recombinant RYR expressed in COS-7 transfected cells causes abnormal cytosolic Ca ²⁺ transients in response to 4-chloro-m-cresol, an agent capable of eliciting <i>in vitro</i> contracture of MH-susceptible muscles. |
| Altafaj X, et al. [85] 2005 | Monkey (COS-7 cells)/ RyR1ΔF7 (3241-3661Del) | Maurocalcine and domain A of the II-III loop of the dihydropyridine receptor Cav 1.1 sub-unit share common binding sites on the skeletal ryanodine receptor. | RyR1 carrying a deletion of fragment 7 shows a loss of interaction with both peptide A and maurocalcine. This deletion abolishes the maurocalcine induced stimulation of [3H] ryanodine binding onto microsomes of transfected COS-7 cells without affecting the caffeine and ATP responses. |
| Vega AV, et al. [269] 2011 | C2C12 (mouse)/ Y524S, I4897T | Calcitonin gene-related peptide restores disrupted excitation-contraction coupling in myotubes expressing central core disease mutations in RyR1 | Changes in excitation-contraction coupling induced by the expression of RyR1 channels bearing CCD mutations Y523S or I4897T can be reversed by calcitonin gene related peptide |
| Lefebvre R, et al. [270] 2011 | Swiss OF1 (mouse)/Y523S, R615C, R2163H, I4897T | Defects in Ca ²⁺ release associated with local expression of pathological ryanodine receptors in mouse muscle fibres | The Y523S, R615C and R2163H RyR1 mutants produce a similar over-sensitive activation of the calcium flux whereas I4897T RyR1 mutants are responsible for a depressed Ca ²⁺ flux. The alterations appear to result from inherent modifications of RyR1 channel function and not from indirect changes in the muscle fibre homeostasis |
| Douris V, et al. [271] 2017 | Drosophila model/G4946V, G4946E, I4790M | Investigation of the contribution of RyR target-site mutations in diamide resistance by CRISPR/Cas9 genome modification in Drosophila | Mutations confer subtle differences on the relative binding affinities of the three diamides at an overlapping binding site on the RyR protein |

Table 7 Other *RYR1* preclinical model systems (Continued)

| Author/Year | Species/ <i>RYR1</i> variant(s) | Title | Conclusions |
|---------------------------------|--|--|---|
| Gao S, et al. [272] 2013 | <i>Drosophila</i> model/ Q3878X, Y4452X, R4305C, E4340K, P2773L | <i>Drosophila</i> ryanodine receptors mediate general anesthesia by halothane | Neurally expressed dRyR mediates a substantial proportion of the anesthetic effects of halothane in vivo, is potently activated by halothane in vitro, and activates an inhibitory conductance |
| Sullivan KM, et al. [273] 2000 | <i>Drosophila</i> model/Ryr16ins | The ryanodine receptor is essential for larval development in <i>Drosophila melanogaster</i> | The ryanodine receptor is required for proper muscle function and may be essential for excitation-contraction coupling in larval body wall muscles Results do not support a role for RyR in normal light responses |
| Wilberger MS, et al. [274] 2015 | Equine model/ C7360G | Prevalence of exertional rhabdomyolysis in endurance horses in the Pacific Northwestern United States | Exertional rhabdomyolysis in this group was not associated with known genetic mutations tied to type 1 PSSM and MH |
| Nieto JE, et al. [275] 2009 | Equine model/ C7360G | A rapid detection method for the ryanodine receptor 1 (C7360G) mutation in Quarter Horses | Genotyping by melting curve analysis with hybridization probes is a rapid and accurate detection method for the RyR1 C7360G mutation that works on both cDNA and gDNA |
| Aleman M, et al. [276] 2009 | Equine model/ C7360G | Malignant hyperthermia associated with ryanodine receptor 1 (C7360G) mutation in Quarter Horses | MH is a potentially fatal disease of Quarter Horses that could be triggered by halogenated anesthetics and other nonanesthetic factors that may include exercise, stress, breeding, illnesses, and concurrent myopathies |
| Aleman M, et al. [277] 2004 | Equine model/ R2454G | Association of a mutation in the ryanodine receptor 1 gene with equine malignant hyperthermia | A missense mutation in RyR1 is associated with MH in the horse, providing a screening test for susceptible individuals. Ryanodine-binding analysis suggests that long-lasting changes in RyR1 conformation persists in vitro after the triggering event |
| Roberts MC, et al. [278] 2001 | Canine model/V547A | Autosomal dominant canine malignant hyperthermia is caused by a mutation in the gene encoding the skeletal muscle calcium release channel (RYR1) | Autosomal dominant canine MH is caused by a mutation in the gene encoding the skeletal muscle calcium release channel The MHS trait in this pedigree of mixed-breed dogs is in perfect co-segregation with the RYR1 V547A mutation |
| Baines KN, et al. [279] 2017 | <i>Caenorhabditis elegans</i> /G341R, R2163H, R2454H, R2458H, R4861H, A4940T, R163C, K3452Q | Aging Effects of <i>Caenorhabditis elegans</i> Ryanodine Receptor Variants Corresponding to Human Myopathic Mutations | Single amino acid modifications in <i>C. elegans</i> also conferred a reduction in lifespan and an accelerated decline in muscle integrity with age, supporting the significance of ryanodine receptor function for human aging |
| Baines KN, et al. [280] 2014 | <i>Caenorhabditis elegans</i> (unc-68)/ G341R, R2163H, R2454H, R2458H, R4861H, A4940T, R163C, K3452Q | <i>Caenorhabditis elegans</i> as a model organism for RYR1 variants and muscle ageing | The ryanodine receptor in <i>Caenorhabditis elegans</i> is UNC-68, which has 40% amino acid identity to the human protein |
| Hamada T, et al. [281] 2002 | <i>Caenorhabditis elegans</i> / <i>kh30</i> , <i>e540</i> , × <i>14</i> , <i>r1161</i> | Molecular dissection, tissue localization and Ca ²⁺ binding of the ryanodine receptor of <i>Caenorhabditis elegans</i> | We propose a model for the functional domains of CeRyR, which agrees well with the model of mammalian skeletal RyR, which is based on proteolysis and cross-linking analysis |
| Maryon EB, et al. [282] 1998 | <i>Caenorhabditis elegans</i> (unc-68)/ <i>r1161</i> , <i>r1162</i> , <i>r1221</i> , <i>e540</i> | Muscle-specific functions of ryanodine receptor channels in <i>Caenorhabditis elegans</i> | Unlike vertebrates, which have at least three ryanodine receptor genes, <i>C. elegans</i> has a single gene encoded by the unc-68 locus. Unc-68 is expressed in most muscle cells, and the phenotypic defects exhibited by unc-68 null mutants result from the loss of unc-68 function in pharyngeal and body-wall muscle cells |
| Sakube Y, et al. [283] 1997 | <i>Caenorhabditis elegans</i> (unc-68)/ <i>e540</i> , unc-68 null | An abnormal ketamine response in mutants defective in the ryanodine receptor gene <i>ryr-1</i> (unc-68) of <i>Caenorhabditis elegans</i> | S1444N substitution is at a putative protein kinase C phosphorylation site in <i>ryr-1</i> unc-68(e540) contains a splice acceptor mutation that creates a premature stop codon in the <i>ryr-1</i> gene |

Table 7 Other *RYR1* preclinical model systems (Continued)

| Author/Year | Species/ <i>RYR1</i> variant(s) | Title | Conclusions |
|---------------------------------|--|---|---|
| Maryon EB, et al. [284] 1996 | <i>Caenorhabditis elegans</i> (unc-68)/ <i>rl151, rl152, rl158, rl160, rl161, rl162, rl167, rl207, rl208, rl209, rl210, rl211, rl212, rDfl, rDf2</i> | unc-68 encodes a ryanodine receptor involved in regulating <i>C. elegans</i> body-wall muscle contraction | The role of RyRs in <i>C. elegans</i> body-wall muscle is to enhance contraction by amplifying a depolarization-coupled Ca ²⁺ transient |
| Airey JA, et al. [285] 1993 | Crooked Neck Dwarf (cn/cn) alpha RyR-null | Failure to make normal alpha ryanodine receptor is an early event associated with the crooked neck dwarf (cn) mutation in chicken | Failure to make normal alpha RyR receptor appears to be an event closely associated with the cn mutation and one which may be largely responsible for development of the cn/cn phenotype in embryonic skeletal muscle |
| Airey JA, et al. [286] 1993 | Crooked Neck Dwarf (cn/cn) alpha RyR-null | Crooked neck dwarf (cn) mutant chicken skeletal muscle cells in low density primary cultures fail to express normal alpha ryanodine receptor and exhibit a partial mutant phenotype | The mutant phenotype observed in ovo is partially expressed under low density culture conditions, and neither beta RyR protein nor its function appear to be capable of preventing the associated changes |
| Ivanenko A, et al. [287] 1995 | Crooked Neck Dwarf (cn/cn) alpha RyR-null | Embryonic chicken skeletal muscle cells fail to develop normal excitation-contraction coupling in the absence of the alpha ryanodine receptor. Implications for a two-ryanodine receptor system | In the absence of alpha RyR there is a failure to develop Ca ²⁺ – independent Ca ²⁺ release and contractions and to sustain Ca ²⁺ – dependent release. Moreover, contributions by the alpha RyR cannot be duplicated by the beta RyR alone |
| Oppenheim RW, et al. [288] 1997 | Crooked Neck Dwarf (cn/cn) alpha RyR-null | Neuromuscular development in the avian paralytic mutant crooked neck dwarf (cn/cn): further evidence for the role of neuromuscular activity in motoneuron survival | It seems likely that the peripheral excitation of muscle by motoneurons during normal development is a major factor in regulating retrograde muscle-derived (or muscle-associated) signals that control motoneuron differentiation and survival |

physiology of excitation-contraction coupling components [205, 225, 232], (c) roles of specific RyR1 structural regions on channel function [216, 222, 235]. The Y524S knock-in mouse has been utilized extensively to investigate the mechanisms behind several phenotypes on the *RYR1*-RM disease spectrum including MH susceptibility [162], statin-induced myopathy [152], and central core disease [158]. Y524S mice have also been used to test potential therapeutics for *RYR1*-RM including the antioxidant *N*-acetylcysteine [145, 160] and the activator of the AMP-activated protein kinase 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) [153].

Other animal model systems

The pathomechanism, diagnosis, and acute treatment of malignant hyperthermia was investigated in 24 publications that used the R615C porcine model system [27, 239–261], Table 6. A number of other preclinical model systems have been described including avian, zebrafish, *C. elegans*, canine, equine, and drosophila, Table 7. Six publications reported on a single recessive zebrafish model system of *RYR1*-RM termed the relatively relaxed (*ryr*^{mi340}) mutant [28, 263–267] which was utilized for high-throughput drug screening [263] and testing of *N*-acetylcysteine as a potential therapeutic to address elevated oxidative stress [264]. A further six publications reported using *Caenorhabditis elegans* (*C. elegans*) with

variants in *unc68*, the *RYR1* ortholog [32, 280–284]. With 40% sequence homology to humans, *C. elegans* have been used to investigate RyR1 functional sites [281] and test the potential impact of *RYR1* mutations on central nervous system function [32]. A single heterozygous canine model system of malignant hyperthermia was reported. The canine model system carried a single missense substitution, V547A, affecting the RyR1 cytosolic shell domain and was characterized by responsiveness to an in vivo halothane-succinylcholine challenge and having a positive in vitro contracture test [278]. Four publications described equine model systems of malignant hyperthermia and exertional rhabdomyolysis that carried variations in the *RYR1* gene [274–277]. Two *RYR1* variants were reported: (a) R2454G associated with fulfilment malignant hyperthermia and a high affinity for ryanodine binding [277] and (b) C7360G associated with both anesthetic-induced malignant hyperthermia and exertional/non-exertional rhabdomyolysis [276]. Three publications reported on drosophila with variations in the equivalent *RYR1* gene (*dRyr*) [271–273]. A total of nine *RYR1* variations were presented comprising eight missense substitutions and one insertion, Table 7. Missense substitutions in drosophila *dRyr* conferred halothane sensitivity [272], and drosophila with CRISPR/Cas9 gene-edited *dRyr* have been used to investigate insecticide resistance [271]. Three publications utilized transfected wild-type rodent cells to generate *RYR1*

model systems with clinically-relevant variations [268–270] and four reported on the avian crooked neck dwarf mutant which lacks the alpha RyR isoform homologous to human RyR1 [285–288].

Discussion

This comprehensive scoping review of MH and *RYR1*-RM preclinical model systems identified 262 relevant published records and serves as a compendium to guide future research. During the period spanning January 1, 1990 to July 3, 2019 a diverse range of preclinical model systems were utilized to investigate the etiology, pathomechanisms, and potential treatments for MH and *RYR1*-RM. There has been sustained research output since 2010 with the predominant model system used varying over time between porcine, cellular, and rodent.

A single missense substitution, R615C, was the sole porcine variant reported. As the first *RYR1* preclinical model system, studies of R615C pigs led to fundamental discoveries including identification of 4-CmC as a potent RyR1 agonist and identification of *RYR1* as a genetic locus for malignant hyperthermia [27, 255]. The R615C porcine model system was also utilized to better understand the mechanism of dantrolene which remains the only approved treatment for MH crises [252].

The number of *RYR1* variations reported in the literature (> 700) has been prohibitive in terms of developing in vivo model systems reflecting each variant. This review has outlined the extent to which cellular model systems, in particular transfected HEK-293 cells and dyspedic myotubes, have been versatile systems through which to investigate the pathogenicity of *RYR1* variations and their impact on intracellular calcium homeostasis. However activity of the RyR1 protein complex is tightly regulated by coupling to the dihydropyridine receptor and by modulators of channel function such as 12-kDa FK506-binding protein (FKBP12) and calmodulin [10]. Absence of these components in the HEK-293 system may therefore affect the reliability of functional data for clinical translation. Epstein-Barr virus-driven immortalization of patient-derived lymphoblasts has also proven a valuable non-recombinant methodology when clinical biospecimens are available, although they also do not contain all elements of the skeletal muscle triad. Both HEK-293 cells and dyspedic myotubes have a standardized and well-characterized background and are therefore less likely, than immortalized patient cells, to be influenced by variations in other genes that may impact RyR1 function. Although patient tissue is not always readily available, it is important to recognize that functional studies of patient-derived primary myotubes can provide valuable supporting evidence of *RYR1* variant pathogenicity. Indeed, such studies have been incorporated within the MH variant scoring matrix developed

by the European Malignant Hyperthermia Group (EMHG) [289]. Despite the above limitations, recent advances in the engineering of skeletal muscle three-dimensional systems using patient-derived induced pluripotent stem cells holds the prospect of providing a physiologically relevant cellular system through which to evaluate and screen potential treatments for skeletal muscle disorders, including *RYR1*-RM [290–292]. Our observation that the most frequently reported variants were localized to MH/CCD hotspot regions is consistent with the initial clinical focus to identify patients with variants in these distinct regions and perform functional characterization [293]. Common functional analyses identified in this review include RyR1 agonist sensitivity (caffeine, 4-CmC), ³[H]-ryanodine binding, halothane and/or isoflurane sensitivity, and intracellular calcium measurements via calcium-sensitive fluorescent dyes such as fluo-4.

The dyspedic mouse was utilized by 47% of publications in the rodent category and its *RYR1*-null myotubes were transfected in 23% of publications in the cellular model category, a testament to importance of the dyspedic mouse for both understanding the fundamental physiology of the ryanodine receptor and as a stable model system to characterize mutant RyR1 channels. Heterozygous knock-in rodent models have formed the basis of in vivo *RYR1*-RM preclinical testing. Y524S, I4895T, and R163C were the most extensively studied knock-in rodent models over the last 30 years. These mice have provided valuable insights into the effects of single missense substitutions on RyR1 dysfunction including channel leak and excitation-contraction uncoupling. Furthermore, these mice have enabled the identification downstream pathologic sequelae in vivo such as elevated oxidative/nitrosative/ER stress and an unfolded protein response. However the abovementioned knock-in mice do not necessarily mirror the phenotypes observed in autosomal dominant patients with equivalent *RYR1* variants (reviewed in detail elsewhere [292]). Two recently published compound heterozygous *RYR1*-RM rodent models recapitulate clinical manifestations observed in recessive *RYR1*-RM patients, including decreased RyR1 protein expression, reduced muscle mass, and progressive muscle weakness [178, 179]. An additional compound heterozygous mouse (T4706M/S1669C + L1716del) included in this review is currently undergoing full characterization [181].

In contrast to rodent model systems, zebrafish are more cost-efficient, have transparent embryos that facilitate visualization of dynamic events, have a shorter life-cycle, have larger hatch sizes, and are easier to maintain [294, 295]. Zebrafish are readily manipulated by chemical approaches because embryos can readily absorb compounds that they are exposed to in solution,

therefore allowing for high-throughput chemical screening [296, 297]. A recessive zebrafish model system of *RYR1*-RM, termed the relatively relaxed (*ryr*^{mi340}) mutant, exhibits weak muscle contractions resulting in slow swimming, dramatically decreased Ca²⁺ transients at the t-tubules of fast muscles due to defective E-C coupling, and small amorphous cores detectable by electron microscopy. Despite the abovementioned advantages over rodent model systems, a consideration is that relatively relaxed (*ryr1b*) zebrafish are homozygous with a truncated RyR1 channel (residual expression = 1–10% of normal RyR1). As such, its genetic defect and pathomechanism do not align with a majority of *RYR1*-RM clinical cases.

Consistent with the findings of this review, over 90% of animals used in research are mice or rats [298]. However, other animal model systems have also been developed and used to study the skeletal muscle ryanodine receptor and the consequences of genetic variations. *C. elegans* and *Drosophila* have been primarily used for genetic and developmental biology studies, whereas the porcine, equine, and canine model systems have focused on understanding and characterizing the etiology of MH in these species. As an alternative to higher order *RYR1*-RM animal model systems, the use of simpler organisms (*C. elegans*, yeast, *Drosophila*) and vertebrates (in particular zebrafish) with sufficient genome sequence homology to humans could be revitalized using more precise genome editing techniques such as prime editing [299]. However, due to evolutionary distance between DNA sequences, results from non-mammalian model systems should undergo further careful validation in mammals such as mice and pigs prior to translation to clinical studies. It is possible that records published in supplementary material may not have been captured by the search strategy used for this review and may be considered a limitation.

Advances in functional genomics coupled with the increase in demand for mice as a primary experimental system are expected to continue driving the need for additional transgenic, gene-edited and combinatorial breeding of different *RYR1*-RM model systems in the near future. Development of conditional targeted animal models (*cre/lox*, *tet*, and other similar approaches) can reduce generation and retention of extraneous animals and also allow for the conduct of developmental studies in late-onset myopathy subtypes in this heterogeneous group of disorders. Determining which murine model most closely represents a majority of either dominant or recessive cases of *RYR1*-RM remains an open question. Funding of research utilizing recently developed model systems is essential to translating these promising advances into clinical trials and treatment discoveries.

Conclusion

Over the past 30 years, there were 262 publications on MH and *RYR1*-RM preclinical model systems featuring more than 200 unique *RYR1* variations tested in a broad range of species. Findings from these studies have set the foundation for therapeutic development for MH and *RYR1*-RM.

Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s13023-020-01384-x>.

Additional file 1.

Abbreviations

4-CmC: 4-Chloromethcathinone; AICAR: 5-aminoimidazole-4-carboxamide ribonucleoside; ApoCaM: Apocalmodulin; ASGS: Allele-specific gene silencing; *C. elegans*: *Caenorhabditis elegans*; CaCaM: Calcium-bound calmodulin; CaM: Calmodulin; CCD: Central Core Disease; CICR: Calcium-induced calcium release; CNMDU1: Congenital neuromuscular disease with uniform type 1 fiber; COS: CV-1 in Origin with SV40 cell line; DHPR: Dihydropyridine receptor; EBV: Epstein-Barr virus; EC: Excitation-contraction; ECCE: Excitation-coupled calcium entry; EMHG: European Malignant Hyperthermia Group; FDB: Flexor digitorum brevis; FKBP12: 12-kDa FK506-binding protein; HEK: Human embryonic kidney cell line; KO: Knock-out; MH: Malignant hyperthermia; MmD: Multimicore disease; NO: Nitrous oxide; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; ROS: Reactive oxygen species; RVIS: Residual variance intolerance score; *RYR1*-RM: *RYR1*-related myopathies; SOICR: Store overload-induced calcium release; SR: Sarcoplasmic reticulum; UPR: Unfolded protein response; VGCR: Voltage-gated calcium release; WT: Wild type

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Authors' contributions

TAL screened articles for eligibility/full text review and reviewed the manuscript; EW developed search criteria, screened articles for eligibility and reviewed the manuscript; NT developed the search strategy, conducted the database search, and reviewed the manuscript; JD reviewed the manuscript; JT conceived of the study design, screened articles for eligibility/full text review, and drafted the manuscript. The author(s) read and approved the final manuscript.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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