Excitation–Contraction Coupling

Mutations in proteins involved in E-C coupling and SOCE and congenital myopathies

Daniela Rossi1,2, Maria Rosaria Catallo1, Enrico Pierantozzi1, and Vincenzo Sorrentino1,2

In skeletal muscle, Ca2+ necessary for muscle contraction is stored and released from the sarcoplasmic reticulum (SR), a specialized form of endoplasmic reticulum through the mechanism known as excitation–contraction (E-C) coupling. Following activation of skeletal muscle contraction by the E-C coupling mechanism, replenishment of intracellular stores requires reuptake of cytosolic Ca2+ into the SR by the activity of SR Ca2+-ATPases, but also Ca2+ entry from the extracellular space, through a mechanism called store-operated calcium entry (SOCE). The fine orchestration of these processes requires several proteins, including Ca2+ channels, Ca2+ sensors, and Ca2+ buffers, as well as the active involvement of mitochondria. Mutations in genes coding for proteins participating in E-C coupling and SOCE are causative of several myopathies characterized by a wide spectrum of clinical phenotypes, a variety of histological features, and alterations in intracellular Ca2+ balance. This review summarizes current knowledge on these myopathies and discusses available knowledge on the pathogenic mechanisms of disease.

Introduction
Calcium ion (Ca2+) represents a central second messenger in eukaryotic cells, where it governs a plethora of cellular processes, including cell proliferation, secretion, and metabolism, among many others (Berridge et al., 2000). In striated and smooth muscle cells, it also plays the fundamental role of regulating muscle contraction by activating the acto-myosin complex (Dulhunty, 2006). To perform all these functions, cells must accurately regulate the intracellular Ca2+ concentration by both controlling Ca2+ exchange with the extracellular environment and establishing intracellular Ca2+ stores for prompt utilization (Bootman and Bultynck, 2020). In skeletal muscle, most of the Ca2+ used during contraction cycles comes from the sarcoplasmic reticulum (SR), a specialized form of endoplasmic reticulum (ER), that forms a complex network of tubules and cisternae wrapping the myofibrils in a sleeve-like structure. The SR is composed of two distinct domains: the longitudinal SR (l-SR) and the junctional SR (j-SR). The l-SR is the main site of Ca2+ uptake from the sarcoplasm, thanks to the presence of sarco/endoplasmic reticulum Ca2+ ATPase (SERCA) pumps, while the j-SR associates with sarcolemma transverse tubules (T-tubules) to form the triads, where the ryanodine receptor type 1 (RYR1) Ca2+ release channels are localized (Franzini-Armstrong, 2018). Triads represent the membrane structures that support the excitation–contraction (E-C) coupling mechanism in skeletal muscle fibers, providing a site where dihydropyridine receptor (DHPR) and RYR1 can physically interact to activate Ca2+ release following membrane depolarization (Meissner and Lu, 1995). A third essential component of the E-C coupling machinery is the adaptor protein SH3 and cysteine-rich domain 3 (STAC3). DHPR is a voltage-dependent L-type Ca2+ channel located on the T-tubules that, following membrane depolarization induced by motor-neuron stimulation, undergoes a conformational change that allows the opening of RYRI. The skeletal muscle DHPR is composed of a heteromultimeric complex that includes the α1s, α2, δ, β1α, and γ1 subunits (Flucher, 2020). The α1s subunit (also referred to as Cav1.1) is an integral membrane protein containing four transmembrane domains, each composed of six α-helices, acting as the pore-forming and the voltage-sensing unit (Hu et al., 2015). Trafficking of the α1s subunit and coupling with RYR1 is regulated by STAC3 (Polster et al., 2016). Opening of RYR1 channels results in massive Ca2+ release from the SR into the myoplasm, which in turn triggers muscle contraction (Dulhunty, 2006). In addition to RYR1, a second isoform, RYR3, is expressed in skeletal muscle, although only RYR1 is essential for E-C coupling activation. At variance with RYR1,
RyR3 channels are mainly expressed in neonatal versus adult muscles and are activated by a Ca^{2+}-induced Ca^{2+}-release (CICR) mechanism, independently of the interaction with DHPR (Rossi and Sorrentino, 2002).

In addition to DHPR, RyRI, and STAC3, which are essential for E-C coupling, other proteins are localized at triads and regulate this mechanism, including junctophillins (JPHs), triadin, junctin, and calsequestrin (CASQ; Rossi et al., 2022a). JPHs mediate triad assembly and maintenance by both establishing the apposition between j-SR cisternae and T-tubules and acting as a scaffold for the assembly of the Ca^{2+} release complex (Phimister et al., 2007; Golinì et al., 2011; Nakada et al., 2018; Rossi et al., 2019a). In mammals, JPH1 and JPH2 are expressed in striated muscles (Takeshima et al., 2000). Triadin and junctin are transmembrane proteins composed by a cytoplasmic N-terminal domain (NTD) and a luminal C-terminal segment (Zhang et al., 1997; Dulhunty et al., 2009; Marty, 2015). They act as functional regulator of E-C coupling by interacting with RyRs (Guo and Campbell, 1995; Zhang et al., 1997; Caswell et al., 1999; Li et al., 2015; Rossi et al., 2022b), CASQ (Kobayashi et al., 2000; Shin et al., 2000; Boncompagni et al., 2012; Rossi et al., 2014; Rossi et al., 2021), and the histidine-rich Ca^{2+}-binding protein (Lee et al., 2001). Triadin also plays a structural role in supporting triad architecture by interacting with the microtubule-binding protein climp-63, also known as cytoskeleton-associated protein 4 (Osseni et al., 2016). CASQ is an intraluminal SR soluble protein with high capacity and low-affinity Ca^{2+}-binding properties. CASQ is the main Ca^{2+} buffering protein of the SR, although recent evidence shows that it can display a more complex regulatory role in Ca^{2+} homeostasis (see below; Beard et al., 2004; Protasi et al., 2011; Beard and Dulhunty, 2015; Manno et al., 2017; Rossi et al., 2021). Two isoforms of CASQ have been identified: CASQ1 is expressed in fast- and slow-twitch skeletal muscle fibers, whereas CASQ2 is expressed in slow-twitch skeletal muscle fibers and cardiac muscle (Biral et al., 1992).

For many years, Ca^{2+} entry from the extracellular environment was believed to play only a marginal role in muscle physiology and contraction. However, new evidence has accumulated indicating that Ca^{2+} influx through the store-operated calcium entry (SOCE) mechanism, mediated by stromal interaction molecule 1 (STIM1), a Ca^{2+} sensor in the SR, and ORAI1, a selective Ca^{2+} channel on the plasma membrane, is also fundamental to refill intracellular Ca^{2+} stores and sustain prolonged activity of skeletal muscle fibers (Launikonis and Rios, 2007; Michelucci et al., 2018). In past decades, while gaining an incredible amount of information on structural and functional aspects that support the mechanisms that govern Ca^{2+} release through the RyRI channels, we have also recognized that mutations in genes that code for proteins involved in Ca^{2+} handling are causative of inherited myopathies. In this review, we provide an overview of the main aspects of muscle diseases linked to mutations in Ca^{2+}-handling proteins in skeletal muscle, focusing on the E-C coupling and SOCE mechanisms.

**E-C coupling and congenital myopathies: RyRI, CACNA1S, and STAC3**

In humans, the RyRI gene codes for a protein of 5,038 amino acids that assembles in tetramers of >2 MD. Structural cryo-EM studies showed that each monomer has a large N-terminal cytoplasmic region, six transmembrane domains that define the pore region, and a small cytoplasmic C-terminal portion (Efremov et al., 2015; Yan et al., 2015; Zalk et al., 2015; des Georges et al., 2016). As reported in Fig. 1, these studies showed that the cytoplasmic region is composed by an α-solenoid scaffold and some globular domains. The α-solenoid scaffold is formed by four segments, and it is capped, at the N-terminus, by two distinct NTDs, NTD-A and NTD-B; these are immediately followed by the first segment, the N-solenoid (N-sol), connected to the SPI/a ryanoide receptor domain (SPRY1) domain. This is followed by a pair of RyR repeats (RY1&2). Two other SPRY domains (SPRY2 and 3) are localized upstream of the second and the third solenoids, the junctional (J-sol) and the bridging (B-sol) solenoids, respectively, that are followed by a second pair of RyR repeats (RY3&4). Finally, the fourth and last solenoid, called the core solenoid (C-sol), precedes an EF-hand domain (Efremov et al., 2015; Yan et al., 2015; Zalk et al., 2015; des Georges et al., 2016). The high flexibility of the α-solenoid scaffold of RyRI facilitates the interaction of the channel with regulatory proteins and molecules, such as Ca^{2+}, Mg^{2+}, ATP, FK-binding protein 12, or calmodulin, and contains consensus sequences for posttranslational modification by kinases and phosphatases (Lanmer et al., 2010; Hernández-Ochoa et al., 2015; Yan et al., 2015; Zalk et al., 2015; des Georges et al., 2016; Woll and Van Petegem, 2022). The transmembrane pore region presents a fold shape with six transmembrane helices (SI–S6), forming a pseudo voltage-sensor domain (pVSD), while SS and S6 helices from the four subunits form the pore of the channel. The S6 helix extends into the cytosol, terminating in the C-terminal domain (CTD), a small α-helical domain that contains a Zn^{2+}-binding domain that contacts the cytosolic region called “thumb-and-forefinger” located within the C-sol (Woll and Van Petegem, 2022).

Mutations in RyRI represent the most frequent cause of nondystrophic congenital muscle diseases, which are thus referred to as RyRI-related myopathies, and of malignant hyperthermia (MH; Kushnir et al., 2018). According to an historical view, mutations in RyRI were described to be clustered in three distinct hotspots, located at the NTD, in the central region, and at the CTD (Rossi and Sorrentino, 2002; Lawal et al., 2018). Nevertheless, over the years, >500 variants have been identified also outside these hotspots, although, currently, not all have been verified as causative (Kushnir et al., 2020). In Fig. 1, the distribution of all known variants identified as of today in the RyRI coding sequence is reported. The position of those mutations validated as causative for MH by the European Malignant Hyperthermia Group (EMHG) is shown in red. Coincidentally, nearly all the mutations validated as causative by the EMHG are within the three hotspot regions. Recent cryo-EM studies revealed that several mutations are positioned near the fourfold symmetry axis, at sites of interaction between the RyRI subunits or at sites of interaction between domains in the same subunit, resulting in destabilization of the closed state of the channel (Tung et al., 2010; des Georges et al., 2016; Gong et al., 2021; Woll et al., 2021; Woll and Van Petegem, 2022).

From a functional point of view, pathological mechanisms due to RyRI mutations have been grouped into three main
categories: (1) mutations causing a gain of function, (2) mutations causing a loss of function, and (3) mutations causing a reduction in RYR1 protein content (Treves et al., 2008). For convenience, in this review, mutations are listed according to their position in the human RYR1 gene and protein.

Gain-of-function mutations in RYR1: in vitro characterization and related mouse models

RYR1 gain-of-function mutations can result in either hypersensitivity of the channels to physiological triggers or Ca\(^{2+}\) leak. Hypersensitivity is typically associated with MH mutations, since exposure to specific triggers, such as halogenated anesthetics, by lowering the threshold for RYR1 activation, induced a massive and uncontrolled Ca\(^{2+}\) efflux from the SR that leads to sustained muscle contraction and a hypermetabolic state (Tong et al., 1997). Among MH-causative mutations, R614C is one of the best characterized, since it is the first mutation identified in humans and is also naturally present in domestic swine populations affected by porcine stress syndrome, an MH-like crisis induced by stress, heat, or volatile anesthetics (Fujii et al., 1991; Gillard et al., 1991; MacLennan and Phillips, 1992). The R614C mutation typically confers hypersensitivity to caffeine and halothane without significantly altering the cytosolic or the luminal Ca\(^{2+}\) concentrations (Tong et al., 1997; Tong et al., 1999; Brini et al., 2005). In addition, studies performed in HEK293T cells showed that this mutation sensitizes RYR1 channels to activation by luminal Ca\(^{2+}\) by lowering the threshold for so-called store overload-induced calcium release (SOICR; Jiang et al., 2008). This mechanism was first described in cardiac muscle, where expression of some mutations in RYR2 associated with catecholaminergic polymorphic ventricular tachycardia resulted in channel opening induced by elevation of luminal Ca\(^{2+}\) (Venetucci et al., 2008). This mechanism was later confirmed in skeletal muscle for the R614C mutation, and also for other RYR1 variants (R2163H, G2434R, R2435H, R2435L, Y2445C, T4826I, L4838V, A4940T, G4943V, and P4973L; Chen et al., 2017).

Other mutations in RYR1, such as R163C or Y522S, resulted in a spontaneous and continuous Ca\(^{2+}\) leak from the SR that caused chronic elevation in resting cytosolic Ca\(^{2+}\) and reduction of the SR Ca\(^{2+}\) content (Tong et al., 1999; Chen et al., 2017). These conditions result in impaired muscle contraction, mitochondrial damage, and appearance of typical cores negative for oxidative enzyme staining, characteristic of central core disease (CCD; Loke and MacLennan, 1998). Over the years, however, the partition between mutations resulting in hypersensitive and leaky channels has become less clear-cut. As an example, low levels of spontaneous Ca\(^{2+}\) release were also observed in dyspedic myotubes expressing RYR1 channels carrying R614C, R2163C, or T4825I mutations, mainly associated with MH (Dirksen and Avila, 2002; Dirksen and Avila, 2004). Similarly, HEK293T cells expressing RYR1 channels carrying R163C,
I403M, Y522S, R2163H, and R2436H mutations, typically associated with CCD, showed lower thresholds for caffeine- and halothane-induced Ca\(^{2+}\) release (Tong et al., 1997; Tong et al., 1999); this suggests the existence of a partial overlap in the functional behavior among different RYRI mutations that, at the clinical level, may explain the existence of mixed MH and CCD phenotypes where a fraction of patients affected by CCD may also show susceptibility to MH.

In the attempt to understand the pathogenic mechanisms leading to either MH or CCD, four knock-in mouse models were generated, with the isogenic mutations corresponding to human RYRI R163C (Yang et al., 2006), Y522S (Chelu et al., 2006), G2434R (Lopez et al., 2018), and T482SI (Yuen et al., 2012) mutations. All four mouse models exhibit anesthetic-triggered MH episodes and environmental heat stress, and analysis of the functional properties of mutant channels confirmed the results previously obtained using cellular models.

In detail, homozygous and heterozygous mice carrying the MH-associated mutations G2434R and T4825T are viable and display all typical hallmarks of MH, with a severity rate dependent on gene dose (homozygous > heterozygous) and sex (male > female; Yuen et al., 2012; Lopez et al., 2018). In contrast, homozygous mice for the CCD-associated mutations, e.g., R163C and Y522S, show a more severe phenotype: Ryr1R163C/R163C mice die in utero at approximately embryonic day 17–18 (Elit et al., 2007; Yang et al., 2006), and Ryr1Y522S/Y522S mice show respiratory failure, skeletal deformities, dysmorphic muscle, likely arising from severe defects in myogenesis and bone formation, and die between E17.5 and postnatal day 1 (Chelu et al., 2006). Heterozygous mice for both R163C and Y522S show the typical hallmarks of MH upon exposure to volatile anesthetics, but unfortunately, they show a different phenotype as it concerns the formation of cores. Indeed, cores are not observed in Ryr1R163C mice in either the homozygous or heterozygous state (Chelu et al., 2006). In contrast, Ryr1Y522S/WT mice show structural alterations similar to those observed in humans (Boncompagni et al., 2009). Characterization of these mice depicted a hypothetical timeline for core formation that (1) starts with mitochondrial damage and formation of early cores at 2–4 mo of age; (2) evolves to formation of contracture cores that completely lack mitochondria and triads and present shortened sarcomeres; and (3) finally results in the development of unstructured cores with larger areas lacking mitochondria and contractile filaments (Boncompagni et al., 2009). The different phenotypes of these two CCD mouse models and the apparent inconsistency in mirroring the typical features of the human disease leave open the question about the nature of causative mechanisms leading to core formation. Differences between species and/or in the genetic background may be considered critical aspects.

The further characterization of these mouse models revealed that, in addition to inducing alterations in muscle contraction and/or structure, these RYRI mutations can impact on other aspects of muscle biology that were not previously considered. For example, extracellular Ca\(^{2+}\) entry was shown to be altered in some of these mouse models, and it was proposed that this may contribute, in the short or long term, to exacerbate muscle impairment (Yang et al., 2007; Cherendnichenko et al., 2008; Bannister et al., 2010; Estève et al., 2010; Elit et al., 2013; Yarotsky et al., 2013; Lambley et al., 2021). In Ryr1Y5225/WT and Ryr1T4826I/WT mice, the decrease in SR Ca\(^{2+}\) content due to channel leakage activates SOCE and increases mitochondrial Ca\(^{2+}\) uptake (Yang et al., 2007; Lambley et al., 2021). This is, initially, translated into activation of a preserving mechanism that leads to increased ATP production to support the greater demand of ATP exerted by SERCA pumps in their continuous activity to counteract Ca\(^{2+}\) leak (Lambley et al., 2021). Nevertheless, in the long term, mitochondria from both Ryr1Y5225/WT and Ryr1T4826I/WT mice showed structural and functional alterations, mainly in fatty acid metabolisms, that resulted in lipotoxicity and an increase in oxidative stress, impacting muscle bioenergetics and muscle performance (Yuen et al., 2012; Chang et al., 2020). Similarly, muscles from Ryr1R163C/WT mice show an increase in excitation contraction calcium entry (ECCE) linked to delayed inactivation of L-type currents through DHPR (Cherendnichenko et al., 2008; Bannister et al., 2010; Estève et al., 2010; both Ryr1R163C/WT and Ryr1Y5225/WT also display an increase in SOCE that may contribute to Ca\(^{2+}\) overload, hypercontractures, heat generation, and rhabdomyolysis (Elit et al., 2013; Yarotsky et al., 2013). Whether these alterations also occur in muscles from patients carrying the same mutations is still to be defined.

Finally, the persistent Ca\(^{2+}\) leak observed in Ryr1Y5225/WT knock-in mice was proposed to lead to increased production of oxidative stress and reactive nitrogen species (RNS) that result in posttranslational modifications of RYRI, which in turn further enhance channel activity. In this way, a destructive feedback cycle is activated, leading (in acute conditions) to sudden death or inducing (over time) mitochondrial damage (Durham et al., 2008; Lanner et al., 2012; Manno et al., 2013; Canato et al., 2019). Interestingly, the use of 5-aminomidazole-4-carboxamide ribonucleoside, a compound that, in this context, can inhibit Ca\(^{2+}\) leakage through RYRI channels, prevented heat-induced death in Ryr1Y5225 mice (Lanner et al., 2012); furthermore, treatment with the antioxidant drug N-acetylcysteine significantly reduced mitochondrial damage, contributing to improved muscle function (Michelucci et al., 2017a).

Loss-of-function mutations in RYRI: in vitro characterization and related mouse model

RYRI loss-of-function mutations result in reduction of Ca\(^{2+}\) release due to either an impairment of RYRI and DHPR functional coupling, through the so-called "uncoupling" mechanism (Avila et al., 2001; Avila et al., 2003), or a reduced channel opening resulting from alterations in sites of interaction with endogenous activators (Yuan et al., 2021). The majority of uncoupling mutations are localized in the C-terminal region of the channel containing the pore domain, within the 4585-4646 amino acid sequence in humans (Gillespie et al., 2014). The first described loss-of-function mutation in RYRI was I4898T, identified in patients affected by CCD (Lynch et al., 1999). Although preliminary experiments performed on heterologous channels expressed in HEK293T cells or lymphocytes from patients carrying the I4898T mutation reported an increase in SR Ca\(^{2+}\) leak (Lynch et al., 1999; Tilgen et al., 2001), further functional characterization gave
opposite and surprising results. Indeed, when expressed in HEK293T cells or dyspedic myotubes, both caffeine- and voltage-induced Ca\textsuperscript{2+} release from homozygous channels were completely abolished, while a significant reduction in caffeine- and voltage-induced Ca\textsuperscript{2+} release was observed in cells expressing heterozygous channels (Avila et al., 2001; Avila et al., 2003; Xu et al., 2008). No alterations in cytosolic or SR Ca\textsuperscript{2+} levels were observed, suggesting that the pathogenic mechanism associated with this mutation had to be different from that proposed for mutations leading to leaky channels (Avila et al., 2003). To further characterize the RYRI I4898T mutation, a mouse model was generated by Zvaritch et al. (2007). Heterozygous Ryr1\textsuperscript{I4898T} mice die after birth and are paralyzed due to complete lack of voltage-induced Ca\textsuperscript{2+} release. Skeletal muscle development is arrested at early stages of myoblast fusion, with impairment of myofibrillogenesis, resembling that observed in dyspedic mice (Takeshima et al., 1994; Zvaritch et al., 2007). Heterozygous Ryr1\textsuperscript{I4898T/WT} mice show a slow progressive congenital myopathy with variable expression of minicores, cores, and rods (Zvaritch et al., 2009), mirroring, in some way, the variable penetrance of this mutation observed in humans. It must be stated, however, that minicores or nemaline rods are not usually observed in humans carrying the RYRI I4898T mutation and that CCD is considered a nonprogressive disease, indicating that this model did not completely fit with the histological and clinical aspects observed in humans carrying the same mutation. A second Ryr1\textsuperscript{I4898T} mouse model generated on a different genetic background developed a mild myopathy that was much more similar to human CCD, suggesting that the genetic background plays a significant role in disease onset and development (Boncompagni et al., 2010; Loy et al., 2011). Indeed, these mice show a preferential involvement of type I fibers that present with areas of Z line streaming and lacking mitochondria, comparable to core regions observed in type I fibers of CCD patients (Boncompagni et al., 2010).

Nevertheless, the possible mechanism of core formation in the Ryr1\textsuperscript{I4898T/WT} mouse models was not completely unraveled. An interesting point concerns the possible involvement of mitochondria in core formation: indeed, unlike Ryr1\textsuperscript{Y522S/WT}, damaged mitochondria and contraction cores were only rarely observed in muscles from the Ryr1\textsuperscript{I4898T/WT} mouse model, although they were found to be displaced within a region of structural alteration; the authors could only suggest that the structural alterations observed in type I fibers may be due to long-term mechanical stress mainly affecting postural muscles (Loy et al., 2011). A third mouse model carrying the RYRI I4898T mutation was generated on yet another genetic background, and these mice also presented with a mild phenotype (Lee et al., 2017). The characterization of this mouse model focused on a novel possible mechanism of disease based on the observation that this mutation correlates, in these mice, with occurrence of a persistent increase in ER stress/unfolded protein response (UPR) that results in a decrease in protein synthesis and an increase in mitochondrial Ca\textsuperscript{2+} uptake, reactive oxygen species (ROS) production, and induction of apoptosis. According to the authors, since residue I4898 is localized in proximity of the RYRI sequence involved in triadin binding, mutation in this residue causes a reduction in triadin binding and content. Given the interaction between triadin and CASQ, this also leads to mislocalization of CASQ, resulting in induction of ER stress/UPR. In support of this hypothesis, the use of the chemical chaperone 4-phenylbutyric acid resulted in a significant improvement of muscle function, suggesting that the ER stress/UPR pathway contributes significantly to development of CCD caused by the I4898T mutation (Lee et al., 2017).

In addition, RYRI mutations L4647P, F4857S, G4891R, R4893W G4899E, G4899R, A4906V R4914G, and D4918N were found to display a loss of voltage-induced Ca\textsuperscript{2+} release, further supporting the idea that the 4895GGIGIDDEG901 amino acid sequence and sequences in close proximity to this region are required for functional E-C coupling (Monnier et al., 2001; Tilgen et al., 2001; Avila et al., 2003; Kraeva et al., 2013; Parker et al., 2017).

RYRI mutations that reduce channel opening by endogenous regulators such as ATP and Ca\textsuperscript{2+} can also be considered among loss-of-function mutations. Indeed, millimolar ATP concentrations enhance Ca\textsuperscript{2+}-dependent activation of RYRI, resulting in increased open probability of the channels (Chan et al., 2000). Micromolar concentrations of cytosolic Ca\textsuperscript{2+} activate RYRI channels, while at millimolar concentrations, Ca\textsuperscript{2+} acts as a channel inhibitor (Meissner et al., 1986). Indeed, the RYRI mutation T4980M, associated with a recessive form of congenital myopathy with cores, is located within the RYRI ATP-binding site (Klein et al., 2011; Maggi et al., 2013). Functional studies showed that this mutation results in reduced channel activation, which may explain the muscle weakness observed in humans (Yuan et al., 2021). Similarly, the Q3969R mutation, linked to a form of CCD, is located close to the Ca\textsuperscript{2+} binding site of RYRI and results in a reduction in Ca\textsuperscript{2+}-dependent channel activation (Yuan et al., 2021).

Mutations causing a reduction in RYRI protein content: related mouse models
A third mechanism of disease for RYRI-related myopathies is associated with a decrease in the overall RYRI protein levels; this is usually correlated with the presence of compound heterozygous mutations, where the first mutation causes a premature termination codon and the second is a missense mutation (Monnier et al., 2008; Bevilacqua et al., 2011; Cacheux et al., 2015; Brennan et al., 2019). Disease severity is linked to the nature of the mutation present in the expressed second allele as well as on the residual expression of the first hypomorphic allele (Brennan et al., 2019; Elbaz et al., 2019). Reduction in RYRI protein content, due to compound heterozygous mutations, has been reported in several RYRI-related myopathies including CCD, multimimicore disease (MmD), centronuclear myopathy (CNM), congenital fiber type disproportion (CFTD), dysty core disease (DuCD), and core rod myopathy (CRM; Ogawara and Nishino, 2021).

A mouse model mimicking the expression of compound heterozygous mutations was generated by introducing a point mutation leading to expression of the T4706M mutation in one RYRI allele and a 16-bp frame-shift deletion resulting in a premature stop codon in the second allele (Ryr1\textsuperscript{TM/Indel}; Brennan et al., 2019). This was intended to most closely mimic the mutation present in patients with RYRI disease.
et al., 2019). These mice show a reduction of ~80% in RYR1 protein levels and present with muscle weakness, hindlimb paralysis, and severe scoliosis but no changes in fiber type and no evidence of cores. Mice have a short lifespan and die probably because of respiratory failure.

A second mouse model carrying a frameshift mutation (Q1970fsX16) together with the missense mutation A4329D (Ryr1Q1970fsX16/A4329D) shows the main features of MmD-affected patients, with a reduction in muscle force and Ca\(^{2+}\) transients, associated with a decrease in RYR1 protein level of ~65% (Elbaz et al., 2019). Interestingly, homozygous expression of the A4329D mutation (Ryr1A4329D) also shows a reduction in RYR1 protein content and muscle performance, but this was limited to slow-twitch muscle fibers. The reason for the selective effect of the homozygous A4329D mutation in slow-twitch fibers is not known; the authors suggested that a different epigenetic regulation of fast- versus slow-twitch muscle fibers may account, at least in part, for these differences (Elbaz et al., 2020). Indeed, an increase in histone deacetylase (HDAC) was observed in soleus muscle of Ryr1A4329D mice and, interestingly, in muscles from patients carrying recessive RYR1 mutations (Zhou et al., 2006; Rokach et al., 2015; Ruiz et al., 2022). Although the correlation between expression of mutant RYR1 channels and epigenetic changes is not clear, the use of an inhibitor of HDAC and DNA methylase significantly improved muscle strength and RYR1 expression (Ruiz et al., 2022).

Finally, a third mouse model with an inducible muscle-specific deletion of one RYR1 allele, leading to a 50% reduction in protein expression levels, showed typical signs of a myopathy with features of CCD and DuCD, progressive muscle weakness, atrophy, and mitochondrial dysfunction (Pelletier et al., 2020). This model is particularly interesting since, unlike the previously described models, it was obtained by exclusive reduction in the expression of the RyR1 gene, thus excluding the existence of possible side effects due to the residual expression of mutant Ryr1 channels. In these mice, the reduction in Ryr1 protein content was sufficient to cause muscle weakness and alterations in E-C coupling. A disorganization of muscle structure was observed, with appearance of lesions resembling those observed in patients with recessive mutations, including mitochondria mislocalization and inhibition of autophagy. Interestingly, an increase in Stim1 and Orai1 was also observed, suggesting a possible role of SOCE in disease onset (Pelletier et al., 2020).

**RYR1-related myopathies**

RYR1-related myopathies are classified in different subtypes mostly based on the histopathological features observed in muscle biopsies of patients (Abath Neto et al., 2017; Garibaldi et al., 2019; Knuiman et al., 2019; Lawal et al., 2020; Table 1). Nevertheless, it must be considered that the histological phenotype associated with RYR1 mutations can differ in individuals with the same variant or change with age in the same patient, and thus classification of RYR1-related myopathies merely based on the histopathological pattern is more complicated than expected. In addition, MH, a nonmyopathic condition, is also strictly linked to RYR1 mutations (Galli et al., 2006).

**CCD**

Core myopathies represent a heterogeneous group of muscle diseases that can differ clinically, pathologically, and genetically; at histological examination, they share a common pathological feature consisting of areas of muscle fibers, called cores, that do not show oxidative enzyme staining because of a reduced number of mitochondria or decreased oxidative enzyme activity. Accordingly, CCD is characterized, at the histopathological level, by centrally located and well-demarcated cores, presenting with reduced oxidative activity, running almost along the entire axis of the fibers, and mostly present in type 1 fibers (Jungbluth, 2007; Jungbluth et al., 2018; Lawal et al., 2020). Cores can be classified as structured or unstructured based on ATPase activity levels and myofibrillar disruption. Additional histopathological features of CCD include increased central nuclei, endomysial fibrosis, and proliferation of sarcotubular membranes (Garibaldi et al., 2019; Lawal et al., 2020). These cores may also contain structural proteins such as desmin, αβ-crystallin, or SR proteins such as RYR1, triadin, and DHPR (Ogasawara et al., 2020). CCD is the most frequent core myopathy, and RYR1 mutations are found in >90% of patients (Wu et al., 2006; Jungbluth et al., 2018; Lawal et al., 2020).

Although in RYR1-related myopathies little or no correlation can be found between the clinical phenotype and localization of mutations in the RYR1 channel, mutations in patients with CCD are more frequently localized in the C-terminal region, containing the pore-forming domain of the channel (Wu et al., 2006). CCD can be inherited with a dominant or recessive transmission. CCD due to dominant RYR1 mutations is the most frequent and is usually associated with a mild condition compared with the more severe cases due to recessive inheritance. Clinically, CCD has a typical onset in infancy or early childhood; patients show nonprogressive hypotonia and motor development delay, congenital dislocation of the hips, scoliosis, myalgia, and muscle stiffness; respiratory, bulbar, and cardiac involvement are less common (Bönneman et al., 2014; Lawal et al., 2018; Jungbluth et al., 2018). Serum creatine kinase levels may be moderately elevated or normal (Klein et al., 2011). CCD due to recessively inherited RYR1 mutations presents more severe features such as marked hypotonia, multiple arthrogryposis, and respiratory failure (Bharucha-Goebel et al., 2013). Some, but not all, CCD patients carrying dominant RYR1 mutations may have an increased risk for MH (Rosenberg et al., 2015). On the other hand, RYR1 mutations found in patients with CCD may be present in individuals with MH susceptibility but no sign of myopathy.

As previously discussed, CCD is associated with both gain of function and loss of function. To unravel the pathogenic mechanisms of core formation due to such functionally different RYR1 mutations, three mouse models of CCD have been generated in the last 15 yr, Ryr1R163C, Ryr1I4898T, and Ryr16o2 mouse models (Chelu et al., 2006; Yang et al., 2006; Zvaritch et al., 2007; Loy et al., 2011; Lee et al., 2017). A model for core formation in CCD murine models proposes that persistent Ca\(^{2+}\) leak from Ryr1 mutant channels induces a chronic condition of ROS/RNS stress, leading to mitochondrial damage, disruption of the sarcotubular system, and thus core formation. These pathogenic mechanisms...
| E-C coupling–related myopathies | Main clinical features | Fiber phenotype | Causative genes | Inheritance | Mechanism (RyR1 channel) |
|---------------------------------|------------------------|-----------------|-----------------|-------------|-------------------------|
| **CCD**                         | ✓ Infantile nonprogressive hypotonia and motor development delay ✓ Mild proximal muscle weakness ✓ Respiratory distress ✓ High arched palate ✓ Craniofacial dysmorphism | ✓ Centrally located, well-demarcated cores, spanning the whole fiber axis ✓ Predominance in type 1 fibers ✓ Increased central nuclei | **RYR1 >90%** | AD or AR | GoF, LoF |
|                                 |                        |                 | **MYH7**        |             | Altered assembly and function of myosin dimers |
| **MmD**                         | ✓ Axial muscle weakness, scoliosis, respiratory insufficiency, and limb joint hyperlaxity ✓ Ophthalmoplegia ✓ Arthrogryposis ✓ Hand amyotrophy | ✓ Numerous cores in a limited area on longitudinal section ✓ Multiple internally located nuclei ✓ Predominance in type 1 fibers | **RYR1 ~20%** (homozygosity or compound heterozygosity) | AR | GoF, LoF, lower protein levels |
|                                 |                        |                 | **SEPNI ~50%**  | AR | Altered redox activity |
|                                 |                        |                 | **TTN** (homozygosity or compound heterozygosity) | AR | M-line alteration |
|                                 |                        |                 | **MYH7**        | AD | Not defined |
|                                 |                        |                 | **ACTA1**       | AR | Not defined |
|                                 |                        |                 | **MEGF10**      | AD or AR | Not defined |
|                                 |                        |                 | **CACNA1S**     | AD | Lower protein levels |
| **CNM**                         | ✓ Not progressive proximal muscle weakness ✓ Not progressive hypotonia | ✓ Centralized and internalized nuclei ✓ Peripheral halos depleted of oxidative activity ✓ Cores | **RYR1 ~15%** (compound heterozygosity) | AR | Lower protein levels |
|                                 |                        |                 | **MTM1**        | XLR | Altered vesicle trafficking |
|                                 |                        |                 | **DNM2**        | AD | Altered membrane fission |
|                                 |                        |                 | **BIN1**        | AD | Altered membrane tubulation |
|                                 |                        |                 | **TTN**         | AR | M-line alterations |
|                                 |                        |                 | **SPEG**        | AR | Altered interaction with MTM1 and desmin |
| **CFTD**                        | ✓ Static or slowly progressive muscle weakness ✓ Respiratory and proximal axial weakness ✓ Ophthalmoplegia ✓ Dysphagia ✓ Facial muscle weakness | ✓ Fiber size disproportion (35–40% of type 1 fibers are smaller in size than type 2 fibers) ✓ Age-related development of rods, cores, and central nuclei | **RYR1 ~20%** | AR | Lower protein levels |
|                                 |                        |                 | **ACTA1**       | AD | Altered interaction with TPM |
|                                 |                        |                 | **TPM2**        | AD or AR | Altered interaction with actin |
|                                 |                        |                 | **TPM3**        | AD or AR | Altered interaction with actin |
|                                 |                        |                 | **SEPNI**       | AR | Altered redox activity |
|                                 |                        |                 | **MYH7**        | AD | LoF, altered interaction with myosin binding protein |
|                                 |                        |                 | **LMNA**        | AD | Not defined |
|                                 |                        |                 | **ZAK**         | AR | LoF |
|                                 |                        |                 | **SPEG**        | AR | LoF |
| **DuCD**                        | ✓ Ocular involvement (eyelid ptosis, ophthalmoplegia) | ✓ Irregularly sized/shaped “dusty” cores (reddish-purple granular material deposition) spanning 10–50 sarcomeres ✓ Myofibrillar disorganization | **RYR1**        | AR | Lower protein levels |

Rossi et al. Journal of General Physiology
Calcium homeostasis and congenital myopathies

https://doi.org/10.1085/jgp.202213115
are further exacerbated by the activation of futile cycles powered by ROS/RNS-dependent RyR1 hyperactivation and increased SOCE to compensate for Ca\(^{2+}\) leak. As concerns core formation in RyR1\(^{-/-}\) mice carrying a RyR1 uncoupling mutation, it is clear that core formation is unlikely to be due to increased intracellular Ca\(^{2+}\). Studies by Lee et al. (2017) proposed that this RyR1\(^{-/-}\) mice carrying a RyR1 uncoupling mutation induces a chronic condition of ER stress/UPR that results in a decrease in protein synthesis and an increase in mitochondrial Ca\(^{2+}\) uptake, ROS production, and induction of apoptosis. According to these models, it can therefore be hypothesized that, although the primary trigger is different, core formation in CCD, due to gain-of-function or loss-of-function mutations in RyR1, can represent the final outcome of the stressful conditions that converge into alterations in activity and/or structure of mitochondria and disruption of SR and myofibrils.

Dominant mutations in the MYH7 gene, which encodes the slow/β-cardiac myosin heavy chain (MyHCI) expressed in type 1 muscle fibers and in the heart, are causative of ~10% of CCD cases (Fananapazir et al., 1993; Romero et al., 2014), as well as of several other myopathies including MmD and CFTD (Tajsharghi et al., 2003; Cullup et al., 2012; Clarke et al., 2013). MYH7 mutations in skeletal myopathies are usually located in the rod domain of the protein (Tajsharghi and Oldfors, 2013; Fiorillo et al., 2016). Although the pathogenic mechanisms associated with mutation in MYH7 are still unclear, mutations in the rod region can affect assembly of functional myosin dimers or their incorporation into thick filaments, causing aberrant accumulation of myosin as in myosin storage myopathy (Tajsharghi and Oldfors, 2013).

**MmD**

Recessive mutations in RYRI represent the second genetic cause of MmD, a myopathy presenting numerous cores, visible as pale spots in oxidative stained muscle sections, sometimes with a moth-eaten appearance and gathered in a limited area on longitudinal section. Multiple internally located nuclei, minimal myofibril disruption, and type 1 fiber predominance are noted in affected muscles, although the histological traits can be extremely variable (Lawal et al., 2018; Ogasawara and Nishino, 2021). Clinical features of MmD are variable and can be classified in four groups: (1) the classic form, with axial muscle weakness, scoliosis, respiratory insufficiency, and limb joint hyperlaxity; (2) the ophthalmoplegia form; (3) the early-onset form with arthrogryposis, and (4) a slowly progressive form with hand amyotrophy (Jungbluth et al., 2005; Jungbluth, 2007; Monnier et al., 2008; Treves et al., 2008; Klein et al., 2012; Romero and Clarke, 2013). MmD patients carrying mutations in RYRI typically present with extracocular muscle involvement and ophthalmoplegia, symptoms not observed in patients with mutations in SEPN1, which are present in the majority of MmD cases (Villar-Quiles et al., 2020). SEPN1 codes for selenoprotein-N, an SR protein with Ca\(^{2+}\)-dependent redox activity (Feerreiro et al., 2002; Petit et al., 2003; Chernorudskiy et al., 2020; Villar-Quiles et al., 2020). The pathogenic mechanisms of RYR1-related...
MmD are variable, including both loss-of-function and gain-of-function mutations, as well as reduction in RYR1 protein content (Jungbluth, 2007).

More rarely, patients with MmD may carry mutations in 
TTN, MYH7, ACTA1, MEGF10, and CACNAIS. Homozygous and compound heterozygous mutations in the TTN gene, which codes for titin, a giant sarcomeric protein, are found in patients with MmD (Chauveau et al., 2014). These mutations are preferentially found in the region of TTN coding for the M-line segment of this protein (Chauveau et al., 2014; Avila-Polo et al., 2018). Mutations in ACTA1, which codes for skeletal muscle α-actin, the principal isoform found in the adult sarcomeres, are rarely found in patients with MmD as well as several other myopathies (Schartner et al., 2017). The pathogenic mechanisms of disease remain to be identified. The MEGF10 gene encodes the multiple EGF-like domain 10 protein, a transmembrane receptor belonging to the multiple epidermal growth factor–like domains family, that is upregulated in activated satellite cells and regulates the progression of the myogenic program (Holterman et al., 2007). Mutations in MEGF10 have been detected in a few cases of MmD (Boyd et al., 2012; Liewluck et al., 2016; Takayama et al., 2018; AlMuhaizea et al., 2021). The CACNAIS gene encodes Cav1.1, the pore-forming subunit of the skeletal muscle voltage-gated Ca2+ channel (DHPR). Recessive and dominant mutations in CACNAIS were identified in patients showing MmD/CNM with structural alterations in T-tubules and SR. Although the pathogenetic mechanism has not been clearly defined, both recessive and dominant mutations correlate with a strong decrease in protein levels, suggesting instability or degradation of DHPR complexes. RYR1-mediated Ca2+ release was also impaired, although no change in cytosolic or SR Ca2+ levels was observed (Schartner et al., 2017).

**CNM**

Recessive mutations in RYR1 are the most common cause of autosomal recessive CNM, accounting for ~15% of patients. At clinical presentation, autosomal CNM most frequently shows delayed motor milestones, nonprogressive muscle weakness and hypotonia, ptosis, ophthalmoplegia, and mild to severe respiratory impairment (Jungbluth et al., 2008; Wilmshurst et al., 2010; Bevilacqua et al., 2011; Romero and Bitoun, 2011; Gómez-Oca et al., 2021). From a histological point of view, CNM shows centralized and internalized nuclei, peripheral halos depleted of oxidative activity, and cores, although based on which gene is mutated, the histological patterns may vary (Nicot et al., 2007; Bevilacqua et al., 2011; Gomez-Oca et al., 2021). RYR1-related CNM often presents as a mixed phenotype between CNM and core myopathies, with the presence of multiple central nuclei and core-like structures with no limited boundaries (Abath Neto et al., 2017). These forms are normally caused by compound heterozygous mutations in RYR1, where one of the two mutations usually cause premature termination of the transcript, resulting in a decrease in RYR1 protein expression levels (Gómez-Oca et al., 2021). Mutations in MTMI account for >50% of CNM cases. This gene encodes the 3′-phosphoinositide phosphatase myotubularin (Hnia et al., 2012; Vandersmissen et al., 2018). Mutations in the gene encoding Dynamin 2 (DNM2) are the most common cause of dominant CNM. DNM2 is a GTPase protein that can bind and organize the microtubular and actin cytoskeleton and associates with nascent vesicles to induce their release (Ferguson and De Camilli, 2012). More rare cases of CNM are due to mutations in BIN1, which encodes amphiphysin 2, a protein implicated in membrane curvature, tubulation, and vesicle trafficking (Peter et al., 2004). MTMI, BINI, and DNM2 encode proteins are functionally interconnected, since they are involved in vesicle trafficking, membrane fission, or autophagy (Durieux et al., 2012). MTMI recruits BIN1 and DNM2 to muscle membranes and enhances BIN1 tubulation. Mutations in MTMI affect triad structure (Royer et al., 2013; Cowling et al., 2017). Given the functional interplay between BIN1, DNM2, and MTMI, a correct balance in their reciprocal expression levels has been shown to be important for muscle physiology. Indeed, overexpression of BIN1 in Dnm2R465W/WT and Dnm2R465W/R465W mice improved muscle atrophy and rescued the perinatal lethality and survival of Dnm2R465W/R465W mice (Lionello et al., 2022). Moreover, the use of antisense oligonucleotides against Dnm2 in Bin1 knockout mice improved muscle force and intracellular architecture (Silva-Rojas et al., 2022). Recently, the role of a microRNA identified within the genomic sequence of DNM2, namely miR-199a-1, was also investigated as a player in the development of CNM (Chen et al., 2020). Recessive TTN mutations result in a variable histological pattern, from cores to typical minicores with a marked type 1 fiber predominance. Multiple centrally located nuclei are also present in a significant proportion, thus accounting for the diagnosis of CNM (Ceyhan-Birsoy et al., 2013). Recessive mutations in the SPEG gene, which encodes the striated muscle enriched protein kinase, a serine/threonine kinase member of the myosin light chain kinases involved in muscle development (Luo et al., 2021), were identified in patients with severe pediatric forms of CNM with cardiac involvement (Agrawal et al., 2014; Wang et al., 2017; Tang et al., 2020).

**CFTD**

CFTD diagnosis is based on the observation that 35–40% of type 1 fibers show a smaller size than type 2 fibers, without other structural defects (Clarke, 2011). However, many patients with age may develop rods, cores, and central nuclei (Garibaldi et al., 2019; Lawal et al., 2020). Recessive mutations in RYR1 are found in ~20% of CFTD patients, while another 40% of cases are associated with mutations in different genes including ACTA1, TPM3, TPM2, SEPN1, MYH7, LMNA, and ZAK; the remaining 40% of CFTD cases have not yet been associated with a genetic cause (Laing et al., 2004; Laing et al., 2009; Lawlor et al., 2010; Ortolano et al., 2011; Kajino et al., 2014; Vasili et al., 2017; Moreno et al., 2020). Clinically, the disease shows static or slowly progressive generalized muscle weakness from infancy as well as respiratory and proximal axial muscle weakness, multiple joint contractures, scoliosis, long thin face, and high arched palate. About 30% of patients also show respiratory involvement. Other common features are ophthalmoplegia, dysphagia, and facial muscle weakness (Clarke, 2011; Lawal et al., 2020). Mutations in ACTA1 account for ~10% of CFTD cases, are often localized on the
surface of ACTA1, and correspond to amino acids that are exposed on the F-actin filament facing the sites of interaction with tropomyosin, suggesting that the mechanism of disease may correlate with disruption or alteration of the actin–tropomyosin interaction (Laing et al., 2004; Laing et al., 2009; Matsumoto et al., 2022). Cardiomyopathy is a rare finding accompanying CFTD with ACTA1 mutation (Laing et al., 2009; Matsumoto et al., 2022). Tropomyosin 2 (TPM2) and TPM3 encode two isoforms of tropomyosin expressed in skeletal muscles. TPM2 codes for β-tropomyosin expressed in type 1 muscle fibers and to a lesser extent in fast-twitch fibers, while TPM3 codes for slow α-tropomyosin expressed exclusively in slow-twitch fiber types (Marttila et al., 2014). Dominant mutations in TPM2 and TPM3 are more frequently associated with CFTD, while recessive mutations are rare (Lawlor et al., 2010; Clarke, 2011; Moreno et al., 2020). Most TPM2 mutations are localized in the coiled-coil domain of the protein; the R133W mutation in TPM2 alters tropomyosin flexibility and disrupts the actin–tropomyosin and actin–myosin interactions (Borovikov et al., 2020). In contrast, mutations in TPM3 are mainly localized in the actin-binding domain of the protein, resulting in either hyper- or hypocontractility (Yuen et al., 2018).

The LMNA gene codes for lamin A and C proteins, structural components of the nuclear lamina, a network underlying the inner nuclear membrane (Fisher et al., 1986). A dominant mutation in LMNA was identified in patients with CFTD who show muscle weakness, hypotonia, and cardiac involvement (Kajino et al., 2014). Mutations in MYH7 were rarely identified in CFTD. A frameshift mutation was proposed to disrupt the stability of the myosin rod domain and introduce a proline residue that may alter chain flexibility and dimerization. Other distal mutations in MYH7 were also identified in a myopathy with predominance of small type I fibers (Muelas et al., 2010; Ortolano et al., 2011; Clarke et al., 2013; Fajusalo et al., 2016). Patients with recessive mutations in the CFL2 gene, encoding the mitogen-activated protein triple kinase ZAK, were identified in three unrelated families diagnosed with CFTD who presented with slowly progressive muscle weakness, developmental delay, and scoliosis (Vasili et al., 2017). All mutations resulted in loss of protein expression due to the presence of premature stop codons in the mRNA. ZAK is a serine-threonine kinase activating the ERK, JNK, and p38 pathways, that, among other functions, regulates myogenesis (Gotoh et al., 2001). Recently, a homozygous mutation in the C-terminal region of SPEG has been described in patients with a mild form of CFTD with severe cardiac involvement, but in whom hypertrophic type II fibers were not observed. The mutation results in the appearance of a premature stop codon in the C-terminal region of SPEG. This mutation does not affect the domain of interaction with MTM1, however, and this may partially explain the mild phenotype of patients (Gurgel-Giannetti et al., 2021).

DuCD

DuCD is defined by irregular areas of myofibrillar disorganization with reddish-purple granular material depositions, devoid of ATPase activity (Bevilacqua et al., 2011). Dusty cores are irregular in size and shape, with no demarked borders, sometime with a star-like appearance (Garibaldi et al., 2019). Unlike CCD, dusty cores are mostly 10–50 sarcomeres in length, with strands of osmophilic material accumulated in specific regions and containing SR or cytoplasmic structures. Patients with DuCD show early disease onset and severe clinical phenotype, with ocular involvement in most of the cases. DuCD is caused by recessive, biallelic RYR3 mutations, resulting in low levels of RYRI expression. It has been speculated that severe RYRI haploinsufficiency may impair integrity of triads, which are often duplicated in patients’ biopsies (Garibaldi et al., 2019).

CRM

As its name suggests, muscles from patients affected by CRM show both central cores and nemaline bodies (rods) that are typical of nemaline myopathy. Rods are mainly composed of actin and α-actinin, probably deriving from Z disks; they can be assembled in clusters or widely distributed along the fibers (Scacheri et al., 2000). Longitudinal sections may also show cores devoid of mitochondria covering a large part of the fiber axis. From a clinical point of view, nonspecific clinical features such as hypotonia, muscle weakness, scoliosis, and respiratory insufficiency are observed. Dominant mutations in RYRI are the main genetic cause of CRM (Monnier et al., 2000; von der Hagen et al., 2008; Hernandez-Lain et al., 2011). Compound heterozygous mutations in RYRI were identified in a patient with fetal akinesia, hypotonia, ophthalmoplegia, and respiratory insufficiency (Kondo et al., 2012).

Other causative genes in CRM have been reported (CFL2, ACTA1, and TPM3), although some of them are typically associated with nemaline myopathy, where cores are not present (Agrawal et al., 2007; Lawal et al., 2018; Pinto et al., 2019). Mutations in coflin 2 (CFL2), coding for the actin-binding protein coflin-2, were identified in patients with nemaline myopathy with minicores and concentric laminated bodies (Agrawal et al., 2007). Dominant mutations in ACTA1 and TPM3 are most frequently associated with nemaline myopathy characterized by early onset and respiratory insufficiency, while recessive forms of nemaline myopathy are most frequently associated with mutations in the nebulin gene (NEB). Mutations in ACTA1 are distributed along the entire gene, affecting stability, function, and conformation of the protein, so a clear correlation with genetic defect and disease onset and development is difficult to discern (Sparrow et al., 2003). Finally, a compound heterozygous mutation in RYR3 coding for the second isoform of RYR expressed in skeletal muscle was identified in a family with nemaline myopathy. Unfortunately, RYR3 mutant channels were not characterized from a functional point of view, and thus no pathogenetic mechanism can be hypothesized (Nilipour et al., 2018).

MH

MH is a pharmacogenetic disorder that results in a hypermetabolic state following exposure to succinylcholine and volatile anesthetics such as halothane, sevoflurane, desflurane, and isoflurane. These may cause a massive Ca2+ release in skeletal muscles, resulting in variable clinical manifestations that can be graded based on symptoms (Larach et al., 1994; Larach et al., 2010). This condition is potentially lethal if not rapidly treated...
with the muscle relaxant dantrolene (Hopkins, 2011; Ellinas and Albrecht, 2020). The incidence of MH is estimated at ~1:100,000 individuals, although the number of people carrying mutations in RYR1 may be estimated at ~12,000–3,000 (Monnier et al., 2001; Riazi et al., 2018).

MH appears to have a higher prevalence in males than females, likely because of smaller muscle mass and a protective effect of estrogens in the latter (Michelucci et al., 2017b). Although ~10% of patients present with no alteration at histological analysis, the remaining 90% show a variable range of alterations, including an increase in fiber size variability, internal nuclei, type 1 fiber predominance, and loss of oxidative stain that is rarely associated with the presence of cores and rods (Knuiman et al., 2019). These histological alterations, although variable, may represent mild features that, in RYR1-related myopathies, evolve to more severe histological and structural alterations. The diagnostic test for MH susceptibility is an in vitro contracture test, which determines the contracture threshold for skeletal muscle bundles treated with caffeine and halothane (Allen et al., 1998; Hopkins, 2011; Hopkins et al., 2015). RYR1 variants account for ~76% of MH events, while only 1% are linked to CACNA1S mutations and <1% are linked to mutations in STAC3 (Johnston et al., 2021). As of today, 48 mutations in RYR1 and 2 mutations in CACNA1S are considered causative for MH according to the EMHG, since they have been characterized at the genetic and functional level (Hopkins et al., 2015). Each of these mutations has been fully characterized at the genetic level, including aspects concerning evolutionary conservation and change in charge, polarity, or structure introduced by the amino acid replacement, cosegregation of the variant with the disease in the families affected, and assessment of the prevalence of the variant in the population. In addition, each mutation has been functionally characterized using one or a combination of test systems including expression of recombinant proteins in muscle or nonmuscle cells or patient-derived myotubes and lymphoblasts (Hopkins et al., 2015). However, hundreds of additional variants in RYR1 have been identified in genetic studies of MH-susceptible individuals, although functional characterization is not yet available for all (Kushnir et al., 2020). Recently, to help in classifying genetic variants in RYR1, a score matrix has been defined based on the identification of pathogenic or benign criteria of the American College of Medical Genetics and Genomics (Johnston et al., 2021).

A small percentage of individuals carrying RYR1 mutations can experience MH-like episodes independently of anesthesia, a condition also referred to as awake MH, which is characterized by skeletal muscle cramping and rigidity, rhabdomyolysis associated with exertional heat illness (the inability to thermoregulate during physical activity), exertional rhabdomyolysis, emotional stress, fatigue, and viral infection (Tobin et al., 2001; Wappler et al., 2001; Capacchione and Muldoon, 2009; Sambuughin et al., 2009; Groom et al., 2011; Dlamini et al., 2013; Molenaar et al., 2014; Timmins et al., 2015; Sambuughin et al., 2018; Zvaritch et al., 2019; Gardner et al., 2020; Laitano et al., 2020; Krujit et al., 2022). Although the mechanisms that trigger awake MH have not yet been defined, the correlation with anesthetic-induced MH is supported by studies in animal models that include pigs carrying the R615C mutation corresponding to the human R614C mutation (Fujii et al., 1991) and mice carrying RYRI mutations equivalent to human Y522S or G2434R mutations, which show susceptibility to undergo MH crisis when exposed to environmental stress (Michelucci et al., 2017b; Michelucci et al., 2017c; Lopez et al., 2018). Interestingly, treatment with dantrolene improves muscle symptoms such as cramps, myalgia, and muscle weakness in humans or prevents or reduces awake MH crisis in animal models, further supporting the idea of a correlation between anesthesia-induced MH and awake MH (Timmins et al., 2015; Michelucci et al., 2017c). More recently, a correlation between MH and increased levels of blood glucose was observed in humans and in the R163C mouse model for MH (Altamirano et al., 2019; Tammineni et al., 2020).

Skeletal muscle represents a primary site for insulin-dependent and -independent glucose uptake (DeFronzo 1988; Jessen and Goodyear, 2005). In myofibers, glucose can be used for energy production, if muscle contraction is activated, or stored as glycogen. The mechanism that integrates muscle activity and glucose processing is regulated by changes in intracellular Ca2+. Along these lines, a chronic elevation of intracellular Ca2+ concentration was reported to correlate with lower expression of the glucose transporter GLUT4 and increased expression of phosphorylated glycogen phosphorylase and glycogen synthase (Park et al., 2009; Tammineni et al., 2020; Uryash et al., 2022). These changes boost glycogen breakdown and reduce glucose uptake, thus promoting insulin resistance and hyperglycemia. Interestingly, treatment with dantrolene improved glucose uptake and tolerance, suggesting that alteration in intracellular Ca2+ plays a central role in MH-associated hyperglycemia (Altamirano et al., 2019; Uryash et al., 2022).

Other RYR1-related myopathies

King–Denborough syndrome (KDS) is characterized by susceptibility to MH, delayed motor development, short stature, cryptorchidism, skeletal abnormalities, and variable dysmorphic features. Resting creatine kinase levels are elevated in some patients, and muscle biopsies show fiber size variation with atrophic type 1 muscle fibers and absence of cores. KDS can present with either a dominant or a recessive inheritance of mutations in RYRI and presents with a high variable penetrance (Isacs and Badenhorst, 1992; Dowling et al., 2011).

Mutations in RYRI have been rarely identified in some other myopathies. For example, three different RYRI mutations were characterized in calf-predominant distal myopathy, a mild dominant distal myopathy characterized by fatty degeneration of medial gastrocnemius, elevated creatine kinase levels, and the presence of cores in muscle biopsies (Savarese et al., 2020). In contrast to early-onset RYRI-related myopathies that affect the medial and anterior thigh compartment, few cases of late-onset axial myopathy have been associated with RYRI mutations. These are characterized by paravertebral and posterior thigh involvement (Jungbluth et al., 2009; Løseth et al., 2013). The real incidence of these myopathies is probably largely underestimated, since clinical manifestation typically occurs in old age and can be confused with normal aging dysfunction.
CACNA1S- and STAC3-related congenital myopathies

CACNA1S mutations are associated with MH and some forms of congenital myopathies (Monnier et al., 1997; Schartner et al., 2017; Mauri et al., 2021). CACNA1S mutations identified in MH affect residues located in the S4 voltage-sensing domain, while those identified in congenital myopathies are associated with a decrease in CACNA1S protein expression and impairment of E-C coupling (Maggi et al., 2021; Brugnoni et al., 2022). More severe cases were reported with fetal akinesia or cognitive delay (Yis et al., 2019; Ravenscroft et al., 2021). Recently, homozygous and compound heterozygous mutations in STAC3 were identified in a rare autosomal recessive congenital myopathy called Native American myopathy, also known as Bailey–Bloch congenital myopathy, a severe myopathy characterized by facial involvement, bone and joint deformities, MH susceptibility, and dermatomyopathy, a severe myopathy characterized by facial involvement, bone and joint deformities, MH susceptibility, and delayed motor milestones (Horstick et al., 2013; Telegrafi et al., 2017). Studies on the Ca2+ affinities of STIM1 and STIM2 have shown that the latter has a lower affinity for Ca2+ than STIM1, supporting a model in which STIM2 is more sensitive to changes in the ER luminal Ca2+ and thus may contribute to activate SOCE at front of minimal decreases in Ca2+ concentration in the intracellular stores (Brandman et al., 2007; Berna-Errro et al., 2009).

Several isoforms generated by different splicing of STIM1 and STIM2 are expressed in different cells. Three spliced isoforms of STIM1 have been described that show tissue-specific patterns of expression and different regulatory properties: STIM1L, expressed in skeletal and cardiac muscle and in the brain (Darbellay et al., 2011); STIMB, a neuron-specific isoform (Ramesh et al., 2021); and STIMA, which appears to negatively modulate SOCE (Knapp et al., 2022). Two splice variants of STIM2 have been identified: STIM 2.1, also called STIM2β, and STIM2.3; the original STIM2 protein is also called STIM2.2 or STIM2α. STIM 2.1 is a strong dominant-negative regulator of SOCE, likely because of a short insert of eight amino acids in the CAD/SAOR region that impairs the association of STIM2.1 with ORAI and the transient receptor potential (TRP) channels. Not much is yet known about the STIM2.3 splice variant, which differs from both STIM2.1 and STIM2.2 in the C-terminal region (Miederer et al., 2015; Rana et al., 2015).

The use of alternative translation initiation sites results in expression of two ORAI isoforms, ORAI1α and ORAI1β. ORAI1α contains 63 additional amino acids in the N-terminal region, not present in ORAI1β (Desai et al., 2015). As concerns the ORAI channels, while the function of ORAI1 has been intensively studied, much less is known about ORAI2 and ORAI3. ORAI2 and ORAI3 share a significant homology to ORAI1 and, when transfected in cells, form CRAC channels that can be activated by STIM proteins, even if they show some regulatory properties distinct from ORAI1 channels (Emrich et al., 2022). Interestingly, knockout of Orai2 and Orai3 in mice and cells has provided evidence that the assembly of heteromeric channels containing ORAI1 with either Orai2 or Orai3 results in the negative regulation of ORAI1 (Yoo et al., 2020).

Additional studies have also shown that SOCE, in addition to ORAI channels, may occur through the recruitment of additional channels such as the nonselective cation channels of the TRP canonical (TRPC) family (Lee et al., 2010). The contribution of TRPC will result in the activation of a store-operated current

SOCE

SOCE is a ubiquitous Ca2+ signaling pathway that, in all cell types, allows regulated entry of Ca2+ from the extracellular environment in response to a decrease in ER Ca2+ content (Putney, 1986). The existence of this mechanism was initially based on the identification of a Ca2+ current activated by depletion of intracellular Ca2+ stores called the Ca2+ release-activated Ca2+ current (ICRAC; Hoth and Penner, 1992). Ten yr of intense work led to the discovery of the two main proteins capable of sustaining SOCE: STIM1 (Liou et al., 2005; Roos et al., 2005) and ORAI1 (Feske et al., 2006). Current evidence supports a model in which STIM1 and ORAI1, given their ubiquitous pattern of expression, represent the main contributors to SOCE in eukaryotic cells (Lewis, 2020).

STIM1 is a single-pass transmembrane protein on the ER/SR membrane that functions as a sensor of intraluminal Ca2+ levels. This function is mediated by two noncanonical Ca2+-binding EF-hand motifs in the N-terminal intraluminal region of STIM1 that, at resting conditions when intracellular Ca2+ stores are full, maintain STIM1 in a Ca2+-bound dimeric conformation in the ER/SR. ORAI1 is a plasma membrane protein containing four transmembrane helices with both N- and C-terminal regions facing the cytosol (Feske et al., 2006; Vign et al., 2006; Zhang et al., 2006). Current evidence supports a model in which STIM1 and ORAI1, given their ubiquitous pattern of expression, represent the main contributors to SOCE in eukaryotic cells (Lewis, 2020).

STIM1 binds to phosphoinositide on the plasma membrane and, through a domain in the cytosolic region termed CRAC-activating domain (CAD) or STIM-ORAI1-activating region (SOAR), interacts with ORAI1. The site in ORAI1 that mediates the interaction with STIM may require multiple separate domains, one of which has been recently proposed to correspond to a peptide in the cytoplasmic C-terminal extension that follows the fourth transmembrane helix in each of the six ORAI1 monomers (Baraniak et al., 2021).

After the initial identification of STIM1 and ORAI1, additional studies identified a second member of the STIM family (STIM2) and two additional ORAI genes, ORAI2 and ORAI3 (Feske et al., 2006). STIM2 shares significant structural homology with STIM1, and the two proteins can form heterodimers (Berna-Errro et al., 2017). Studies on the Ca2+ affinities of STIM1 and STIM2 have shown that the latter has a lower affinity for Ca2+ than STIM1, supporting a model in which STIM2 is more sensitive to changes in the ER luminal Ca2+ and thus may contribute to activate SOCE at front of minimal decreases in Ca2+ concentration in the intracellular stores (Brandman et al., 2007; Berna-Errro et al., 2009).

Several isoforms generated by different splicing of STIM1 and STIM2 are expressed in different cells. Three spliced isoforms of STIM1 have been described that show tissue-specific patterns of expression and different regulatory properties: STIM1L, expressed in skeletal and cardiac muscle and in the brain (Darbellay et al., 2011); STIMB, a neuron-specific isoform (Ramesh et al., 2021); and STIMA, which appears to negatively modulate SOCE (Knapp et al., 2022). Two splice variants of STIM2 have been identified: STIM 2.1, also called STIM2β, and STIM2.3; the original STIM2 protein is also called STIM2.2 or STIM2α. STIM 2.1 is a strong dominant-negative regulator of SOCE, likely because of a short insert of eight amino acids in the CAD/SAOR region that impairs the association of STIM2.1 with ORAI and the transient receptor potential (TRP) channels. Not much is yet known about the STIM2.3 splice variant, which differs from both STIM2.1 and STIM2.2 in the C-terminal region (Miederer et al., 2015; Rana et al., 2015).

The use of alternative translation initiation sites results in expression of two ORAI isoforms, ORAI1α and ORAI1β. ORAI1α contains 63 additional amino acids in the N-terminal region, not present in ORAI1β (Desai et al., 2015). As concerns the ORAI channels, while the function of ORAI1 has been intensively studied, much less is known about ORAI2 and ORAI3. ORAI2 and ORAI3 share a significant homology to ORAI1 and, when transfected in cells, form CRAC channels that can be activated by STIM proteins, even if they show some regulatory properties distinct from ORAI1 channels (Emrich et al., 2022). Interestingly, knockout of Orai2 and Orai3 in mice and cells has provided evidence that the assembly of heteromeric channels containing ORAI1 with either Orai2 or Orai3 results in the negative regulation of ORAI1 (Yoo et al., 2020).

Additional studies have also shown that SOCE, in addition to ORAI channels, may occur through the recruitment of additional channels such as the nonselective cation channels of the TRP canonical (TRPC) family (Lee et al., 2010). The contribution of TRPC will result in the activation of a store-operated current
The existence of two STIM and three ORAI genes and alternative spliced isoforms, some of which have dominant-negative effects on the I_{CRAC} current, is strongly suggestive of the importance of fine-tuning SOCE-mediated Ca^{2+} signaling to regulate the variety of different functions that operate simultaneously in eukaryotic cells. That SOCE plays signaling functions in addition to refilling the empty Ca^{2+} stores is now supported by several studies that link modification of SOCE function with activation of specific transcription factors and signaling pathways, resulting in the regulation several specific cell functions, including regulation of metabolism. Indeed, SOCE has been shown to contribute to activation of T cell proliferation by triggering calcineurin-mediated activation of the NFAT transcription factor and of stimulation of the PI3K-AKT-mTOR pathways, resulting in upregulation of glucose transporters and glycolytic and mitochondrial enzymes that provide the metabolic support necessary for T cell expansion and activation of adaptive immune functions (Vaeth et al., 2017). Studies with Orai1- or Stim1/Stim2-deficient mice revealed a role of SOCE in regulating fatty acid metabolism in liver, skeletal, and cardiac muscle (Maus et al., 2017). A role of SOCE in regulating mitochondria and glycolysis has also been observed in studies using inducible smooth muscle-specific STIM1 knockout mice (Johnson et al., 2022).

**SOCE regulation, mitochondria, and metabolism**

While the mechanisms responsible for SOCE activation are quite well defined, less is known about the mechanisms that terminate CRAC channel activity. Two mechanisms responsible for CDI of CRAC channel activity have been described (Zweifach and Lewis, 1995a; Zweifach and Lewis, 1995b). Fast CDI occurs within tens of milliseconds, and it has been proposed that direct Ca^{2+} binding to Orai1 inactivates the channel (Zweifach and Lewis, 1995a); Ca^{2+} and Ca^{2+}-calmodulin (CaM) binding to STIM1 (Litjens et al., 2004; Mullins et al., 2009) and Orai1 have been described to also have a role in fast CDI (Litjens et al., 2004; Srikanth et al., 2010). Interestingly, Orai1β has a lower fast inactivation rate than Orai1α, suggesting also that the 63-aa insert present in Orai1α might be involved in fast inactivation (Parekh, 2017). On the other hand, it has been suggested that a slow, Ca^{2+}-dependent conformational change in the STIM1/Orai1 complex due to store refilling or reversible biochemical changes may be responsible for slow CDI (Parekh, 2017).

Mitochondria, although not directly involved in SOCE activation, can attenuate the slow phase of CDI by lowering cytosolic Ca^{2+} levels in proximity to activated Orai channels by transporting Ca^{2+} to the mitochondrial matrix through the mitochondrial calcium uniporter (MCU; Parekh 2008; Mammucari et al., 2018). Indeed, if mitochondria are depolarized, Ca^{2+} entry into the mitochondrial matrix is reduced and cytosolic Ca^{2+} remains high, resulting in CRAC channel inactivation. Because fast CDI is not affected by mitochondrial depolarization, it has been suggested that mitochondria are involved in regulation of slow CDI (Glitsch et al., 2002). Increases in mitochondrial Ca^{2+} concentration regulate ATP production by enhancing the synthesis of NADH and FADH2 (Rossi et al., 2019b), thus linking ATP production to muscle demand. Moreover, the dynamics of Ca^{2+} transport in and out of mitochondria and the effects of Ca^{2+} on the enzymes that govern ATP synthesis activate additional mechanisms by which mitochondria can modulate SOCE (Muallem, 2007; Ben-Kasus Nissim et al., 2017; Walters and Usachev, 2022).

More recently, however, the role of mitochondria in regulating SOCE by preventing CDI has been challenged by studies based on knockout and knockdown of the MCU. These studies revealed that, against expectations, deletion of the MCU stimulated an increase in SOCE-mediated Ca^{2+} entry, even if CDI of these channels was promoted by MCU deletion (Yoast et al., 2021). Using mathematical simulations, those authors showed that mitochondrial Ca^{2+} transport can act on different pathways to finely regulate Ca^{2+} homeostasis. Altogether, it appears that the role of mitochondria in regulating SOCE must occur through additional mechanisms other than simply removing Ca^{2+} from the cytosol and prolonging SOCE current by delaying inactivation (Yoast et al., 2021).

An additional regulator of SOCE is SARAF, an ER-resident protein that can exert activating and inactivating effects on CRAC channels by interacting with the SOAR domain or the inhibitory domain in STIM1 (Palty et al., 2012; Dagan and Paltry, 2021; Zomot et al., 2021). Several other proteins interact and regulate SOCE, including STIMATE (STIM-activating enhancer; Lopez et al., 2016) and CASQ1, the main Ca^{2+} buffer of the SR (Shin et al., 2003; Zhang et al., 2016).

**SOCE in skeletal muscle**

In recent years, it has become evident that also skeletal muscle fibers depend on SOCE mechanism to replenish SR Ca^{2+} reserves (Kurebayashi and Ogawa, 2001; Launikis and Rios, 2007). In mammalian skeletal muscle, STIM1 and STIM2 are expressed together with some of their alternative spliced isoforms. STIMIL is a longer splice variant of STIM1 that, in the C-terminal region, contains an additional 106 amino acids encoding an actin-binding domain that allows STIM1 to interact with the subcortical actin filaments (Darbellay et al., 2011; Lilliu et al., 2021). This interaction supports the permanent assembly of STIMIL clusters in proximity to Orai1 channels on the plasma membrane. Based on this stable position, STIMIL was proposed to be mainly responsible for the faster activation kinetics of SOCE observed in skeletal muscle (Darbellay et al., 2011). Nevertheless, it is not clear if this occurs through Orai1 activation, since STIMIL appears to be less efficient than STIM1 at activating Orai1, while it shows better interaction and functional activation of TRPC1 and TRPC4 (Dyrd et al., 2020). Indeed, the expression of STIMIL together with TRPC1 and TRPC4 has been reported to be required for fusion and differentiation of myoblasts, and biophysical studies demonstrated that all three proteins are required for optimal SOCE kinetics in myotubes (Antigny et al., 2017; Dyrd et al., 2020). STIM2.1, an alternatively spliced isoform of STIM2, is also expressed in skeletal muscle. The STIM2.1 isoform can assemble with other STIM isoforms, resulting in heterodimers that negatively regulate SOCE. The resulting modulation of Ca^{2+} homeostasis appears to
stimulate myogenic differentiation by increasing the Ca^{2+}-regulated expression of NFAT4 and MEF2C transcription factors (Kim et al., 2019). Accordingly, muscle-specific Stim1 knockout mice show a reduction in Ca^{2+}-dependent signal transduction pathways involved in muscle growth, thus resulting in growth delay and postnatal lethality (Li et al., 2012).

In adult skeletal muscle, SOCE was found to be activated at triads by single action potentials immediately after Ca^{2+} was released by RYR1 following activation by DHPRs. Detailed studies demonstrated that, despite global SR Ca^{2+} content remaining constant during E-C coupling, Ca^{2+} concentrations at the j-SR drop significantly; it has been proposed that local, but consistent, reduction in SR Ca^{2+} content rapidly activates SOCE well before global SR Ca^{2+} depletion (Launikis et al., 2009; Koenig et al., 2018; Pearce et al., 2022). Studies on SOCE in skeletal muscle have been also extended to its role in maintaining the SR Ca^{2+} stores following repetitive stimulation of muscle contraction, and especially after intense prolonged activity (Boncompagni et al., 2017; Michelucci et al., 2018; Michelucci et al., 2019; Michelucci et al., 2020; Lilliu et al., 2020). Interestingly, intense exercise was shown to induce the assembly of new intracellular junctions between T tubules and l-SR, where STIM1 and ORAI1 are colocalized. These newly identified structures, named Ca^{2+} entry units (CEUs), are formed by elongations of T tubules that run parallel to the l-SR. CEUs support increased Ca^{2+} entry via ORAI1 and contribute to improve fatigue resistance under continued muscle activity (Michelucci et al., 2019; Michelucci et al., 2020). In contrast to studies in which activation of SOCE was observed only at triads (Cully et al., 2017), CEUs appear to connect ORAI1 on the T tubules with STIM1 on the l-SR (Boncompagni et al., 2017; Michelucci et al., 2020), suggesting that Ca^{2+} entry may occur at different sites in skeletal muscle fibers. To avoid cytosolic Ca^{2+} overload and help preserving energy for muscle contraction and reducing fatigue, STIM1 can be phosphorylated by the SAMP-activated kinase (AMPK) during exercise, resulting in a decrease in SOCE, which may avoid cytosolic Ca^{2+} overload, help preserve energy for muscle contraction, and reduce fatigue (Nelson et al., 2019).

Evidence of an additional role of STIM1, but not of ORAI1, in skeletal muscle has recently been observed in response to the high energy demand that occurs during exercise. Experiments with inducible knockout of STIM1 showed that the reduction in muscle mass and exercise capacity following STIM1 ablation was due not only to a direct impact on Ca^{2+} availability for muscle contraction, but also to alterations in muscle metabolism and increase in ER stress/UPR (Wilson et al., 2022). Because these effects were not observed in Orai1-deficient mice, they may result from a direct interplay between STIM1 and mitochondria, independent of SOCE. Indeed, STIM1 ablation resulted in an increase in lactate production, mainly due to an increase in glucose use associated with a reduction in the functional activity of Ca^{2+}-regulated mitochondrial enzymes such as pyruvate dehydrogenase. In addition, biochemical analysis showed that deletion of STIM1 resulted in the selective dampening of pathways linked to growth, while presumably protein synthesis showed an increase. These apparent opposite findings can be explained by a model in which skeletal muscle of Stim1-knockout mice adapts to moderate ER stress levels by slowing growth and augmenting protein quality control mechanisms, resulting in more protein synthesis and turnover with a lower muscle mass (Wilson et al., 2022).

**Altered SOCE mechanism in skeletal muscle fibers and congenital myopathies**

Mutations in ORAI1 and STIM1 genes can severely affect the SOCE mechanism in different cell types and, consequently, cause a range of human genetic diseases. Patients carrying recessive loss-of-function mutations in STIM1 or ORAI1 develop a life-threatening immune deficiency, leading to recurrent severe infections accompanied by nonprogressive muscular hypotonia, anhidrotic ectodermal dysplasia, defective dental enamel formation, and mydriasis (McCarl et al., 2009; Fuchs et al., 2012; Wang et al., 2014; Lacruz and Feske, 2015; Silva-Rojas et al., 2020; Conte et al., 2021a).

Recessive loss-of-function mutations in STIM1 and ORAI1 genes can result from either frameshift mutations or single missense point mutations that lead to either loss of STIM1 and ORAI1 protein or poorly functional proteins (Fig. 2). As a result, at the functional level, loss-of-function mutations induce a strong reduction or completely abrogate CRAC channel activity, hence the name CRAC channelopathy. Although skeletal muscle is also affected by CRAC channelopathies, the clinically most relevant affected cells are cells of the immune system and especially T lymphocytes (Hoeh et al., 2000; Weidinger et al., 2013; Lacruz and Feske, 2015; Silva-Rojas et al., 2020).

Mice knocked out for Stim1 and Orai1 die around birth (Gwack et al., 2008; Oh-Hora et al., 2008); in contrast, mice with selective skeletal muscle-specific knockout of Stim1 and Orai1 are viable and fertile. Skeletal muscle-specific Stim1 and Orai1 knockout mice, however, show reduced muscle mass, reduced muscle contractility and force production, and increased susceptibility to fatigue, which can be explained by alterations in both cytosolic and SR Ca^{2+} levels. At the histological and ultrastructural level, muscles revealed a significant reduction in fiber size with appearance of swollen mitochondria (Stiber et al., 2008; Li et al., 2012; Carrell et al., 2016). Muscle-specific conditional Orai1 knockout mice show a more significant reduction in type I fibers in soleus muscles that may be explained by alterations in transition from fast to slow myosin expression during perinatal muscle remodeling (Carrell et al., 2016).

Similar alterations in terms of muscle mass, SR Ca^{2+} levels, and resistance to fatigue were also observed in mice carrying the dominant-negative mutation E108Q in ORAI1 (E108Q dnOrai1 mice; Wei-Lapierre et al., 2013). Nevertheless, these mice, unlike Stim1 and Orai1 knockout mice, are viable and have a normal lifespan; despite a reduction in muscle mass and complete loss of SOCE, they do not show significant myopathic alterations at the histological level. According to those authors, the differences between STIM1 knockout mice and E108Q dnOrai1 mice may be explained by the fact that STIM1 also regulates other intracellular targets such as TRPC, adaptor proteins, ER chaperones, and second messenger enzymes (Wei-Lapierre et al., 2013).
Gain-of-function mutations in STIM1 and ORAI1: tubular aggregate myopathy (TAM)/Stormorken syndrome

Gain-of-function mutations that result in constitutive and/or overactivation of SOCE are mainly associated with TAM and Stormorken syndrome (Chevessier et al., 2004; Chevessier et al., 2005; Morin et al., 2020). TAM is a rare genetic disease that selectively affects skeletal muscle. At the clinical level, TAM patients present a range of symptoms that may include muscle weakness, myalgia, cramps, and increased creatine kinase levels and exercise intolerance and can start in infancy and worsen over time, although diagnosis at adult age is also reported. Proximal muscles of lower limbs are predominantly affected, but other skeletal muscles can also be affected (Böhm et al., 2013, 2017; Nesin et al., 2014; Endo et al., 2015; Böhm and Laporte, 2018; Silva-Rojas et al., 2020). Patients with gain-of-function mutations in STIM1 and ORAI1 may also present with a rare, severe multisystem disorder, Stormorken syndrome, that shares with TAM the skeletal muscle involvement but, in line with the wide cell and tissue expression patterns of ORAI1 and STIM1, is characterized by a variety of additional symptoms that affect other tissues. As a whole, TAM and Stormorken syndrome are actually considered a clinical continuum of manifestations characterized by muscle weakness, myalgia, and cramps, mostly at the level of the lower limbs, that in some patients may be accompanied by a variable range of additional symptoms such as miosis, ichthyosis, thrombocytopenia, short stature, and dyslexia (Stormorken et al., 1985; Misceo et al., 2014; Böhm and Laporte, 2018; Silva-Rojas et al., 2020).

At the histological level, muscle biopsies from TAM/Stormorken patients present a prevalence of type I fibers and a characteristic pattern, predominantly found in type 2 fibers, consisting of an accumulation of highly ordered and packed membrane tubules that appear bright red with the modified Gomori trichrome technique and stain positive with periodic acid–Schiff (PAS) and NADH-tetrazolium reductase, but are negative for succinate dehydrogenase or cytochrome c oxidase staining (Chevessier et al., 2005). When visualized by EM, they appear as single- or double-walled tubules arranged as honeycomb-like structures (Salviati et al., 1985; Schiaffino, 2012; Böhm et al., 2013; Chevessier et al., 2005). These tubular aggregates are positive in immunofluorescence for several SR proteins such as CASQ1, SERCA, triadin, RYR1, and STIM1 (Chevessier et al., 2005; Morin et al., 2020; Silva-Rojas et al., 2020). What leads to the development of tubular aggregates is not clear, although they are likely to represent the final stage of a protective mechanism aimed to prevent muscle hypercontraction and damage caused by altered Ca²⁺ homeostasis that, by inducing protein misfolding and aggregation, causes...
morphological changes in the SR that ends in formation of tubular aggregates (Chevessier et al., 2004; Schiaffino, 2012; Chevessier et al., 2005; Morin et al., 2020).

Several gain-of-function mutations in STIM1 have been identified, the majority of which affect different amino acids in the canonical and noncanonical EF-hand motifs (Böhm et al., 2013; Böhm and Laporte, 2018; Fahrner et al., 2018; Morin et al., 2020; Silva-Rojas et al., 2020) or the R304 residue in the coiled-coil domain I (C1) of the protein (Miscoe et al., 2014; Nesin et al., 2014; Harris et al., 2017; Peche et al., 2020). In the Ca\(^{2+}\)-bound state of STIM1, the canonical and noncanonical EF-hand motifs and the sterile α-motif domain are tightly packed together (Enamoto et al., 2020). Mutations in the EF-end motifs essentially alter or disrupt Ca\(^{2+}\) binding, resulting in constitutive active SOCE due to domain unfolding (Böhm et al., 2013, Böhm et al., 2017; Sallinger et al., 2020). As observed in muscle cells from mouse models or in myoblasts from patients carrying these mutations, the mutations induce constitutive STIM1 clustering, independently of SR Ca\(^{2+}\) depletion, that results in constitutive activation of SOCE, leading to increased Ca\(^{2+}\) levels in both the cytosol and the SR (Cordero-Sánchez et al., 2019; Silva-Rojas et al., 2019; Conte et al., 2021b).

Mutations in STIM1 have been extensively studied (Nesin et al, 2014; Fahrner et al, 2018; Rathner et al, 2021; Silva-Rojas et al, 2021). This mutation affects one conserved residue in CCI, unlocking the inhibitory state of STIM1 and constitutive SOCE activation (Morin et al., 2020). In addition, the R304W mutation suppresses fast CDI of ORAI, further enhancing chronic Ca\(^{2+}\) influx from the extracellular space (Nesin et al., 2014).

Gain-of-function mutations in ORAI1 were identified in the transmembrane domains. Mutations in M1, M2, and M3 transmembrane domains appear to induce constitutive channel activity, independently of STIM1 activation (Nesin et al., 2014; Endo et al., 2015; Böhm et al., 2017; Garibaldi et al., 2017; Bulla et al., 2019; Peche et al., 2020), while the ORAI1 P245L mutation in TM4, identified in a patient with a Stormorken-like syndrome, appears to lengthen the duration of the Ca\(^{2+}\) entry current, following activation by store depletion, by removing the slow CDI mechanism, while the fast CDI is preserved (Nesin et al., 2014).

Mouse models for gain-of-function mutations in STIM1 have been generated and found to present with a variable combination of the clinical signs of TAM/Stormorken syndrome in humans. Stimi\(^{DSAG}\) mice mainly present with alteration in platelet activation and bleeding (Grosse et al., 2007). Stimi\(^{H155F}\) mice have histological and functional alterations in skeletal muscle tissue and display an increased susceptibility to fatigue. They also present with hematological defects with thrombocytopenia and altered differentiation in the myeloid lineage and natural killer cells (Cordero-Sanchez et al., 2019). Stim1\(^{R304W}\) mice showed defective skeletal muscle function, thrombocytopenia, spleen alteration, anomalies of the eye and skin, altered bone structure, and dysregulation of immune system (Silva-Rojas et al., 2019). Characterization of skeletal muscle of Stim1\(^{R304W}\) mice revealed evidence of apoptosis, muscle degeneration, and overexpression of ER stress/UPR accompanied by downregulation of RYRI, DHPR, and SERCA1, which could represent a protective mechanism to limit SR Ca\(^{2+}\) overload (Silva-Rojas et al., 2021). However, none of these mouse models develops defined tubular aggregates, suggesting that additional factors might be required in mice for development of these structures (Gamage et al., 2018; Silva-Rojas et al., 2019).

**Mutations in CASQ1 and RYR1 in patients with mild forms of TAM**

Mutations in STIM1 and ORAI are detected in only a fraction of patients diagnosed TAM/Stormorken syndrome (Morin et al., 2020; Silva-Rojas et al., 2020; Conte et al., 2021a). Studies aimed at identifying additional genes causative of TAM/Stormorken syndrome resulted in the identification of three different causative mutations in the CASQ1 gene in 11 patients with a TAM diagnosis from 6 unrelated families (Barone et al., 2017; Böhm et al., 2018). CASQ1 is the major Ca\(^{2+}\)-buffering protein in the SR that, thanks to its ability to bind Ca\(^{2+}\) with low affinity and high capacity, plays a key role in providing the high Ca\(^{2+}\) storage capacity of the SR necessary for activation of muscle contraction (Rossi et al., 2021). The ability of CASQ1 to bind Ca\(^{2+}\) is closely linked to its ability to polymerize and form long ribbon-like structures that allow Ca\(^{2+}\) storage within the SR (Park et al., 2003; Sanchez et al., 2012). CASQ1, alone or in combination with junctin and triadin, can regulate RYR1 opening and thus contribute to regulation of Ca\(^{2+}\) release (Beard et al., 2002; Gaburjakova et al., 2013; Manno et al., 2017; Rossi et al., 2021).

Accordingly, altered polymerization due to mutations in CASQ1 may affect channel gating, resulting in leaky RYR1 channels. Initial evidence of a possible regulatory role of CASQ1 on the SOCE mechanism was provided by experiments based on knockdown of CASQ1 in muscle fibers that resulted in increased Ca\(^{2+}\) entry across the sarcolemma (Zhao et al., 2010) and by experiments indicating that CASQ1 can bind both STIM1 and STIM2 and inhibit SOCE (Shin et al., 2003; Wang et al., 2015; Zhang et al., 2016; Jeong et al., 2021). More recently, Casq1 knockout mice were shown to have an increased expression of Stimi and Orai1 associated with enhanced SOCE and preformed CEUs, possibly reflecting a compensatory mechanism to maintain the SR Ca\(^{2+}\) levels sufficient to ensure muscle contraction in the absence of CASQ1 (Michelucci et al., 2020).

At the clinical level, CASQ1 mutations were detected in patients that reported progressive muscle weakness and exercise-induced myalgia with fatigability predominantly involving the proximal limb muscles. Only one patient reported muscle stiffness. Serum creatine kinase levels were normal in most patients. As expected, given its selective skeletal muscle expression, TAM patients with CASQ1 mutations presented signs of only a mild
myopathy and, except for one patient who reported ichthyosis, no other symptoms of Stormorken syndrome (Barone et al., 2017; Böhmi et al., 2018). All three CASQ1 mutations identified in these TAM patients altered Ca\(^{2+}\)-dependent polymerization and reduced Ca\(^{2+}\) storage content when transfected in cells. Two of the mutants were shown to have lost the ability to inhibit SOCE, although one mutant was still able to inhibit Ca\(^{2+}\) influx (Barone et al., 2017).

A recent study reported the identification of two different missense RYR1 mutations in two unrelated patients with a mild form of myopathy, where the presence of tubular aggregates in muscle fibers was the only alteration observed on histological examination, thus identifying RYR1 as the fourth causative gene in TAM (Vattemi et al., 2022). The two patients came to medical examination reporting symptoms since childhood and early adulthood, respectively. Only one patient reported significant muscle weakness and presented with myopathic changes in four limbs by needle electromyography. Both patients had a history of persistent increase of serum creatine kinase levels (two- to fourfold the normal values). Interestingly, the two patients complained of suffering muscle stiffness after repetitive movements, a symptom not previously associated with TAM, but they did not report symptoms related to Stormorken syndrome. Therefore, considering the quite mild phenotype reported, patients with TAM associated with RYR1 mutations may represent the less severe side of the TAM/Stormorken syndrome. At the functional level, both RYR1 mutations detected in these patients were shown to alter the properties of the RYR1 channels and have been reported as pathogenic and causative for MH susceptibility based on EMHG guidelines (https://www.emhg.org).

One of the two mutations has been also found in patients with CCD. The identification of mutations in the RYR1 gene in TAM patients is of interest, since RYR1 mutations may represent the genetic cause of myopathy in at least a fraction of the patients affected by TAM that do not carry mutations in STIM1, ORAI1, or CASQ1 and therefore represent a new diagnostic target for these patients. Moreover, since the phenotype of both patients is rather mild, it is possible that RYR1-related TAM may be currently underdiagnosed in the population.

Concluding remarks

The development of fast and cost-effective DNA sequencing technologies has tremendously increased our knowledge of the genetic basis of skeletal muscle pathologies. This has provided further evidence that mutations in one gene, as in RYR1-related disorders, result in several different myopathies characterized by a spectrum of clinical and histopathological phenotypes. At the same time, as in a mirror image, we have seen an increase in the number of genes that can be associated with the same disease (Jungbluth et al., 2018; Lawal et al., 2018; Garibaldi et al., 2019; Lawal et al., 2020). The recent identification of tubular aggregates, a hallmark of TAM/Stormorken syndrome, in patients carrying RYR1 mutations represents only the latest evidence of how complex it is to associate genetic data and histopathological patterns (Vattemi et al., 2022).

Although it is easy to understand that mutations in RYR1 or CACNA1S can be found in patients with MH or CCD and that mutations in STIM1 or ORAI1 are associated with TAM/Stormorken syndrome, less obvious are the cases where mutations in genes not involved in Ca\(^{2+}\)-handling pathways, such as MYH7, TTN, or MEGF10, are detected in patients presenting with clinical symptoms and histopathological alterations like those present in RYR1-related myopathies. This apparent incongruency can be rationalized by evidence that, at least in some cases, mutations in genes apparently not directly connected with Ca\(^{2+}\) signaling may indirectly affect regulation of Ca\(^{2+}\) homeostasis, as proposed for SEPN1 (Chernorudskiy et al., 2020) or BNI, MTMI, or DNM2 (Gómez-Oca et al., 2021). However, more work is needed in this direction to explain how genes not known to affect Ca\(^{2+}\) signaling mat induce a myopathy. Future advancements in studying functions, regulatory properties, and even more the network of interactions participated by proteins encoded by causative genes of interest will certainly help answer these questions. In this perspective, a significant contribution can be also provided by the identification of genetic or drug modifiers (Bazrafshan et al., 2021; Volpatti et al., 2020). It can be expected that future investigation will address the identification of the pathogenic pathways activated by different genes, and how these may translate in the development of the different histological findings that characterize these myopathies. A better understanding of these pathogenic mechanisms will improve our knowledge, contribute to the classification of these myopathies, and likely identify novel targets for pharmacological and/or genetic intervention to cure these diseases.

Acknowledgments

Eduardo Ríos served as editor.

This study was supported by a grant from Fondazione Telethon (grant number GGP19291) to V. Sorrentino.

The authors declare no competing financial interests.

Author’s contribution: D. Rossi and V. Sorrentino designed the organization of the manuscript; D. Rossi, M.R. Catallo, E. Pierantozzi, and V. Sorrentin wrote the test; E. Pierantozzi prepared the figures and table; D. Rossi and V. Sorrentin revised the manuscript.

Submitted: 31 January 2022

Accepted: 21 July 2022

References

Abath Neto, O., C.A.M. Moreno, E. Malufatti, S. Donkervoort, J. Böhmi, J.B. Guimarães, A.R. Foley, P. Mohassel, J. Dastgir, D.X. Barron-Goebl, et al. 2017. Common and variable clinical, histological, and imaging findings of recessive RYR1-related centronuclear myopathy patients. Neuromuscul. Disord. 27:975–985. https://doi.org/10.1016/j.nmd.2017.05.016

Agrawal, P.B., R.S. Greenleaf, K.K. Tomczak, V.-L. Lehtokari, C. Wallgren-\textit{Pettersson}, W. Wallefeld, N.G. Laing, B.T. Darras, S.K. Maciver, P.R. Dormitzer, and A.H. Beggs. 2007. Nemaline myopathy with minicores caused by mutation of the CFL2 gene encoding the skeletal muscle actin-binding protein, cofilin-2. Am. J. Hum. Genet. 80:162–167. https://doi.org/10.1086/510402

Agrawal, P.B., C.R. Pierson, M. Joshi, X. Liu, G. Ravenscroft, B. Mogha-\textit{dazadeh}, T. Talabere, M. Viola, L.C. Swanson, G. Halligulu, et al. 2014.
SPEG interacts with myotubulin, and its deficiency causes centronuclear myopathy with dilated cardiac myopathy. Am. J. Hum. Genet. 95:523–526. https://doi.org/10.1016/j.ajhg.2014.07.004

Allen, G.C., M.G. Larach, and A.R. Kunselman. 1998. The sensitivity and specificity of the caffeine-halothane contracture test: A report from the north American malignant hyperthermia registry the north American malignant hyperthermia registry of MAHAS. Anesthesiology. 88: 579–588. https://doi.org/10.1097/00000542-199803000-00006

Almazrui, M., O. Dabbagh, H. AlQuadiy, A. AlHattaibi, R. Sami, R. Alotaibi, M.M. Ali, H. Alhindti, D. Colak, and N. Kaya. 2021. Phenotypic variability of MEGF10 variants causing congenital myopathy: Report of two unrelated patients from a highly consanguineous population. Genes 12:783. https://doi.org/10.3390/genes12060783

Altamirano, F., S. Riazi, I.B. Ibarro Moreno, A.F. Dulhunty, and D.R. Laver. 2002. Calsequestrin and the sarcomeric scaffolding as a common baseline histopathologic lesion in sarcoglycan-related myopathies. J. Neuropathol. Exp. Neurol. 61:277–288. https://doi.org/10.1097/00005072-200206000-00004

Avila, G., J.J. O’Brien, and R.T. Dirksen. 2001. Excitation–contraction uncoupling by a human central core disease mutation in the ryanodine receptor. Proc. Natl. Acad. Sci. USA. 98:4215–4220. https://doi.org/10.1073/pnas.071048198

Avila, G., K.M.S. O’Connell, and R.T. Dirksen. 2003. The pore region of the skeletal muscle ryanodine receptor is a primary locus for excitation–contraction uncoupling in central core disease. J. Gen. Physiol. 121: 277–286. https://doi.org/10.1085/jgp.200308791

Ávila-Polo, R., E. Malfatti, X. Lornage, C. Cheraud, I. Nelson, J. Nectoux, J. Böhm, R. Schneider, C. Hedberg-Oldfors, B. Eymard, et al. 2018. Loss of sarcomeric scaffolding as a common baseline histopathologic lesion in titin-related myopathies. J. Neuropathol. Exp. Neurol. 77:1101–1114. https://doi.org/10.1093/jn/nej095

Bannister, R.A., E. Estèvre, J.M. Ettin, I.N. Pessah, D.L. Anderson, and K.G. Avila, G., J.M. Stenson, A.L. Whitesell, Y. Wang, M. Trebek, and D.L. Gilli. 2021. Orai channel C-terminus peptide are key modulators of STIM1-Orai coupling and calcium signal generation. Cell Rep. 35:10932. https://doi.org/10.1016/j.celrep.2021.109322

Barone, V., V. Del Re, A. Gamberucci, V. Polverino, L. Galli, D. Rossi, E. Cazzato, L. Tomassi, G. Berti, A. Malandreti, et al. 2017. Identification and characterization of three novel mutations in the CASQ1 gene in four patients with tubular aggregate myopathy. Hum. Mutat. 38:1761–1773. https://doi.org/10.1002/humu.23338

Bazrafshan, S., H. Kushfal, M. Kakroo, J. Quinlan, R.C. Becker, and S. Dayyappan. 2021. Genetic modifiers of hereditary neuromuscular disorders and cardiomypathy. Cells 10:349. https://doi.org/10.3390/cells10030349

Beard, N.A., A.M. Sullivan, A.F. Dulhunty, and D.R. Laver. 2002. Calsequestrin is an inhibitor of skeletal muscle ryanodine receptor calcium release channels. Biophys. J. 82:310–320. https://doi.org/10.1016/S0006-3495(02)75396-4

Beard, N.A., D.R. Laver, and A.F. Dulhunty. 2004. Calsequestrin and the calcium release channel of skeletal and cardiac muscle. Prog. Biophys. Mol. Biol. 85:33–69. https://doi.org/10.1016/j.pbiomolbio.2003.07.001

Beard, N.A., and A.F. Dulhunty. 2015. C-terminal residues of skeletal muscle calsequestrin are essential for calcium binding and for skeletal ryanodine receptor inhibition. Skelet. Muscle. 5:6. https://doi.org/10.1186/s13395-015-0029-7

Ben-Kasus Nissim, T., X. Zhang, A. Elazar, S. Roy, J.A. Stulwijk, Y. Zhou, R.K. Mottiani, M. Guzman, N. Hempel, M. Herschkunl, et al. 2017. Mitochondria control store-operated Ca2+ entry through Na+ and redox signals. EMBO J. 36:975–815. https://doi.org/10.15252/embj.201592481

Berna-Enro, A., A. Braun, R. Kraft, C. Kleinschnitz, M.K. Schuhmann, D. Stegner, T. Wultsch, J. Eilers, S.G. Meuth, G. Stoll, and B. Nieswandt. 2009. STIM1 regulates capacitive Ca2+ entry in neurons and plays a key role in hyperexcitable neuronal cell death. Sci. Signal. 2:a67. https://doi.org/10.1126/scisignal.20050522

Berna-Enro, A., I. Jardin, G.M. Salido, and J.A. Rosado. 2017. Role of STIM2 in cell function and physiopathology. J. Physiol. 595:3111–3128. https://doi.org/10.1113/JP273889

Berridge, M.J., P. Lipp, and M.D. Bootman. 2000. The versatility and universality of calcium signalling. Nat. Rev. Mol. Cell Biol. 1:1–21. https://doi.org/10.1038/35063035
expressing recombinant ryanodine receptors harboring malignant hyperthermia and central core disease mutations. J. Biol. Chem. 280: 15380–15389. https://doi.org/10.1074/jbc.M410421200

Brugnoni, R., E. Canioni, M. Filosto, A. Pini, P. Tonin, T. Rossi, C. Canavese, M. Eoli, G. Siciliano, G. Laura, et al. 2022. Mutations associated with hypokalemic periodic paralysis: From hotspot regions to complete analysis of CACNA1S and SCN4A genes. Neurogenetics. 23:19–25. https://doi.org/10.1007/s10476-021-00673-2

Bull, M., G. Gyimesi, J.H. Kim, R. Bhardwaj, M.A. Hediger, M. Frieden, and C. Chan, W.M., W. Welch, and R. Sitsapesan. 2000. Structural factors that determine the ability of adenosine and related compounds to activate the cardiac ryanodine receptor. Br. J. Pharmacol. 130:1618–1626. https://doi.org/10.1038/sj.bjp.0703459

Brugnoni, R., E. Canioni, M. Filosto, A. Pini, P. Tonin, T. Rossi, C. Canavese, M. Eoli, G. Siciliano, G. Laura, et al. 2022. Mutations associated with hypokalemic periodic paralysis: From hotspot regions to complete analysis of CACNA1S and SCN4A genes. Neurogenetics. 23:19–25. https://doi.org/10.1007/s10476-021-00673-2

Buonocore, J.F., and S.M. Muldoon. 2009. The relationship between exercise heat, exertional rhabdomyolysis, and malignant hyperthermia. Anesth. Analg. 109:1065–1069. https://doi.org/10.1213/ane.0b013e31819d8949

Cai, S.Y., Y.-Q. Gao, Y.-Y. Zheng, W. Wang, P. Wang, J. Liang, W. Zhao, T. Tao, J. Sun, L. Wei, et al. 2020. The intragenic microRNA miR199A1 in the dynamin 2 gene contributes to the pathology of X-linked centronuclear myopathy. J. Biol. Chem. 295:15226–15237. https://doi.org/10.1074/jbc.RA120000337

Chakraborty, D., I. N. Pessah, P. Zhang, M.B. Bhattacharjee, R.T. Dirksen, and S.L. Allen. 2020. Bioenergetic defects in muscle fibers of RYR1 mutant knock-in mice associated with malignant hyperthermia. Anesth. Analg. 121:961–969. https://doi.org/10.1093/anae/rtaa378

Chakraborty, D., I. N. Pessah, P. Zhang, M.B. Bhattacharjee, R.T. Dirksen, and S.L. Allen. 2020. Bioenergetic defects in muscle fibers of RYR1 mutant knock-in mice associated with malignant hyperthermia. Anesth. Analg. 121:961–969. https://doi.org/10.1093/anae/rtaa378

Chevessier, F., I. Marty, M. Pasti, K. Jouas, D. Hantai, and M. Verdillé-Sahau. 2004. Tubular aggregates are from whole sarcoplasmic reticulum origin: Alterations in calcium binding protein expression in mouse skeletal muscle during aging. Neuromuscul. Disord. 14:208–216. https://doi.org/10.1016/j.nmd.2003.11.007

Chevessier, F., S. Bauché-Godard, J.-P. Leroy, J. Koenig, M. Paturneau-Jouas, B. Eyman, D. Hantai, and M. Verdillé-Sahau. 2005. The origin of tubular aggregates in human myopathies. J. Pathol. 207:315–323. https://doi.org/10.1002/path.1832

Clarke, N.F. 2012. Alteration of STIM1/Orai-mediated SOCE in skeletal muscle: Impact in genetic muscle diseases and beyond. Cells. 10: 2722. https://doi.org/10.3390/cells1002222

Conte, E., A. Pannunziolo, P. Imbrici, G.M. Camerino, L. Maggi, M. Mora, S. Gibbertini, O. Capparalli, A. De Luca, M. Coluccia, and A. Liantonio. 2021b. Gain-of-Function STIM1 L96V mutation causes myogenesis alteration in muscle cells from a patient affected by tubular aggregate myopathy. Front. Cell Dev. Biol. 9:635063. https://doi.org/10.3389/fcell.2021.635063

Cordero-Sanchez, C., B. Riva, S. Reano, N. Clemente, I. Zaggia, F.A. Ruffinatti, A. Potenzieri, T. Pirali, S. Raffa, S. Sangalenti, et al. 2019. A luminal EF-hand mutation in STIM1 in mice causes the clinical hallmarks of tubular aggregate myopathy. Dis. Model. Mech. 13:dnm04111. https://doi.org/10.1242/dmm.041111

Cowling, B.S., I. Prokic, H. Tasfaout, A. Rabai, B. Rinaldi, A.-S. Nicot, C. Kretz, S. Friant, A. Roux, and J. Laporte. 2017. Amphiphysin (BIN1) negatively regulates dynamin 2 for normal muscle maturation. J. Clin. Invest. 127:4477–4487. https://doi.org/10.1172/JCI90542

Culley, T.P., J.F. Lamont, S. Cirak, M.S. Damian, W. Wallefeld, R. Gooding, S.V. Tan, J. Sheehan, F. Muntoni, S. Abb, et al. 2012. Mutations in MYH7 cause Multi-minicore Disease (MmD) with variable cardiac involvement. Neuromuscul. Disord. 22:1096–1104. https://doi.org/10.1016/j.nmd.2012.06.007

Culley, T.R., R.M. Murphy, L. Roberts, T. Raastad, R.G. Fassett, J.S. Coombes, J. Darbellay, B., S. Arnould, C. R. Bader, S. König, and L. Bernheim. 2011. STIM1 L1863P causes central core and Miyoshi–MINO myopathies. J. Biol. Chem. 286:4363–4369. https://doi.org/10.1074/jbc.M110.192424

Dagan, L. and R. Palty. 2021. Regulation of store-operated Ca2+ entry by STIM1. Sci. Signal. 14:eaaz862. https://doi.org/10.1126/scisignal.aaa862

Darbella, B., S. Arnaudeau, C.R. Bader, S. König, and L. Bernheim. 2011. STIM1L is a new actin-binding splice variant involved in fast repetitive Ca2+ release. J. Cell Biol. 194:335–346. https://doi.org/10.1083/jcb.201012157

DeFrancesco, R.A. 1988. Lilly lecture 1987. The triunvimate—Beta-cell, muscle, liver. A collusion responsible for NIDDM. Diabetes. 37:667–687. https://doi.org/10.2337/dbah.37.6.667

Desai, P.N., X. Zhang, W. Wu, A. Janoshazi, S. Bolimuntha, J.W. Putney, and M. Trebak. 2015. Multiple types of calcium channels arising from alternative translation initiation of the Ornai message. Sci. Signal. 8:aqa7. https://doi.org/10.1126/scisignal.aqa7

Dirksen, R.T., and G. Avila. 2002. Altered ryanodine receptor function in central core disease: Leaky or uncoiled Ca2+ release channels? Trends Cardiovasc. Med. 12:189–197. https://doi.org/10.1016/s1050-1738(02)00163-9

Dirksen, R.T., and G. Avila. 2004. Distinct effects on Ca2+ handling caused by malignant hyperthermia and central core disease mutations in RyR1. Cell. 115:189–197. https://doi.org/10.1016/s0092-8674(04)00166-3

Desai, P.N., X. Zhang, W. Wu, A. Janoshazi, S. Bolimuntha, J.W. Putney, and M. Trebak. 2015. Multiple types of calcium channels arising from alternative translation initiation of the Ornai message. Sci. Signal. 8:aqa7. https://doi.org/10.1126/scisignal.aqa7
Calcium homeostasis and congenital myopathies

Ferguson, S.M., and P. De Camilli. 2012. Dynamin, a membrane-remodelling GTPase. Nat. Rev. Mol. Cell Biol. 13:75–83. https://doi.org/10.1038/nrm3266

Ferreiro, A., S. Quijano-Roy, C. Pichereau, B. Moghadaszadeh, N. Goemans, C. Boncompagni, D.L. Galvan, C.P. Gilman, M.R. Baker, N. Shirokova, et al. 2008. RyR1 S-nitrosylation underlies environmental heat stroke and sudden death in Y2552R RyR1 knockin mice. Cell. 133:53–65. https://doi.org/10.1016/j.cell.2008.02.042

Frisnes, J., and M.B. Pedersen. 2009. Junctin—the quiet achiever. J. Physiol. 587:3135–3137. https://doi.org/10.1113/jphysiol.2009.179599

Fukushima, D., Z. Osada, and T. Nakamura. 2016. A mutation in Orai1 causes immune deficiency by antagonizing CRAC channel function. Nature. 441:179–185. https://doi.org/10.1038/nature04702

Furillo, C., G. Astrea, M. Savarese, D. Cassandrini, G. Brisa, F. Trucou, M. Pedemonte, R. Trotta, L. Ruggiero, L. Vercelli, et al. 2016. MYH7-related myopathies: Clinical, histopathological and imaging findings in a cohort of Italian patients. Orphanet J. Rare Dis. 11:91. https://doi.org/10.1186/s13023-016-0234-8

Fushi, S., A. Rensing-Ehl, C. Speckmann, B. Bengsch, A. Schmitt-Graeff, I. Bondiazo, A. M-Pavlic, T. Biss, T. Vraetz, B. Strahm, et al. 2012. Antiviral and regulatory T cell immunity in a patient with stromal interaction molecule 1 deficiency. J. Immunol. 188:1523–1533. https://doi.org/10.4049/jimmunol.1102507

Gaburjakova, M., N.C. Bal, J. Eckhardt, J. Biro, and S. Periasamy. 2013. Functional interaction between calsequerin and ryrodyne channel in the heart. Cell. Mol. Life Sci. 70:2935–2945. https://doi.org/10.1007/s00018-012-1199-7

Gali, L., A. Orrico, S. Lorenzini, S. Censini, M. Falciani, A. Covacci, V. Teazzin, G. Dessimone, and V. Sorrentino. 2006. Frequency and localization of the mutations in 106 exons of the RYR1 gene in 50 individuals with malignant hyperthermia. Hum. Mutat. 27:830. https://doi.org/10.1002/humu.20226

Garibaldi, M., F. Fattori, B. Riva, C. Labasse, G. Brochiere, P. Ottaviani, S. Sacconi, E. Vizzaccaro, F. Laschena, N.B. Romero, et al. 2017. A novel gain-of-function mutation in Orai1 causes late-onset tubular aggregate myopathy and congenital cataract. Clin. Genet. 91:783–786. https://doi.org/10.1111/cge.12888

Gardi, L., M. Rinuenda, T. Brocard, E. Lacene, J. Fauré, G. Brochiere, M. Beuvin, C. Labasse, A. Madeleine, E. Malfatti, et al. 2019. “Dusty core disease” (DuCD): Expanding morphological spectrum of RYR1 recessive myopathies. Acta Neuropathol. Commun. 7:3. https://doi.org/10.1186/s40478-018-0635-5

Gardner, L., A.D. Wilson, A. Alhussni, T. Humberstone, et al. 2019. Quantitative RyR1 reduction and loss of calcium sensitivity of RyR1Q1970fs-102134. https://doi.org/10.1016/j.celrep.2019.102134

Gillespie, D., L. Xu, and G. Meissner. 2014. Selecting ions by size in a calcium channel: The ryrodyne channel case study. Biophys. J. 107:2263–2273. https://doi.org/10.1016/j.bpj.2014.09.031

Gibbs, L., S. De Lorenzo, P. Natarajan, and C. Sambandamurthy. 2016. RyR1 channel regulation by Ca2+-induced shifts in the beta-microglobulin–histone H1 complex. J. Biol. Chem. 291:20949–20959. https://doi.org/10.1074/jbc.M115.691036

Gómez-Oca, R., B.S. Cowling, and J. Laporte. 2021. Common pathogenic mechanisms in centronuclear and myotubular myopathies and latest treatment advances. Int. J. Mol. Sci. 22:11377. https://doi.org/10.3390/ijms22111377

Gong, D., N. Yan, and H.A. Ledford. 2021. Structural basis for the modulation of ryrodyne channels. Trends Biochem. Sci. 46:489–501. https://doi.org/10.1016/j.ydbio.2020.11.009

González-Oca, R., B.S. Cowling, and J. Laporte. 2021. Common pathogenic mechanisms in centronuclear and myotubular myopathies and latest treatment advances. Int. J. Mol. Sci. 22:11377. https://doi.org/10.3390/ijms22111377

Gong, D., N. Yan, and H.A. Ledford. 2021. Structural basis for the modulation of ryrodyne channels. Trends Biochem. Sci. 46:489–501. https://doi.org/10.1016/j.ydbio.2020.11.009

González-Oca, R., B.S. Cowling, and J. Laporte. 2021. Common pathogenic mechanisms in centronuclear and myotubular myopathies and latest treatment advances. Int. J. Mol. Sci. 22:11377. https://doi.org/10.3390/ijms22111377

Gong, D., N. Yan, and H.A. Ledford. 2021. Structural basis for the modulation of ryrodyne channels. Trends Biochem. Sci. 46:489–501. https://doi.org/10.1016/j.ydbio.2020.11.009
Gotto, I., M. Adachi, and E. Nishida. 2001. Identification and characterization of a novel MAP kinase kinase kinase, MLTK. J. Biol. Chem. 276: 4126–4126. https://doi.org/10.1074/jbc.M00591201

Groom, L., S.M. Muldoon, Z.Z. Tang, B.W. Brandon, M. Bayarsaikhan, S. Bina, H.-S. Lee, X. Qiu, N. Sambuaghin, and R.T. Dirksen. 2011. Identiﬁc de novo mutation in the type 1 ryanodine receptor gene associated with familial, stress-induced malignant hyperthermia in two unrelated families. Anesth. Analg. 113:938–945. https://doi.org/10.1213/ANE.0b013e3182230068

Grosse, J., A. Braun, D. Varga-Szabo, N. Beyendorf, B. Schneider, L. Zeitlmann, P. Hanke, P. Schropp, L. Mühlstedt, C. Zorn, et al. 2007. An EF hand mutation in Stn1 causes premature platelet activation and bleeding in mice. J. Clin. Invest. 117:3540–3550. https://doi.org/10.1172/JCI33212

Grzybowski, M., A. Schanzer, A. Pepler, C. Heller, B.A. Neubauer, and A. Hahn. 2011. New STAC3 mutations in the ﬁrst non–American patient with native American myopathy. Neuropediatrics. 48:451–455. https://doi.org/10.1007/s00121-011-1868-y

Guo, W., and K.P. Campbell. 1995. Association of triadin with the ryanodine receptor and calsequestrin in the lumen of the sarcoplasmic reticulum. J. Biol. Chem. 270:9027–9030. https://doi.org/10.1074/jbc.270.16.9027

Gurgel-Giannetti, J., L.S. Souza, G.F. Messina de Padua Andrade, M.F. Der- Groom, L., S.M. Muldoon, Z.Z. Tang, B.W. Brandon, M. Bayarsaikhan, S. Bina, H.-S. Lee, X. Qiu, N. Sambuaghin, and R.T. Dirksen. 2011. Identiﬁc de novo mutation in the type 1 ryanodine receptor gene associated with familial, stress-induced malignant hyperthermia in two unrelated families. Anesth. Analg. 113:938–945. https://doi.org/10.1213/ANE.0b013e3182230068

Grosse, J., A. Braun, D. Varga-Szabo, N. Beyendorf, B. Schneider, L. Zeitlmann, P. Hanke, P. Schropp, L. Mühlstedt, C. Zorn, et al. 2007. An EF hand mutation in Stn1 causes premature platelet activation and bleeding in mice. J. Clin. Invest. 117:3540–3550. https://doi.org/10.1172/JCI33212

Grzybowski, M., A. Schanzer, A. Pepler, C. Heller, B.A. Neubauer, and A. Hahn. 2011. New STAC3 mutations in the ﬁrst non–American patient with native American myopathy. Neuropediatrics. 48:451–455. https://doi.org/10.1007/s00121-011-1868-y

Guo, W., and K.P. Campbell. 1995. Association of triadin with the ryanodine receptor and calsequestrin in the lumen of the sarcoplasmic reticulum. J. Biol. Chem. 270:9027–9030. https://doi.org/10.1074/jbc.270.16.9027

Gurgel-Giannetti, J., L.S. Souza, G.F. Messina de Padua Andrade, M.F. Der-
Loy, R.E., M. Orynbayev, L. Xu, Z. Andronache, S. Apostol, E. Zvaritch, D.H. MacLennan, G. Meissner, W. Melzer, and R.T. Dirksen. 2011. Muscle weakness in RyR1<sup>76STOP</sup>/WT knock-in mice as a result of reduced ryanoide receptor Ca<sup>2+</sup> ion permeation and release from the sarcoplasmic reticulum. J. Gen. Physiol. 137:43–57. https://doi.org/10.1085/jgp.201010523

Luo, S., Q. Li, J. Lin, Q. Murphy, I. Marty, Y. Zhang, S. Kazerounian, and P.B. Agrawal. 2021. SPEG binds with desmin and its deficiency causes defects in triad and focal adhesion protein. Hum. Mol. Genet. 29:3892–3891. https://doi.org/10.1093/hmg/ddab276

Lynch, P.J., J. Tong, M. Lehane, A. Mallet, L. Giblin, J.J. Heffron, P. Vaughan, G. Zafra, D.H. MacLennan, and T.V. McCarthy. 1999. A mutation in the transmembrane/luminal domain of the ryanodine receptor is associated with abnormal Ca<sup>2+</sup> release channel function and severe core disease. Proc. Natl. Acad. Sci. USA. 96:4164–4169. https://doi.org/10.1073/pnas.96.7.4164

MacLennan, D.H., and M.S. Phillips. 1992. Malignant hyperthermia. Science 256:789–794. https://doi.org/10.1126/science.1589759

Maggi, L., T. Melone, S. Miradoli, A. Balistreri, F. Tagliabue, R. Caruso, M. Rizzuto. 2018. Mitochondrial calcium uptake in organ physiology: From molecular mechanism to animal models. Pfugers Arch. 470:1165–1179. https://doi.org/10.1007/s00424-018-2123-2

Manno, C., L. Figueroa, L. Royer, S. Pouvreau, C.S. Lee, P. Volpe, A. Nori, J. Matsumoto, A., H. Tsuda, S. Furui, M. Kawada-Nagashima, T. Anzai, M. Seki, M. Cuk, B. Patel, J. Lian, M. Ouimet, U. Kaufmann, J. Yang, R. Meissner, G., E. Darling, and J. Eveleth. 1986. Kinetics of rapid Ca<sup>2+</sup> release by dihydropyridine receptor-ryanodine receptor complex depleted in the sarcoplasmic reticulum of working muscle. Hum. Mutat. 25:988–972. https://doi.org/10.1002/humu.23899

Manno, C., A. Paolini, S. Boncompagni, M. Canato, C. Reggiani, and F. Protasi. 2017c. Strenuous exercise triggers a life-threatening response in mice susceptible to malignant hyperthermia. FASEB J. 31:3469–3462. https://doi.org/10.1096/fj.201610297R

Michelucci, A., M. García-Castañeda, S. Boncompagni, and R.T. Dirksen. 2018. Role of STIM1/ORAI1-mediated store-operated Ca<sup>2+</sup> entry in skeletal muscle physiology and disease. Cell Calcium. 76:101–115. https://doi.org/10.1016/j.ceca.2018.10.004

Michelucci, A., S. Boncompagni, L. Pietrangelo, M. García-Castañeda, T. Takanu, S. Malik, R.T. Dirksen, and F. Protasi. 2019. Transverse tubule remodeling enhances Orai1-dependent Ca<sup>2+</sup> entry in skeletal muscle. Elife. 8:e47576. https://doi.org/10.7554/eLife.47576

Michelucci, A., S. Boncompagni, L. Pietrangelo, T. Takano, F. Protasi, and R.T. Dirksen. 2020. Pre-assembled Ca<sup>2+</sup> entry units and constitutively active Ca<sup>2+</sup> entry in skeletal muscle of caalsequestrin-1 knockout mice. J. Gen. Physiol. 152:202012617. https://doi.org/10.1085/jgp.202012617

Miederer, A.-M., D. Alansary, G. Schäfer, P.H. Lee, M. Jung, V. Helms, and B.A. Niemeyer. 2015. A STIM2 splice variant negatively regulates store-operated calcium entry. Nat. Commun. 6:6899. https://doi.org/10.1038/ncomms7899

Misceo, D., A. Holmgren, W.E. Louch, P.A. Holme, M. Mizobuchi, R.J. Moles, A.M. De Paula, A. Stray-Pedersen, R. Lyle, B. Dalsbu, et al. 2014. A dominant STIM1 mutation causes stromorken syndrome. Hum. Mutat. 35:556–564. https://doi.org/10.1002/humu.22544

Molenar, J.P., N.C. Voermans, B.J. van Hoeve, E.J. Kamsteeg, L.A. Kluitmans, B. Kusters, H.J. Jungbluth, and B.G. van Engelen. 2014. Fever-induced recurrent rhabdomyolysis due to a novel mutation in the ryanodine receptor type 1 gene. Intern. Med. J. 44:819–820. https://doi.org/10.1111/imj.12498

Monnier, N., V. Procaccio, P. Stiegitz, and J. Lunardi. 1997. Malignant hyperthermia susceptibility is associated with a mutation of the alpha 1-subunit of the human dihydropyridine-sensitive L-type voltage-dependent calcium-channel receptor in skeletal muscle. Am. J. Hum. Genet. 60:1316–1325. https://doi.org/10.1086/315454

Monnier, N., N.B. Romero, J. Lerale, Y. Nivoche, D. Qi, D.H. MacLennan, M. Fardeau, and J. Lunardi. 2000. An autosomal dominant congenital myopathy with cores and rods is associated with a neomutation in the RYR1 gene encoding the skeletal muscle ryanodine receptor. Hum. Mutat. 19:259–268. https://doi.org/10.1002/humu.912599

Monnier, N.P., L. D’Ambrosio, S. Paggi, S. Moggio, M. Ripolone, R. Violano, P. Marcorelles, D. Maréchal, et al. 2014. Familial and sporadic forms of central core disease are associated with mutations in the C-terminal domain of the skeletal muscle ryanodine receptor. Hum. Mol. Genet. 10.1093/hmg/ddu257. 10.2551. https://doi.org/10.1093/hmg/ddu257

Monnier, N., I. Marty, J. Jaure, C. Castiglioni, C. Desnuelle, S. Saccone, B. Esbours, R. Ferreiro, N. Romero, A. Laquerriere, et al. 2008. Null mutations causing depletion of the type 1 ryanodine receptor (RYR1) are commonly associated with recessive structural congenital myopathies with cores. Hum. Mutat. 29:670–678. https://doi.org/10.1002/humu.20696

Moro, C.A.M., E.P. Estephan, A. Fappi, S. Monges, F. Lubieniecki, O. Lopes Abath Neto, U.C. Reed, S. Donkeroolvoort, M.B. Harms, C. Bonnemann, and E. Zanotelli. 2020. Congenital fiber type disproportion caused by TPM3 mutation: A report of two atypical cases. Neuro muscular. Disord. 30:54–58. https://doi.org/10.1016/j.nd.2019.11.001

Morin, G., V. Biancalana, A. Echaniz-Laguna, J.-B. Noury, L. Xornage, M. Moggio, M. Ripolone, R. Violano, P. Marcorelles, D. Marechal, et al. 2020. Tubular aggregate myopathy and stormorken syndrome: Mutation spectrum and genotype/phenotype correlation. Hum. Mutat. 41:17–37. https://doi.org/10.1002/humu.23989

Mualem, S. 2007. Calcium signaling: Pyruvate and CRAC meet at the crossroads. Curr. Biol. 17:R549–R551. https://doi.org/10.1016/j.cub.2007.05.037

Muelas, N., P. Hackman, H. Luque, M. Garcés-Sánchez, I. Azorin, T. Suominen, T. Sevilla, F. Mayordo, L. Gómez, F. Martí, et al. 2010. MFH7 gene tail mutation causes myofibrillar profiles beyond laing distal myopathy. Neurology. 75:732–741. https://doi.org/10.1212/01.wnl.0b013e3181ee4e64

Mullins, F.M., C.Y. Park, R.E. Dolmetsch, and R.S. Lewis. 2009. STIM1 and calmodulin interact with ORAI1 to induce Ca<sup>2+</sup>-dependent inactivation of
entry: Critical regulators of Ca\textsuperscript{2+} content and function in skeletal muscle. J. Physiol. https://doi.org/10.1113/JP279512
Pecher, C.A., C. Spiegelhalter, R. Silva-Rojas, J. Laporte, and J. Böhm. 2020. Functional analyses of STIM1 mutations reveal a common patho-
mechanism for tubular aggregate myopathy and Stormorken syndrome. Neuropathol. 40:559–569. https://doi.org/10.1111/neup.12692
Pelletier, L., A. Petitot, J. Brocard, B. Giannesini, D. Giovannini, C. Sanchez, L. Travard, M. Chivet, M. Beaufils, C. Kutchukian, et al. 2020. In vivo RyR1
rescues in muscle fibers of a core-like myopathy. Acta Neuropathol. Commun. 8:192. https://doi.org/10.1038/s41428-020-01068-4
Perni, S., M. Lavorato, and K.G. Beam. 2017. De novo reconstitution reveals the proteins required for skeletal muscle voltage-induced Ca\textsuperscript{2+} release. Proc. Natl. Acad. Sci. USA. 114:13822–13827. https://doi.org/10.1073/pnas.1716641115
Peter, B.J., H.M. Kent, I.G. Mills, Y. Vallis, P.J.G. Butler, P.R. Evans, and H.T. McMahon. 2004. BAR domains as sensors of membrane curvature: The
amphiphrin BAR structure. Science. 303:495–499. https://doi.org/10.1126/science.1092586
Pettit, N., A. Lesercue, M. Rederstorff, A. Krol, B. Moghadzahadeh, U.M. Wewer, and P. Guicheney. 2003. Selenoprotein N: An endoplasmic
reticulum glycoprotein with an early developmental expression pattern. Hum. Mol. Genet. 12:1045–1053. https://doi.org/10.1093/hmg/ddg115
Phimister, A.J., R. Jango, E.H. Lee, M.A. Ernst-Russell, H. Takahisa, J. Ma, P.D. Allen, and I.N. Pessah. 2007. Conformation-dependent stability of
ryanodine receptor type 1 (RyR1) channel complex is mediated by their hyper-reactive thiols. J. Biol. Chem. 282:1067–1076. https://doi.org/10.1074/jbc.
M300120200
Pinto, M.M., S. Monges, E. Malfatti, F. Lubieniecki, X. Lornage, L. Abbas, C. Labas, M. Adelaine, M. Farude, J. Laporte, et al. 2019. Sarcomeric
disorganization and nemaline bodies in muscle biopsies of patients with EXOSC3-related type 1 pontocerebellar hypoplasia. Muscle Nerve. 59:
137–141. https://doi.org/10.1002/mus.26305
Polster, A., B.R. Nelson, E.N. Olson, and K.G. Beam. 2016. Stac has a direct role in skeletal muscle-type excitation-contraction coupling that is
disrupted by a myopathy-causing mutation. Proc. Natl. Acad. Sci. USA. 113:10986–10991. https://doi.org/10.1073/pnas.1612441113
Protasi, F., C. Paolini, M. Canato, C. Reggiani, and M. Quarta. 2018. Malignant hyperthermia in the post-genomics era: New perspectives on an old concept.
Muscle Nerve 59:940–947. https://doi.org/10.1002/mus.26679
Putney, J.W. 1986. A model for receptor-regulated calcium entry. Cell Calcium. 7:1–12. https://doi.org/10.1016/0143-4160(86)90026-6
Ramesh, G., L. Jarzembowski, Y. Schwarz, V. Poth, M. Konrad, M.L. Knapp, G. Schwär, A.A. Lauer, M.O.W. Grimm, D. Alansary, et al. 2021. A short
isoform of STIM1 confers Ca\textsuperscript{2+}-dependent mitochondrial enhancement. Cell Rep. 34:108844. https://doi.org/10.1016/j.celrep.2021.108844
Rana, A., M. Yen, A.M. Sadaghiani, S. Malmersjö, C.Y. Park, R.E. Dolmetsch, and R.S. Lewis. 2015. Alternative splicing converts STIM2 from an
activator to an inhibitor of store-operated calcium channels. J. Cell Biol. 209:653–669. https://doi.org/10.1083/jcb.201412060
Rathner, P., M. Fahrner, L. Cerofolini, H. Grabmayr, F. Hlavsaith, H. Kroboth, A. Motii, E. Ravera, M. Fragnol, M. Beachmann, et al. 2021. Interhelical
interactions within the STIM1 CCI domain modulate CRAC channel activation. Nat. Chem. Biol. 17:196–204. https://doi.org/10.1038/s41589-
020-00672-8
Ravenscroft, G.A., C. Spiegelhalter, R. Silva-Rojas, J. Laporte, and J. Böhme. 2020. Calcium homeostasis and congenital myopathies.
Calcium homeostasis and congenital myopathies

Savoressi, M., J. Sarpantara, A. Vihola, P.H. Jonson, M. Johari, S. Ruspanen, P. Hackman, and B. Udd. 2020. Panorama of the distal myopathies. Acta Myol. 39:245-265. https://doi.org/10.26185/2532-9900-028

Scherifer, P.C., E.P. Hoffman, J.D. Fratkin, C. Semino-Mora, A. Senchak, M.R. Davis, N.G. Laing, V. Vedanarayanan, and S.H. Subramony. 2000. A novel ryanodine receptor gene mutation causing both cores and rods in congenital myopathy. Neurology. 55:1689–1696. https://doi.org/10.1212/wnl.55.11.1689

Scharnagl, V., N.B. Romero, S. Donkervoort, S. Treves, P. Munot, T.M. Pierson, J. Dabaj, E. Malaffi, I.T. Zaharieva, F. Zorozto, et al. 2017. Di-hydropyridine receptor (DHPR, CACNA1S) congenital myopathy. Acta Neuropathol. 133:517–533. https://doi.org/10.1007/s00401-016-1656-8

Schiaffino, S. 2012. Tubular aggregates in skeletal muscle: Just a special type of protein aggregates?. Neuromuscul. Disord. 22:199–207. https://doi.org/10.1016/j.nmd.2011.10.005

Shin, D.W., J. Ma, and D.H. Kim. 2000. The asp-rich region at the carboxyl-terminus of calsequestrin binds to Ca(2+) and interacts with triadin. FEBs Lett. 486:178–182. https://doi.org/10.1016/s0014-5793(00)02424-6

Shin, D.W., Z. Pan, E.K. Kim, J.M. Lee, M.B. Bhat, J. Parness, D.H. Kim, and J. Ma. 2003. A retrograde signal from calsequestrin for the regulation of store-operated Ca2+ entry in skeletal muscle. J. Biol. Chem. 278: 3286–3292. https://doi.org/10.1074/jbc.M209045200

Silva-Rojas, R., S. Treves, H. Jacobs, P. Kessler, N. Messaddeg, J. Laporte, and J. Böh m. 2019. STIM1 over-activation generates a multi-systemic phenotype affecting the skeletal muscle, spleen, eye, skin, bones and immune system in mice. Hum. Mol. Genet. 28:1579–1593. https://doi.org/10.1093/hmg/ddy144

Silva-Rojas, R., J. Laporte, and J. Böh m. 2020. STIM1/ORA1 loss-of-function and gain-of-function mutations inversely impact on SOCE and calcium homeostasis and cause multi-systemic mirror diseases. Front. Physiol. 11: 60491. https://doi.org/10.3389/fphys.2020.60491

Silva-Rojas, R., A.-L. Charles, S. Djeddi, B. Geny, J. Laporte, and J. Böhm. 2021. Pathophysiological effects of overactive STIM1 on murine muscle function and structure. Cells. 10:1730. https://doi.org/10.3390/cells10071730

Sparrow, J.C., K.J. Nowak, H.J. Durling, A.H. Beggs, C. Wallgren-Pettersson, N. Romero, I. Nonaka, and N.G. Laing. 2003. Muscle disease caused by mutations in the skeletal muscle alpha-actin gene (ACTA1). Muscle Nerve. 28:367-374. https://doi.org/10.1002/mus.10071730

Tajsharghi, H., L.-E. Thorrell, C. Lindberg, B. Lindvall, K.-G. Henriksson, and A. Oldfors. 2003. Myosin storage myopathy associated with a heterozygous missense mutation in MYH7. Ann. Neurol. 54:494–500. https://doi.org/10.1002/ana.10693

Tajsharghi, H., and A. Oldfors. 2013. Myosinopathies: Pathology and mechanisms. Acta Neuropathol. 125:3–18. https://doi.org/10.1007/s00401-012-1024-z

Takayama, K., S. Mitsuhashi, J.-Y. Shin, R. Tanaka, T. Fujii, R. Tsukubayra, S. Mukaida, S. Noguchi, I. Nonaka, and I. Nishino. 2016. Japanese multiple epidermal growth factor 10 (MEGF10) myopathy with novel mutations: A phenotype-genotype correlation. Neurobiol. Disord. 85:1149–1160. https://doi.org/10.1016/j.nbd.2016.06.005

Takeshima, H., M. Iino, H. Takekura, M. Nishi, J. Kuno, O. Minowa, H. Tanaka, and T. Noda. 1994. Excitation-contraction uncoupling and muscular degeneration in mice lacking functional skeletal muscle ryanodine-receptor gene. Nature. 369:556–559. https://doi.org/10.1038/369556a0

Rosenberg, R., J. Menguß, P. Grieb, H. Wenzel, R. Dörner, and C. Döttling. 2007. Calcium, mitochondria and cell metab-olism: A review. Orphanet J. Rare Dis. 10:93. https://doi.org/10.1186/1750-1172-2-93

Rosenblum, N., N. Pollock, A. Schiemann, T. Bulger, and K. Stowell. 2015. Maligned hyperthermia: A review. Orphanet J. Rare Dis. 10:31–10. https://doi.org/10.1186/s13023-015-0310-1

Rosi et al. | Journal of General Physiology https://doi.org/10.1525/jgp.20221115
Venetucci, L.A., A.W. Trafford, S.C. O’Neill, and D.A. Eisner. 2008. The sarcoplasmic reticulum and arrhythmogenic calcium release. Circ. Res. 107:285–292. https://doi.org/10.1161/circresaha.107.15728

Vig, M., C. Peinelt, A. Beck, D.L. Koomoa, D. Rabah, M. Koblan-Huberson, S. Kraft, H. Turner, A. Fleg, R. Penner, and J.-P. Kinet. 2006. CRACM1 is a plasma membrane protein essential for store-operated Ca2+ entry. Sci. 312:1220–1223. https://doi.org/10.1126/science.1127883

Villar-Quiles, R.N., M. von der Hagen, C. Méty, V. González, S. Donkervoort, E. Bertini, C. Castiglioni, D. Clainghe, J. Colomer, M.L. Cuadrado, et al. 2020. The clinical, histologic, and genotypic spectrum of SEPN1-related myopathy: A case series. Neurology. 95:e515–e527. https://doi.org/10.1212/wnl.0000000000008227

Volpatti, J.R., Y. Endo, J. Knox, L. Groom, S. Brennan, R. Roche, W.J. Zuercher, P. Roy, R.T. Dirksen, and J.J. Dowling. 2020. Identification of drug modifiers for RYR1-related myopathy using a multi-species discovery pipeline. Elife. 9:e52946. https://doi.org/10.7554/eLife.52946

von der Hagen, M., W. Kress, G. Hahn, K.S. Brocke, P. Mitzscherling, A. Huebner, C. Müller-Reible, G. Stoltenburg-Dídinger, and A.M. Kaindl. 2008. Novel RYR1 missense mutation causes core rod myopathy. Eur. J. Neurol. 15:e31–e32. https://doi.org/10.1111/j.1468-1331.2008.02094.x

Walters, G.C., and Y.M. Usachov. 2022. MCU (mitochondrial Ca2+ unipor) makes the calcium go round. J. Biol. Chem. 298:101604. https://doi.org/10.1074/jbc.T200216.101604

Wang, S., M. Choi, A.S. Richardson, B.M. Reid, F. Seymour, M. Yildirim, E. Tuna, K. Gencay, J.P. Simmer, and J.C. Hu. 2014. STIM1 and SLC2A4 are critical for enamel maturation. J. Dent. Res. 93:945–1005. https://doi.org/10.5210/jb.20203451452791

Wang, L., Z. Zhang, S. Li, Y. Zheng, X. Yan, M. Chen, H. Wang, J.W. Putney, and D. Luo. 2015. Retrograde regulation of STIM1-Orai1 interaction and store-operated Ca2+ entry by calsequestrin. Sci. Rep. 5:11349. https://doi.org/10.1038/srep11349

Wang, H., C. Castiglioni, A. Kaçar Bayram, F. Fattori, S. Pekus, D. Araneda, H. Per, R. Erazo, H. Gümüş, S. Zorluemir, et al. 2017. Insights from genotype-phenotype correlations by novel SPEG mutations causing centronuclear myopathy. Neuromuscul. Disord. 27:836–842. https://doi.org/10.1016/j.nmd.2017.05.014

Wappler, F., M. Fiege, M. Steinath, K. Agarwal, J. Scholz, S. Singh, J. Matschke, and J. Schulte Am Esch. 2001. Evidence for susceptibility to malignant hyperthermia in patients with exercise-induced rhadomyolysis. Anesthesiology. 94:95–100. https://doi.org/10.1095/jbc.2001203451452791

Weidinger, C., J.P. Shaw, and S. Feske. 2013. STIM1 and STIM2-mediated Ca2+ influx regulates antinurnour immunity by CD4(+) T cells. EMBO Mol. Med. 5:1311–1322. https://doi.org/10.1002/emmm.201202989

Weir-Apierre, L., E.M. Carrell, S. Boncompari, F. Protasi, and R.T. Dirksen. 2013. Orai-dependent calcium entry promotes skeletal muscle growth and limits fatigue. Nat. Commun. 4:2805. https://doi.org/10.1038/ncomms3805

Wilmont, M., J.M., S. Lilliss, H. Zhou, K. Pillay, H. Henderson, W. Kress, C.R. Müller, A. Ndevu, V. Cloke, T. Cullup, et al. 2010. RYR1 mutations are a common cause of congenital myopathies with central nuclei. Neurol. 68:717–726. https://doi.org/10.1212/wnl.0b013e3181eed246

Wilson, R.J., S.P. Lyons, T.R. Koves, V.G. Bryson, H. Zhang, T.I., S.B. Crown, J.-D. Ding, P.A. Grimsrud, P.B. Rosenberg, and D.M. Muoio. 2022. Disruption of STIM1-mediated Ca2+ sensing and energy metabolism in adult skeletal muscle compromises exercise tolerance, proteostasis, and lean mass. Mol. Metab. 37:101429. https://doi.org/10.1016/j.molmet.2021.101429

Woll, K.A., O. Haji-Ghassemi, and F. Van Petegem. 2021. Pathological consequences of disease mutations RYR1 Receptors revealed by cryo-EM. Nat. Commun. 12:807. https://doi.org/10.1038/s41467-021-21141-3

Woll, K.A., and F. Van Petegem. 2022. Calcium-release channels: Structure and function of IP3 receptors and ryanodine receptors. Physiol. Rev. 102:209–268. https://doi.org/10.1152/physrev.00033.2020

Wu, S., M.C.A. Ibarra, M.C.V. Malidiyan, K. Murayama, Y. Ichihara, H. Kikuchi, I. Nonaka, S. Noguchi, Y.K. Hayashi, and I. Nishino. 2006. Central core disease is due to RYR1 mutations in more than 90% of patients. Brain. 129:1470–1480. https://doi.org/10.1093/brain/aws107

Xu, L., Y. Wang, N. Yamaguchi, D.A. Pasek, and G. Meissner. 2008. Single channel properties of heterotetrameric mutant RyR1 ion channels linked to core myopathies. J. Biol. Chem. 283:6321–6329. https://doi.org/10.1074/jbc.M703753200

Yang, Z., X. Bai, C. Yan, J. Wu, Z. Li, X. Weng, C. Yin, X. Li, S.H.W. Shcheres, et al. 2015. Structure of the rabbit ryanodine receptor RyR1 at near-atomic resolution. Nature. 517:50–55. https://doi.org/10.1038/nature14063

Yang, T., J. Riehl, E. Esteve, K.I. Matthaei, S. Goth, P.D. Allen, I.N. Pessah, and J.R. Lopez. 2006. Pharmacologic and functional characterization of
malignant hyperthermia in the R163C RyR1 knock-in mouse. Anesthesiology. 105:1164–1175. https://doi.org/10.1097/01.ANE.00005422-200612000-00016

Yang, T., P.D. Allen, I.N. Pessah, and J.R. Lopez. 2007. Enhanced excitation-coupled calcium entry in myotubes is associated with expression of RyR1 malignant hyperthermia mutations. J. Biol. Chem. 282:37471-37478. https://doi.org/10.1074/jbc.M701379200

Yarotsky, V., F. Protasi, and R.T. Dirksen. 2013. Accelerated activation of SOCE current in myotubes from two mouse models of anesthetic- and heat-induced sudden death. PLoS One. 8:e7763. https://doi.org/10.1371/journal.pone.0077633

Yiğ, U., S. Hiz, S. Güneş, G. Diniz, F. Baydan, A. Topf, E. Sonmezler, H. Lochmüller, R. Horvath, and Y. Oktay. 2019. Dihydropyridine receptor congenital myopathy in a consanguineous Turkish family. J. Neuromuscul. Dis. 6:37–384. https://doi.org/10.3233/JND-190383

Yoast, R.E., S.M. Emrich, X. Zhang, P. Xin, M.T. Johnson, A.J. Fike, V. Walter, N. Hempel, D.I. Yule, J. Sneyd, et al. 2020. The native ORAI channel trio underlies the diversity of Ca2+ signaling events. Nat. Commun. 11:2444. https://doi.org/10.1038/s41467-020-16232-6

Yoast, R.E., S.M. Emrich, X. Zhang, P. Xin, V. Arige, T. Pathak, J.C. Benson, M.T. Johnson, A.E. Abdelnaby, N. Lakomski, et al. 2021. The Mito-ordinated calcium entry in myotubes is associated with expression of calsequestrin-1 regulation. J. Biol. Chem. 296:101174. https://doi.org/10.1074/jbc.B21.101174

Yuan, Q., H. Dridi, O.B. Clarke, S. Reiken, Z. Melville, A. Wronska, A. Kushnir, R. Zalk, L. Sittenfeld, and A.R. Marks. 2021. RyR1-related myopathy mutations in ATP and calcium binding sites impair channel regulation. Acta Neuropathol. Commun. 9:186. https://doi.org/10.1186/s40478-021-02187-3

ZALK, R., O.B. Clarke, A. des Georges, R.A. Grasso, S. Reiken, F. Mancia, W.A. Hendrickson, J. Frank, and A.R. Marks. 2015. Structure of a mammalian ryanodine receptor. Nature. 517:44–49. https://doi.org/10.1038/nature13950

Zhao, X., C.-K. Min, J.-K. Ko, J. Parness, D.H. Kim, N. Weisleder, and J. Ma. 2010. Increased store-operated Ca2+ entry in skeletal muscle with reduced calsequestrin-1 expression. Biophys. J. 99:1556–1564. https://doi.org/10.1016/j.bpj.2010.06.050

Zhou, H., M. Brockington, H. Jungbluth, D. Monk, P. Stanier, C.A. Sewry, G.E. Moore, and F. Muntoni. 2006. Epigenetic allele silencing unveils recessive RyR1 mutations in core myopathies. Am. J. Hum. Genet. 79:859–868. https://doi.org/10.1086/508300

Zomot, E., H. Achillidiev Cohen, I. Dagan, R. Militsin, and R. Palry. 2021. Bidirectional regulation of calcium release-activated calcium (CRAC) channel by SARAF. J. Cell Biol. 220:e202104007. https://doi.org/10.1083/jcb.202104007

Zvaritch, E., F. Depreux, N. Kraeva, R.E. Loy, S.A. Goonasekera, S. Boncompagni, S. Boncompagni, A. Kraev, A.O. Gramolini, R.T. Dirksen, et al. 2007. An Ryr1I4895T mutation abolishes Ca2+ release channel function and delays development in homozygous offspring of a mutant mouse line. Proc. Natl. Acad. Sci. USA. 104:18537–18542. https://doi.org/10.1073/pnas.0709312104

Zvaritch, E., N. Kraeva, E. Bombardier, R.A. McCloy, F. Depreux, D. Holmyard, A. Kraev, C.E. Seidman, J.G. Seidman, A.R. Tupling, and D.H. MacLennan. 2009. Ca2+ dysregulation in Ryr1(I4895T/wt) mice causes congenital myopathy with progressive formation of minicores, cores, and nemaline rods. Proc. Natl. Acad. Sci. USA. 106:21813–21818. https://doi.org/10.1073/pnas.0912126106

Zvaritch, E., R. Gillies, N. Kraeva, M. Richer, H. Jungbluth, and S. Riazi. 2019. Fatal awake malignant hyperthermia episodes in a family with malignant hyperthermia susceptibility: A case series. Can J. Anaesth. 66:540–545. https://doi.org/10.1002/cja.21320

Zweifach, A., and R.S. Lewis. 1995b. Slow calcium-dependent inactivation of RYR1. J. Physiol. 49:186. https://doi.org/10.1113/jphysiol.1995.sp023223

Zweifach, A., and R.S. Lewis. 1995a. Rapid inactivation of depletion-activated calcium current. J. Gen. Physiol. 105:209–226. https://doi.org/10.1085/jgp.105.2.209

Zweifach, A., and R.S. Lewis. 1995b. Slow calcium-dependent inactivation of depletion-activated calcium current. Store-dependent and -independent mechanisms. J. Biol. Chem. 270:14445–14451. https://doi.org/10.1074/jbc.270.24.14445