Ryanodinopathies: Muscle Disorders Linked to Mutations in Ryanodine Receptors

John D. Lueck, Sanjeewa A. Goonasekera and Robert T. Dirksen

University of Rochester Medical Center, Department of Pharmacology and Physiology, Rochester, USA

Abstract

Excitation-contraction (EC) coupling in skeletal and cardiac muscle depends on bi-directional signaling interactions between sarcolemmal L-type Ca^{2+} channels (or dihydropyridine receptors, DHPRs) and Ca^{2+} release channels (or ryanodine receptors, RyRs) of the sarcoplasmic reticulum (SR). Mutations in the skeletal muscle RyR (RyR1) result in several clinically distinct muscle disorders including malignant hyperthermia (MH), central core disease (CCD), multi-minicore disease (MmD), and nemaline rod myopathy (NM). Similarly, two inherited exercise-induced arrhythmogenic disorders, catecholaminergic polymorphic ventricular tachycardia (CPVT) and arrhythmogenic right ventricular dysplasia type-2 (ARVD2), are linked to mutations in the cardiac isoform of the RyR (RyR2). This review examines the genetics, pathophysiology, and clinical/diagnostic characteristics of the "ryanodinopathies," an emerging and eclectic array of clinically distinct muscle disorders that arise from genetic and functional defects in the skeletal and cardiac muscle RyRs.

Key words: calcium channel, cardiac, excitation-contraction coupling, muscular dystrophy, ryanodine receptor, skeletal muscle.

Basic Appl Myol 14 (5): 345-358, 2004

Skeletal and cardiac muscle excitation-contraction (EC) coupling involves complex signaling interactions between sarcolemmal L-type Ca^{2+} channels (or dihydropyridine receptors, DHPRs) and Ca^{2+}-release channels (or ryanodine receptors, RyRs) located in the terminal cisternae of the sarcoplasmic reticulum (SR). During skeletal muscle EC coupling, action potentials in the transverse tubules induce rapid conformational alterations in DHPRs that trigger activation of mechanically-coupled RyRs (RyR1) [97, 103], ultimately resulting in a massive release of SR Ca^{2+} into the myoplasm (a process termed "orthograde coupling," [84]). Interestingly, the DHPR-RyR1 interaction is bi-directional in skeletal muscle since the Ca^{2+} channel activity of the DHPR is strongly influenced by its interaction with RyR1 (termed "retrograde coupling;" [3, 45, 85]. The central importance of this bi-directional DHPR-RyR1 mechanical interaction for skeletal muscle EC coupling is emphasized by the observation that point mutations in both of these proteins result in several clinically distinct human congenital muscle diseases including hypokalemic periodic paralysis, malignant hyperthermia (MH), central core disease (CCD), and specific variants of both multi-minicore disease (MmD) and nemaline rod myopathy (NM).

SR Ca^{2+} release channels in cardiac muscle are comprised of four identical cardiac RyR monomers (RyR2), which exhibit ~66% identity with RyR1 [91]. Similar to its skeletal muscle counterpart, RyR2 Ca^{2+} release channels also mediate SR calcium release during EC coupling in cardiac muscle [10, 99, 104]. However, unlike the skeletal isoform, release channel activation in the heart requires Ca^{2+} influx through sarcolemmal L-type calcium channels, via a process termed "calcium-induced calcium release" [26, 27, 83]. Recently, attention has focused on the roles of altered RyR2 function in a subset of autosomal-dominantly inherited arrhythmogenic disorders including catecholaminergic polymorphic ventricular tachycardia (CPVT) and arrhythmogenic right ventricular dysplasia type-2 (ARVD2). Both of these arrhythmogenic disorders are associated with missense mutations in RyR2 and are characterized by stress-induced bi-directional or polymorphic ventricular tachycardia [56, 95, 112].

Genetic Diseases Linked to Mutations in the Skeletal Muscle Ryanodine Receptor (RYR1)

Central Core Disease (CCD) and Malignant Hyperthermia (MH)

CCD, the first described human congenital myopathy [108], is inherited primarily in an autosomal dominant manner, although autosomal recessive forms have also been confirmed [51, 79]. Disease progression is either mild or static and typically presents with hypotonia and proximal muscle weakness during infancy (floppy infant syndrome). Clinical severity in adulthood is variable from normal to significant muscle weakness and can be accompanied by musculoskeletal features, including kyphoscoliosis, pes cavus, clubfoot, thoracic deformities, and congenital hip dislocation [107]. CCD is diagnosed through histochemical identification of amorphous central areas (cores) that can extend down the length of type 1 muscle fibers. The core regions can be central, peripheral or eccentric, are clearly circumscribed, and lack mitochondria and oxidative enzymatic activity. Electron microscopic analysis of core regions reveals disintegration of the contractile machinery and alterations in the structure and content of SR and t-tubule membranes [47, 67, 106]. CCD is often, though not always, associated with malignant hyperthermia (MH), a pharmacogenetic disorder of skeletal muscle in which depolarizing muscle relaxants (e.g. succinyl choline) and inhalation anesthetics (e.g. halothane) trigger uncontrolled Ca^{2+} release in skeletal muscle. The incidence of MH is estimated to be ~1 in 15,000 anaesthetized children and ~1 in 50,000-100,000 anaesthetized adults. MH episodes are characterized by hypermetabolism, skeletal muscle rigidity, hypoxia, extreme elevation in body temperature, and can be fatal if corrective therapy is not initiated promptly [67]. Standardized diagnostic *in vitro* contracture tests (IVCT) for assessing human MH susceptibility have been developed and implemented in both North America and Europe.

MH and CCD are caused by mutations in the gene encoding the skeletal muscle ryanodine receptor (RyR1) [25, 54, 61]. The RyR1 protein forms a large tetrameric Ca^{2+} release channel located in the terminal cisternae of the SR in skeletal muscle that is activated to rapidly increase myoplasmic Ca^{2+} levels during EC coupling. Currently, at least 67 different dominant missense mutations and small deletions in the RyR1 protein are known to be associated with MH (43 mutations) and CCD (36 mutations). Although at least 12 of these RyR1 mutations unequivocally lead to both CCD and MH [25, 87], increased MH susceptibility has not been confirmed for many of the other CCD mutations. Moreover, three additional RyR1 mutations have recently been shown to be linked to a recessive form of CCD [30, 51, 69, 79]. While CCD is primarily associated with mutations in RyR1 (but see also [28, 52]), only ~50-80% of MH families can be linked to the RyR1 gene. Consequently, alternate candidate genes for MH have also been proposed (including *SCN4A, CACNA2*, *CACNA1S*). However, only two amino acid substitutions of a single, highly conserved residue in *CACNA1S* gene (which encodes the α_{1S}-subunit of the skeletal muscle DHPR) have been identified among these other candidate loci [54, 80]. All of the dominant MH/CCD mutations/deletions are clustered in three distinct areas of the RyR1 protein: MH/CCD region 1 (N-terminal region, residues 35-614), MH/CCD region 2 (central region, residues 2129-2458) and MH/CCD region 3 (C-terminal region, residues 3916-4973). Ikemoto and colleagues have hypothesized that MH/CCD regions 1 and 2 fold together in the 3-dimensional structure of the channel to form a key regulatory domain of the channel [49], while others have suggested that MH/CCD region 3 contributes to the transmembrane and pore-forming region of the channel [120].

The demonstration that both MH and CCD largely arise from mutations in RyR1 has sparked considerable efforts to characterize the functional effects of these mutations on Ca^{2+} release channel activity and EC coupling in skeletal muscle. Initial studies were performed on the porcine model of MH, which arises from an R615C mutation in RyR1. Skeletal myotubes and fiber bundles obtained from MH pigs exhibit enhanced sensitivity to activation by calcium, caffeine, 4-chloro-m-cresol, halothane, and reduced inhibition by Mg^{2+} and Ca^{2+} (see [76] for review). Furthermore, voltage-gated SR Ca^{2+} release in porcine MH muscle is activated at more negative potentials while the magnitude and voltage dependence of the L-type Ca^{2+} channel activity is unaltered compared to that of control muscle [24, 36, 37]. These abnormalities presumably sensitize release channel activation by inhalation anesthetics and depolarizing skeletal-muscle relaxants (for excellent reviews see [54, 61, 74]. A similar increased sensitization to ligand-activated Ca^{2+} release has also been reported for human myotubes [17] and immortalized B-lymphocytes derived from MH susceptible individuals [41].

Effects of MH mutations on resting Ca^{2+} levels remain controversial. Some studies using porcine MHS skeletal muscle fibers report significant elevations in resting Ca^{2+} [2, 62] while others have failed to observe a difference compared to wild-type [48]. Similar discrepancies exist with regard to human MHS muscle as Censier et al. (1998) and Brinkmeier et al. (1999) found that resting Ca^{2+} levels were unaltered in MH susceptible skeletal muscle, while Lopez et al. (1992) reported an elevation in resting myoplasmic Ca^{2+} levels in MH patients [15, 17, 63].

Functional effects of disease mutations located in RyR1 MH/CCD regions 1-3 have been explored following both heterologous expression in HEK293 or COS-7 cells [64, 82, 113-115] and homologous expression in either cultured C_2C_{12} cells [90] or skeletal myotubes derived from RyR1-knockout (dyspedic) mice [122]. Tre-

ves et al. (1994) were the first to demonstrate enhanced 4-chloro-m-cresol sensitivity for MH mutant RyR1 channels (R615C) heterologously expressed in COS-7 cells [115]. In the same year, Otsu et al. (1994) reported that R615C mutant release channels expressed in C_2C_{12} cells also exhibited increased sensitivity to activation by caffeine and halothane [90]. Tong and colleagues (1997, 1999) performed an extensive functional analysis on 15 different MH and CCD mutants heterologously expressed in HEK293 cells [113, 114]. All of these mutants exhibited enhanced sensitivity to activation by both caffeine and halothane. In addition, cells expressing the CCD mutants exhibited reduced ER Ca^{2+} store content and enhanced resting Ca^{2+} levels. These results indicate that CCD mutations are unique in being able to significantly promote Ca^{2+} leak from intracellular stores. Since RyR1 function in HEK-293 cells may not accurately reflect activity within a muscle environment, a similar line of experimentation was conducted following homozygous and heterozygous expression of CCD mutant RyR1 proteins in skeletal myotubes derived from RyR1-knockout (dyspedic) mice [4, 6]. These studies confirmed that many of the CCD mutations in RyR1 (R164C, I404M, Y523S, R2163H, R2435H, and Y4795C) also promote SR Ca^{2+} leak and store depletion following homologous expression in dyspedic myotubes [4, 6]. In addition, dyspedic myotubes expressing these CCD mutant release channels also exhibited increased sensitivity to activation and a decrease in the magnitude of voltage-gated Ca^{2+} release during EC coupling. Remarkably, strong correlations were found between the increased sensitivity to activation by the voltage sensor and the 1) increase in resting Ca^{2+}, 2) degree of SR Ca^{2+} store depletion, and 3) magnitude of voltage-gated Ca^{2+} release [4]. Results from these studies clearly indicate that MH/CCD mutations located in regions 1 and 2 (and Y4795C in MH/CCD region 3) promote varying degrees of Ca^{2+} leak through the release channel, which ultimately leads to a significant reduction in releasable SR/ER Ca^{2+}.

Recently, Allen and colleagues (2003) conducted a series of similar homologous expression experiments using several MH-selective mutations in RyR1 (G342R, R615C, R2163C, V2168M, R2458H, and T4825I) [122]. This study found that release channel sensitivity to activation by KCl depolarization, caffeine, and 4-chloro-m-cresol were each increased following expression of the MH mutants in dyspedic myotubes. However, while the magnitude of caffeine and 4-chloro-m-cresol-induced Ca^{2+} release was reduced, maximal depolarization-induced release was increased by each of the MH mutations tested; a result fundamentally different from that observed for the CCD mutations in RyR1 that promote SR Ca^{2+} leak [4].

Originally, RyR1 disease mutations were reported only in MH/CCD regions 1 and 2. However, Lynch et al. (1999) identified a mutation in the extreme C-terminal region of RyR1 (I4898T) that results in an unusually severe and highly penetrant form of CCD [64], but did not appear to result in clinical MH. As a result, several subsequent genetic studies recruited CCD kindreds based solely on clinically and morphologically expressed CCD rather than MH susceptibility [22, 79, 81, 102, 110]. These studies identified an additional 27 CCD mutations in the C-terminal region of RyR1 (MH/CCD region 3). Thus, MH/CCD region 3 represents a primary molecular hot-spot for CCD. In addition, several MH-selective mutations have now been identified in this region [16, 38, 92].

Similar to that observed for CCD mutations in MH/CCD regions 1 and 2, ER Ca^{2+} leak was also enhanced following expression of I4897T mutant rabbit RyR1 proteins (analogous to the human I4898T mutation) in HEK293 cells [64]. In contrast, homologous expression of I4897T-containing release channels in dyspedic myotubes did not promote SR Ca^{2+} leak. However, voltage-gated Ca^{2+} release was either absent or reduced by 50% following expression in dyspedic myotubes of I4897T alone or in combination with wild-type RyR1, respectively [5]. Thus, the I4897T mutation exerts a dominant-negative reduction in voltage-gated SR Ca^{2+} release, consistent with the autosomal-dominant nature of this disorder. The reduction in Ca^{2+} release during skeletal muscle EC coupling is mechanistically distinct from the behavior of leaky CCD mutant channels and reflects a functional uncoupling of excitation from the efficient release of SR Ca^{2+} (termed EC uncoupling) [5]. Importantly, these results indicate that I4897T-containing release channels in skeletal muscle are not excessively leaky to Ca^{2+}, as found by Lynch et al. (1999) in HEK-293 cells, but rather, release calcium inefficiently following activation by sarcolemmal DHPRs. Thus, as RyR1 function is strongly influenced by numerous relatively muscle-specific factors (e.g. skeletal DHPR, triadin, calsequestrin, FKBP12), caution should be made with regard to effects of disease mutations on RyR1 function assessed exclusively using *in vitro* and heterologous expression approaches.

Interestingly, single channel measurements of mutations in I4897 and neighboring amino acids have provided strong evidence that this region of the protein comprises the pore-lining region of the channel [39, 123]. Consistent with this idea, several other CCD mutations in this sub-region of MH/CCD region 3 (including G4890R, R4892W, G4898E, G4898R, A4905R, R4913G) all result in varying degrees of EC uncoupling, suggesting that the pore region of RyR1 represents a primary molecular locus of the EC uncoupling mechanism [6]. It will be important for future work to determine the functional effects (e.g. leaky or EC uncoupling) of mutations in MH/CCD region 3 that are located clearly outside the channel's narrow selectivity filter.

While considerable advancements in understanding MH and CCD pathophysiology have been made over the past several years, many questions still remain unanswered. For one, why are resting Ca^{2+} levels elevated following expression of CCD and/or MH mutants that promote SR Ca^{2+} leak if long-term resting Ca^{2+} levels are ultimately determined by net Ca^{2+} transport across the sarcolemma? One possible explanation could be that SR Ca^{2+} depletion activates a store-operated Ca^{2+} entry (SOC) pathway that may contribute to an overall increase in net Ca^{2+} entry. In fact, recent evidence supports the presence of a SOC pathway activated by graded store depletion in skeletal muscle [57]. Alternatively, changes in myoplasmic ATP levels secondary to disrupted mitochondrial function within core regions might result in reduced sarcolemmal Ca^{2+}-ATPase activity and a reduction in net sarcolemmal Ca^{2+} efflux. Finally, additional work will be required in order to determine whether or not elevations in myoplasmic Ca^{2+} occur uniformly within the muscle cell or if subcellular Ca^{2+} gradients exist within structurally defined intracellular sub-domains (e.g. a central core).

A second question that requires significant attention is to what degree, if at all, alterations in muscle Ca^{2+} homeostasis caused by CCD mutations in RyR1 contribute to the formation and development of core regions. MacLennan and colleagues originally suggested that elevated resting Ca^{2+} levels may cause Ca^{2+}-mediated destruction of mitochondria in the center of the muscle fiber and that cores represent a cellular defense mechanism designed to protect the rest of the cell from further Ca^{2+}-induced damage [61]. However, cores in CCD are not always centrally located and can also be eccentric or located in peripheral regions of the fiber. More importantly, homologous expression studies indicate that resting Ca^{2+} levels are not elevated by CCD mutations that result in EC uncoupling. Therefore, it is unlikely that an elevation in resting Ca^{2+} is absolutely required for core formation. Alternatively, a significant reduction in Ca^{2+} release during EC coupling is a common feature of both leaky and EC uncoupled channels. Close structural and functional Ca^{2+} signaling microdomains between SR Ca^{2+} release and mitochondria are known to permit very rapid Ca^{2+} uptake by mitochondria under physiologic conditions [98]. Thus, changes in this SR-mitochondrial Ca^{2+} signaling microdomain during EC coupling may contribute to mitochondrial dysfunction and core development in CCD. In addition, Sheu and colleagues have suggested that RyR1 may also function as an important rapid mitochondrial Ca^{2+} uptake pathway [12, 13]. Under such a scenario, CCD mutations in RyR1 could more directly alter mitochondrial Ca^{2+} homeostasis by altering the properties of a key mitochondrial Ca^{2+} transport mechanism. Functional measurements of mitochondrial matrix Ca^{2+} levels in normal and diseased skeletal muscle will be required to determine whether or not mitochondrial Ca^{2+} dynamics during EC coupling is altered by MH and/or CCD mutations in RyR1.

Minicore Myopathy (MmD)

Minicore myopathy (multi-minicore disease, MmD) is a clinically heterogeneous, autosomal recessively-inherited muscle disorder. MmD is currently classified into three major groups; a classical form (including an ophthalmoplegic subgroup), an early onset form with arthrogryposis, and a moderate form with hand involvement [29]. The most prevalent is the classical form (~75%), which is associated with neonatal hypotonia, delayed motor milestones, and axial muscle weakness that often occurs with scoliosis and respiratory failure [53, 79]. MmD is diagnosed morphologically by the presence of multi-focal, poorly circumscribed and short core-like lesions. Like central cores in CCD, minicores also exhibit sarcomere disorganization, depletion of mitochondria, and reduced oxidative enzyme activity in both type I and II skeletal muscle fibers. In contrast, cores observed in CCD patients are predominantly found in type I fibers, possess sharply defined borders, and are typically singular and extend throughout a considerable length of the muscle fiber. Thus, based on differences in both histological and clinical presentation, MmD and CCD are typically regarded as two separate entities. However, minicores and central cores have occasionally been observed in the same muscle biopsy and in different individuals from the same family [11, 30, 51, 117]. Thus, the association of cores and minicores in select cases may involve a common or related pathogenic mechanism. Additionally, Ferriero et al. (2002) reported a family in which individuals that only exhibited minicores early in adulthood were found to possess abnormalities characteristic of CCD when biopsied 20 years later, consistent with a pathological variant of CCD with transitory presentation of MmD [30]. Finally, the recent identification of recessively inherited mutations in the *RYR1* gene provides a genetic explanation for the clinical overlap between MmD and CCD in certain families [30, 51, 79].

Currently, MmD has been linked to mutations in two genes, *SEPN1* and *RYR1*, which code for selenoprotein N (SEPN1) and skeletal muscle type ryanodine receptor (RyR1), respectively. A large percentage of classical MmD cases are attributed to nine identified mutations in SEPN1 [31]. While the function of SEPN1 is not known, selenoproteins are thought to function as catalysts for redox reactions. Additionally, SEPN1 contains a conserved EF hand-like calcium-binding domain and is also known to associate with intracellular ER/SR Ca^{2+} storage compartments [78, 94].

MmD has recently been linked to mutations in the *RYR1* gene. To date, three separate recessive mutations in RyR1 (V4849I, S3572S, and an intronic splicing site mutation) are known to result in a variant of MmD that also presents as a recessive form of CCD [30, 51, 79]. No *RYR1* mutations have been identified to date that re-

sult in MmD in the absence of CCD involvement, while all identified *SEPN1* mutations result solely in MmD. Therefore, the precise relationship between these diseases still remains undefined. The V4849I MmD/CCD mutation in RyR1 is located in MH/CCD region 3 and therefore may affect ion conduction of the SR Ca^{2+} release channel [51]. Interestingly, the P3527S MmD/CCD mutation is the only currently identified RyR1 disease mutation located clearly outside MH/CCD regions 1-3. Ferreiro et al. (2002) suggested that the P3527S mutation may alter channel regulation either by introducing a novel casein kinase phosphorylation site or disrupting putative calmodulin (3614-3643) and/or redox (C3635) regulatory sites just downstream from the proline mutation [30]. However, effects of the recessive V4849I and P3527S MmD/CCD mutations in RyR1 on SR Ca^{2+} release channel function and EC coupling have not been reported.

As discussed above, genetic studies have linked MmD to mutations in both selenoprotein N and RyR1. While its specific function(s) in skeletal muscle is unknown, selenoprotein N localizes to SR membranes, contains a highly conserved Ca^{2+} binding domain, and may act to regulate the local cellular redox state [78, 94]. On the other hand, the functional role of RyR1 in muscle is more thoroughly understood [33]. RyR1 tetramers function as SR Ca^{2+} release channels that provide the Ca^{2+} ions used for contraction during EC coupling. In addition, RyR1 channels are macromolecular signaling complexes whose activity reflects the integrated effects of numerous factors including phosphorylation, regulatory small molecules (including Ca^{2+}, Mg^{2+}, NO, and ATP), interaction with several key regulatory proteins [66], and the local redox environment around the channel [75]. Thus, it is tempting to speculate that MmD/CCD co-incidence may arise from altered redox regulation of the SR Ca^{2+} release channel and that mutations in either RyR1 or selenoprotein N alter this important regulatory control of release channel activity. Clearly, further work will be required to determine whether or not the RyR1 redox state, and consequently muscle Ca^{2+} homeostasis, is influenced by selenoprotein N and if so, if MmD mutations in RyR1 and/or selenoprotein N mutations disrupt this regulatory mechanism.

A novel and unexpected mutation in the RyR1 gene was very recently identified from a patient diagnosed with a particularly severe form of classic MmD associated with opthalmoplegia (the proband presented with severe scoliosis, respiratory insufficiency, and lost gait at 12 years of age). After screening for mutations in *RYR1*, a cryptic splice-site mutation +2990 bp from exon 101 was identified that results in a frameshift and premature stop codon prior to the last transmembrane domain [79]. Although the splice site intronic mutation allowed production of both normal and truncated transcripts, normal RyR1 mRNA was reduced by 90%. A similar quantitative reduction in functional SR Ca^{2+} channels would likely result in a large reduction in functioning EC coupling "units" and thus represents a unique form of "EC uncoupling" (reduced depolarization-induced Ca^{2+} release in the absence of increased SR Ca^{2+} leak) [79]. Additionally, it is also possible that if sufficiently stable, a large fraction of truncated RyR1 monomers might also exert a dominant-negative effect on release channel function by inhibiting wild-type tetramer formation. Functional studies of the effects of the truncated construct upon co-expression with wild-type RyR1 will be required to more rigorously test this hypothesis.

Core-Rod Myopathy

Nemaline rod myopathy (NM) is a rare congenital neuromuscular disorder affecting 1 in every 50,000 live births that can range clinically from a severe fatal neonatal form to a slowly progressive adult form [46]. NM is typically characterized by skeletal muscle weakness at birth (floppy infant syndrome), hypotonia, small muscles, slender extremities and proximal muscle weakness [102]. NM is also characterized by the presence of nemaline bodies (or rods) composed mostly of the Z-line protein α-actinin arranged in irregular clusters in the periphery and/or at the Z-line [19]. However, the pathology of rod formation and its relationship to disease phenotype are unknown. Nemaline rods, visualized using Gomori's trichrome stain, are present in both type I and II skeletal muscle fibers. Nemaline myopathy is known to arise from mutations in α-tropomyosin-3 (*TPM3*, autosomal dominant and recessive forms), α-actin (*ACTA1*, autosomal dominant and recessive forms), nebulin (*NEB*, autosomal recessive), β-tropomyosin (*TPM2*, autosomal recessive) and troponin T (*TNNT1*, autosomal recessive). Several studies have also reported the simultaneous occurrence of both central cores and nemaline rods in the same muscle biopsy [1, 11, 43, 82, 93, 102, 105, 117], suggesting a "core-rod myopathy" that represents a clinical overlap between CCD and NM. As in CCD, cores in core-rod myopathy are characterized as well-demarcated regions devoid of oxidative enzymes and mitochondria that extend throughout type I fibers. Until recently, the genetic basis of core-rod myopathy was only linked to mutations in the gene that encodes the skeletal muscle ryanodine receptor (*RYR1*), while classic NM has been associated with mutations to genes that encode thin filament proteins of the sarcomere [118]. However, Gommans and colleagues (2003) reported a family with core-rod myopathy that was linked to chromosome 15q21-q23, indicating the existence of an alternate genetic locus of core-rod myopathy [43].

Currently, three mutations in the RyR1 gene are associated with core-rod myopathy. Monnier et al. (2000) identified a Y4796C mutation in MH/CCD region 3 in one family [82]. The proband in this family tested strongly positive for MH susceptibility in a standardized IVCT. Additionally, resting Ca^{2+} levels were increased

and SR Ca^{2+} content was reduced following either heterologous expression of Y4796C channels in HEK293 cells [82] or homologous expression in dyspedic myotubes [6]. In addition, the sensitivity of voltage-gated SR Ca^{2+} release increased while its magnitude decreased in Y4796C-expressing dyspedic myotubes [6], similar to that observed previously for CCD mutations in MH/CCD regions 1 and 2 that result in increased SR Ca^{2+} leak [4]. Thus, the Y4796C mutation represents the first CCD mutation in MH/CCD region 3 shown so far to lead to increased SR Ca^{2+} leak following expression in dyspedic myotubes, demonstrating that the leaky channel mechanism is not linked solely to mutations in MH/CCD regions 1 and 2. Interestingly, separate studies have identified two different mutations of another amino acid (T4637I [23] and T4637A [102]) that result in a rather mild core-rod clinical phenotype. For example, the T4637I mutation resulted in the occurrence of rods in only ~10% of muscle fibers. Additionally, many patients possessing the T4637A mutation were given general anesthesia without experiencing an MH episode, therefore this mutation is unlikely to be strongly associated with MH unlike that observed for the Y4796C core-rod mutation [23, 102]. The functional effects of the Y4637A/I mutations on SR Ca^{2+} release channel function and EC coupling (leaky or EC uncoupling) have not been reported. In addition, it will be important for future work to determine the mechanisms by which mutations to T4637 and Y4796 promote rod pathology while nemaline rods are not observed for other CCD mutations in RyR1.

The identity of rod myopathy associated with mutations in RyR1 raises the question of how mutations in functionally distinct proteins (e.g. α-tropomyosin$_{slow}$, α-actin, nebulin, β-tropomyosin, troponin T, and RYR1) can result in a single common structural phenotype (rod formation). Based on extensive expression profiling of skeletal muscle biopsies obtained from patients with presumed mutations in *NEB or ACTA1,* it was suggested that Ca^{2+} homeostasis is likely to be altered in skeletal muscle of NM patients [101]. If NM mutations in genes coding for thin filament proteins result in elevations in resting Ca^{2+} levels similar to that documented for the Y4796C core-rod mutation in RyR1, then a possible common mechanism for rod formation may involve alterations in Ca^{2+}-regulated gene transcription (i.e. α-actin). However, if changes in Ca^{2+}-regulated gene transcription initiate nemaline rod biogenesis, then it is not clear why nemaline rods are not also observed for CCD mutations in MH/CCD regions 1 and 2, which also increase myoplasmic resting Ca^{2+} levels. Clearly, more work will be required to address this question.

Genetic diseases linked to mutations in the cardiac ryanodine receptor (RYR2)

Although a rich history of literature suggests an indirect involvement of RyR2 in cardiac diseases [42, 59, 72, 77, 88, 100, 121], recent evidence has indicated a more direct role of RyR2 in causing cardiac arrhythmias that lead to sudden cardiac death. CPVT and ARVD2 are autosomal dominant clinical disorders that are associated with missense mutations in RyR2 that confer a "gain of function" phenotype by promoting the trigger of extrasystoles and arrhythmogenesis [56, 95, 112]. Although the clinical phenotype for both disorders has been appreciated for a number of years now, genetic linkage of these disorders to the cardiac ryanodine receptor gene (*RYR2*) has only recently been demonstrated.

Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT)

Coumel et al. first described CPVT in 1978 as a novel clinical entity in which catecholaminergic-induced ventricular tachyarrhythmias occur in juveniles in the absence of structural heart disease [20]. A follow up study of these patients 16 years later showed that while resting ECGs and echocardiograms along with other clinical parameters remained normal in these individuals, physical exercise and other methods designed to increase heart rate triggered premature ventricular complexes that could precipitate into polymorphic and bidirectional ventricular tachycardia [58]. Interestingly, the ECG patterns in affected individuals closely resembled those of patients suffering from digitalis toxicity in which calcium overload triggers oscillatory SR Ca^{2+} release, activation of a Ca^{2+}-dependent sarcolemmal conductance (e.g. electrogenic Na^+/Ca^{2+} exchange), and the subsequent development of delayed after depolarizations (DADs) [32], [65].

Through genetic analysis of affected individuals in two families, Swan et al. (1999) mapped the gene locus of CPVT to chromosome 1q42-43 while pedigree analysis confirmed an autosomal dominant mode of inheritance [109]. Several genes have been mapped to this locus including RyR2 (*RYR2*), G-protein γ_4 subunit (*GNG4*), and the type-3 muscarinic receptor (*CHMR3*) [55, 89]. However, based on the observation that ECG recordings were consistent with arrhythmias triggered by oscillatory SR calcium release, the RyR2 gene was considered a leading candidate. This hypothesis was later confirmed in a study of 30 individuals showing symptoms of CPVT from four different families [56]. This study refined the genetic locus on chromosome 1q42-43 and identified *RYR2* as the defective gene causing the clinical phenotype. Three missense mutations (P2328S, Q4201R and V4653F) in RyR2 were identified from three of the four families investigated [56]. In an independent study published by Priori et al. (2001) of 30 CPVT probands, four additional single amino acid substitutions (S2246L, R2474S, N4104K and R4497C) were identified from a cohort of four families with a history of syncope and sudden death associated with physical or emotional stress [95]. Three of these mutations were sporadic mutations consistent with the conclusions

of Leenhardt et al. (2001) that many patients may represent the only affected individuals within a particular family [58]. However, the fourth mutation was found in five heterozygous members of the same family confirming an autosomal dominant mode of inheritance [95]. To date, a total of 15 missense mutations in RyR2 linked to CPVT have been identified (S2246L, E2311D, P2328S, R2474S, E3778F, G3946S, N4104K, Q4201R, R4497C, V4653F, V4711I, A4860G, I4867M, N4895D, and E4950K).

The cellular and ionic mechanisms by which CPVT mutations in RyR2 promote catecholaminergic-induced arrhythmias are controversial and are only now beginning to be unraveled. One hypothesis is based on functional studies of one of these mutants (R4497C) following heterologous expression in HEK293 cells [50]. This study found that compared to wild-type RyR2, R4497C channels exhibit increased activity at very low levels of Ca^{2+} that results in an increased frequency of spontaneous basal Ca^{2+} oscillations. These findings led the authors to suggest that CPVT mutations in RyR2 promote stress-induced ventricular arrhythmias by enhancing spontaneous RyR2 activity. Under non-stress conditions, normal Ca^{2+} homeostatic mechanisms may compensate for this defect. However, under conditions of SR Ca^{2+} overload induced during catecholaminergic stimulation, the mutant channels may support unwarranted diastolic SR Ca^{2+} release, thus resulting in the occurrence of stress-induced DADs [50].

An alternate arrhythmogenic mechanism proposed by Marks and colleagues involves a disruption of FKBP12.6 binding/regulation of RyR2 by the combined effects of PKA phosphorylation of RyR2 during adrenergic stimulation and intrinsic effects of the CPVT mutations. FKBP12.6 is an important modulator of RyR2 channel gating by acting to stabilize the channel in its closed state during diastole [14]. Under normal adrenergic stimulation, PKA phosphorylation of one or two RyR2 monomers increases release channel activity, and thus, SR Ca^{2+} release and contractility during cardiac EC coupling. However, during chronic hyperadrenergic disease states (e.g. heart failure), RyR2 release channels become hyperphosphorylated. Hyperphosphorylation of RyR2 promotes FKBP12.6 dissociation, increased release channel open probability during diastole and the development of DADs [70-72]. Marks and colleagues have proposed a similar mechanism for arrhythmogenesis caused by CPVT mutations in RyR2 during even normal levels of adrenergic stress. According to this hypothesis, CPVT mutations themselves reduce the affinity of FKBP12.6 for RyR2 such that an additional reduction in affinity resulting from a modest degree of PKA phosphorylation during normal stress is sufficient to fully dissociate FKBP12.6 from RyR2. Consistent with this hypothesis, Wehrens et al. (2003) found that FKBP12.6 binding to RyR2 is reduced by three different CPVT mutations (S2246L, R2474S, R4497C) and that PKA phosphorylation of each mutant resulted in a significantly higher channel open probability than that of wild-type RyR2 channels [119]. The authors suggested that the CPVT mutations coupled with even moderate PKA phosphorylation during normal stress would be sufficient to fully dissociate FKBP12.6 and lead to increased channel open probability, diastolic SR Ca^{2+} release, and the generation of arrhythmogenic DADs [119].

One potential problem with the unifying hypothesis proposed by Marks and colleagues is that identified CPVT mutations are widely dispersed across three very disparate regions of RyR2 (analogous to the MH/CCD regions 1-3 of RyR1). As it is somewhat unlikely that each region contributes equally to FKBP12.6 binding, it will be important for future work to more rigorously test this hypothesis by correlating the severity and clinical phenotype associated with each mutation with quantitative effects on the affinity of RyR2 for FKBP12.6. In addition, although residues in the central cytoplasmic region of RyR2 strongly influence FKBP12.6 binding to RyR2 [35], other regions of the protein have also been shown to contribute to FKBP12.6 binding [73, 124]. Thus, precise identification of all RyR2 regions important for determining FKBP12.6 binding would help to explain how mutations in vastly different regions of the primary sequence of RyR2 might influence the affinity for FKBP12.6 binding. Finally, additional uncertainty with regard to the possible role of altered FKBP12.6 regulation of RyR2 in the pathogenesis of CPVT comes from a recent study by George et al. (2003) who characterized Ca^{2+} release in HL-1 cardiomyocytes transfected with either wild-type RyR2 or one of several different CPVT-linked RyR2 mutants (S2246L, N4104K, R4497C). This study found that CPVT RyR2-expressing cardiomyocytes exhibited increased Ca^{2+} release and enhanced sensitivity to agonist activation (caffeine, 4-chloro-m-cresol and isoproterenol) and that these functional effects occurred in the absence of altered FKBP12.6 binding to RyR2 [40].

Arrhythmogenic Right Ventricular Dysplasia Type-2 (ARVD2)

A second clinical entity associated with mutations in RyR2 is arrhythmogenic right ventricular dysplasia type-2 (ARVD2), a subtype of arrhythmogenic right ventricular cardiomyopathies (ARVC). ARVCs are a group of diseases characterized by progressive degeneration of the right myocardial ventricle resulting from fibrous and fatty tissue deposition [9]. Another primary clinical manifestation of this group of diseases include exercise-induced arrhythmias that lead to syncope and sudden death [85]. Diagnosis of ARVC has been difficult due to the absence of a single test that could establish or exclude this disorder in individuals [21]. Several distinct forms of ARVC have been identified and ARVD2 stands out from the rest due to distinct stress-induced ventricular tachycardia and a high penetrance in

individuals independent of gender [85]. Pedigree analysis of ARVD2 affected families has demonstrated an autosomal dominant pattern of inheritance [112] and ARVD2 is now definitively linked to the gene for RyR2 on chromosome 1q42-43 [96]. Currently, a total of 6 missense mutations in RyR2 have been linked to ARVD2 (R176Q, R420W, L433P, N2386I, Y2392C, and T2504M).

Although functional studies have not yet been reported for ARVD2 mutations, pathophysiological mechanisms similar to those proposed for CPVT may contribute to stress-induced arrhythmogenesis in ARVD2 [112]. In this regard, a recent study used a yeast two-hybrid analysis to show that ARVD2 mutations exert a negative effect on the association of RyR2 and FKBP12.6 [111]. The ventricular myopathy observed in ARVD2 may result from erroneous Ca^{2+} signaling events that trigger death signals leading to apoptosis [68, 116], although the precise mechanisms that underlie this process are poorly understood. Finally, it is unknown how point mutations in RyR2 lead to a myopathy that localizes primarily to the right ventricular myocardium.

All 21 CPVT and ARVD2 mutations discussed here are located in three highly conserved regions of RyR2 (N-terminal, central, and C-terminal), suggesting involvement of these regions in regulating channel function. Interestingly these regions loosely correspond to three homologous regions in RyR1 shown to harbor the MH and CCD mutations [111]. Although both CPVT and ARVD2 exhibit stress-induced cardiac arrhythmias, the inevitable question still remains as to why mutations in the same gene that may exhibit similar arrhythmogenic mechanisms can result in a structural myocardial abnormality in one case and not in the other. One possibility is that CPVT and ARVD2 mutations may tend to localize to different functional domains. In fact, all identified ARVD2 mutations localize to only the N-terminal and central regions, while the CPVT mutations localize to the central and C-terminal domains. A similar genotype-phenotype dichotomy is also found for the CCD mutations in RyR1. CCD mutations located in the cytoplasmic region of RyR1 are associated with a "leaky channel" phenotype, while only mutations in the C-terminal pore-lining domain have been shown to exhibit "EC uncoupling" [4-6]. However, whether differences in clinical phenotype (e.g. ARVD2 versus CPVT) arise from mechanistic differences resulting from mutations to functionally distinct domains will require comparative functional studies of CPVT and ARVD2 mutant channels reconstituted within transgenic animals and/or muscle cell culture models where cardiac EC coupling can be assessed [44].

Conclusions and Perspectives

This review has examined the genetics, clinical properties, pathophysiology, and genotype-phenotype correlations for the currently identified ryanodinopathies; an assorted collection of inherited muscle disorders that arise from mutations in genes that encode the skeletal (*RYR1*) and cardiac (*RYR2*) muscle ryanodine receptors. In both cases, identified mutations are primarily located in three distinct regions in the linear RyR sequence (N-terminal, central, and C-terminal domains). Another similarity between the cardiac and skeletal muscle ryanodinopathies is that mutations are identified that result in two clinically distinct disorders: MH and CCD for RyR1 and CPVT and ARVD2 for RyR2. Moreover, only one of these disorders for each tissue (CCD and ARVD2) is known to result in a significant myopathic phenotype. Finally, although the cellular pathophysiologic mechanisms by which disease mutations in RyR1 and RyR2 lead to altered skeletal and cardiac muscle function are just beginning to be unraveled, recent evidence suggests that at least some of the RyR1 and RyR2 mutations lead to increased SR Ca^{2+} leak. However, as arrhythmogenesis resulting from RyR2 mutations most likely involves increased incidence of DADs secondary to uncontrolled diastolic Ca^{2+} release, it seems unlikely that any of the RyR2 mutations will operate via an "EC uncoupling" mechanism like that identified for CCD mutations in the pore region of RyR1. Consistent with this notion, although 11 different CPVT mutations have been identified in the C-terminal region of RyR2, none of these mutations appear to involve residues that comprise the putative pore-forming region (residues 4816-4841, [120]) or selectivity filter (residues 4820-4829, [123]).

Currently, the ryanodinopathies are only associated with inherited disorders in skeletal and cardiac muscle. However, given the widespread tissue distribution and functional roles of RyR Ca^{2+} release channels in neurons, glia, smooth muscle, and non-excitable cells (including pancreatic beta/acinar cells and lymphocytes), it would seem reasonable to also expect manifestations of the ryanodinopathies to extend to certain non-muscle related phenotypes/disorders. For example, since RyR2 is the most prominent RyR isoform expressed in the brain, CPVT and ARVD2 mutations in RyR2 (and possibly other mutations in RyR2 that do not lead to increased arrhythmogenesis) might also lead to altered CNS activity. A similar argument could also be made for disease mutations in RyR1 since these release channels are also expressed at lower levels in a variety of neuronal cells including cerebellar Purkinje cells, dentate gyrus of the hippocampus, CA1 and CA3 cells of the Ammons' horn and the olfactory bulb [99]. In addition, localized Ca^{2+} release through RyR2 channels (Ca^{2+} sparks) leads to local activation of Ca^{2+}-activated K^+ (BK) channels, hyperpolarization of vascular smooth muscle cells, and ultimately arteriolar vasodilation [86]. Thus, future work will be required to determine whether blood pressure control during adrenergic stimulation is also altered by CPVT/ARVD2 disease mutations in RyR2. Finally, the type III ryanodine receptor (RyR3) is known to contribute to Ca^{2+} release in the diaphragm

and during early developmental stages of skeletal muscle [18]. In addition, RyR3 also plays a role in certain forms of synaptic plasticity in the hippocampus [7, 34] and in regulating Ca^{2+} spark frequency and BK channel activation in vascular smooth muscle [60]. Given the vast array of RyR3 functional roles and its high conservation with RyR1 (~65% identity) and RyR2 (~68% identity), it would not be surprising if future genetic studies identified other inherited disorders that are linked to mutations in the gene for RyR3. For example, mutations in RyR3 (or even RyR2) that promote SR Ca^{2+} depletion in vascular smooth muscle might result in an inherited form of essential hypertension (by reducing Ca^{2+} spark mediated activation of BK channels). In addition, RyR mutations that lead to an increased frequency of spontaneous Ca^{2+} release within particular neuronal circuits could conceivably contribute to or exacerbate certain forms of epilepsy or other seizure disorders. However, one thing that seems certain is that future advances in identifying additional RyR-linked disorders and the fundamental mechanisms by which these mutations alter RyR function will increase our understanding of the pathophysiological mechanisms that underlie disease progression and clinical presentation of the inherited ryanodinopathies.

Address correspondence to:

Dr. Robert T. Dirksen; University of Rochester Medical Center; Department of Pharmacology and Physiology; 601 Elmwood Avenue; Rochester, NY 14642 U.S.A.; Tel: 585-275-4824; Fax: 585-273-2652; E-mail:Robert_Dirksen@urmc.rochester.edu

Non-Standard Abbreviations:

Excitation-contraction (EC); Dihydropyridine receptor (DHPR); Sarcoplasmic reticulum (SR); Ryanodine receptor (RyR); Malignant hyperthermia (MH); Central core disease (CCD); Multi-minicore disease (MmD); Nemaline myopathy (NM); Catecholaminergic polymorphic venticular tachycardia (CPVT); Arrhythmogenic right ventricular dysplasia (ARVD); *in vitro* contracture test (IVCT); Delayed after depolarization (DAD); Arrythmogenic right ventricular cardiomyopathies (ARVC); Ca^{2+}-activated K^+ channels (BK).

References

[1] Afifi AK, Smith JW, Zellweger H: Congenital non-progressive myopathy. *Neurology* 1965; 15: 371-381.

[2] Allen PD, Lopez JR, Sanchez V, Ryan JF, Sreter FA: EU 4093 decreases intracellular Ca2+ in skeletal muscle fibers from control and malignant hyperthermia-susceptible swine. *Anesthesiology* 1992; 76: 132-138.

[3] Avila G, Dirksen RT: Functional impact of the ryanodine receptor on the skeletal muscle L-type Ca^{2+} channel. *J Gen Physiol* 2000; 115: 467-480.

[4] Avila G, Dirksen RT: Functional effects of central core disease mutations in the cytoplasmic region of the skeletal muscle ryanodine receptor. *J Gen Physiol* 2001; 118: 277-290.

[5] Avila G, O'Brien JJ, Dirksen RT: Excitation-contraction uncoupling by a human central core disease mutation in the ryanodine receptor. *Proc Natl Acad Sci USA* 2001; 98: 4215-4220.

[6] Avila G, O'Connell KM, Dirksen RT: The pore region of the skeletal muscle ryanodine receptor is a primary locus for excitation-contraction uncoupling in central core disease. *J Gen Physiol* 2003; 121: 277-286.

[7] Balschun D, Wolfer DP, Bertocchini F, Barone V, Conti A, Zuschratter W, Missiaen L, Lipp HP, Frey JU, Sorrentino V: Deletion of the ryanodine receptor type 3 (RyR3) impairs forms of synaptic plasticity and spatial learning. *EMBO J* 1999; 18: 5264-5273.

[8] Balshaw D, Gao L, Meissner G: Luminal loop of the ryanodine receptor: a pore-forming segment? *Proc Natl Acad Sci USA* 1999; 96: 3345-3347.

[9] Basso C, Thiene G, Corrado D, Angelini A, Nava A, Valente M: Arrhythmogenic right ventricular cardiomyopathy. Dysplasia, dystrophy, or myocarditis? *Circulation* 1996; 94: 983-991.

[10] Bers DM: Cardiac excitation-contraction coupling. *Nature* 2002; 415: 198-205.

[11] Bethlem J, Arts WF, Dingemans KP: Common origin of rods, cores, miniature cores, and focal loss of cross-striations. *Arch Neurol* 1978; 35: 555-566.

[12] Beutner G, Sharma VK, Dirksen R, Sheu SS. Mitochondrial calcium regulation and heart disease. *Biophys J* 2003; 84: 206a-206a.

[13] Beutner G, Sharma VK, Giovannucci DR, Yule DI, Sheu SS: Identification of a ryanodine receptor in rat heart mitochondria. *J Biol Chem* 2001; 276: 21482-21488.

[14] Brillantes AB, Ondrias K, Scott A, Kobrinsky E, Ondriasova E, Moschella MC, Jayaraman T, Landers M, Ehrlich BE, Marks AR: Stabilization of calcium release channel (ryanodine receptor) function by FK506-binding protein. *Cell* 1994; 77: 513-523.

[15] Brinkmeier H, Kramer J, Kramer R, Iaizzo PA, Baur C, Lehmann-Horn F, Rudel R: Malignant hyperthermia causing Gly2435Arg mutation of the ryanodine receptor facilitates ryanodine-induced calcium release in myotubes. *Br J Anaesth* 1999; 83: 855-861.

[16] Brown RL, Pollock AN, Couchman KG, Hodges M, Hutchinson DO, Waaka R, Lynch P, McCarthy TV, Stowell KM: A novel ryanodine receptor mutation and genotype-phenotype correlation in a large malignant hyperthermia New Zealand Maori pedigree. *Hum Mol Genet* 2000; 9: 1515-1524.

[17] Censier K, Urwyler A, Zorzato F, Treves S: Intracellular calcium homeostasis in human primary muscle cells from malignant hyperthermia-susceptible and normal individuals. Effect Of overexpression of recombinant wild-type and Arg163Cys mutated ryanodine receptors. *J Clin Invest* 1998; 101: 1233-1242.

[18] Conti A, Gorza L, Sorrentino V: Differential distribution of ryanodine receptor type 3 (RyR3) gene product in mammalian skeletal muscles. *Biochem J* 1996; 316: 19-23.

[19] Corbett MA, Robinson CS, Dunglison GF, Yang N, Joya JE, Stewart AW, Schnell C, Gunning PW, North KN, Hardeman EC: A mutation in alpha-tropomyosin (slow) affects muscle strength, maturation and hypertrophy in a mouse model for nemaline myopathy. *Hum Mol Genet* 2001; 10: 317-328.

[20] Coumel P, Fidelle J, Lucet F, Attuel P, Bouvrain Y: Catecholamine Induced Severe Ventricular Arrhythmias with Stokes Adams Syndrome in Children: Report of Four Cases. *Br Heart J* 1978; 40: S28-S37.

[21] Danieli GA, Rampazzo A: Genetics of arrhythmogenic right ventricular cardiomyopathy. *Curr Opin Cardiol* 2002; 17: 218-221.

[22] Davis M, Brown R, Dickson A, Horton H, James D, Laing N, Marston R, Norgate M, Perlman D, Pollock N, Stowell K: Malignant hyperthermia associated with exercise-induced rhabdomyolysis or congenital abnormalities and a novel RYR1 mutation in New Zealand and Australian pedigrees. *Br J Anaesth* 2002; 88: 508-515.

[23] Davis MR, Haan E, Jungbluth H, Sewry C, North K, Muntoni F, Kuntzer T, Lamont P, Bankier A, Tomlinson P, Sanchez A, Walsh P, Nagarajan L, Oley C, Colley A, Gedeon A, Quinlivan R, Dixon J, James D, Muller CR, Laing NG: Principal mutation hotspot for central core disease and related myopathies in the C-terminal transmembrane region of the RYR1 gene. *Neuromuscul Disord* 2003; 13: 151-157.

[24] Dietze B, Henke J, Eichinger HM, Lehmann-Horn F, Melzer W: Malignant hyperthermia mutation Arg615Cys in the porcine ryanodine receptor alters voltage dependence of Ca^{2+} release. *J Physiol* 2000; 526: 507-514.

[25] Dirksen RT, Avila G: Altered ryanodine receptor function in central core disease: leaky or uncoupled Ca^{2+} release channels? *Trends Cardiovasc Med* 2002; 12: 189-197.

[26] Fabiato A: Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. *Am J Physiol* 1983; 245: C1-14.

[27] Fabiato A, Fabiato F: Calcium and cardiac excitation-contraction coupling. *Annu Rev Physiol* 1979; 41: 473-484.

[28] Fananapazir L, Dalakas MC, Cyran F, Cohn G, Epstein ND: Missense mutations in the beta-myosin heavy-chain gene cause central core disease in hypertrophic cardiomyopathy. *Proc Natl Acad Sci USA* 1993; 90: 3993-3997.

[29] Ferreiro A, Fardeau M: 80th ENMC International Workshop on Multi-Minicore Disease: 1st International MmD Workshop. 12-13th May, 2000, Soestduinen, The Netherlands. *Neuromuscul Disord* 2002; 12: 60-68.

[30] Ferreiro A, Monnier N, Romero NB, Leroy JP, Bonnemann C, Haenggeli CA, Straub V, Voss WD, Nivoche Y, Jungbluth H, Lemainque A, Voit T, Lunardi J, Fardeau M, Guicheney P: A recessive form of central core disease, transiently presenting as multi-minicore disease, is associated with a homozygous mutation in the ryanodine receptor type 1 gene. *Ann Neurol* 2002; 51: 750-759.

[31] Ferreiro A, Quijano-Roy S, Pichereau C, Moghadaszadeh B, Goemans N, Bonnemann C, Jungbluth H, Straub V, Villanova M, Leroy JP, Romero NB, Martin JJ, Muntoni F, Voit T, Estournet B, Richard P, Fardeau M, Guicheney P: Mutations of the selenoprotein N gene, which is implicated in rigid spine muscular dystrophy, cause the classical phenotype of multiminicore disease: reassessing the nosology of early-onset myopathies. *Am J Hum Genet* 2002; 71: 739-749.

[32] Fozzard HA: Afterdepolarizations and triggered activity. *Basic Res Cardiol* 1992; 87 Suppl 2: 105-113.

[33] Franzini-Armstrong C, Protasi F: Ryanodine receptors of striated muscles: a complex channel capable of multiple interaction. *Physiol Rev* 1997; 77: 699-729.

[34] Futatsugi A, Kato K, Ogura H, Li ST, Nagata E, Kuwajima G, Tanaka K, Itohara S, Mikoshiba K: Facilitation of NMDAR-independent LTP and spatial learning in mutant mice lacking ryanodine receptor type 3. *Neuron* 1999; 24: 701-713.

[35] Gaburjakova M, Gaburjakova J, Reiken S, Huang F, Marx SO, Rosemblit N, Marks AR: FKBP12 binding modulates ryanodine receptor channel gating. *J Biol Chem* 2001; 276: 16931-16935.

[36] Gallant EM, Jordan RC: Porcine malignant hyperthermia: genotype and contractile threshold of immature muscles. *Muscle & Nerve* 1996; 19: 68-73.

[37] Gallant EM, Lentz LR: Excitation-contraction coupling in pigs heterozygous for malignant hyperthermia. *Am J Physiol* 1992; 262: C422-C426.

[38] Galli L, Orrico A, Cozzolino S, Pietrini V, Tegazzin V, Sorrentino V: Mutations in the RYR1 gene in Italian patients at risk for malignant hyperthermia: evidence for a cluster of novel mutations

in the C-terminal region. *Cell Calcium* 2002; 32: 143-151.

[39] Gao L, Balshaw D, Xu L, Tripathy A, Xin C, Meissner G: Evidence for a role of the lumenal M3-M4 loop in skeletal muscle Ca^{2+} release channel (ryanodine receptor) activity and conductance. *Biophys J* 2000; 79: 828-840.

[40] George CH, Higgs GV, Lai FA: Ryanodine Receptor Mutations Associated With Stress-Induced Ventricular Tachycardia Mediate Increased Calcium Release in Stimulated Cardiomyocytes. *Circ Res* 2003; *In Press*.

[41] Girard T, Cavagna D, Padovan E, Spagnoli G, Urwyler A, Zorzato F, Treves S: B-lymphocytes from malignant hyperthermia-susceptible patients have an increased sensitivity to skeletal muscle ryanodine receptor activators. *J Biol Chem* 2001; 276: 48077-48082.

[42] Gomez AM, Valdivia HH, Cheng H, Lederer MR, Santana LF, Cannell MB, McCune SA, Altschuld RA, Lederer WJ: Defective excitation-contraction coupling in experimental cardiac hypertrophy and heart failure. *Science* 1997; 276: 800-806.

[43] Gommans IM, Davis M, Saar K, Lammens M, Mastaglia F, Lamont P, van Duijnhoven G, ter Laak HJ, Reis A, Vogels OJ, Laing N, van Engelen BG, Kremer H: A locus on chromosome 15q for a dominantly inherited nemaline myopathy with core-like lesions. *Brain* 2003; 126: 1545-1551.

[44] Goonasekera S, Dirksen RT. Molecular Reconstitution of Cardiac EC Coupling in Dyspedic Myotubes. *Biophys J* 2003; 84: 433a.

[45] Grabner M, Dirksen RT, Suda N, Beam KG: The II-III loop of the skeletal muscle dihydropyridine receptor is responsible for the Bi-directional coupling with the ryanodine receptor. *J Biol Chem* 1999; 274: 21913-21919.

[46] Gurgel-Giannetti J, Reed U, Bang ML, Pelin K, Donner K, Marie SK, Carvalho M, Fireman MA, Zanoteli E, Oliveira AS, Zatz M, Wallgren-Pettersson C, Labeit S, Vainzof M: Nebulin expression in patients with nemaline myopathy. *Neuromuscul Disord* 2001; 11: 154-162.

[47] Hayashi K, Miller RG, Brownell AK: Central core disease: ultrastructure of the sarcoplasmic reticulum and T-tubules. *Muscle & Nerve* 1989; 12: 95-102.

[48] Iaizzo PA, Klein W, Lehmann-Horn F: Fura-2 detected myoplasmic calcium and its correlation with contracture force in skeletal muscle from normal and malignant hyperthermia susceptible pigs. *Pflugers Arch* 1988; 411: 648-653.

[49] Ikemoto N, Yamamoto T: Postulated role of interdomain interaction within the ryanodine receptor in Ca^{2+} channel regulation. *Trends Cardiovasc Med* 2000; 10: 310-316.

[50] Jiang D, Xiao B, Zhang L, Chen SR: Enhanced basal activity of a cardiac Ca^{2+} release channel (ryanodine receptor) mutant associated with ventricular tachycardia and sudden death. *Circ Res* 2002; 91: 218-225.

[51] Jungbluth H, Muller CR, Halliger-Keller B, Brockington M, Brown SC, Feng L, Chattopadhyay A, Mercuri E, Manzur AY, Ferreiro A, Laing NG, Davis MR, Roper HP, Dubowitz V, Bydder G, Sewry CA, Muntoni F: Autosomal recessive inheritance of RYR1 mutations in a congenital myopathy with cores. *Neurology* 2002; 59: 284-287.

[52] Jungbluth H, Sewry CA, Brown SC, Nowak KJ, Laing NG, Wallgren-Pettersson C, Pelin K, Manzur AY, Mercuri E, Dubowitz V, Muntoni F: Mild phenotype of nemaline myopathy with sleep hypoventilation due to a mutation in the skeletal muscle alpha-actin (ACTA1) gene. *Neuromuscul Disord* 2001; 11: 35-40.

[53] Jungbluth H, Sewry CA, Muntoni F: What's new in neuromuscular disorders? The congenital myopathies. *Eur J Paediatr Neurol* 2003; 7: 23-30.

[54] Jurkat-Rott K, McCarthy T, Lehmann-Horn F: Genetics and pathogenesis of malignant hyperthermia. *Muscle & Nerve* 2000; 23: 4-17.

[55] Kalyanaraman S, Copeland NG, Gilbert DG, Jenkins NA, Gautam N: Structure and chromosomal localization of mouse G protein subunit gamma 4 gene. *Genomics* 1998; 49: 147-151.

[56] Laitinen PJ, Brown KM, Piippo K, Swan H, Devaney JM, Brahmbhatt B, Donarum EA, Marino M, Tiso N, Viitasalo M, Toivonen L, Stephan DA, Kontula K: Mutations of the cardiac ryanodine receptor (RyR2) gene in familial polymorphic ventricular tachycardia. *Circulation* 2001; 103: 485-490.

[57] Launikonis BS, Barnes M, Stephenson DG: Identification of the coupling between skeletal muscle store-operated Ca^{2+} entry and the inositol trisphosphate receptor. *Proc Natl Acad Sci USA* 2003; 100: 2941-2944.

[58] Leenhardt A, Lucet V, Denjoy I, Grau F, Ngoc DD, Coumel P: Catecholaminergic polymorphic ventricular tachycardia in children. A 7-year follow-up of 21 patients. *Circulation* 1995; 91: 1512-1519.

[59] Lindner M, Erdmann E, Beuckelmann DJ: Calcium content of the sarcoplasmic reticulum in isolated ventricular myocytes from patients with terminal heart failure. *J Mol Cell Cardiol* 1998; 30: 743-749.

[60] Lohn M, Lauterbach B, Haller H, Pongs O, Luft FC, Gollasch M: b(1)-Subunit of BK channels regulates arterial wall Ca^{2+} and diameter in mouse cerebral arteries. *J Appl Physiol* 2001; 91: 1350-1354.

[61] Loke J, MacLennan DH: Malignant hyperthermia and central core disease: disorders of Ca^{2+} release channels. *Am J Med* 1998; 104: 470-486.

[62] Lopez JR, Alamo LA, Jones DE, Papp L, Allen PD, Gergely J, Sreter FA: $[Ca^{2+}]_i$ in muscles of malignant hyperthermia susceptible pigs determined in vivo with Ca^{2+} selective microelectrodes. *Muscle & Nerve* 1986; 9: 85-86.

[63] Lopez JR, Gerardi A, Lopez MJ, Allen PD: Effects of dantrolene on myoplasmic free Ca^{2+} measured in vivo in patients susceptible to malignant hyperthermia. *Anesthesiology* 1992; 76: 711-719.

[64] Lynch PJ, Tong J, Lehane M, Mallet A, Giblin L, Heffron JJ, Vaughan P, Zafra G, MacLennan DH, McCarthy TV: A mutation in the transmembrane/luminal domain of the ryanodine receptor is associated with abnormal Ca^{2+} release channel function and severe central core disease. *Proc Natl Acad Sci USA* 1999; 96: 4164-4169.

[65] Ma G, Brady WJ, Pollack M, Chan TC: Electrocardiographic manifestations: digitalis toxicity. *J Emerg Med* 2001; 20: 145-152.

[66] MacKrill JJ: Protein-protein interactions in intracellular Ca^{2+}-release channel function. *Biochem J* 1999; 337: 345-361.

[67] MacLennan DH, Phillips MS: Malignant hyperthermia. *Science* 1992; 256: 789-794.

[68] Mallat Z, Tedgui A, Fontaliran F, Frank R, Durigon M, Fontaine G: Evidence of apoptosis in arrhythmogenic right ventricular dysplasia. *N Engl J Med* 1996; 335: 1190-1196.

[69] Manzur AY, Sewry CA, Ziprin J, Dubowitz V, Muntoni F: A severe clinical and pathological variant of central core disease with possible autosomal recessive inheritance. *Neuromuscul Disord* 1998; 8: 467-473.

[70] Marks AR: Cardiac intracellular calcium release channels: role in heart failure. *Circ Res* 2000; 87: 8-11.

[71] Marks AR: Ryanodine receptors/calcium release channels in heart failure and sudden cardiac death. *J Mol Cell Cardiol* 2001; 33: 615-624.

[72] Marx SO, Reiken S, Hisamatsu Y, Jayaraman T, Burkhoff D, Rosemblit N, Marks AR: PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell* 2000; 101: 365-376.

[73] Masumiya H, Wang R, Zhang J, Xiao B, Chen SR: Localization of the 12.6-kDa FK506-binding protein (FKBP12.6) binding site to the NH2-terminal domain of the cardiac Ca^{2+} release channel (ryanodine receptor). *J Biol Chem* 2003; 278: 3786-3792.

[74] McCarthy TV, Quane KA, Lynch PJ: Ryanodine receptor mutations in malignant hyperthermia and central core disease. *Hum Mutat* 2000; 15: 410-417.

[75] Meissner G: Regulation of mammalian ryanodine receptors. *Front Biosci* 2002; 7: d2072-d2080.

[76] Mickelson JR, Louis CF: Malignant hyperthermia: excitation-contraction coupling, Ca^{2+} release channel, and cell Ca^{2+} regulation defects. *Physiol Rev* 1996; 76: 537-592.

[77] Milnes JT, MacLeod KT: Reduced ryanodine receptor to dihydropyridine receptor ratio may underlie slowed contraction in a rabbit model of left ventricular cardiac hypertrophy. *J Mol Cell Cardiol* 2001; 33: 473-485.

[78] Moghadaszadeh B, Petit N, Jaillard C, Brockington M, Roy SQ, Merlini L, Romero N, Estournet B, Desguerre I, Chaigne D, Muntoni F, Topaloglu H, Guicheney P: Mutations in SEPN1 cause congenital muscular dystrophy with spinal rigidity and restrictive respiratory syndrome. *Nat Genet* 2001; 29: 17-18.

[79] Monnier N, Ferreiro A, Marty I, Labarre-Vila A, Mezin P, Lunardi J: A homozygous splicing mutation causing a depletion of skeletal muscle RYR1 is associated with multi-minicore disease congenital myopathy with ophthalmoplegia. *Hum Mol Genet* 2003; 12: 1171-1178.

[80] Monnier N, Procaccio V, Stieglitz P, Lunardi J: 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* 1997; 60: 1316-1325.

[81] Monnier N, Romero NB, Lerale J, Landrieu P, Nivoche Y, Fardeau M, Lunardi J: 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* 2001; 10: 2581-2592.

[82] Monnier N, Romero NB, Lerale J, Nivoche Y, Qi D, MacLennan DH, Fardeau M, Lunardi J: 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 Mol Genet* 2000; 9: 2599-2608.

[83] Nabauer M, Callewaert G, Cleemann L, Morad M: Regulation of calcium release is gated by calcium current, not gating charge, in cardiac myocytes. *Science* 1989; 244: 800-803.

[84] Nakai J, Dirksen RT, Nguyen HT, Pessah IN, Beam KG, Allen PD: Enhanced dihydropyridine receptor channel activity in the presence of ryanodine receptor. *Nature* 1996; 380: 72-75.

[85] Nava A, Canciani B, Daliento L, Miraglia G, Buja G, Fasoli G, Martini B, Scognamiglio R, Thiene G: Juvenile sudden death and effort ventricular tachycardias in a family with right ventricular cardiomyopathy. *Int J Cardiol* 1988; 21: 111-126.

[86] Nelson MT, Cheng H, Rubart M, Santana LF, Bonev AD, Knot HJ, Lederer WJ: Relaxation of arterial smooth muscle by calcium sparks. *Science* 1995; 270: 633-637.

[87] Nelson TE: Malignant hyperthermia: a pharmacogenetic disease of Ca^{2+} regulating proteins. *Curr Mol Med* 2002; 2: 347-369.

[88] Ohkusa T, Ueyama T, Yamada J, Yano M, Fujumura Y, Esato K, Matsuzaki M: Alterations in cardiac sarcoplasmic reticulum Ca^{2+} regulatory proteins in the atrial tissue of patients with chronic atrial fibrillation. *J Am Coll Cardiol* 1999; 34: 255-263.

[89] Otsu K, Fujii J, Periasamy M, Difilippantonio M, Uppender M, Ward DC, MacLennan DH: Chromosome mapping of five human cardiac and skeletal muscle sarcoplasmic reticulum protein genes. *Genomics* 1993; 17: 507-509.

[90] Otsu K, Nishida K, Kimura Y, Kuzuya T, Hori M, Kamada T, Tada M: The point mutation Arg615-->Cys in the Ca^{2+} release channel of skeletal sarcoplasmic reticulum is responsible for hypersensitivity to caffeine and halothane in malignant hyperthermia. *J Biol Chem* 1994; 269: 9413-9415.

[91] Otsu K, Willard HF, Khanna VK, Zorzato F, Green NM, MacLennan DH: Molecular cloning of cDNA encoding the Ca^{2+} release channel (ryanodine receptor) of rabbit cardiac muscle sarcoplasmic reticulum. *J Biol Chem* 1990; 265: 13472-13483.

[92] Oyamada H, Oguchi K, Saitoh N, Yamazawa T, Hirose K, Kawana Y, Wakatsuki K, Tagami M, Hanaoka K, Endo M, Iino M: Novel mutations in C-terminal channel region of the ryanodine receptor in malignant hyperthermia patients. *Jpn J Pharmacol* 2002; 88: 159-166.

[93] Pallagi E, Molnar M, Molnar P, Dioszeghy P: Central core and nemaline rods in the same patient. *Acta Neuropathologica* 1998; 96: 211-214.

[94] Petit N, Lescure A, Rederstorff M, Krol A, Moghadaszadeh B, Wewer UM, Guicheney P: Selenoprotein N: an endoplasmic reticulum glycoprotein with an early developmental expression pattern. *Hum Mol Genet* 2003; 12: 1045-1053.

[95] Priori SG, Napolitano C, Tiso N, Memmi M, Vignati G, Bloise R, Sorrentino V, Danieli GA: Mutations in the Cardiac Ryanodine Receptor Gene (hRyR2) Underlie Catecholaminergic Polymorphic Ventricular Tachycardia. *Circulation* 2001; 103: 196-200.

[96] Rampazzo A, Nava A, Erne P, Eberhard M, Vian E, Slomp P, Tiso N, Thiene G, Danieli GA: A new locus for arrhythmogenic right ventricular cardiomyopathy (ARVD2) maps to chromosome 1q42-q43. *Hum Mol Genet* 1995; 4: 2151-2154.

[97] Rios E, Brum G: Involvement of dihydropyridine receptors in excitation-contraction coupling in skeletal muscle. *Nature* 1987; 325: 717-720.

[98] Rizzuto R, Pinton P, Carrington W, Fay FS, Fogarty KE, Lifshitz LM, Tuft RA, Pozzan T: Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca^{2+} responses. *Science* 1998; 280: 1763-1766.

[99] Rossi D, Sorrentino V: Molecular genetics of ryanodine receptors Ca^{2+}-release channels. *Cell Calcium* 2002; 32: 307-319.

[100] Sainte BC, Allen PD, Dambrin G, Rannou F, Marty I, Trouve P, Bors V, Pavie A, Gandgjbakch I, Charlemagne D: Cardiac calcium release channel (ryanodine receptor) in control and cardiomyopathic human hearts: mRNA and protein contents are differentially regulated. *J Mol Cell Cardiol* 1997; 29: 1237-1246.

[101] Sanoudou D, Haslett JN, Kho AT, Guo S, Gazda HT, Greenberg SA, Lidov HG, Kohane IS, Kunkel LM, Beggs AH: Expression profiling reveals altered satellite cell numbers and glycolytic enzyme transcription in nemaline myopathy muscle. *Proc Natl Acad Sci USA* 2003; 100: 4666-4671.

[102] Scacheri PC, Hoffman EP, Fratkin JD, Semino-Mora C, Senchak A, Davis MR, Laing NG, Vedanarayanan V, Subramony SH: A novel ryanodine receptor gene mutation causing both cores and rods in congenital myopathy. *Neurology* 2000; 55: 1689-1696.

[103] Schneider MF, Chandler WK: Voltage dependent charge movement of skeletal muscle: a possible step in excitation-contraction coupling. *Nature* 1973; 242: 244-246.

[104] Scoote M, Williams AJ: The cardiac ryanodine receptor (calcium release channel): emerging role in heart failure and arrhythmia pathogenesis. *Cardiovasc Res* 2002; 56: 359-372.

[105] Seitz RJ, Toyka KV, Wechsler W: Adult-onset mixed myopathy with nemaline rods, minicores, and central cores: a muscle disorder mimicking polymyositis. *J Neurol* 1984; 231: 103-108.

[106] Sewry CA, Muller C, Davis M, Dwyer JS, Dove J, Evans G, Schroder R, Furst D, Helliwell T, Laing N, Quinlivan RC: The spectrum of pathology in central core disease. *Neuromuscul Disord* 2002; 12: 930-938.

[107] Shuaib A, Paasuke RT, Brownell KW: Central core disease. Clinical features in 13 patients. *Medicine* 1987; 66: 389-396.

[108] Shy GM, Magee KR: A new congenital nonprogressive myopathy. *Brain* 1956; 79: 610-621.

[109] Swan H, Piippo K, Viitasalo M, Heikkila P, Paavonen T, Kainulainen K, Kere J, Keto P, Kontula K, Toivonen L: Arrhythmic disorder mapped to chromosome 1q42-q43 causes malignant polymorphic ventricular tachycardia in structurally normal hearts. *J Am Coll Cardiol* 1999; 34: 2035-2042.

[110] Tilgen N, Zorzato F, Halliger-Keller B, Muntoni F, Sewry C, Palmucci LM, Schneider C, Hauser E, Lehmann-Horn F, Muller CR, Treves S: Identification of four novel mutations in the C-terminal

membrane spanning domain of the ryanodine receptor 1: association with central core disease and alteration of calcium homeostasis. *Hum Mol Genet* 2001; 10: 2879-2887.

[111] Tiso N, Salamon M, Bagattin A, Danieli GA, Argenton F, Bortolussi M: The binding of the RyR2 calcium channel to its gating protein FKBP12.6 is oppositely affected by ARVD2 and VTSIP mutations. *Biochem Biophys Res Commun* 2001; 299: 594-598.

[112] Tiso N, Stephan DA, Nava A, Bagattin A, Devaney JM, Stanchi F, Larderet G, Brahmbhatt B, Brown K, Bauce B, Muriago M, Basso C, Thiene G, Danieli GA, Rampazzo A: Identification of mutations in the cardiac ryanodine receptor gene in families affected with arrhythmogenic right ventricular cardiomyopathy type 2 (ARVD2). *Hum Mol Genet* 2001; 10: 189-194.

[113] Tong J, McCarthy TV, MacLennan DH: Measurement of resting cytosolic Ca^{2+} concentrations and Ca^{2+} store size in HEK-293 cells transfected with malignant hyperthermia or central core disease mutant Ca^{2+} release channels. *J Biol Chem* 1999; 274: 693-702.

[114] Tong J, Oyamada H, Demaurex N, Grinstein S, McCarthy TV, MacLennan DH: Caffeine and halothane sensitivity of intracellular Ca^{2+} release is altered by 15 calcium release channel (ryanodine receptor) mutations associated with malignant hyperthermia and/or central core disease. *J Biol Chem* 1997; 272: 26332-26339.

[115] Treves S, Larini F, Menegazzi P, Steinberg TH, Koval M, Vilsen B, Andersen JP, Zorzato F: Alteration of intracellular Ca^{2+} transients in COS-7 cells transfected with the cDNA encoding skeletal-muscle ryanodine receptor carrying a mutation associated with malignant hyperthermia. *Biochem J* 1994; 301: 661-665.

[116] Valente M, Calabrese F, Thiene G, Angelini A, Basso C, Nava A, Rossi L: In vivo evidence of apoptosis in arrhythmogenic right ventricular cardiomyopathy. *Am J Pathol* 1998; 152: 479-484.

[117] Vallat JM, de Lumley L, Loubet A, Leboutet MJ, Corvisier N, Umdenstock R: Coexistence of minicores, cores, and rods in the same muscle biopsy. A new example of mixed congenital myopathy. *Acta Neuropathol(Berl)* 1982; 58: 229-232.

[118] Wallgren-Pettersson C, Laing NG: Report of the 83rd ENMC International Workshop: 4th Workshop on Nemaline Myopathy, 22-24 September 2000, Naarden, The Netherlands. *Neuromuscul Disord* 2001; 11: 589-595.

[119] Wehrens XH, Lehnart SE, Huang F, Vest JA, Reiken SR, Mohler PJ, Sun J, Guatimosim S, Song LS, Rosemblit N, D'Armiento JM, Napolitano C, Memmi M, Priori SG, Lederer WJ, Marks AR: FKBP12.6 deficiency and defective calcium release channel (ryanodine receptor) function linked to exercise-induced sudden cardiac death. *Cell* 2003; 113: 829-840.

[120] Williams AJ, West DJ, Sitsapesan R: Light at the end of the Ca^{2+}-release channel tunnel: structures and mechanisms involved in ion translocation in ryanodine receptor channels. *Q Rev Biophys* 2001; 34: 61-104.

[121] Yamamoto T, Yano M, Kohno M, Hisaoka T, Ono K, Tanigawa T, Saiki Y, Hisamatsu Y, Ohkusa T, Matsuzaki M: Abnormal Ca^{2+} release from cardiac sarcoplasmic reticulum in tachycardia-induced heart failure. *Cardiovasc Res* 1999; 44: 146-155.

[122] Yang T, Ta TA, Pessah IN, Allen PD: Functional defects in six ryanodine receptor isoform-1 (RyR1) mutations associated with malignant hyperthermia and their impact on skeletal excitation-contraction coupling. *J Biol Chem* 2003; 278: 14211-14218.

[123] Zhao M, Li P, Li X, Zhang L, Winkfein RJ, Chen SR: Molecular identification of the ryanodine receptor pore-forming segment. *J Biol Chem* 1999; 274: 25971-25974.

[124] Zissimopoulos S, Lai A: Evidence for a FKBP12.6 binding site at the C-terminus of thecardiac ryanodine receptor. *Biophys J* 2002; 82: 59a.