Journal of Neuromuscular Diseases 10 (2023) 135–154
DOI 10.3233/JND-221609
IOS Press

Meeting Report

RYR-1-Related Diseases International Research Workshop: From Mechanisms to Treatments Pittsburgh, PA, U.S.A., 21-22 July 2022

Thomas N. O'Connor[a,1], Luuk R. van den Bersselaar[b,c,1], Yu Seby Chen[d,1], Stefan Nicolau[e,1], Brentney Simon[f], Andrew Huseth[f], Joshua J. Todd[g], Filip Van Petegem[d], Anna Sarkozy[h], Michael F. Goldberg[f], Nicol C. Voermans[b] and Robert T. Dirksen[a,*] on behalf of the RYR1 Myopathy Consortium[2]

[a]*Department of Pharmacology and Physiology, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA*
[b]*Department of Neurology, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen, the Netherlands*
[c]*Malignant Hyperthermia Investigation Unit, Department of Anaesthesia, Canisius Wilhelmina Hospital, Nijmegen, the Netherlands*
[d]*Department of Biochemistry and Molecular Biology, The Life Sciences Institute, The University of British Columbia, Vancouver, BC, Canada*
[e]*Center for Gene Therapy, Nationwide Children's Hospital, Columbus, OH, USA*
[f]*The RYR-1 Foundation, Pittsburgh, PA, USA*
[g]*National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, USA*
[h]*The Dubowitz Neuromuscular Centre, Institute of Child Health and Great Ormond Street Hospital for Children, London, UK*

Pre-press 16 November 2022
Published 3 January 2023

ABBREVIATIONS

CCD	central core disease
CNM	centronuclear myopathy
CFTD	congenital fiber type disproportion
CM	congenital myopathy
EC	excitation-contraction
ER	endoplasmic reticulum
ENMC	European Neuromuscular Centre
ERM	exertional rhabdomyolysis
MH	malignant hyperthermia
MHS	malignant hyperthermia susceptibility
MmD	multi-minicore disease
RYR1	type 1 ryanodine receptor
RYR1-RD	RYR1-related disease
RYR1-RM	RYR1-related myopathy

[1]Contributed equally to this work.

[2]Members listed at the end of this article as workshop participants.

*Correspondence to: Robert T. Dirksen, Department of Pharmacology and Physiology, University of Rochester, 601 Elmwood Avenue, Rochester, NY 14642, USA. E-mail: Robert_Dirksen@URMC.Rochester.edu.

ISSN 2214-3599/$35.00

SERCA1	type 1 sarco-endoplasmic reticulum Ca^{2+} ATPase
SR	sarcoplasmic reticulum
sgRNA	single-guide RNA
T-tubule	transverse tubule
NAC	N-acetylcysteine

INTRODUCTION AND OVERVIEW

Preparation for and aims of research workshop

The "RYR-1-Related Diseases International Research Workshop: From Mechanisms to Treatments," held from July 21 – July, 22, 2022, was the first-ever patient-led international research workshop devoted exclusively to RYR1-related diseases (RYR1-RD). The workshop organizing committee was led by Robert Dirksen and included: Andrew Huseth, Anna Sarkozy, Brentney Simon, Filip Van Petegem, and Nicol Voermans. The committee held regular meetings to discuss the aims, prepare the program, and reach a consensus on clinical and research priorities.

The overall scientific goal of the workshop was to provide a forum that united leading international RYR1 disease experts (researchers, clinicians, and geneticists) with affected individuals, family members, and patient advocates to share knowledge, exchange ideas, form collaborations, and develop new strategies for finding effective therapies. Additional objectives were to develop consensus recommendations for clinical/research priorities, create actionable items needed to move the field forward, and provide a platform for trainees to engage with established leaders in the RYR1 field and individuals affected or afflicted by RYR1-related diseases.

RYR1-related diseases

Congenital myopathies (CMs) result from pathogenic variants in over 40 genes that encode proteins involved in skeletal muscle Ca^{2+} homeostasis, excitation–contraction (EC) coupling, and sarcomere assembly/function, with pathogenic variants in the gene that encodes the type I ryanodine receptor (*RYR1*) representing the most frequent cause [1–4].

The *RYR1* gene product (RYR1) is a Ca^{2+} release channel in the terminal cisternae of the sarcoplasmic reticulum (SR) of skeletal muscle that is required for EC coupling [5]. Pathogenic variants in the *RYR1* gene result in a wide range of muscle disorders that includes malignant hyperthermia susceptibility (MHS), central core disease (CCD), multi-minicore disease (MmD), centronuclear myopathy (CNM), and congenital fiber type disproportion (CFTD). These conditions collectively comprise the most common genetic cause of non-dystrophic myopathy [1–4]. The subclass of all RYR1-RD that exhibit significant muscle weakness are referred to as RYR1-related myopathies (RYR1-RM).

The most severe cases of RYR1-RM exhibit a recessive pattern of inheritance, present during infancy with skeletal muscle hypotrophy and weakness, involve respiratory insufficiency, short stature, and are characterized by a marked reduction in RYR1 protein expression in skeletal muscle [6]. Many of these children are non-ambulant, require ventilator assistance, and experience severe disability and occasionally premature death [7]. Despite its severity, relatively high prevalence, and association with significant disability and early mortality, there are no treatments or disease-modifying therapies for RYR1-RM and care is strictly supportive [8, 9]. Thus, there is a clear unmet need to develop, test, and validate new treatments for these diseases [10].

RYR1-RD International Research Workshop: From Mechanisms to Treatments

The scientific program of this workshop focused on several exciting new clinical and basic research advancements in the RYR1-RD field since the previous related international workshop, co-hosted by European Neuromuscular Centre (ENMC) and The RYR-1 Foundation, held in Naarden, the Netherlands, in January 2016 [11]. These recent advances include: 1) development of the first mouse models of severe recessive RYR1 myopathy [12, 13]; 2) establishment of expert panels to assess RYR1 variant pathogenicity [14]; 3) development of comprehensive Clinical Care Guidelines translated into eight languages (www.ryr1.org/ccg); 4) the wide availability of diagnostic next generation sequencing resulting in a further expansion in understanding of the complex clinical spectrum of RYR1-RD; 5) the completion of a series of cross-sectional, retrospective and short term prospective natural history studies on RYR1-RM; 6) completion of the first phase I/II clinical trial in RYR1-RM patients (NCT02362425) [15]; and 7) recent completion of a second phase I clinical trial (NCT04141670).

These advancements presented an opportune time to bring together leading RYR1-RD researchers, geneticists and clinicians with affected individuals,

family members and patient advocates to not only discuss these advances, but also determine and assess current needs/opportunities in the field, set clinical/research priorities, and develop an action plan to move the field forward toward a more comprehensive understanding of the pathogenesis and treatment of RYR1-RD. It is this exciting progress and need for future planning that provided the basis for this workshop.

RYR-1 International Family Conference

For a rare condition like RYR1-RD, providing a forum for affected individuals and families to meet others like them has significant psychological, social and medical value. When an affected individual or their family members seek to find others who understand what they are experiencing, a family conference becomes an invaluable and necessary resource. Family conferences help to connect patients and family members with each other, with resources provided by The RYR-1 Foundation, and with clinicians, geneticists, and researchers with expertise in RYR1-RD. A major goal of The RYR-1 Foundation is to enhance and enrich these relationships within the RYR1 community by hosting International Family Conferences on a biennial basis. The RYR-1 Foundation held the inaugural RYR-1 International Family Conference in Baltimore, MD in July 2016 and the second RYR-1 International Family Conference was held in Pittsburgh, PA in July 2018. Combined, the first two family conferences included 388 attendees, representing 75 RYR1-RD affected families from 31 states and eight countries. These conferences provide unique opportunities for affected individuals and families to meet, form friendships, and develop a genuinely connected RYR1 community. Unfortunately, the planned 2020 RYR-1 International Family Conference was canceled due to the COVID-19 pandemic. Thus, there was significant anticipation within the RYR1 community for the 2022 RYR-1 International Family Conference, which was held over two days immediately following the RYR-1 International Workshop. Many workshop participants also attended the family conference.

AFFECTED INDIVIDUAL SURVEY AND TESTIMONIALS

Affected individual survey

Drew Huseth (Mesa, USA) and **Brentney Simon** (North Charleston, USA) presented a survey of individuals affected by RYR1-RD that was initiated by patient representatives of The RYR-1 Foundation. Based on an idea from **Jeni Ryan** (Denver, USA), Director of The RYR-1 Foundation Board of Directors, Mr. Huseth and Ms. Simon led a patient-based workshop group to develop a survey that consisted of 21 questions divided into the following 4 subject areas: Demographics and Diagnosis, Lifestyle and General Functioning, Physical Activity and Exercise, and Clinical Trials and Studies. The goal of the survey was to provide the RYR1 community an opportunity to communicate their needs, hopes, and experiences with researchers and medical experts. The results serve as a guide to researchers and clinicians as they continue to develop new and effective treatments for RYR1-RD.

The survey was launched on various social media platforms by The RYR-1 Foundation. A total of 226 patients across a wide range of age groups participated; 63.3% were female and 36.7% were male. The three most commonly reported RYR1-RD subclassifications among the participants in the survey were CCD (53.2%), followed by MHS (16.2%) and CNM (5.2%). The most common forms of inheritance were reported as autosomal dominant (27.0%), followed by autosomal recessive (26.1%), and *de novo* (9.3%), with 32.7% of participants reporting their form of inheritance as being unknown. Most participants (64.2%) reported being able to walk unassisted, with 11.9% reporting needing assistance to walk and 5.8% requiring wheelchair assistance. Respondents reported experiencing significant physical (88.9%), emotional (63.7%), and social (58%) challenges. Physical symptoms were self-reported as progressive (47.1%), non-progressive (33.9%), or being unsure about progression of their symptoms (7.5%). An overwhelmingly high willingness was expressed among patients to participate in a clinical trial, as 86% of respondents reported being either willing (40%) or likely willing (46%) to participate.

As indicated above, many respondents classified their symptoms as being progressive. Beyond muscle weakness and fatigue, other physical symptoms reported included fixed or stiff joints, heat intolerance, muscle tightness and cramping, difficulty walking and running, and hip dislocation. Many individuals reported taking part in some type of physical exercise on a weekly basis: 30.5% exercise at least 1-2 times per week for more than 15 minutes and are able to maintain their strength and endurance while exercising. Aerobic training and physical therapy, such as walking, swimming, bicycling, and weight resistance

training, were reported as popular choices among those who exercise.

Respondents to the survey also raised several questions including: How can I relieve everyday symptoms (e.g., muscle pain)? How can I improve my physical well-being (e.g., fatigue, endurance)? How can I improve my emotional well-being (e.g., anxiety)? How can my nutritional well-being be improved (e.g., dietary supplements)? What holistic approaches are beneficial? How does the climate and environment (e.g. temperature, humidity) impact my symptoms?

Patient testimonials

A group of twelve individuals who were either directly or indirectly affected with an RYR1-RD provided brief testimonials regarding their perspective of what it's like to live with or care for someone with an RYR1-RD. These testimonials also included personal questions, concerns, and ideas for researchers and medical experts. A number of common themes emerged from these testimonials. Several individuals recounted prolonged diagnostic odysseys and misdiagnoses before a diagnosis of RYR1-RD was finally established. Many individuals reported the benefits that exercise and regular activity had on their symptoms, while also expressing concern and uncertainty regarding which types of activities may be harmful and how frequently to engage in these activities to avoid excessive pain and muscle damage. Several individuals inquired about supplementation and nutritional approaches that might promote muscle health. Others shared approaches that they reported were beneficial to them, including supplementation with creatine and magnesium. The most common complaints that emerged from the patient testimonials were both physical (pain and fatigue) and social/emotional, noting both the physical and mental barriers that RYR1-RD introduces in their life. Finally, many individuals expressed their sincere gratitude for the work of the clinicians and researchers in the field, while also imploring them to continue to make strides in the area of therapy development.

PHENOTYPIC VARIABILITY

Heinz Jungbluth (London, United Kingdom) discussed the expanding clinical spectrum of RYR1-RD. The *RYR1* gene encodes the principal SR Ca^{2+} release channel in skeletal muscle, which plays a crucial role in skeletal muscle Ca^{2+} homeostasis and EC coupling. Since the original implication of dominant *RYR1* variants in MHS [16] and CCD [17] in the early 1990s, the genetic and phenotypic spectrum of RYR1-RD has continuously expanded [18]. Pathogenic *RYR1* variants are now widely recognized as one of the most common causes of non-dystrophic neuromuscular disorders and MH-associated phenotypes [2, 19].

RYR1-RMs include dominantly-inherited CCD [17] and recessively-inherited MmD [20], CNM [21] and CFTD [22]. CCD is characterized by predominantly proximal weakness pronounced in the hip girdle, (exertional) myalgia, and is often associated with orthopedic complications (in particular congenital hip dislocation and scoliosis). Despite differences in histopathological appearance, RYR1-related MmD, CNM, and CFTD share the same clinical appearance. In contrast to CCD, this clinical presentation is characterized by extraocular muscle involvement and more generalized weakness, including more frequent respiratory and bulbar manifestations. Less common primary neuromuscular manifestations include more severe presentations within the fetal akinesia [23], congenital muscular dystrophy [24], and Limb Girdle Muscular Dystrophy spectra [25]. CCD is typically due to pathogenic *RYR1* missense variants localized to the C-terminal region of the protein, whereas recessive *RYR1* pathogenic variants are often truncating and distributed throughout the *RYR1* coding sequence. While MHS is well-recognized as being associated with CCD, this association is less clear for MmD, CNM and CFTD. In all forms of RYR1-RM, consistent and specific muscle imaging findings on ultrasound and magnetic resonance imaging may aid the (differential) diagnosis in cases with only mild or non-specific histopathological abnormalities [26].

RYR1-related MHS describes a pharmacogenetic predisposition to severe adverse reactions characterized by hyperthermia, muscle breakdown and metabolic decompensation in response to administration of volatile anesthetics and the depolarizing neuromuscular blocking agent succinylcholine [27]. In addition to CCD, MH is associated with a number of other specific myopathies including King-Denborough syndrome [28] and late-onset axial myopathy [29], as well as episodic presentations such as exertional rhabdomyolysis (ERM) [19, 30] and (atypical) periodic paralysis [31]. The recognition of exercise and pyrexia as predisposing factors for MH [32] and of subtle neuromuscular signs and symptoms in MH patients [33] suggests a previously

underappreciated continuum between RYR1-RMs and MHS-associated phenotypes [34].

Systemic manifestations of RYR1-RD include mild abnormal bleeding (characterized by frequent epistaxis and menorrhagia/post-partum hemorrhage in women), as well as bowel and bladder dysfunction, most likely due to disrupted smooth muscle function [35].

Together, these observations highlight the extreme range of manifestations associated with primary RYR1 dysfunction, thus presenting significant diagnostic challenges and important considerations for patient care.

Anna Sarkozy (London, United Kingdom) described the variability in disease presentation and progression in early-onset forms of RYR1-RM. Large cross-sectional studies in CM and RYR1-RM provided valuable phenotypic information on various genetic forms of RYR1-RM. In particular, these studies highlighted clinical and pathological differences between dominant and recessive forms of RYR1-RM [7, 36–39]. Of note, neonatal hypotonia, ptosis and ophthalmoplegia are more frequently observed in recessive RYR1-RM, along with greater motor impairment and difficulty standing and transferring as measured using 32-item Motor Function Measure functional assessment. Amburgey et al. [7] also highlighted a higher frequency of severe disease and ophthalmoplegia with hypomorphic variants in recessive RYR1-RM.

However, comprehensive longitudinal natural history studies are needed to provide quantitative understanding of disease progression, to plan interventions, and improve trial readiness. These studies will inform possible study endpoints, statistical variability of disease, sample size estimation and feasibility, as well as identify optimal outcome measures specific to disease subcategories. Thus far, only a limited number of retrospective and/or prospective studies have been published. Dr. Sarkozy and the teams at the Dubowitz Neuromuscular Centre and the Evelina Children's Hospital recently completed a retrospective longitudinal study on 69 pediatric patients with dominant and recessive RYR1-RM, with various motor abilities (personal observation). Patients were followed up for a median of 6.2 years (range 0-15 years) with an average of six visits per patient (range 1-18 visits). The study evidenced a number of key findings. In particular, observational data on motor abilities highlighted a relatively high prevalence of patients with no/reduced ambulation (21%) within the recessive cohort. Furthermore, a slowly progressive reduction in respiratory abilities, as measured by forced vital capacity in patients above the age of five years old, and mostly due to respiratory muscle weakness, was also observed. The study also indicated a higher disease severity in patients with recessive or dominant *de novo* inheritance (personal observation). Recently, a 6-month natural history study completed at the National Institutes of Health on 34 ambulant patients of various ages showed that functional assessments and graded timed tests are able to detect motor impairment in RYR1-RM patients [40]. This motor impairment remained stable over six months. Thus, possible therapeutic interventions, if successful, should demonstrate improvement in these measures [40].

Overall, these studies confirm major variability in disease presentation and progression between individuals with dominant and recessive RYR1-RM, which can only partly be explained by genetic differences. This single prospective study indicates short-term disease stability in ambulant patients. However, longer prospective natural history studies for larger cohorts of patients with different abilities and clinical severities are needed to assess disease progression over a longer period of time and to identify disease-specific outcome measures and biomarkers. In the meantime, it is of utmost importance for future clinical trial readiness that patients receive optimal care following international standards. Looking forward, international collaborations should be prioritized to increase study sample size and improve our understanding of the disease spectrum.

Luuk van den Bersselaar and **Nicol Voermans** (Nijmegen, the Netherlands) presented preliminary results of a recently completed clinical study on neuromuscular symptoms in patients with RYR1-related ERM and MHS [34]. These two phenotypes have long been considered episodic phenotypes occurring in healthy individuals in response to an external trigger. However, recent studies demonstrated a clinical and histopathological continuum between RYR1-related MHS and/or ERM and CM [30, 33, 41, 42]. Based on results from previous studies in smaller or selected cohorts [30, 42], patients with RYR1-related MHS and/or ERM were hypothesized to frequently suffer from neuromuscular symptoms in between MH and/or ERM episodes.

In a prospective, cross-sectional, observational, clinical study using questionnaires on neuromuscular symptoms, a comprehensive neuromuscular assessment and review of all relevant ancillary diagnostic

tests was performed up to the point of inclusion. A total of 40 patients with a history of RYR1-related MHS and/or ERM were recruited from the MH-unit in the Canisius Wilhelmina Hospital and the neuromuscular outpatient clinic in the Radboud University Medical Center, Nijmegen, the Netherlands.

The proportion of patients with a history of RYR1-related MHS and/or ERM who exhibited neuromuscular symptoms, including myalgia, cramps and exertional myalgia/cramps was higher compared to healthy controls. These symptoms frequently result in consultation of health care professionals and may require additional ancillary investigations including muscle ultrasound, magnetic resonance imaging, and electromyography. Apart from mild abnormalities in muscle biopsies and elevated creatine kinase levels, ancillary investigations were normal in most patients. Most patients displayed normal function during neuromuscular assessment as determined by the Medical Research Council grading score, spirometry and results of other functional measurements. Three patients included in the present study suffered from late-onset proximal muscle weakness as reported previously [43].

These observations have important implications for the diagnosis and management of patients with a history of RYR1-related MHS and/or ERM. First, all healthcare professionals overseeing patients with MHS should be aware that episodic and fixed neuromuscular symptoms can be part of the MH disease spectrum and do not necessarily reflect a second pathology. When diagnosed with MHS, patients should be informed about these neuromuscular manifestations with the goal of reassuring patients and reducing unnecessary invasive diagnostic investigations. Second, although cramps and myalgia are common and non-specific neuromuscular symptoms, MH-associated RYR1 variants may represent an underappreciated cause of cramps and myalgia. Thus, healthcare professionals should consider RYR1 variants in patients presenting with myalgia, cramps, elevated creatine kinase levels and/or ERM in whom other more obvious causes have been excluded [44–46].

Sheila Riazi (Toronto, Canada) presented results of a recently published retrospective study of pre-operative exercise and pyrexia as modifying factors in MH [32]. This study found a remarkable discrepancy between the prevalence of risk genotypes and actual MH incidence. This observation can partly be explained by a lack of exposure of individuals with MHS to MH triggering agents. However, several individuals with MHS experienced uneventful exposure to triggering agents before suffering an MH reaction [47]. This observation suggests the presence of modifying factors in the occurrence of an MH reaction as a possible contribution to the observed low penetrance. Both pyrexia and exercise are well-established risk factors for the occurrence of rhabdomyolysis and heat stroke, two other episodic phenotypes related to RYR1 variants. Based on these observations and a previously reported fatal MH reaction in a patient exposed to MH-triggering agents following intense exercise [48], MH was hypothesized to represent a multifactorial event, determined not only by genotype but also non-genetic factors, in particular exercise and pyrexia.

Riazi and colleagues conducted a retrospective study that included patients from the MH-units in Antwerp (Belgium), Lund (Sweden), Melbourne (Australia), Nijmegen (the Netherlands), and Toronto (Canada). Inclusion criteria were: 1) clinical features suggestive of an MH reaction, 2) confirmation of MHS by *in vitro* contracture test or caffeine-halothane contracture test and/or *RYR1* gene testing [49] and, 3) a history of strenuous exercise within 72 hours and/or pyrexia $> 37.5^{\circ}$C prior to triggering agent exposure. The characteristics of the general anesthesia associated with an MH event were compared to those of general anesthesia without an MH event in the same patients and/or relatives that possess the same variant. Statistical analyses were done using logistic regression. Adjustment for clustering on patient visit was used to assess risk factors for the development of MH using a backwards elimination process.

A total of 41 cases from 40 families fulfilled the inclusion criteria. These cases represent 8.6% of the index cases who were referred to one of the participating MH centers and in whom MHS was confirmed. Pre-operative exercise and/or pyrexia, trauma and acute abdomen as surgery indications, emergency surgery and use of succinylcholine were all more common in the group of individuals that experienced a triggering agent with an MH event.

These findings suggest that pre-operative strenuous exercise and/or pyrexia may increase the risk of experiencing an MH episode and provide at least a partial explanation for the variable response to the same triggering agent in genetically-susceptible individuals. These findings also provide additional evidence for the evolving continuum between RYR1-related neuromuscular phenotypes, and in particular, ERM and MHS.

RYR1 GENETICS AND VARIANT CLASSIFICATION

Leslie Biesecker (Bethesda, USA) presented a solution towards comprehensive interpretation of RYR1-related MHS variants. Genomic variants are reported using a standardized process described by the American College of Medical Genetics and Association of Molecular Pathologists that evaluates the probability of pathogenicity of a variant, based on attributes of the variant [50]. The probability of pathogenicity of a variant is classified as pathogenic (P,>99%), likely pathogenic (90–99%), variant of uncertain significance (10-90%), likely benign (1-10%) and benign (<1%). These classifications are based on a Bayesian probabilistic analysis. Bayes law, also called the rule of inverse probability, dictates the likelihood of a proposition before and after accounting for evidence. For variant classification, one assesses variant attributes, using a prior probability of pathogenicity and four levels of evidence of pathogenicity [51]. The prior probability is set at 10%, which is derived from the above recommendations. This estimate is reasonable for single gene testing scenarios where it is common to encounter on the order of ten variants, one of which is causative. The conditional probabilities specify the odds of observing the evidence, given that the variant is pathogenic. These probabilities were back-calculated from the American College of Medical Genetics and Association of Molecular Pathologists recommendations. Through the ClinGen consortium, an international group of RYR1 experts collaborated to assess the pathogenicity of all RYR1 variants previously associated with MHS [51]. This work involved developing adapted classification rules for RYR1-related MHS and applying them to 335 variants. This analysis resulted in classification of 86 variants as pathogenic or likely pathogenic, 219 as variants of uncertain significance, and 30 variants as benign or likely benign. These classifications are endorsed by the Food and Drug Administration and serve as an international standard for clinical laboratories.

A key distinction is that the pathogenicity of the variant is not the validity of the clinico-molecular diagnosis of the patient. These two concepts are crucial to distinguish. The validity of the clinico-molecular diagnosis crucially depends on the likelihood of the diagnosis prior to the test result. In diagnostic testing, this is generally high because the clinician is ordering the test because there is a strong clinical suspicion of the disease. In healthy population screening, the likelihood of disease prior to testing is low – it is essentially equal to the general population risk of the disease. The effect of these differing prior probabilities of disease on the interpretation of test results is profound. A pathogenic/likely pathogenic variant in a clinical testing scenario often leads to a 99% or greater validity of a clinico-molecular diagnosis, while it can lead to a clinico-molecular diagnosis of $\leq 10\%$ during population screening. In this setting, the clinician is obliged to evaluate the individual and their family to establish or refute the diagnosis. In addition, given the high pre-test probability in diagnostic testing, a variant of uncertain significance can lead to a high validity of a clinico-molecular diagnosis. As genomic testing can be highly useful in both diagnostic and population screening, clinicians are obligated to be informed in the proper interpretation and application of this testing.

Susan Treves (Basel, Switzerland) presented recently published work from the Zorzato/Treves team where they investigated biochemical and epigenetic changes in muscles of patients with RYR1 variants. RYR1 disease-causing variants result mainly in four types of RYR1 channel defects [52]. The first type of pathogenic variant (dominant, MH-associated) cause RYR1 channels to become hypersensitive to activation by electrical and pharmacological stimuli [53]. The second type of variant (dominant, CCD-associated) result in "leaky" RYR1 channels leading to depletion of Ca^{2+} from SR stores [54]. The third type (dominant, CCD-associated) result in "EC uncoupling," whereby voltage-dependent activation results in reduced Ca^{2+} release from a non-depleted SR Ca^{2+} store [55]. The fourth type (recessive, CM-associated) encompasses recessive variants that are accompanied by decreased expression of RYR1 channels in the SR membrane [56]. In addition to the reduction of RYR1 protein, muscles of patients with recessive RYR1 pathogenic variants exhibit striking epigenetic changes including altered expression of microRNAs, increased levels of HDAC4 and HDAC5, and hypermethylation of more than 3600 CpG genomic sites [57–59]. Thus, epigenetic modifying enzymes may constitute a valid therapeutic target for RYR1-RM. Consistent with this, inhibiting DNA methyltransferases and class II histone deacetylases improves muscle function in a mouse model of recessive RYR1-RM [60], providing a proof-of-concept for the pharmacological treatment of patients with CM linked to recessive RYR1 variants. In recently published work,

the Zorzato/Treves team investigated the expression of different transcripts, including those encoding epigenetic enzymes, in human muscle biopsies from patients with genetically diverse CMs. These findings may be helpful for classifying patients in the future, as well as aiding in the development of new and effective therapeutic strategies.

DRUG DEVELOPMENT AND VALIDATION

Oliver Clarke (New York, USA) provided an update on recent advances in cryo-electron microscopy and how this approach is being used for structural characterization of small molecule binding to RYR1. Advances in cryo-electron microscopy instrumentations and data processing software have significantly improved resolution, making it possible to unambiguously identify binding sites for small molecule ligands. For example, in 2016, a collaborative effort at Columbia University revealed the binding sites of several RYR1 activating ligands (Ca^{2+}, ATP, and caffeine) [61]. These ligands bind to the central region of the protein at interdomain interfaces of the C-terminal domain, promoting conformational changes that prime the channel pore for opening. With an improved purification workflow that includes an additional anion-exchange chromatography step and an improved cryo-electron microscopy workflow that includes focused reconstructions of multiple regions, his team is able to consistently obtain a combined reconstruction with high-resolution details ($2.6 - 3\mathring{A}$) across the entire tetrameric RYR1 channel complex. These improved maps enable construction of atomic models of peripheral regions of RYR1 with previously unassigned residues, such as the Repeat12 and bridging solenoid domains. These high-resolution reconstructions also allow for identification of the binding sites of various small molecules that either enhance or inhibit channel opening, furthering our understanding of their effect on the structure and function of RYR1. Cryo-electron microscopy also paves the way for structure-based drug design of novel therapeutics to regulate RYR1 structure and function. For instance, this approach enabled identification of the binding site of S48168 (ARM210), a second generation Rycal currently in a clinical trial for patients with RYR1-RM [62]. Specifically, S48168 (ARM210) binds cooperatively with ATP to the Repeat12 domain to stabilize the RYR1 closed state. In addition to being a powerful tool that complements X-ray crystallography in drug discovery, cryo-electron microscopy provides a unique advantage for studying RYR1 in native lipid environments, such as in liposomes [63], which provides an additional avenue for studying the structure and dynamics of RYR1 under a chemical gradient.

Razvan Cornea (Minneapolis, USA) presented his group's work on developing high-throughput screening methods to identify therapeutic compounds that target skeletal muscle disorders associated with dysregulation of intracellular calcium. Ca^{2+} dysregulation in skeletal muscle most often manifests as an elevation in intracellular Ca^{2+}, which prevents normal muscle relaxation and can be associated with a depletion of SR Ca^{2+} stores needed for contraction. Therefore, his team seeks to identify compounds that either inhibit Ca^{2+} leak through RYR1 channels or enhance Ca^{2+} reuptake into the SR by the ATP-dependent Ca^{2+} pump (SERCA1a). With this goal in mind, his team developed several high throughput screening methods that combine high-precision structural biology, medicinal chemistry, and physiological assays to identify potentially useful compounds. To discover inhibitors of resting RYR1 leak, the method uses a biosensor system based on fluorescence resonance energy transfer between FKBP12 and calmodulin bound to RYR1 [64]. Using measurements of fluorescence resonance energy transfer lifetime, compounds that allosterically inhibit Ca^{2+} leak from RYR1 channels can be identified. The fluorescence lifetime signal is 30x more precise than the classic intensity signal. High-throughput screening of chemical libraries of more than 50,000 compounds were conducted, and new drug-like agents that mitigate RYR1 Ca^{2+} leak in isolated SR membranes were identified. Conversely, Ca^{2+} reuptake by SERCA1a can be enhanced either through activating SERCA1a directly or reversing the inhibitory effect of sarcolipin on SERCA1a. For screening SERCA1a activators, the approach leverages previous studies of SERCA2a (cardiac), with fluorescence resonance energy transfer biosensors expressed in HEK cells [65–67]. The method uses an intramolecular GFP-RFP SERCA biosensor (2-color SERCA) to identify compounds that bind to SERCA1a, alter its structure, and modulate its activity. To screen for compounds that reverse the effect of sarcolipin, a method is being developed that uses an intermolecular biosensor – with a donor on SERCA1a and an acceptor on sarcolipin. Cellular and *in vitro* muscle physiological assays of RYR1 Ca^{2+} leak, as well as force generation and relaxation are integrated in the screening funnel to select compounds that

reverse undesirable RYR1 Ca^{2+} leak, eventually transitioning to *in vivo* animal testing of the most promising identified compounds [64, 68, 69]. Combined, these methods provide drug discovery platforms for large-scale high-throughput campaigns to identify small-molecules that target RYR1-linked pathological states for therapeutic development.

Takashi Murayama (Tokyo, Japan) presented their recent efforts to establish high-throughput screening platforms for small molecules that modify (inhibit or activate) RYR1-mediated Ca^{2+} release. Currently, except for dantrolene in MH, there are no targeted treatments for other RYR1-RD. To discover novel drug candidates, his team established high-throughput screening methods using ER $[Ca^{2+}]$ as a readout. To screen for inhibitors, RYR1 carrying a pathogenic MH variant (R2163C) and R-CEPIA1er, a genetically-encoded fluorescent Ca^{2+} indicator, were stably expressed in HEK293 cells [70]. In these cells, Ca^{2+} leak from mutant RYR1 channels reduces ER $[Ca^{2+}]$. Addition of an RYR1 inhibitor prevents Ca^{2+} leak, thereby restoring the ER $[Ca^{2+}]$, which is monitored fluorometrically. A library containing 1,500 drugs was screened. Oxolinic acid was identified as a novel RYR1-selective inhibitor. A series of studies on structural analogs resulted in the discovery of Cpd1, which exhibits 60-fold higher potency than oxolinic acid [71]. Cpd1 prevents and rescues isoflurane-induced MH crises in a RYR1 MH knock-in mouse model (R2509C mice) [72]. Additionally, Cpd1 exhibits favorable properties of improved water solubility and rapid *in vivo* clearance compared to dantrolene. These findings indicate that Cpd1 is a promising candidate to treat MH and possibly other RYR1-RD.

In an effort to explore RYR1 activators that could be used to address effects of loss-of-function RYR1 pathogenic variants, his team recently established a novel high-throughput platform for reconstituted EC coupling in non-muscle cells. Five genes (encoding $Ca_v1.1$, β1a, Stac3, JP2, and Kir2.1) were expressed using baculovirus infection into HEK293 cells with stable expression of RYR1 and R-CEPIA1er [73]. Stimulation with high $[K^+]$ solution successfully triggered depolarization-induced Ca^{2+} release in a $[K^+]$-dependent manner. Removal of each essential component completely abolished or severely impaired depolarization-induced Ca^{2+} release activity. RYR1 inhibitors dantrolene and Cpd1 both suppressed depolarization-induced Ca^{2+} release, whereas perchlorate, a twitch potentiator, enhanced the sensitivity of depolarization-induced Ca^{2+} release. Ca^{2+}-induced Ca^{2+} release activity, evaluated using caffeine, was inhibited by dantrolene and Cpd1, but not by perchlorate. These results reproduced previous findings observed in muscle cells. This platform of reconstituted depolarization-induced Ca^{2+} release will be useful for future high-throughput drug screens designed to identify potential therapeutic agents for RYR1-RD. Of course, the overall utility of such drug discovery platforms depends strongly on the number and structural diversity of the compounds in the chemical libraries used in these screens.

PREVALENCE AND PATHOPHYSIOLOGY OF RYR1-RD

Heinz Jungbluth (London, United Kingdom) provided an update on the status of the international RYR1-RD prevalence study. Despite pathogenic RYR1 variants being one of the most common causes of CM and MHS, precise epidemiological data concerning RYR1-RD are currently lacking, but are urgently needed, with therapy development on the horizon.

A small number of epidemiological studies from Northern Sweden [74], Northern Ireland [75], Northern England [76], and the United States (Michigan) [1] suggest a prevalence of between 1 in 22,480-135,000 for CM overall and between 1 in 90,000-249,000 for myopathies with confirmed or probable RYR1 involvement. However, these studies were regionally limited, mostly conducted before widespread diagnostic RYR1 sequencing was available, predominantly included pediatric patients, and focused on CM but not other RYR1-RD.

To address the urgent need for more precise epidemiological data concerning the full spectrum of RYR1-RD, an international prevalence study was proposed. This study focuses on this important group of neuromuscular disorders while considering several general and specific challenges. Specifically, the proposed study aims to determine the: 1) point prevalence and incidence of RYR1-RD in 4 different countries, 2) relative proportion of different CM and MHS-associated phenotypes, and 3) relative frequency of specific RYR1 genotypes. Inclusion criteria include: 1) confirmed (likely) pathogenic variant(s) in RYR1 and 2) clinical features of a recognized RYR1-RD (i.e. CM, MHS or related phenotype), defined on clinical and histopathological grounds and/or through *in vitro* contracture testing. In

addition to genotype and basic demographic information, the study will also collect information regarding the specific RYR1-RD (e.g. CCD, MmD, CNM, CFTD, King-Denborough Syndrome) and CM with non-specific histopathological features (e.g. MHS, and ERM). Requirements for the countries participating in this study include: 1) availability of centralized RYR1 testing with population-wide coverage, 2) presence of national expertise centers for CM and MHS, and 3) corresponding population-wide patient database/registry coverage.

Point prevalence and incidence rates for RYR1-RD as a group (as well as the frequency of specific subgroups) will be calculated for each country in comparison to national population numbers. Considering the likelihood of pauci-symptomatic presentations, a multiple source capture-recapture approach will be applied [77].

This prevalence study will be the largest proposed epidemiological study to date focused on the entire spectrum of RYR1-RD. It is expected that this study will document the true societal burden of RYR1-RD and will provide the basis for larger natural history studies, ultimately stimulating increased industry interest for therapy development.

Isabelle Marty (Grenoble, France) presented ongoing work from her group screening various molecules for therapeutic efficacy in alleviating the consequences of reduced RYR1 expression in recessive RYR1-RM. Many identified RYR1 variants associated with CM (including CCD and MmD) result in a marked reduction in muscle Ca^{2+} release by different pathophysiological mechanisms. Besides alterations to RYR1 Ca^{2+} release channel function, a frequently identified pathogenic mechanism in individuals with recessive RYR1-RM involves a marked reduction in RYR1 protein expression [78]. Work from Dr. Marty's lab aims to identify molecules that are able to increase Ca^{2+} release in the presence of reduced levels of RYR1 protein, and therefore, help to reverse the downstream effects of RYR1 reduction, regardless of the RYR1 variant involved. Their group first developed and characterized the tools needed to screen for such molecules including various cellular models and an inducible RYR1 knockout mouse model. They were the first to develop an inducible, muscle-specific RYR1 knockout mouse model. The characterization of this mouse model demonstrated that reduction in RYR1 expression in adult mice results in muscle weakness that correlates directly with RYR1 protein levels [79], as is observed in individuals with recessive RYR1-RM [78]. Their lab also developed human immortalized myoblasts from patient biopsies with various pathogenic variants and mouse primary muscle cultures with reduced RYR1 expression generated from the RYR1-null mouse model. A reduction in both RYR1 expression and Ca^{2+} release was confirmed in these cell models. Using these tools, a drug screening pipeline was developed to identify small molecules capable of increasing RYR1-mediated Ca^{2+} release. Identified molecules will be further tested and validated *in vivo* using the inducible, muscle-specific RYR1-knockout mice.

James Dowling (Toronto, Canada) presented results from his collaborative work with **Robert Dirksen** (Rochester, USA) aimed at identifying and translating new potential therapies for RYR1-RD. Dr. Dowling highlighted recent therapies uncovered for other CM with secondary RYR1 dysfunction, including tamoxifen, anti-sense oligonucleotide-mediated knockdown of DNM2, and epigenetic modulation with HDAC inhibitors. He also presented results from a recent drug screen for RYR1-RD performed using a multi-species drug discovery pipeline (nematodes, zebrafish, mice, and mammalian myotubes). In this study, his team performed large scale drug screens in ryr1 deficient C. elegans and zebrafish. Positive hits were tested across species, and then brought to mice and cultured mammalian cells. In addition to establishing a screening platform, this work also identified p38 inhibitors as potential modifiers of RYR1 related cellular phenotypes. Lastly, Dr. Dowling outlined a strategy for identifying therapeutic targets in a mouse model of recessive RYR1-RM and discussed preliminary results on the first therapies being tested in these mice. This work started through the generation and characterization of newly developed mice with compound heterozygous variants in RYR1, and led to the elucidation of the disease, as well as the identification progression in these mice, as well as identification of outcome measures suitable for drug testing. Based on these data, candidate therapeutics, such as tamoxifen and p38 inhibitors, are now being tested for their ability to increase survival and improve motor function in mouse models of RYR1-RM.

Johann Böhm (Strasbourg, France) discussed how RYR1 disease-causing variants are implicated in CNM. CM affects children and adults in all populations and usually involves stable or progressive muscle weakness. Individual CM subtypes can be distinguished by the predominance of particular histological anomalies on muscle biopsies as distinguished by an abnormal nuclear positioning in CNM

or the presence of areas devoid of mitochondrial activity in MmD [2, 80]. However, CM is characterized by diverse clinical and genetic heterogeneity, and histopathological hallmarks may not be restricted to a specific subtype.

The skeletal muscle triad is composed of transverse tubules (T-tubules) flanked by SR cisternae that contain the EC coupling machinery. The main CNM forms are typically due to pathogenic variants in genes that encode proteins involved in T-tubule biogenesis (*MTM1*, *BIN1*, and *DNM2*). Meanwhile, MmD can be caused by pathogenic variants in RYR1, which is located in the SR [81–84]. Of note, patients sometimes show overlapping histological features of CNM and MmD. This is presumably due to the co-localization of RYR1 and the CNM-associated proteins at the triad coupled with the interconnection of pathways regulating membrane remodeling (CNM proteins) and the Ca^{2+} release complex (RYR1) [21, 85, 86]. Store-operated calcium entry is another process that occurs at the skeletal muscle triad and individuals with tubular aggregate myopathy and pathogenic variants in the *STIM1* and *ORAI1* genes can exhibit histopathological characteristics similar to those observed for CNM and MmD [87, 88]. This congruence is underscored by a recent study describing the presence of tubular aggregates in individuals carrying RYR1 variants [89]. Conversely, patients with RYR1 pathogenic variants may not always exhibit cores on muscle sections [90].

To ensure that RYR1 variants are not overlooked in CM patients and to provide a rapid and precise molecular diagnosis for the affected families, the Strasbourg diagnostic laboratory performs panel sequencing that covers the 238 known genes implicated in neuromuscular disorders. Depending on the medical and/or histological indications, it is possible to focus on the genes associated with CNM, MmD, or tubular aggregate myopathy in the initial analysis and subsequently assess all other neuromuscular genes as a secondary analysis. While exome or genome sequencing may be a next step, the interpretation of variants in novel genes requires functional investigations on cells and animal models to make conclusions regarding pathogenicity.

THERAPEUTIC PIPELINE

Jacques Tremblay (Québec, Canada) presented preclinical data from his group focused on correction of RYR1 pathogenic variants using a CRISPR/Cas9 prime editing approach. Traditional CRISPR/Cas9 systems rely on repair of double-stranded DNA breaks induced by a Cas9 nuclease. The Cas9 enzyme is directed to the target site by a single-guide RNA (sgRNA), which recognizes a 20 nucleotide sequence adjacent to a short protospacer adjacent motif in the target genomic DNA [91–93].

Prime editing technology is also based on CRISPR/Cas9, but it can introduce small changes in the genomic DNA without the need for a double strand break. Prime editing employs a SpCas9 nickase, which induces a cut only in a single DNA strand. The SpCas9 nickase is fused to a reverse transcriptase and delivered along with an engineered prime editing guide RNA [94]. This is a modified sgRNA containing the scaffold and the spacer sequences of the sgRNA, along with a primer binding site, a reverse transcriptase template, and a pseudoknot sequence.

Dr. Tremblay's group designed prime editing guide RNAs capable of either inserting or correcting a T4709M RYR1 variant in human cells or correcting the analogous T4706M variant in a knock-in mouse model. After optimization of the system, PCR amplification of *RYR1* exon 96 and Sanger sequencing of the amplicons demonstrated that the T4709M variant was introduced in 63% of HEK293T cells by a single prime editing treatment. The group then attempted to correct the analogous variant in cells (a mixture of fibroblasts and myoblasts) derived from muscle of T4706M knock-in mice. Unfortunately, this approach did not result in significant correction, likely due to the low transfection efficiency of these cells.

The main challenge for a prime editing therapy in RYR1-RD is efficient delivery of the gene editing machinery to a large percentage of the muscle fibers. To overcome this challenge, Dr. Tremblay's group plans to test three delivery methods for *in vivo* correction of the T4706M variant: 1) a dual AAV delivery system [95–98], with one AAV coding for a SpCas9 nickase-intein and the other for an intein-reverse transcriptase plus an engineered prime editing guide RNA; 2) plasma-purified extracellular vesicles containing the full length SpCas9 nickase-reverse transcriptase protein and an engineered prime editing guide RNA [99]; and 3) lipid nanoparticles containing the SpCas9 nickase-reverse transcriptase mRNA and an engineered prime editing guide RNA [100–103]. By altering the engineered prime editing guide RNA sequence, the prime editing strategy may not only be useful for other RYR1 variants, but also a multitude of pathogenic variants responsible for other hereditary diseases.

Nanna Witting (Copenhagen, Denmark) summarized data supporting the benefits of exercise in patients with CMs. Exercise is generally recommended for patients with myopathies to increase fitness and function in activities of daily living, as well as to reduce the risk of diseases associated with a sedentary lifestyle. Solid evidence indicates that exercise is safe, well-tolerated, and improves physiological parameters for individuals that exhibit a wide number of different myopathies [104, 105].

The 2012 consensus statement on standard of care recommended exercise for individuals with CM, but acknowledged that there is no good evidence to recommend a particular frequency or intensity [8]. Fatigue appears to be a significant limiting factor for many of these patients. One of the few studies examining exercise in CMs found that aerobic training led to an increase in maximal oxygen uptake without an elevation in creatine kinase levels. Only seven of sixteen total patients however completed the study with fatigue being the most common cause for dropout. Those who dropped out included three of the four RYR1-RD patients in the study [106]. Fatigue is also limiting for training in Kennedy disease. In this condition, there is evidence that patients perform better with high intensity interval training [107]. This approach improves maximal oxygen uptake without inducing the same degree of fatigue as conventional training programs. High intensity interval training may therefore be a valid alternative for patients with CM.

Tokunbor Lawal (Bethesda, USA) discussed preclinical data implicating lipid peroxidation and redox imbalance in the pathophysiology of RYR1-RD. This association was corroborated by the recent clinical trial of N-acetylcysteine (NAC) [15], which confirmed baseline increases in oxidative stress markers (including lipid urine 15-F_{2t} isoprostane and GSH:GSSG ratio) in RYR1-RM patients. Unfortunately, NAC treatment did not improve these abnormalities, likely owing to metabolic degradation preventing sufficient target engagement in muscle. Nevertheless, addressing the pathological sequelae of RYR1 dysfunction (including Ca^{2+} leak and oxidative stress) remains a priority for the field. It is possible that a combination of channel stabilizing compounds and targeted antioxidant therapy could yield significant therapeutic benefit.

Antioxidant compounds with potential for target engagement in RYR1-RD include prescription-grade n-3 polyunsaturated fatty acids (eicosapentaenoic acid, docosahexaenoic acid [LOVAZA]) and mitoquinol mesylate). It has been shown that n-3 polyunsaturated fatty acids inhibit SR Ca^{2+} release and alter muscle lipid composition [108, 109]. Meanwhile, mitoquinol mesylate is a neuroprotective mitochondria-targeted coenzyme Q_{10} analog that is able to permeate the mitochondrial membrane, leading to intra-mitochondrial concentrations that are 100-500-fold higher than coenzyme Q_{10} [110]. Previous studies suggested a link between mitochondrial Ca^{2+} accumulation and redox imbalance in RYR1-RD [111]. As such, mitoquinol mesylate has the potential to address redox imbalance associated with core formation at its source. Both mitoquinol mesylate and n-3 polyunsaturated fatty acids exhibit a favorable safety profile. In addition, their availability and relatively low cost render them amenable to rapid translation to clinical trials. Dr. Lawal and colleagues are conducting a study to test the effects of these compounds at physiologically-relevant levels, alone and in combination, on lipid peroxidation, redox balance and muscle function in two murine models of RYR1-RM: recessive T4706M/S1669C+L1716del knock-in mice and dominant Y524S knock-in mice [112]. The aim of this study is to generate preclinical data to support a phase II clinical trial in RYR1-RD.

RYR1 CLINICAL TRIALS

Eva Michael (Gothenburg, Sweden) presented the design of COMPIS (NCT05099107), a recently initiated clinical trial of oral salbutamol for CMs. A possible benefit of salbutamol in these disorders is suggested by preclinical evidence of impaired neuromuscular transmission in several CMs [113–115], as well as by the role of beta-adrenergic signaling in muscle physiology [116, 117]. Prior studies reported that salbutamol enhanced muscle strength and function in CMs [118, 119]. In the absence of a large randomized study, however, no consensus has been reached and salbutamol is not included in the standard of care. The aim of the COMPIS study is to examine the effects of 6 months of salbutamol treatment on muscle function and strength in CM patients. COMPIS is a prospective, randomized-controlled study with a crossover design. Twenty patients from 6-31 years of age with a genetically confirmed CM are being recruited in Sweden. The study includes five visits over nineteen months and has three time periods: a baseline and two treatment periods. After the baseline period, patients are randomized to either salbutamol or no treatment. An initial evaluation

occurs after six months, followed by a washout period of one month. The groups will then cross over for an additional six months. The same battery of tests and evaluations are performed at each study visit. The primary outcome measure of the study is an increase of at least three points on the 32-item Motor Function Measure functional assessment scale. The six-minute walk test, several timed function tests, myometry, forced vital capacity and the nine-hole peg test serve as secondary outcomes. The clinical evaluators (physiotherapists and occupational therapists) are blinded to treatment group assignment. The study will also examine changes in quality of life. COMPIS was initiated in October 2021 and is expected to be completed in August 2024.

Joshua J. Todd (Bethesda, USA) presented results from the first clinical trial of NAC, a precursor to the ubiquitous antioxidant glutathione, in patients with RYR1-RM [15]. The study evaluated the impact of NAC treatment on oxidative stress and endurance in ambulatory individuals with a genetic diagnosis of RYR1-RM. This phase II, double-blind, randomized, placebo-controlled trial was conducted at the National Institutes of Health between 2015 and 2017. The trial design comprised a six-month lead in (natural history) phase, followed by a six-month treatment phase. The primary endpoints were levels of urine 15-F_{2t} isoprostane, an oxidative stress marker, and six-minute walk distance as a measure of endurance. In total, 150 individuals were screened, 53 were enrolled in the natural history phase, and 33 were randomized (1 : 1) to receive either NAC or placebo in effervescent tablet formulation (30 mg/kg/day not to exceed 2700 mg/day) for six months. NAC exhibited a favorable safety and tolerability profile. All trial participants were found to exhibit an elevated baseline concentration of urine 15-F_{2t} isoprostane, a stable biomarker of lipid peroxidation, during the natural history phase compared to normal control individuals. This elevation, however, was not corrected following NAC treatment. Likewise, NAC treatment did not significantly increase distance of the six-minute walk test.

Although this clinical trial did not meet its primary endpoint, it provided important insights into trial readiness and lessons for future clinical trials in RYR1-RM. Key lessons included stringent eligibility criteria to avoid floor and ceiling effects on efficacy endpoints, establishing a robust collaborative framework between government, industry, academia, and patient advocacy, early regulatory interactions, and building an operational infrastructure in advance of trial launch. This trial led to further discussion on the added value of obtaining a breadth of data and biospecimens for future research. Indeed, 1) owing to methodological advances, the study team is now positioned to re-evaluate the original primary endpoint (systemic reduced-to-oxidized glutathione ratio) in banked whole blood samples; 2) data was submitted to the Rare Disease Cures Accelerator Data Analytics Platform, an initiative supported by the U.S. Food and Drug Administration [120]; and 3) muscle biopsy specimens obtained for exploratory analyses were used to provide *ex vivo* proof-of-concept data to support the phase I trial of Rycal S48168 (ARM210) in RYR1-RD individuals [121]. This experience also highlighted the importance of obtaining feedback from trial participants on their experience to inform future studies.

Payam Mohassel (Baltimore, USA) presented the results of the first clinical trial of S48168 (ARM210), a Rycal compound, in individuals with RYR1-RM (NCT04141670). In preclinical studies, Dr. Andrew Marks and colleagues previously reported that decreased RYR1-calstabin1 association exacerbates SR Ca^{2+} leak, with detrimental downstream effects on muscle function [122]. Rycal compounds bind to RYR1 to stabilize the RYR1 closed state [62]. Studies of muscle biopsies from individuals with RYR1-RM found that *ex vivo* addition of Rycals mitigates aberrant Ca^{2+} leak [121]. Based on these findings, Dr. Mohassel and colleagues, in collaboration with ARMGO Pharma (the study sponsor), conducted a phase I clinical trial of S48168 (ARM210) in ambulatory adults with RYR1-RM (NCT04141670). The primary endpoint of the trial was safety and tolerability over a one-month dosing period. Pharmacokinetic studies, muscle biopsies, motor/muscle function tests, and a patient-reported fatigue questionnaire were also included. Seven eligible individuals were enrolled and received either a low (120 mg, $n = 3$) or high (200 mg, $n = 4$) daily dose of S48168 (ARM210). S48168 (ARM210) was well tolerated; three treatment-emergent adverse events grade 2 or above were reported, all unrelated to S48168 (ARM210), and there were no serious adverse events. S48168 (ARM210) showed a dose-dependent pharmacokinetic profile and peak plasma concentration remained within pre-defined safety margins. At one-month, self-reported fatigue scores decreased and shoulder abduction strength trended higher, in the high dose group. Other preliminary efficacy results were mixed. Given the favorable safety profile and early encouraging efficacy trends, fur-

ther clinical development of S48168 (ARM210) for RYR1-RM is warranted.

CONSENSUS RECOMMENDATIONS FOR BASIC/TRANSLATION AND CLINICAL PRIORITIES

During the RYR1 international research workshop, several recurring basic/translational and clinical priorities emerged (Table 1). These priorities were reviewed by the organizing committee and consolidated into the following consensus recommendations and action plan for the field.

Basic/translational

Continued development and testing of pre-clinical models of RYR1-RD

Drs. Dowling, Treves, and Marty provided new information regarding the pathogenesis of RYR1-RD and muscle dysfunction due to RYR1 deficiency [13, 79, 123]. These and other RYR1 pre-clinical models of RYR1-RD provide tremendous opportunities to identify key underlying disease pathomechanisms (e.g. RYR1 Ca^{2+} leak, ER/SR stress, reduced RYR1 expression, epigenetic modifications), as well as providing valuable models to test the efficacy of mechanism-based therapeutic interventions. Thus, the availability of validated pre-clinical models that faithfully reproduce key aspects of RYR1-RD are needed to test the efficacy of exciting advances in RYR1 drug discovery and genetic-based therapeutic interventions discussed below.

Expand the drug discovery pipeline

Drs. Cornea and Murayama presented new findings using high-throughput screening approaches to identify modifiers of RYR1 activity [64], SERCA1 function [65], and depolarization-induced Ca^{2+} release. These and other high-throughput drug screens (and subsequent medicinal chemistry efforts to improve drug delivery/bioavailability) are needed to identify new therapeutic agents that can then be tested for efficacy in the different pre-clinical models of RYR1-RD.

Development of RNA- and DNA-based therapies

Dr. Tremblay's group capitalized on recent advances in CRISPR/Cas9 prime editing to correct pathogenic variants in dystrophin that cause Duchenne muscular dystrophy [124] and is optimizing this approach to correct *RYR1* pathogenic variants. Additional genetic-based therapeutic interventions, some of which are being pursued with varying levels of success for other genetic disorders, could also be operationalized for RYR1-RD. For example, antisense oligonucleotides designed to alter RNA splicing events have shown remarkable translational promise for the treatment of spinal muscular atrophy [125]. In addition, trans-splicing approaches are currently being developed in pre-clinical models of Duchenne muscular dystrophy [126, 127]. siRNA-mediated mutant allele-specific gene silencing can mitigate disease phenotypes in autosomal dominant disorders and have been used previously in a mouse model of MHS with cores [128]. Anti-codon edited tRNAs are being optimized to suppress nonsense variants or premature termination codons that result in cystic fibrosis [129, 130]. Thus, an important area of future basic/translational development involves optimizing these and other RNA- and DNA-based therapies. Again, the availability of appropriate pre-clinical models of RYR-RD are essential tools needed for successful clinical translation of these and other novel therapeutic interventions.

Pathogenicity classification of RYR1 variants for RYR1-RD

Dr. Biesecker summarized findings of the ClinGen MHS curation expert panel for *RYR1* pathogenicity classifications in MH using American College of Medical Genetics and Genomics guidelines adapted for MHS. Similar ClinGen variant curation expert panels are needed to adapt these guidelines to provide objective quantitative evidence for the pathogenicity of RYR1 variants linked to other forms of RYR1-RD (e.g. CCD, CFTD, CNM, and MmD).

Launch a comprehensive database for RYR1-RD (with patient community input)

Susan Hamilton (Houston, USA) raised the importance of making genetic and phenotypic data on RYR1-RD centrally available for patients, family members, clinicians, and researchers. To address this concern, attendees advocated for the continued refinement and public launch of the RYR1-RD database project, initially funded by The RYR-1 Foundation. This comprehensive database represents a critical unmet need for the field. Dr. Todd provided a status update on the project and outlined next steps.

Table 1
Consensus recommendations to emerge from the workshop regarding both basic/translational and clinical priorities for the RYR1-RD field

Consensus recommendations	
Basic/translational priorities	Clinical priorities
Preclinical model development and testing	Analyze and follow up on patient surveys
Drug discovery pipeline expansion	Expand and centralize natural history data
RNA and DNA-based therapy development	Evaluate salbutamol efficacy
RYR1 variant pathogenicity classification	Clinical trial advancement of Rycal S48168
Establish patient-facing RYR1-RD database	

Clinical

Analyze patient-led survey data and continue to engage in patient-initiated research

Mr. Huseth and Ms. Simon provided an overview of a patient-initiated online survey. The survey results highlight patient perspectives on factors that positively and negatively impact quality of life and revealed that a vast majority of respondents are eager to participate in future research and clinical studies.

Improve clinical understanding of the complex histopathological spectrum and dynamics in RYR1-RM and provide MHS neuromuscular symptom information on patient-facing websites

New insights into the age-dependent histopathological spectrum and neuromuscular manifestations of RYR1-RM were presented by Dr. Voermans (Radboud, the Netherlands) and Dr. Jungbluth (London, United Kingdom). Thus, a continued appreciation for the ever-expanding clinical spectrum of RYR1-RM is warranted [34]. Given this continued evolution, it will be important to make this information available on multiple patient-facing websites (e.g. The RYR-1 Foundation, MDA, ENMC, MHAUS, and EMHG).

Centralize existing (retrospective) natural history data to facilitate clinical trial readiness

Dr. Todd reviewed natural history data obtained from the NAC trial made available to the research community through the Rare Disease Cures Accelerator Data Analytics Platform, a program funded through the Food and Drug Administration. This platform provides a centralized and standardized infrastructure to support and accelerate rare disease characterization, with the goal of accelerating therapy development across rare diseases. The platform is already being utilized for Duchenne muscular dystrophy, Huntington's disease, Friedreich's ataxia and polycystic kidney disease. Researchers in the field are encouraged to submit their RYR1-RM natural history data to the Rare Disease Cures Accelerator Data Analytics Platform [120].

Develop a collaborative framework for future prospective natural history data collection

The workshop highlighted that the majority of natural history data obtained to date were collected retrospectively and without consistency in outcome measures and assessments. Developing a framework for prospective natural history data collection either through an international multi-center study with common data elements will be important for maximizing clinical trial readiness across the spectrum of RYR1-RM.

Continued clinical evaluation of salbutamol as a potential therapeutic for congenital myopathies, including RYR1-RM

Dr. Michael provided an overview of an ongoing investigator-initiated clinical trial of oral salbutamol in individuals with congenital myopathies (COMPIS, NCT05099107). This open label, randomized, crossover trial was designed to determine whether a six-month course of oral salbutamol treatment improves muscle function in affected individuals. Encouraging observations from some previously published cases warrant continued investigation into the potential benefits of salbutamol therapy for RYR1-RM.

Advance clinical development of Rycal S48168 (ARM210) for RYR1-RD

Dr. Mohassel presented early results from an industry-sponsored phase I dose-escalation trial of Rycal S48168 (ARM210) in RYR1-RM-affected individuals (ARMGO Pharma Inc., NCT04141670).

The compound was found to exhibit a favorable safety and tolerability profile. Thus, attendees agreed that further clinical development of S48168 (ARM210) for RYR1-RM is warranted. Full results from the clinical trial are being prepared for publication.

WORKSHOP PARTICIPANTS (RYR1 MYOPATHY CONSORTIUM)

Leslie Biesecker (Bethesda, US)
Johann Böhm (Strasbourg, FR)
Carsten Bönnemann (Bethesda, US)
Oliver Clarke (New York, US)
Razvan Cornea (Minneapolis, US)
Robert Dirksen (Rochester, US)
James Dowling (Toronto, CA)
Michael Goldberg (Pittsburgh, US)
Susan Hamilton (Houston, US)
Drew Huseth (Mesa, US)
Heinz Jungbluth (London, UK)
Tokunbor Lawal (Bethesda, US)
Andrew Marks (New York, US)
Isabelle Marty (Grenoble, FR)
Livija Medne (Philadelphia, US)
Eva Michael (Gothenburg, SE)
Payam Mohassel (Bethesda, US)
Takashi Murayama (Tokyo, JP)
Sheila Riazi (Toronto, CA)
Anna Sarkozy (London, UK)
Brentney Simon (North Charleston, US)
Joshua Todd (Bethesda, US)
Jacques Tremblay (Québec, CA)
Susan Treves (Basel, CH)
Luuk van den Bersselaar (Nijmegen, NL)
Filip Van Petegem (Vancouver, CA)
Nicol Voermans (Nijmegen, NL)
Nanna Witting (Copenhagen, DK)

ACKNOWLEDGMENTS

This workshop was hosted by The RYR-1 Foundation (www.ryr1.org). It was supported by grants from the National Institutes for Health (R13AR08169) and the Muscular Dystrophy Association (www.mda.org), donations from numerous benefactors of The RYR-1 Foundation, and additional administrative and financial resources from The RYR-1 Foundation. The RYR-1 International Family Conference (https://ryr1.org/family-conferences) held immediately following this workshop, was supported by the Schooner Foundation (www.schoonercapital.com/the-foundation). In addition, several companies co-financed this workshop and the Family Conference, including Eagle Pharmaceuticals, Ingenious Targeting Laboratory, Invitae, DF Dent, Cindy Leonard Consulting, Prevention Genetics, ARMGO, Strassburger McKenna Gutnick & Gefsky, and Susan A. Ott, Esq.

Several authors of this publication are supported by the National Institutes for Health (R01AR078000 to RTD; R01GM135633 to FVP) or are members of the European Reference Network for rare neuromuscular diseases (EURO-NMD). Dr. Andrew Marks owns equity in ARMGO Pharma Inc. and is a member of the company's Board of Directors and Chair of the Scientific Advisory Board of The RYR-1 Foundation. We are grateful to the ENMC for the fruitful discussion during the preparation of this workshop.

REFERENCES

[1] Amburgey K, McNamara N, Bennett LR, McCormick ME, Acsadi G, Dowling JJ. Prevalence of congenital myopathies in a representative pediatric united states population. Ann Neurol. 2011;70(4):662-5.
[2] Jungbluth H, Treves S, Zorzato F, Sarkozy A, Ochala J, Sewry C, et al. Congenital myopathies: Disorders of excitation-contraction coupling and muscle contraction. Nat Rev Neurol. 2018;14(3):151-67.
[3] Westra D, Schouten MI, Stunnenberg BC, Kusters B, Saris CGJ, Erasmus CE, et al. Panel-Based Exome Sequencing for Neuromuscular Disorders as a Diagnostic Service. J Neuromuscul Dis. 2019;6(2):241-58.
[4] Huang K, Bi FF, Yang H. A Systematic Review and Meta-Analysis of the Prevalence of Congenital Myopathy. Front Neurol. 2021;12:761636.
[5] Santulli G, Lewis DR, Marks AR. Physiology and pathophysiology of excitation-contraction coupling: The functional role of ryanodine receptor. J Muscle Res Cell Motil. 2017;38(1):37-45.
[6] Zhou H, Rokach O, Feng L, Munteanu I, Mamchaoui K, Wilmshurst JM, et al. RyR1 deficiency in congenital myopathies disrupts excitation-contraction coupling. Hum Mutat. 2013;34(7):986-96.
[7] Amburgey K, Bailey A, Hwang JH, Tarnopolsky MA, Bonnemann CG, Medne L, et al. Genotype-phenotype correlations in recessive RYR1-related myopathies. Orphanet J Rare Dis. 2013;8:117.
[8] Wang CH, Dowling JJ, North K, Schroth MK, Sejersen T, Shapiro F, et al. Consensus statement on standard of care for congenital myopathies. J Child Neurol. 2012;27(3):363-82.
[9] Dowling JJ, H DG, Cohn RD, Campbell C. Treating pediatric neuromuscular disorders: The future is now. Am J Med Genet A. 2018;176(4):804-41.
[10] Lawal TA, Todd JJ, Meilleur KG. Ryanodine Receptor 1-Related Myopathies: Diagnostic and Therapeutic Approaches. Neurotherapeutics. 2018;15(4):885-99.
[11] Jungbluth H, Dowling JJ, Ferreiro A, Muntoni F, Consortium RYRM. 217th ENMC International Workshop:

RYR1-related myopathies, Naarden, The Netherlands, 29-31 January 2016. Neuromuscul Disord. 2016;26(9):624-33.
[12] Brennan S, Garcia-Castaneda M, Michelucci A, Sabha N, Malik S, Groom L, et al. Mouse model of severe recessive RYR1-related myopathy. Human Molecular Genetics. 2019;28(18):3024-36.
[13] Elbaz M, Ruiz A, Bachmann C, Eckhardt J, Pelczar P, Venturi E, et al. Quantitative RyR1 reduction and loss of calcium sensitivity of RyR1Q1970fsX16+A4329D cause cores and loss of muscle strength. Hum Mol Genet. 2019;28(18):2987-99.
[14] Johnston JJ, Dirksen RT, Girard T, Gonsalves SG, Hopkins PM, Riazi S, et al. Variant curation expert panel recommendations for RYR1 pathogenicity classifications in malignant hyperthermia susceptibility. Genet Med. 2021;23(7):1288-95.
[15] Todd JJ, Lawal TA, Witherspoon JW, Chrismer IC, Razaqyar MS, Punjabi M, et al. Randomized controlled trial of N-acetylcysteine therapy for RYR1-related myopathies. Neurology. 2020;94(13):e1434-e44.
[16] Fujii J, Otsu K, Zorzato F, de Leon S, Khanna VK, Weiler JE, et al. Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science. 1991;253(5018):448-51.
[17] Zhang Y, Chen HS, Khanna VK, De Leon S, Phillips MS, Schappert K, et al. A mutation in the human ryanodine receptor gene associated with central core disease. Nat Genet. 1993;5(1):46-50.
[18] Snoeck M, van Engelen BG, Kusters B, Lammens M, Meijer R, Molenaar JP, et al. RYR1-related myopathies: A wide spectrum of phenotypes throughout life. Eur J Neurol. 2015;22(7):1094-112.
[19] van den Bersselaar LR, Hellblom A, Gashi M, Kamsteeg EJ, Voermans NC, Jungbluth H, et al. Referral Indications for Malignant Hyperthermia Susceptibility Diagnostics in Patients without Adverse Anesthetic Events in the Era of Next-generation Sequencing. Anesthesiology. 2022;136(6):940-53.
[20] Jungbluth H, Zhou H, Hartley L, Halliger-Keller B, Messina S, Longman C, et al. Minicore myopathy with ophthalmoplegia caused by mutations in the ryanodine receptor type 1 gene. Neurology. 2005;65(12):1930-5.
[21] Wilmshurst JM, Lillis S, Zhou H, Pillay K, Henderson H, Kress W, et al. RYR1 mutations are a common cause of congenital myopathies with central nuclei. Ann Neurol. 2010;68(5):717-26.
[22] Clarke NF, Waddell LB, Cooper ST, Perry M, Smith RL, Kornberg AJ, et al. Recessive mutations in RYR1 are a common cause of congenital fiber type disproportion. Hum Mutat. 2010;31(7):E1544-50.
[23] Todd EJ, Yau KS, Ong R, Slee J, McGillivray G, Barnett CP, et al. Next generation sequencing in a large cohort of patients presenting with neuromuscular disease before or at birth. Orphanet Journal of Rare Diseases. 2015;10.
[24] Brackmann F, Turk M, Gratzki N, Rompel O, Jungbluth H, Schroder R, et al. Compound heterozygous RYR1 mutations in a preterm with arthrogryposis multiplex congenita and prenatal CNS bleeding. Neuromuscul Disord. 2018;28(1):54-8.
[25] Topf A, Johnson K, Bates A, Phillips L, Chao KR, England EM, et al. Sequential targeted exome sequencing of 1001 patients affected by unexplained limb-girdle weakness. Genet Med. 2020;22(9):1478-88.
[26] Jungbluth H, Davis MR, Muller C, Counsell S, Allsop J, Chattopadhyay A, et al. Magnetic resonance imaging of muscle in congenital myopathies associated with RYR1 mutations. Neuromuscul Disord. 2004;14(12):785-90.
[27] Rosenberg H, Pollock N, Schiemann A, Bulger T, Stowell K. Malignant hyperthermia: A review. Orphanet J Rare Dis. 2015;10:93.
[28] Dowling JJ, Lillis S, Amburgey K, Zhou HY, Al-Sarraj S, Buk SJA, et al. King-Denborough syndrome with and without mutations in the skeletal muscle ryanodine receptor (RYR1) gene. Neuromuscular Disorders. 2011;21(6):420-7.
[29] Loseth S, Voermans NC, Torbergsen T, Lillis S, Jonsrud C, Lindal S, et al. A novel late-onset axial myopathy associated with mutations in the skeletal muscle ryanodine receptor (RYR1) gene. J Neurol. 2013;260(6):1504-10.
[30] Dlamini N, Voermans NC, Lillis S, Stewart K, Kamsteeg EJ, Drost G, et al. Mutations in RYR1 are a common cause of exertional myalgia and rhabdomyolysis. Neuromuscul Disord. 2013;23(7):540-8.
[31] Matthews E, Neuwirth C, Jaffer F, Scalco RS, Fialho D, Parton M, et al. Atypical periodic paralysis and myalgia: A novel RYR1 phenotype. Neurology. 2018;90(5):e412-e8.
[32] Riazi S, van den Bersselaar L, Islander G, Heytens L, Snoeck M, Bjorksten A, et al. Pre-operative exercise and pyrexia as modifying factors in malignant hyperthermia. Neuromuscular Disorders. 2021;31:S49-S.
[33] Knuiman GJ, Kusters B, Eshuis L, Snoeck M, Lammens M, Heytens L, et al. The histopathological spectrum of malignant hyperthermia and rhabdomyolysis due to RYR1 mutations. Journal of Neurology. 2019;266(4):876-87.
[34] van den Bersselaar LR, Kruijt N, Scheffer GJ, van Eijk L, Malagon I, Buckens S, et al. The neuromuscular and multisystem features of RYR1-related malignant hyperthermia and rhabdomyolysis: A study protocol. Medicine (Baltimore). 2021;100(33):e26999.
[35] Lopez RJ, Byrne S, Vukcevic M, Sekulic-Jablanovic M, Xu LF, Brink M, et al. An RYR1 mutation associated with malignant hyperthermia is also associated with bleeding abnormalities. Science Signaling. 2016;9(435).
[36] Klein A, Lillis S, Munteanu I, Scoto M, Zhou H, Quinlivan R, et al. Clinical and genetic findings in a large cohort of patients with ryanodine receptor 1 gene-associated myopathies. Hum Mutat. 2012;33(6):981-8.
[37] Colombo I, Scoto M, Manzur AY, Robb SA, Maggi L, Gowda V, et al. Congenital myopathies: Natural history of a large pediatric cohort. Neurology. 2015;84(1):28-35.
[38] Todd JJ, Sagar V, Lawal TA, Allen C, Razaqyar MS, Shelton MS, et al. Correlation of phenotype with genotype and protein structure in RYR1-related disorders. J Neurol. 2018;265(11):2506-24.
[39] Natera-de Benito D, Ortez C, Jou C, Jimenez-Mallebrera C, Codina A, Carrera-Garcia L, et al. The Phenotype and Genotype of Congenital Myopathies Based on a Large Pediatric Cohort. Pediatr Neurol. 2021;115:50-65.
[40] Witherspoon JW, Vuillerot C, Vasavada RP, Waite MR, Shelton M, Chrismer IC, et al. Motor function performance in individuals with RYR1-related myopathies. Muscle Nerve. 2019;60(1):80-7.
[41] van Ruitenbeek E, Custers JAE, Verhaak C, Snoeck M, Erasmus CE, Kamsteeg EJ, et al. Functional impairments, fatigue and quality of life in RYR1-related myopathies: A

questionnaire study. Neuromuscul Disord. 2019;29(1):30-8.

[42] Witting N, Laforet P, Voermans NC, Roux-Buisson N, Bompaire F, Rendu J, et al. Phenotype and genotype of muscle ryanodine receptor rhabdomyolysis-myalgia syndrome. Acta Neurol Scand. 2018;137(5):452-61.

[43] de Souza A. Adult-onset selective quadriceps femoris weakness in RYR1-related myopathy. Neurol Sci. 2022;43(5):3453-5.

[44] Scalco RS, Snoeck M, Quinlivan R, Treves S, Laforet P, Jungbluth H, et al. Exertional rhabdomyolysis: Physiological response or manifestation of an underlying myopathy? BMJ Open Sport Exerc Med. 2016;2(1):e000151.

[45] Kyriakides T, Angelini C, Schaefer J, Mongini T, Siciliano G, Sacconi S, et al. EFNS review on the role of muscle biopsy in the investigation of myalgia. Eur J Neurol. 2013;20(7):997-1005.

[46] Kyriakides T, Angelini C, Schaefer J, Sacconi S, Siciliano G, Vilchez JJ, et al. EFNS guidelines on the diagnostic approach to pauci- or asymptomatic hyperCKemia. Eur J Neurol. 2010;17(6):767-73.

[47] Riazi S, Larach MG, Hu C, Wijeysundera D, Massey C, Kraeva N. Malignant hyperthermia in Canada: Characteristics of index anesthetics in 129 malignant hyperthermia susceptible probands. Anesth Analg. 2014;118(2):381-7.

[48] Forrest KM, Foulds N, Millar JS, Sutherland PD, Pappachan VJ, Holden S, et al. RYR1-related malignant hyperthermia with marked cerebellar involvement - a paradigm of heat-induced CNS injury? Neuromuscul Disord. 2015;25(2):138-40.

[49] Hopkins PM, Ruffert H, Snoeck MM, Girard T, Glahn KP, Ellis FR, et al. European Malignant Hyperthermia Group guidelines for investigation of malignant hyperthermia susceptibility. Br J Anaesth. 2015;115(4):531-9.

[50] Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-24.

[51] Johnston JJ, Dirksen RT, Girard T, Hopkins PM, Kraeva N, Ognoon M, et al. Updated variant curation expert panel criteria and pathogenicity classifications for 251 variants for RYR1-related malignant hyperthermia susceptibility. Hum Mol Genet. 2022.

[52] Treves S, Jungbluth H, Muntoni F, Zorzato F. Congenital muscle disorders with cores: The ryanodine receptor calcium channel paradigm. Curr Opin Pharmacol. 2008;8(3):319-26.

[53] MacLennan DH, Phillips MS. Malignant hyperthermia. Science. 1992;256(5058):789-94.

[54] Lawal TA, Todd JJ, Witherspoon JW, Bonnemann CG, Dowling JJ, Hamilton SL, et al. Ryanodine receptor 1-related disorders: An historical perspective and proposal for a unified nomenclature. Skelet Muscle. 2020;10(1):32.

[55] Avila G, O'Connell KMS, Dirksen RT. The pore region of the skeletal muscle ryanodine receptor is a primary locus for excitation-contraction uncoupling in central core disease. Journal of General Physiology. 2003;121(4):277-86.

[56] Monnier N, Marty I, Faure J, Castiglioni C, Desnuelle C, Sacconi S, et al. Null mutations causing depletion of the type 1 ryanodine receptor (RYR1) are commonly associated with recessive structural congenital myopathies with cores. Hum Mutat. 2008;29(5):670-8.

[57] Rokach O, Sekulic-Jablanovic M, Voermans N, Wilmshurst J, Pillay K, Heytens L, et al. Epigenetic changes as a common trigger of muscle weakness in congenital myopathies. Human Molecular Genetics. 2015;24(16):4636-47.

[58] Bachmann C, Noreen F, Voermans NC, Schar PL, Vissing J, Fock JM, et al. Aberrant regulation of epigenetic modifiers contributes to the pathogenesis in patients with selenoprotein N-related myopathies. Hum Mutat. 2019;40(7):962-74.

[59] Zhou H, Brockington M, Jungbluth H, Monk D, Stanier P, Sewry CA, et al. Epigenetic allele silencing unveils recessive RYR1 mutations in core myopathies. Am J Hum Genet. 2006;79(5):859-68.

[60] Ruiz A, Benucci S, Duthaler U, Bachmann C, Franchini M, Noreen F, et al. Improvement of muscle strength in a mouse model for congenital myopathy treated with HDAC and DNA methyltransferase inhibitors. Elife. 2022;11.

[61] des Georges A, Clarke OB, Zalk R, Yuan Q, Condon KJ, Grassucci RA, et al. Structural Basis for Gating and Activation of RyR1. Cell. 2016;167(1):145-57 e17.

[62] Melville Z, Dridi H, Yuan Q, Reiken S, Wronska A, Liu Y, et al. A drug and ATP binding site in type 1 ryanodine receptor. Structure. 2022;30(7):1025-34 e4.

[63] Melville Z, Kim K, Clarke OB, Marks AR. High-resolution structure of the membrane-embedded skeletal muscle ryanodine receptor. Structure. 2022;30(1):172-80 e3.

[64] Rebbeck RT, Singh DP, Janicek KA, Bers DM, Thomas DD, Launikonis BS, et al. RyR1-targeted drug discovery pipeline integrating FRET-based high-throughput screening and human myofiber dynamic Ca2+assays. Scientific Reports. 2020;10(1).

[65] Gruber SJ, Cornea RL, Li J, Peterson KC, Schaaf TM, Gillispie GD, et al. Discovery of Enzyme Modulators via High-Throughput Time-Resolved FRET in Living Cells. Journal of Biomolecular Screening. 2014;19(2):215-22.

[66] Schaaf TM, Kleinboehl E, Yuen SL, Roelike LN, Svensson B, Thompson AR, et al. Live-Cell Cardiac-Specific High-Throughput Screening Platform for Drug-Like Molecules That Enhance Ca2+Transport. Cells. 2020;9(5).

[67] Stroik DR, Yuen SL, Janicek KA, Schaaf TM, Li J, Ceholski DK, et al. Targeting protein-protein interactions for therapeutic discovery via FRET-based high-throughput screening in living cells. Scientific Reports. 2018;8.

[68] Zhang JY, Singh DP, Ko CY, Nikolaienko R, Yuen SMWK, Schwarz JA, et al. Cardiac ryanodine receptor N-terminal region biosensors identify novel inhibitors via FRET-based high-throughput screening. Journal of Biological Chemistry. 2022;298(1).

[69] Lindsay A, Baumann CW, Rebbeck RT, Yuen SL, Southern WM, Hodges JS, et al. Mechanical factors tune the sensitivity of mdx muscle to eccentric strength loss and its protection by antioxidant and calcium modulators. Skeletal Muscle. 2020;10(1).

[70] Murayama T, Kurebayashi N, Ishigami-Yuasa M, Mori S, Suzuki Y, Akima R, et al. Efficient High-Throughput Screening by Endoplasmic Reticulum Ca2+Measurement to Identify Inhibitors of Ryanodine Receptor Ca2+-Release Channels. Molecular Pharmacology. 2018;94(1):722-30.

[71] Mori S, Iinuma H, Manaka N, Ishigami-Yuasa M, Murayama T, Nishijima Y, et al. Structural development of a type-1 ryanodine receptor (RyR1) Ca2+-release chan-

nel inhibitor guided by endoplasmic reticulum Ca2+assay. European Journal of Medicinal Chemistry. 2019;179:837-48.

[72] Yamazawa T, Kobayashi T, Kurebayashi N, Konishi M, Noguchi S, Inoue T, et al. A novel RyR1-selective inhibitor prevents and rescues sudden death in mouse models of malignant hyperthermia and heat stroke. Nature Communications. 2021;12(1).

[73] Murayama T, Kurebayashi N, Kobayashi T, Okazaki S, Yamashiro K, Sakurai T. High-throughput platform of reconstituted skeletal muscle depolarization-induced Ca2+release in HEK293 cells. Biophysical Journal. 2022;121(3):88.

[74] Darin N, Tulinius M. Neuromuscular disorders in childhood: A descriptive epidemiological study from western Sweden. Neuromuscular Disorders. 2000;10(1):1-9.

[75] Hughes MI, Hicks EM, Nevin NC, Patterson VH. The prevalence of inherited neuromuscular disease in Northern Ireland. Neuromuscular Disorders. 1996;6(1): 69-73.

[76] Norwood FLM, Harling C, Chinnery PF, Eagle M, Bushby K, Straub V. Prevalence of genetic muscle disease in Northern England: In-depth analysis of a muscle clinic population. Brain. 2009;132:3175-86.

[77] Deenen JCW, Arnts H, van der Maarel SM, Padberg GW, Verschuuren JJGM, Bakker E, et al. Population-based incidence and prevalence of facioscapulohumeral dystrophy. Neurology. 2014;83(12):1056-9.

[78] Garibaldi M, Rendu J, Brocard J, Lacene E, Faure J, Brochier G, et al. "Dusty core disease' (DuCD): Expanding morphological spectrum of RYR1 recessive myopathies. Acta Neuropathologica Communications. 2019;7.

[79] Pelletier L, Petiot A, Brocard J, Giannesini B, Giovannini D, Sanchez C, et al. In vivo RyR1 reduction in muscle triggers a core-like myopathy. Acta Neuropathologica Communications. 2020;8(1).

[80] Romero NB, Clarke NF. Congenital myopathies. Pediatric Neurology, Pt Iii. 2013;113:1321-36.

[81] 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. Human Molecular Genetics. 2003;12(10):1171-8.

[82] Bitoun M, Maugenre S, Jeannet PY, Lacene E, Ferrer X, Laforet P, et al. Mutations in dynamin 2 cause dominant centronuclear myopathy. Nature Genetics. 2005;37(11):1207-9.

[83] Laporte J, Hu LJ, Kretz C, Mandel JL, Kioschis P, Coy JF, et al. A gene mutated in X-linked myotubular myopathy defines a new putative tyrosine phosphatase family conserved in yeast. Nature Genetics. 1996;13(2):175-82.

[84] Nicot AS, Toussaint A, Tosch V, Kretz C, Wallgren-Pettersson C, Iwarsson E, et al. Mutations in amphiphysin 2 (BIN1) disrupt interaction with dynamin 2 and cause autosomal recessive centronuclear myopathy. Nature Genetics. 2007;39(9):1134-9.

[85] Neto OA, Heise CO, Moreno CDM, Estephan ED, Mesrob L, Lechner D, et al. Nonlethal CHRNA1-Related Congenital Myasthenic Syndrome with a Homozygous Null Mutation. Canadian Journal of Neurological Sciences. 2017;44(1):125-7.

[86] Jungbluth H, Zhou H, Sewry CA, Robb S, Treves S, Bitoun M, et al. Centronuclear myopathy due to a de novo dominant mutation in the skeletal muscle ryanodine receptor (RYR1) gene. Neuromuscular Disorders. 2007;17(4):338-45.

[87] Schartner V, Laporte J, Bohm J. Abnormal Excitation-Contraction Coupling and Calcium Homeostasis in Myopathies and Cardiomyopathies. Journal of Neuromuscular Diseases. 2019;6(3):289-305.

[88] Morin G, Biancalana V, Echaniz-Laguna A, Noury JB, Lornage X, Moggio M, et al. Tubular aggregate myopathy and Stormorken syndrome: Mutation spectrum and genotype/phenotype correlation. Human Mutation. 2020;41(1):17-37.

[89] Vattemi GNA, Rossi D, Galli L, Catallo MR, Pancheri E, Marchetto G, et al. Ryanodine receptor 1 (RYR1) mutations in two patients with tubular aggregate myopathy. European Journal of Neuroscience. 2022;56(3):4214-23.

[90] Biancalana V, Rendu J, Chaussenot A, Mecili H, Bieth E, Fradin M, et al. A recurrent RYR1 mutation associated with early-onset hypotonia and benign disease course. Acta Neuropathologica Communications. 2021;9(1).

[91] Cong L, Ran FA, Cox D, Lin SL, Barretto R, Habib N, et al. Multiplex Genome Engineering Using CRISPR/Cas Systems. Science. 2013;339(6121):819-23.

[92] Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F. Genome engineering using the CRISPR-Cas9 system. Nature Protocols. 2013;8(11):2281-308.

[93] Jinek M, East A, Cheng A, Lin S, Ma EB, Doudna J. RNA-programmed genome editing in human cells. Elife. 2013;2.

[94] Nelson JW, Randolph PB, Shen SMP, Everette KA, Chen PTJ, Anzalone AV, et al. Engineered pegRNAs improve prime editing efficiency (Oct, 10.1038/s41587-021-01039-7, 2021). Nature Biotechnology. 2021.

[95] Truong DJJ, Kuhner K, Kuhn R, Werfel S, Engelhardt S, Wurst W, et al. Development of an intein-mediated split-Cas9 system for gene therapy. Nucleic Acids Research. 2015;43(13):6450-8.

[96] Villiger L, Grisch-Chan HM, Lindsay H, Ringnalda F, Pogliano CB, Allegri G, et al. Treatment of a metabolic liver disease by *in vivo* genome base editing in adult mice. Nature Medicine. 2018;24(10):1519-+.

[97] Levy JM, Yeh WH, Pendse N, Davis JR, Hennessey E, Butcher R, et al. Cytosine and adenine base editing of the brain, liver, retina, heart and skeletal muscle of mice via adeno-associated viruses. Nature Biomedical Engineering. 2020;4(1):97-110.

[98] Wang D, Zhang F, Gao GP. CRISPR-Based Therapeutic Genome Editing: Strategies and In Vivo Delivery by AAV Vectors. Cell. 2020;181(1):136-50.

[99] Majeau N, Fortin-Archambault A, Gerard C, Rousseau J, Yameogo P, Tremblay JP. Serum extracellular vesicles for delivery of CRISPR-CAS9 ribonucleoproteins to modify the dystrophin gene. Molecular Therapy. 2022;30(7):2429-42.

[100] Ediriweera GR, Chen LY, Yerbury JJ, Thurecht KJ, Vine KL. Non-Viral Vector-Mediated Gene Therapy for ALS: Challenges and Future Perspectives. Molecular Pharmaceutics. 2021;18(6):2142-60.

[101] Xu CF, Chen GJ, Luo YL, Zhang Y, Zhao G, Lu ZD, et al. Rational designs of *in vivo* CRISPR-Cas delivery systems. Advanced Drug Delivery Reviews. 2021;168:3-29.

[102] Yan JY, Kang DN, Dong YZ. Harnessing lipid nanoparticles for efficient CRISPR delivery. Biomaterials Science. 2021;9(18):6001-11.

[103] Taha EA, Lee J, Hotta A. Delivery of CRISPR-Cas tools for *in vivo* genome editing therapy: Trends and challenges. Journal of Controlled Release. 2022;342:345-61.
[104] Alemdaroglu I, Karaduman A, Yilmaz OT, Topaloglu H. Different Types of Upper Extremity Exercise Training in Duchenne Muscular Dystrophy: Effects on Functional Performance, Strength, Endurance, and Ambulation. Muscle & Nerve. 2015;51(5):697-705.
[105] Vissing CR, Hedermann G, Vissing J. Moderate-Intensity Aerobic Exercise Improves Physical Fitness in Bethlem Myopathy. Muscle & Nerve. 2019;60(2):183-7.
[106] Hedermann G, Vissing CR, Heje K, Preisler N, Witting N, Vissing J. Aerobic Training in Patients with Congenital Myopathy. Plos One. 2016;11(1).
[107] Heje K, Andersen G, Buch A, Andersen H, Vissing J. High-intensity training in patients with spinal and bulbar muscular atrophy. Journal of Neurology. 2019;266(7):1693-7.
[108] Swan JS, Dibb K, Negretti N, O'Neill SC, Sitsapesan R. Effects of eicosapentaenoic acid on cardiac SR Ca2+-release and ryanodine receptor function. Cardiovascular Research. 2003;60(2):337-46.
[109] McGlory C, Galloway SDR, Hamilton DL, McClintock C, Breen L, Dick JR, et al. Temporal changes in human skeletal muscle and blood lipid composition with fish oil supplementation. Prostaglandins Leukotrienes and Essential Fatty Acids. 2014;90(6):199-206.
[110] Apostolova N, Victor VM. Molecular Strategies for Targeting Antioxidants to Mitochondria: Therapeutic Implications. Antioxidants & Redox Signaling. 2015;22(8):686-729.
[111] Canato M, Capitanio P, Cancellara L, Leanza L, Raffaello A, Reane DV, et al. Excessive Accumulation of Ca2+in Mitochondria of Y522S-RYR1 Knock-in Mice: A Link Between Leak From the Sarcoplasmic Reticulum and Altered Redox State. Frontiers in Physiology. 2019;10.
[112] Chelu MG, Goonasekera SA, Durham WJ, Tang W, Lueck JD, Riehl J, et al. Heat- and anesthesia-induced malignant hyperthermia in an RyR1 knock-in mouse. Faseb Journal. 2006;20(2):329-30.
[113] Dowling JJ, Joubert R, Low SE, Durban AN, Messaddeq N, Li XL, et al. Myotubular myopathy and the neuromuscular junction: A novel therapeutic approach from mouse models. Disease Models & Mechanisms. 2012;5(6):852-9.
[114] Robb SA, Sewry CA, Dowling JJ, Feng L, Cullup T, Lillis S, et al. Impaired neuromuscular transmission and response to acetylcholinesterase inhibitors in centronuclear myopathies. Neuromuscular Disorders. 2011;21(6):379-86.
[115] Gibbs EM, Clarke NF, Rose K, Oates EC, Webster R, Feldman EL, et al. Neuromuscular junction abnormalities in DNM2-related centronuclear myopathy. Journal of Molecular Medicine-Jmm. 2013;91(6):727-37.
[116] Lynch GS, Ryall JG. Role of beta-adrenoceptor signaling in skeletal muscle: Implications for muscle wasting and disease. Physiological Reviews. 2008;88(2):729-67.
[117] Jessen S, Reitelseder S, Kalsen A, Kreiberg M, Onslev J, Gad A, et al. beta(2)-Adrenergic agonist salbutamol augments hypertrophy in MHCIIa fibers and sprint mean power output but not muscle force during 11 weeks of resistance training in young men. Journal of Applied Physiology. 2021;130(3):617-26.
[118] Messina S, Hartley L, Main M, Kinali M, Jungbluth H, Muntoni F, et al. Pilot trial of salbutamol in central core and multi-minicore diseases. Neuropediatrics. 2004;35(5):262-6.
[119] Schreuder LTW, Nijhuis-van der Sanden MWG, de Hair A, Peters G, Wortmann S, Bok LA, et al. Successful use of albuterol in a patient with central core disease and mitochondrial dysfunction. Journal of Inherited Metabolic Disease. 2010;33:S205-S9.
[120] Larkindale J, Betourne A, Borens A, Boulanger V, Crider VT, Gavin P, et al. Innovations in Therapy Development for Rare Diseases Through the Rare Disease Cures Accelerator-Data and Analytics Platform. Ther Innov Regul Sci. 2022;56(5):768-76.
[121] Kushnir A, Todd JJ, Witherspoon JW, Yuan Q, Reiken S, Lin H, et al. Intracellular calcium leak as a therapeutic target for RYR1-related myopathies. Acta Neuropathologica. 2020;139(6):1089-104.
[122] Bellinger AM, Reiken S, Carlson C, Mongillo M, Liu XP, Rothman L, et al. Hypernitrosylated ryanodine receptor calcium release channels are leaky in dystrophic muscle. Nature Medicine. 2009;15(3):325-30.
[123] Brennan S, Garcia-Castaneda M, Michelucci A, Sabha N, Malik S, Groom L, et al. Mouse model of severe recessive RYR1-related myopathy. Hum Mol Genet. 2019;28(18):3024-36.
[124] Happi Mbakam C, Rousseau J, Tremblay G, Yameogo P, Tremblay JP. Prime Editing Permits the Introduction of Specific Mutations in the Gene Responsible for Duchenne Muscular Dystrophy. Int J Mol Sci. 2022;23(11).
[125] Reilly A, Chehade L, Kothary R. Curing SMA: Are we there yet? Gene Ther. 2022.
[126] Li J, Sun W, Wang B, Xiao X, Liu XQ. Protein trans-splicing as a means for viral vector-mediated *in vivo* gene therapy. Hum Gene Ther. 2008;19(9):958-64.
[127] Koo T, Popplewell L, Athanasopoulos T, Dickson G. Triple trans-splicing adeno-associated virus vectors capable of transferring the coding sequence for full-length dystrophin protein into dystrophic mice. Hum Gene Ther. 2014;25(2):98-108.
[128] Loy RE, Lueck JD, Mostajo-Radji MA, Carrell EM, Dirksen RT. Allele-specific gene silencing in two mouse models of autosomal dominant skeletal myopathy. Plos One. 2012;7(11):e49757.
[129] Ko W, Porter JJ, Sipple MT, Edwards KM, Lueck JD. Efficient suppression of endogenous CFTR nonsense mutations using anticodon-engineered transfer RNAs. Mol Ther Nucleic Acids. 2022;28:685-701.
[130] Porter JJ, Heil CS, Lueck JD. Therapeutic promise of engineered nonsense suppressor tRNAs. Wiley Interdiscip Rev RNA. 2021;12(4):e1641.