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Treatments for RYR1-related disorders (Protocol)

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[Intervention Protocol]

Treatments for RYR1-related disorders

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ABSTRACT

Objectives

This is a protocol for a Cochrane Review (intervention). The objectives are as follows:

Primary objective

To analyse the benefits and harms of pharmacological or other interventions (e.g. special diet, exercise programme) compared with placebo or standard care for *RYR1*-related disorders, including both permanent myopathies and intermittent (episodic) presentations (exertional myalgia and rhabdomyolysis), with the aim to improve motor and respiratory function and/or to reduce the frequency of episodes, respectively.

Secondary objectives

1. To assess whether the interventions, compared with placebo or standard of care, change the outcome of *RYR1*-related diseases.
2. To assess whether the interventions, compared with placebo or usual care, change the expression of the disease state in patients with *RYR1*-related diseases.
3. To identify a set of standardised outcome tools to be used in future studies.

BACKGROUND

Description of the condition

The skeletal muscle ryanodine receptor (RyR1) is the main sarcoplasmic reticulum calcium release channel and plays a crucial role in excitation-contraction coupling (ECC), the process whereby a neuronal electrical impulse is translated into calcium release inside the myofibre and, ultimately, muscle contraction. The RyR1 protein is encoded by the *RYR1* gene, which spans 105 exons and more than 15,000 base pairs of exonic sequencing on chromosome 19q13.2.

Dominant and recessive *RYR1* pathogenic variants are associated with a wide range of inherited myopathies, including central core disease (CCD), multi-minicore disease (MmD), centronuclear myopathy (CNM), congenital fibre type disproportion (CFTD), King-Denborough syndrome, late-onset axial myopathy, recurrent exertional rhabdomyolysis/myalgia and atypical periodic paralysis. Non-skeletal muscle-associated manifestations such as a mild bleeding disorder are also increasingly recognised. Dominant *RYR1* mutations may also cause malignant hyperthermia (MH), an altered pharmacogenetic response to halogenated anaesthetics and muscle relaxants in susceptible but otherwise healthy individuals. Similarly, dominant mutations in *RYR1* are found as a cause of 'awake' MH-like events triggered by exercise and/or heat and including heat stroke. Consequently, *RYR1*-related 'induced' disorders/phenotypes should enter the differential diagnosis in patients presenting with exertional myalgia, exertional rhabdomyolysis, anaesthetic complications consistent with an MH reaction and exertional heat illness. The clinical phenotype of diseases due to *RYR1* mutations is highly heterogenous in terms of onset, inheritance, key clinical features and key clinical biomarkers. Table 1 summarises the diseases and the aforementioned factors associated with dominant and recessive *RYR1* gene mutations.

While the exact prevalence of *RYR1*-related diseases is currently uncertain, existing estimates of their pooled prevalence are 0.2 per 100,000 across all ages and 2.76 per 100,000 in the paediatric population [1]. Pathogenic variants may conform to either autosomal recessive or dominant patterns of inheritance. *RYR1* is a large gene, which is usually sequenced in diagnostic centres that use next-generation sequencing (NGS). Massive parallel sequencing of different candidate genes has demonstrated that *RYR1*-related disorders are not uncommon, although precise prevalence data are still missing. In addition, patients with pathogenic *RYR1* variants may be asymptomatic until exposed to specific triggers, potentially resulting in an underestimation of their frequency.

Description of the intervention and how it might work

The purpose of this review is to critique the strength of evidence supporting treatment interventions that specifically target patients with *RYR1* mutations and associated disease manifestations.

Identified interventions will assist in developing our understanding of the treatments available. As efficacy and safety measures will be documented via the available outcome tools, this will enable critique of these measures to support consistency in future clinical trials when delineating outcome measures.

Management of RYR1-related disorders that manifest with chronic weakness is currently mainly restricted to supportive care, focussing on mobility, orthopaedic needs, respiratory function and bulbar issues as per existing consensus care guidelines for congenital myopathies. The phenotypic spectrum of *RYR1*-related disorders greatly varies from congenital myopathy (where weakness is present chronically) to dynamic conditions such as rhabdomyolysis, exertional myalgias or malignant hyperthermia susceptibility [2]. Treatment approaches and outcome measures will differ accordingly. In this review, we will include treatments relating to the whole spectrum of *RYR1*-related disorders, including the most common phenotypes related to static myopathy (i.e. congenital myopathy presentations) and those related to dynamic presentations (such as rhabdomyolysis). Rhabdomyolysis is a medical emergency that poses a substantial burden to public health systems, as it often requires hospital admission, and can include costly intensive care treatment. Rhabdomyolysis-related complications can also have a subsequent medical and financial impact, as acute kidney injury may require both intensive care unit treatment and dialysis, and compartment syndrome often necessitates emergency surgery. The burden of care in *RYR1*-related disorders falls on the patient and their family, and has both economic and clinical resource impacts. In settings where the risk of dynamic phenotypes (rhabdomyolysis, exertional heat illness, malignant hyperthermia) is known or presumed (i.e. in patients with previous episodes or with *RYR1* variants associated with these phenotypes), primary prevention by avoiding triggering exposures is a key interventional strategy.

Interventions in the management of *RYR1*-related disorders may include both pharmacological and non-pharmacological modalities. Targeted interventions focus on normalisation of the *RYR1* channel function. Specific pharmacological treatments under investigation for *RYR1* dominant, 'hyperactive' variants include dantrolene, a pharmacological compound that inhibits calcium release from the sarcoplasmic reticulum by direct and specific action on the skeletal muscle ryanodine (RyR1) receptor [3]. N-acetylcysteine (NAC), an antioxidant, is under investigation for both dominant and recessive *RYR1*-related myopathies. Studies in vertebrate disease models (zebra fish that model recessive *RYR1* and mice that model dominant *RYR1*) and in human myotubes revealed that exposure to NAC reduced oxidative stress and promoted phenotypic rescue (improved survival of pro-oxidant exposed myotubes and improved muscle function in *RYR1* zebra fish). Other compounds that reduce calcium leak from the sarcoplasmic reticulum by enhancing *RYR1*-calstabin interactions, a channel-stabilising protein, include Rycals and AICAR. Rycals reduces Ca^{2+} leak by stabilising the RyR channels through preserving the RyR-calstabin interaction, thereby improving contractile function in both heart and skeletal muscle [4]. AICAR activates adenosine monophosphate (AMP)-activated protein kinase (AMPK), which functions as a cellular energy sensor that is activated by increases in the AMP to adenosine triphosphate (ATP) ratio [5], thereby improving muscle endurance without exercise [5]. Salbutamol has been explored in a small six-month pilot study of patients with CCD and MmD (not all of them genetically resolved), where it was found to be well tolerated and to produce a significant increase in muscle strength (as measured by myometry and manual muscle testing) and pulmonary function (as measured by forced vital capacity (FVC)), suggesting that it may be beneficial in *RYR1* myopathy and central core and minicore histotypes. Exon skipping, an RNA-based genetic strategy to treat *RYR1*-related myopathy was successfully tested in cells for one recessive case of *RYR1*-related myopathy [6]; however, the translatability of this approach may be limited due to the lack of recurrent *RYR1* variants that would be amenable to a single RNA

therapeutic, the current high cost of developing and implementing such therapies, and existing challenges related to the delivery and toxicity of exon skipping molecules (see Table 2).

We will analyse the benefits and harms of both pharmacological and other interventions (e.g. special diet, exercise programme) compared with placebo or standard care for *RYR1*-related disorders. These will include both permanent myopathies and intermittent (episodic) presentations, namely exertional myalgia and rhabdomyolysis, with the aim of the intervention being to improve motor and respiratory function and/or to reduce the frequency of episodes, respectively. In addition, we aim to assess the effects of the interventions on the outcome of *RYR1*-related diseases, to determine whether the interventions result in a change in the expression of the disease state in patients with RYR1-related diseases and to identify a set of standardised outcome tools to be used in future studies. Details of the existing tools used and reported in clinical practice can be found in Table 3. We understand that there is currently no consistency in the outcome measures used. During the review process, we will identify the most commonly used outcome measures, whilst ensuring these are reproducible, translatable and relevant to the disease. Existing drug treatments may be pre-clinical (mouse or zebra fish) or clinical, specifically in children with CCD or MmD. Further details regarding existing treatments/interventions may be found in Table 2.

Why it is important to do this review

RYR1-related disorders are the most common cause of congenital myopathy. This is a highly heterogenous group of conditions and early interventions have an impact on outcomes. This review will set a framework for future studies as they become available. A consistent format for inclusion criteria and outcome measures will assist in the design of standardised studies that can strengthen future meta-analysis.

Currently, no cure or significant disease-modifying therapies exist for *RYR1*-related disorders. There is no international standard of care in terms of drug treatment prescription, and standardised guidelines for the management and treatment of *RYR1*-related disorders have not been developed. A consensus statement on standard of care for congenital myopathies was published over 10 years ago, although this is not specific to *RYR1*-related disorders [7]. In addition, the tools used to measure outcomes are not consistent, limiting potential comparison across studies (Table 3).

This review will enhance the understanding of existing treatment interventions. Standardisation of outcome measures will increase consistency and directness in future studies, as well as improve comparison of future studies.

OBJECTIVES

Primary objective

To analyse the benefits and harms of pharmacological or other interventions (e.g. special diet, exercise programme) compared with placebo or standard care for *RYR1*-related disorders, including both permanent myopathies and intermittent (episodic) presentations (exertional myalgia and rhabdomyolysis), with the aim to improve motor and respiratory function and/or to reduce the frequency of episodes, respectively.

Secondary objectives

1. To assess whether the interventions, compared with placebo or standard of care, change the outcome of *RYR1*-related diseases.
2. To assess whether the interventions, compared with placebo or usual care, change the expression of the disease state in patients with *RYR1*-related diseases.
3. To identify a set of standardised outcome tools to be used in future studies.

METHODS

Criteria for considering studies for this review

Types of studies

We will include randomised controlled trials (RCTs) and quasi-RCTs. Quasi-randomised trials are parallel-group studies that allocate participants using an approximation of randomisation, such as the use of alternation, case record number or date of attendance.

We anticipate that there will be no or few clinical trials. Therefore, we will consider cluster-randomised, cross-over and non-randomised studies according to previous recommendations [8]. We will handle cross-over and cluster-randomised trials according to Chapter 23 of the *Cochrane Handbook for Systematic Reviews of Interventions* (henceforth called the *Cochrane Handbook*) [9]. We will include non-randomised studies with a comparison arm, as in previous Cochrane protocols [10]. We will also consider observational studies reporting an intervention with a comparison group. Clinical studies are expected to be published in the future by clinically experienced teams and may be included in future updates of the review.

The rationale for including non-randomised studies is “to provide evidence of the effects (benefit or harm) of interventions that can feasibly be studied in randomized trials, but for which only a small number of randomized trials is available (or likely to be available)” as suggested by the *Cochrane Handbook* [11].

We will identify standardised outcome tools during the process of reviewing articles. We will not use reporting of outcome measures as a criterion for including studies. Details of existing tools used and reported in clinical practice can be found in Table 3. Currently, there is no consistency in the outcome measures used. We will identify the most commonly used outcome measures, whilst ensuring these are reproducible, translatable and relevant to the disease, during the review process. Multiple outcomes/measures will be scored, independent of the result. Comparability across tools is challenging; however, an objective of this review is to explore the tools that are used. This will be documented and may be used as a framework to enable consistency in future studies.

For non-randomised studies of interventions (NRSI), we will consider the following confounding factors and co-interventions: for example, age (children versus adults), non-confirmed molecular diagnosis, co-morbidities, antioxidant treatments (unless these are the test intervention), patients taking acetaminophen, nitroglycerine, carbamazepine and β2-adrenergic agonist use as adjuvants.

We will review studies independently of their reporting status, including full text, those published as an abstract only and unpublished data, to explore potentially viable reports. We will only

include studies with no comparison group (case reports, single-arm design, etc.) for the background or discussion of the review and not for the actual analysis.

There will be no restrictions as to language of publication.

Types of participants

We will include both children and adults of any age, male and female, from any clinical setting with (pathogenic or likely pathogenic) variants in *RYR1* defined according to the American College of Medical Genetics and Genomics (ACMG) criteria [12]. If there are no genetic results, we will include the study in the background or discussion section and not in the formal analysis or recommendations.

We will classify participants according to their phenotype, to include:

1. (Congenital) myopathies – autosomal dominant/autosomal recessive diseases causing CCD, MmD, CNM, CFTD, late-onset axial myopathy and King-Denborough syndrome.
2. (Exertional) rhabdomyolysis (presenting with at least one episode of rhabdomyolysis, defined by muscle pain, weakness and/or swelling in association with an acute > 5-fold rise and subsequent fall in serum creatine kinase (CK) levels).
3. Exertional myalgia (defined as muscle pain elicited by exercise, with a discrepancy between the exercise intensity/frequency and pain severity/frequency (in comparison to peers with a similar level of physical fitness)).

If the studies include additional non-eligible groups of patients, we will only collect data for the subgroup of interest. If the studies include both eligible and non-eligible participants in the same group, we will collect data only if the majority (≥ 50%) of the patients are eligible. We will perform a sensitivity analysis excluding studies with mixed populations, to investigate the robustness of our conclusions.

Types of interventions

Any pharmacological agent specifically targeting patients with *RYR1* mutations. We will analyse treatments separately by drug and by phenotype. Interventions will be compared with *RYR1* mutation managed with standard of care, defined as treatment accepted by experts as evidence-based and widely used by healthcare professionals.

Comparisons will be made according to the phenotype of permanent and episodic disease states.

Permanent

- Symptomatic support, e.g. salbutamol.
  - Targeted interventions that focus on normalisation of the *RYR1* channel function, e.g. salbutamol, dantrolene, N-acetylcysteine Rycals and AICAR, exercise regimens (rehabilitation targeted).
- Disease-modifying interventions, e.g. gene therapy.
- Other interventions, such as special diet and exercise programmes.

Episodic disease states – rhabdomyolysis/(external) myalgia

- Interventions to prevent recurrence as compared to placebo.
- Pharmacological treatments (active: new drugs; control: old drugs)/non-pharmacological interventions (avoidance of triggers for rhabdomyolysis).
- Rhabdomyolysis/exertional myalgia: placebo/other pharmacological treatments (active: new drugs; control: old drugs)/non-pharmacological intervention (avoiding triggers for exercise-related muscle pain).

Outcome measures

We will manage multiplicity of outcome measures by applying a hierarchy based on the frequency of use of the measures among the included studies [13]. We will only consider measures obtained with validated methods or scales [13]. We will only include data with the highest rank of the measure when a study reports the same outcome based on data acquired from multiple measures [13].

Primary and secondary outcome measures are described below.

Critical outcomes

From baseline up to 12 months.

1. Permanent: improvement in:
   a. motor function, evaluated by verified tools such as MRC and Hammersmith scores [14, 15], respectively, within one, three and six months of treatment; and/or
   b. respiratory function, evaluated by verified tools such as spirometry within one, three and six months of treatment, respectively.
2. Episodic: rhabdomyolysis/exertional myalgia: reduction in recurrence measured by "number of episodes" in 12 months of treatment.

Important outcomes

1. Mean change in total walked distance (for the duration of the trial) for ambulant children and adults.
2. Mean change in respiratory function assessed by % expected FVC standardised by year.
3. Mean change in ventilator support as measured by duration of support needed in 24-hour periods.
4. Any adverse events: adverse events that lead to discontinuation of treatment and serious adverse events (SAEs), which are those that are fatal, life-threatening or require prolonged hospitalisation for the duration of the trial. SAEs are defined according to the US Food and Drug Administration (FDA) website (www.fda.gov).
5. Survival at six months or longer.

Search methods for identification of studies

Electronic searches

A Medical Librarian will search the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE and Embase. No limits or filters will be applied. We will search all databases from inception to the present, and we will impose no restriction on language of publication. We will use the highly sensitive search strategy designed by Cochrane for identifying randomised controlled trials and controlled clinical trials [16].

We will search the following databases (see Supplementary material 1):

- PubMed from inception (1946) to present;
- Embase via Ovid (1974 to present);
- Cochrane Central Register of Controlled Trials (CENTRAL; latest issue).

Some search terms were taken from previous protocols [3, 2]. We are using some terms from these search strategies to increase the comprehensiveness of our search strategy as recommended by the *Cochrane Handbook*, which states that "a third approach for identifying text words consists of checking search strategies from other systematic reviews on a similar topic" [16].

Searching other resources

We will also conduct a search of the US National Institutes for Health Clinical Trials Registry, ClinicalTrials.gov (www.ClinicalTrials.gov) and the WHO International Clinical Trials Registry Portal (ICTRP). We will search all databases from inception to the present, and we will impose no restriction on language of publication [16]. Searches will include the reference lists of included trials and relevant reviews.

We will use the highly sensitive search strategy to identify randomised controlled trials (Supplementary material 1).

We will search for retractions and expressions of concern about the included studies through MEDLINE/PubMed and the Retraction Watch database; we will report the date of these searches.

Data collection and analysis

Selection of studies

We will manage studies with Covidence [17].

Groups of two review authors will work independently to screen titles and abstracts of all the potential studies identified from the search under each sub-heading (rhabdomyolysis and myopathy subtypes). We will check review articles for additional studies not captured by the initial search. We will code potential studies as retrieve (definitely or potentially eligible) and do not retrieve (exclude as does not meet eligibility criteria). Two review authors will assess full-text articles independently. Differences in assessments will be resolved through independent review by a separate member of the team.

We will separate studies into:

- include and analyse - RCTs, cross-over, cluster-randomised and non-randomised trials, observational studies;
- exclude after full read - does not meet eligibility criteria.

The unit of interest for the review is treatments for *RYR1* mutations. We will group multiple reports and papers related to a single study under a single reference ID.

We will document the selection process in a PRISMA flow diagram and provide a 'Characteristics of excluded studies' table [18].

Population: humans with *RYR1* mutations, sub-categorised into disease subgroups (permanent and episodic).

Intervention:

Permanent

1. Symptomatic - pyridostigmine, salbutamol, albuterol, drug, drug therapy, pharmacological, modulation of neuromuscular junction, myostatin inhibitor
2. Disease-modifying - gene transfer therapy, gene therapy
3. Other - special diet and exercise programme

Episodic disease states

1. Interventions to prevent recurrence as compared to placebo
2. Pharmacological and non-pharmacological treatments for rhabdomyolysis or exertional myalgia

Comparison: population with *RYR1* mutation, i.e. placebo-controlled arms (all participants are presumed to be managed with standard of care).

Outcome: evidence of safety and efficacy, standardisation of optimal outcome measures.

Data extraction and management

Seven authors (SR, JW, NV, LS, JD, GB, HJ) will extract data in pairs varied for each *RYR1* disease subtype. Disagreements will be resolved by an independent person from the group who will further cross-check the data for duplications and erroneous inclusions. A piloted form will be trialled by each pair for a randomly allocated study. We will contact key groups invested in the research of *RYR1* mutations to ensure that additional data are not missed, e.g. TREAT NMD task force for congenital myopathies.

We will extract information on the study design, setting, participant characteristics, study eligibility criteria, intervention details, outcomes assessed, source of study funding and conflicts of interest stated by the researchers [13].

We will import data into a database that will be shared with all study collaborators. SR/JW will verify the content and discuss discrepancies with the group.

We will construct the review based on consensus of definitions of the diseases associated with *RYR1* mutations, following understanding of the pathological process that results in the spectrum of phenotypes. We will separate the interventions into symptomatic and disease-modifying. We will assess the outcome measures under the primary outcomes: improvement in motor and/or respiratory function and reduction in the recurrence of episodes of exertional myalgia and rhabdomyolysis. Secondary outcomes expand on this in relation to the mean change in total distance walked, mean change in respiratory function, mean change in ventilator support, adverse events and survival at six months or longer. We will assess the studies assessing these factors in line with GRADE for directness and consistency.

We will state the certainty of the existing evidence and the strength of statements about the safety and efficacy of the interventions. The review will close with recommendations for where further studies are needed. Where there are multiple outcomes or measures, as defined in Table 3, we will include all these outcomes in the analysis. We will present data on participants with permanent and episodic disease states in separate summary of findings tables.

We will present the results as a poster session at several medical conferences, such as the *World Muscle Society* congress and the UK MRC Translational Research Conference. In addition, the

information will be relayed on related social media platforms and to disease-relevant patient support groups.

Risk of bias assessment in included studies

Groups of two review authors (SR, JW, NV, LS, JD, GB, HJ) will independently assess the risk of bias for each study using the criteria outlined in the *Cochrane Handbook* [19]. We will resolve any disagreements by discussion and by involving another author from the review author group (SR, JW, NV, JD, LS, GB, HJ).

We will use the Cochrane risk of bias tool version 2 (RoB 2) for RCTs [20], RoB 2 variants for cluster-randomised and cross-over studies (https://www.riskofbias.info/) and the Risk of Bias in Non-randomized Studies of Interventions (ROBINS-I) for non-randomised studies [21].

For both RCTs and NRSIs, we will assess the risk of bias for the critical outcome results, measured at the longest follow-up (see Critical outcomes and Measures of treatment effect).

Using RoB 2, assessment will include the following domains [20]:

- bias arising from the randomisation process;
- bias due to deviations from intended interventions;
- bias due to missing outcome data;
- bias in measurement of the outcome; and
- bias in selection of the reported result.

The RoB 2 variants for cluster-randomised studies and cross-over trials include one additional domain each: bias arising from the timing and recruitment of participants, and bias arising from period and carryover effects, respectively.

We will assess the risk of bias in each domain and the overall risk of bias as 'low risk', 'some concerns' or 'high risk' using the signalling questions/tool algorithms. We will consider that some of the signalling questions and guidance for RoB 2 for cluster-RCTs differ from those for parallel RCTs (i.e. considerations for missing data and baseline imbalances). We will document the risk of bias using Excel templates (available at https://www.riskofbias.info/). Answers to signalling questions will be made publicly available online through the Open Science Framework (https://osf.io).

ROBINS-I, in turn, includes seven domains for assessment: domains 2 to 5 of RoB 2 in addition to confounding, selection bias and measurement classification of interventions. This tool categorises studies as 'low risk', 'moderate risk', 'serious risk' or 'critical risk' of bias, for each outcome.

When considering treatment effects, we will take into account the risk of bias for the studies that contribute to that outcome.

We will summarise risk of bias judgements for each outcome across different studies for each of the domains listed, where the overall risk of bias for the result will be the least favourable assessment across the domains of bias. We will analyse the effect of assignment to the intervention at baseline, regardless of whether the interventions are received as intended (the 'intention-to-treat effect').

We will include a figure with risk of bias assessments in each domain, along with a rationale for our decisions, to illustrate risk of bias, and we will add this information to figures showing meta-analysis when possible. This information will be available for each outcome included in the review.

We will make summary assessments of the risk of bias for each outcome (across domains) within and across studies. The assessments will inform the GRADE assessments of the certainty of the evidence [19], and will be included in the summary of findings tables. The timing and measures for assessment of each outcome will be consistent with the Outcome measures section.

We will use the results of the risk of bias assessments (using both RoB 2 and ROBINS-I) to feed directly into the GRADE assessment domain risk of bias and the summary of findings tables. 'Low' risk of bias would indicate 'no limitation'; 'some concerns' would indicate either 'no limitation' or 'serious limitation'; and 'high' risk of bias would indicate either 'serious limitation' or 'very serious limitation'. 'Critical' risk of bias on ROBINS-I would indicate extremely serious limitations in GRADE.

We will assess reports for (non-pharmaceutical) interventions outside the main study intervention arm that could impact on outcome. This may include additional physiotherapy, surgical intervention such as Nissen fundoplication/gastrostomy and initiation of non-invasive ventilation.

For meta-analysis, we will perform sensitivity analyses excluding studies showing a high risk of bias. In addition, we will only include the results of non-randomised trials if their overall risk of bias is low to moderate.

Assessment of bias in conducting the systematic review

We will report any deviations from this published protocol in the systematic review.

Measures of treatment effect

We will analyse dichotomous data (adverse events, survival) as risk ratios (RR) and continuous data (improvement in motor and or respiratory function, reduction in recurrence of rhabdomyolysis/exertional myalgia, change in total walked distance, change in respiratory function, change in ventilator support) as mean differences, or as standardised mean differences for results across studies with outcomes that are conceptually the same but measured in different ways. We will enter data presented as a scale with a consistent direction of effect.

We will undertake meta-analyses only where this is meaningful, i.e. if the treatments, participants and the underlying clinical question are similar enough for pooling to make sense.

If data are not reported in a format that we can enter directly into a meta-analysis, we will convert the data to the required format using the information in Chapter 6 of the *Cochrane Handbook* [22].

Where trials do not report time-to-event estimates, we will extract data from papers using defined methods [23, 24]. We will report summary estimates as hazard ratios (HRs) with 95% confidence intervals (CIs).

Unit of analysis issues

Where multiple trial arms are reported in a single trial, we will note all trial arms but only include data for arms relevant to the review topic. If two comparisons (e.g. drug A versus placebo and drug B

versus placebo) are combined in the same meta-analysis, we will follow the guidance in Section 16.5.4 of the *Cochrane Handbook* to avoid double-counting [9]. Our preferred approach will be to combine intervention groups, if clinically appropriate, or halve a control group.

To avoid unit of analysis errors by incorporating cluster-randomised trials, the unit of analysis for these studies will be the cluster. For cross-over trials, we will include data from the first period only because of the dynamic course of *RYR1*-related diseases.

Dealing with missing data

We will contact investigators or study sponsors in order to verify key study characteristics and obtain missing numerical outcome data where possible (e.g. when a study is available as an abstract only). This is a highly specialised area of research, and the review authors expect to be able to access all investigators involved in the field to ensure completeness of data and to avoid bias of restricted inclusion of results. Should the data not be accessible, this will be acknowledged in the discussion.

Reporting bias assessment

We do not expect to have more than 10 studies across the review and, as such, will not utilise funnel plots [25].

Synthesis methods

We will use Review Manager (RevMan) to conduct the analyses. One author will enter the data into RevMan and another author will check it for accuracy.

If a meta-analysis is deemed appropriate, we will use a fixed-effect model. We will not choose a model based on heterogeneity tests; only if we find that the true effect size does vary across studies will we opt for a random-effects model. We do not feel that this approach will need us to undertake a sensitivity analysis, but we will do so if required.

We will apply the inverse variance method for continuous data summarised by arithmetic means and standard deviations. We will pool continuous outcomes measured with different units or scales using the standardised mean difference (SMD); we will use defined methods for SMD interpretation, to convert and interpret the pooled SMDs as mean differences with 95% confidence intervals in the original units of a scale with the most clinical relevance and impact [26]. We will synthesise dichotomous data using the Mantel-Haenszel method.

We will perform separate meta-analyses for RCTs and NRSIs; these studies will not be pooled together.

We will perform subgroup analyses to explore potential sources of heterogeneity if we find this. We will present the summarised results of the meta-analysis in forest plots. We will only pool the results of non-randomised trials if the overall risk of bias is low to moderate.

Any data that cannot be included in meta-analysis will be reported narratively and in a tabular format, using the Synthesis Without Meta-analysis (SWiM) recommendations [27].

We will investigate different types of heterogeneity, including clinical diversity (participants, interventions, etc. within the RCTs), methodological diversity (study design, outcome measurement tools used, etc.) and statistical heterogeneity (variability in the numerical effect estimates resulting from clinical and methodological diversity). We will summarise key clinical and methodological characteristics and effect modifiers for the included studies to inform the discussion and conclusions. We will summarise data on clinical and methodological variability in the 'Characteristics of included studies' table.

We will use the I^2 statistic to measure heterogeneity among the trials in each analysis [28]. If we identify substantial unexplained heterogeneity, we will report it and explore possible causes by prespecified subgroup analysis. We will use the rough guide to interpretation outlined in Chapter 10 of the *Cochrane Handbook* [29], as follows:

- 0% to 40%: might not be important;
- 30% to 60%: may represent moderate heterogeneity*;
- 50% to 90%: may represent substantial heterogeneity*;
- 75% to 100%: considerable heterogeneity*.

*We will avoid using simple thresholds to interpret heterogeneity. The importance of the observed value of the I^2 statistic depends on 1) the magnitude and direction of effects, and 2) the strength of evidence for heterogeneity (e.g. P value from the Chi^2 test, or a CI for the I^2 statistic: uncertainty in the value of the I^2 statistic is substantial when the number of studies is small).

Investigation of heterogeneity and subgroup analysis

For the analysis, we will divide the groups under disease headings for *RYR1* subtypes (Table 1) into responses to symptomatic interventions and responses to disease-modifying interventions.

We will perform the following subgroup analysis:

- Possible versus confirmed mutations.

We will use the formal test for subgroup interactions in Review Manager [30].

We will consider the limitations of subgroup analyses when interpreting results, including their observational nature and their power to detect differences with fewer than 10 studies per category.

Equity-related assessment

We will extract data relating to participant factors that may result in inequitable access to interventions using the PROGRESS framework [31], including place of residence, race/ethnicity, language, occupation, gender, religion, socioeconomic status, social capital and data related to personal characteristics potentially associated with discrimination (e.g. age or disability).

We will also evaluate baseline imbalance across PROGRESS factors, including health inequity components, in summary of findings tables. We will interpret findings related to health equity in the discussion (including the impact on intervention and review outcomes), consider health inequity in research and clinical practice in the recommendations provided, and use the Equity Checklist for Systematic Review Authors.

Sensitivity analysis

We plan to carry out the following sensitivity analyses:

- Characteristics of participants (e.g. participants in some RCTs meet the age range criteria of the review and other RCTs include some younger or some older participants).
- Characteristics of publication status (e.g. RCTs published as abstract only and RCTs published in full).
- Characteristics of the outcome (e.g. time point of assessment or means of measurement; imputed data).
- Characteristics of the comparator (e.g. variations in what is considered treatment as usual, or control).
- Risk of bias (e.g. excluding studies showing a high risk of bias).

Certainty of the evidence assessment

We will assess the certainty of the evidence for both RCTs and non-randomised (either clinical trial or observational) studies using GRADE according to previous recommendations [8]. We will create summary of findings table(s) using GRADEpro GDT software [32], presenting the following outcomes:

1. Improvement in motor function evaluated by verified tools within the first 12 months of treatment.
2. Mean change in respiratory function assessed by % expected FVC within the first 12 months of treatment.
3. Reduction in rhabdomyolysis/exertional myalgia recurrence measured by 'number of episodes' in 12 months of treatment.
4. Mean change in total walked distance (for the duration of the trial) for ambulant children.
5. Mean change in ventilator support as measured by duration of support needed in 24-hour periods.
6. Any adverse events: adverse events that lead to discontinuation of treatment and SAEs, which are those that are fatal, life-threatening or require prolonged hospitalisation for the duration of the trial (www.fda.gov).
7. Survival at six months or longer.

We will prepare a separate summary of findings table for each comparison.

Groups of two review authors (SR, JW, NV, LS, JD, GB, HJ) will use the five GRADE considerations (study limitations, consistency of effect, imprecision, indirectness and publication bias) to independently assess the certainty of the body of evidence (studies that contribute data for the prespecified outcomes). We will use the methods and recommendations described in Chapter 14 of the *Cochrane Handbook* [33]. We will resolve any disagreements by discussion or by involving another review author (SR, JW, NV, LS, JD, GB, HJ).

RCTs will start as high certainty, but may be downgraded if valid reasons exist among the following: risk of bias, imprecision, inconsistency, indirectness and publication bias. We will consider evidence from the included studies as high certainty if the five factors above are not present to any serious degree, but may downgrade the level of certainty to moderate, low or very low.

NRSIs assessed with ROBINS-I will also start as high-certainty evidence, but will be automatically downgraded by two levels due to their non-randomised allocation.

We will downgrade the certainty of evidence once if a GRADE consideration is serious and twice if very serious. We will justify all decisions to downgrade or upgrade the certainty of evidence using footnotes. We will justify, document and incorporate judgements into the reporting of results for each outcome.

Evidence from NRSIs could be upgraded if there is a large effect, or a dose-response effect.

Consumer involvement

We will identify stakeholders through various channels to ensure a comprehensive representation:

- Patients and caregivers: identified via patient advocacy groups and online communities.
- Healthcare professionals: engaged through professional networks and clinical associations.
- Researchers: selected based on their expertise and contributions to the field.
- Funders: included based on their interest and investment in the topic

Selected stakeholders will assist with specific tasks such as literature search, data extraction and interpretation of findings. We will maintain regular contact through email updates. We will provide opportunities for stakeholders to review draft protocols and interim findings. Stakeholders will be involved in reviewing the final report and planning dissemination strategies.

We will inform stakeholders about how their input was used via thank-you communications. We will publicly recognise the contributions of stakeholders in the acknowledgements sections of our publications and during public presentations.

SUPPLEMENTARY MATERIALS

Supplementary materials are available with the online version of this article: 10.1002/14651858.CD014439.

Supplementary material 1 Search strategies

ADDITIONAL INFORMATION

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The Information Specialist of Cochrane Neuromuscular, Farhad Shokraneh, developed the search strategy in consultation with the review authors.

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Editorial and peer reviewer contributions

The following people conducted the editorial process for this article:

- Sign-off Editor (final editorial decision): Joshua Burns, St. Jude Children's Research Hospital, Memphis, TN;
- Managing Editor (selected peer reviewers, provided editorial guidance to authors, edited the article): Anupa Shah, Cochrane Central Editorial Service;
- Editorial Assistant (conducted editorial policy checks, collated peer reviewer comments and supported the editorial team): Lisa Wydrzynski, Cochrane Central Editorial Service;

- Copy Editor (copy editing and production): Jenny Bellorini, Cochrane Central Production Service;
- Peer reviewers (provided comments and recommended an editorial decision): Joshua J. Todd, Ph.D. (clinical review); Jennifer Hilgart, Cochrane (methods review); Jo Platt, Central Editorial Information Specialist (search review). One additional peer reviewer provided clinical peer review but chose not to be publicly acknowledged.

Contributions of authors

Conception of the protocol: Raga S, Wilmshurst J

Design of the protocol: Raga S, Wilmshurst J, Voermans N, Jungbluth H, Perez-Neri I

Co-ordination of the protocol: Raga S, Wilmshurst J, Voermans N, Jungbluth H, Tillema A

Writing of the protocol: Raga S, Wilmshurst J, Voermans N, Jungbluth H, Perez-Neri I, Dowling J, Baranello G, Servais L, Tillema A

Declarations of interest

SR: received a grant through the Medical Research Council (UK) from 01/01/2020 to 31/12/2023 and a SAMRC researcher development award from 01/03/24 to 28/02/2025.

JW: national South African advisory board for Novartis; national South African advisory board for Sanofi Pasteur.

NV: member of advisory board for Dynacure until 01/01/2020; principal investigator for Unite-CNM trial 2020 to 2022.

IP: none known.

JD: received a grant through the National Institutes of Health from 29/07/2020 to 31/05/2025; scientific advisory board member for RYR1 foundation.

GB: advisory board for AveXis 31/12/2020, advisory board for Biogen from 08/01/2020; contract with Hoffmann-La Roche from 05/01/2023; consultant of advisory board Novartis Gene Therapies; advisory consultant for Pfizer; advisory board for PTC therapeutics 31/10/2016; advisory board for Sarepta Therapeutics 31/03/2018.

LS: consultant for Astellas Pharma; consultant and board member of Biogen; consultant for Dyne therapeutic; steering committee and board member of Hoffman-La Roche; chair of the DSMB FibroGen; chair of the DSMB Lupin Pharmaceuticals Inc; consultant for Novartis; consultant for PTC therapeutics; board member of regenexBio; board member for Santhera; consultant for Sarepta therapeutics; consultant for sysnav.

HJ: advisory role on RYR1-related disorders for Armgo Pharma Inc; scientific advisory board for Astellas Pharma.

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Registration and protocol

Cochrane approved the proposal for this review in July 2021.

Data, code and other materials

Data sharing is not applicable to this article as it is a protocol, so no datasets were generated or analysed.

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ADDITIONAL TABLES

Table 1. Clinical phenotype of diseases due to *RYR1* mutations

Centronuclear myopathy disease	Inheritance	Prevalence/incidence/regional populations?	Age range	Key clinical features Neuromuscular	Other systems	Key biomedical markers: CK/histology
Inherited myopathies						
Central core disease	AD	Regional studies in Northern Island and Western Sweden suggest a prevalence of 3.5 to 5.0 per 100,000 paediatric population	Neonatal period Later infancy Adulthood	Hypotonia and muscle weakness (variable) proximal > distal Mild facial weakness DTR N/$^{-}$ Age-related severity	Musculoskeletal: congenital hip dislocation, kyphoscoliosis, joint contractures and foot deformities	Cores of degenerated myofibrils in muscle fibres
Multi-minicore	AR		Birth Early childhood	Classic: neonatal hypotonia, delayed motor development, axial/proximal muscle weakness	Classic: feeding difficulties, failure to thrive, scoliosis, respiratory impairment Secondary cardiac impairment	Minicores on light microscopy

Table 1. Clinical phenotype of diseases due to *RYR1* mutations *(Continued)*

				Moderate: hand weakness, joint hypermobility Distal legs relatively spared Antenatal: polyhydramnios and poor fetal movement, arthrogryposis multiplex congenita	Varying degrees of spinal rigidity Moderate: minimal scoliosis and respiratory involvement	
Centronuclear myopathy	AR/AD		Birth Early infancy	Facial weakness, ptosis and extraocular muscle weakness	Impaired bulbar function	CK N/ Centrally placed nuclei
Congenital fibre type disproportion			Birth Early infancy	Generalised hypotonia and weakness of the limbs, neck, trunk and facial muscles Ophthalmoplegia uncommon Elongated face, high arched palate	Musculoskeletal: Scoliosis, multiple contractures, congenital hip dislocation, torticollis, foot deformities	Increased proportion and small size of type 1 fibres
Rhabdomyolysis		USA: incidence 26,000 per annum		Broad spectrum: asymptomatic to excessive myalgia, myoglobinuria with pigmenturia	Compartment syndrome, acute kidney injury, DIC, cardiac arrhythmias, multiorgan failure and death	Elevated CK: mild-profound

AR: autosomal recessive; AD: autosomal dominant; CK: creatine kinase; DIC: disseminated intravascular coagulation; DTR: deep tendon reflexes

Table 2. Existing treatments and or interventions targeting RYR1 related pathology (including preclinical studies)

Treatment	Study type	Dose	Details	Endpoint	Outcome
N-acetylcysteine (NAC)[1]	Randomised, double-blind, placebo-controlled	30 mg/kg/day orally for 6 months	NAC (n = 16) vs placebo (n = 17)	Primary: urine 15-F2t isoprostane concentration Co-primary: 6MWT	Oral NAC does not reduce oxidative stress as measured by 15-F2t isoprostane
Pyridostigmine[2]	Mouse models	Intraperitoneal injections	Pyridostigmine vs placebo daily for 4 weeks	Grip fatigue and treadmill endurance	Modest improvement in grip fatigue and treadmill endurance
Salbutamol in CCD and multi-minicore disease (MmD)[4]	Children (total 13, 8 CCD, 5 MmD)	2 mg 4 times daily	Change in baseline at 3 and 6 months	MRC score, myometry, functional measures and forced vital capacity	Significant increases in myometry, MRC scores and FVC between baseline and 6 months. Myometry and MRC difference already significant at 3 months. Significant increase in functional abilities based on structured functional scale.

Table 2. Existing treatments and or interventions targeting RYR1 related pathology (including preclinical studies) *(Continued)*

Albuterol and aerobic exercise[5]	Case report	2 mg daily for 1 year and aerobic exercise 20 minutes 3 times/week	Functionality independence and spontaneous movements measured at 6 weeks, 6 months and 1 year	"Striking increase in strength" after 6 months. After 1 year, significant further improvement, including fine-motor development, activity and speech.

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Abbreviations: 6MWT: six-minute walk test; CCD: central core disease; FVC: forced vital capacity; MRC: Medical Research Council; NAC: N-acetylcysteine

Table 3. Standard tools used in studies and clinical practice to assess muscle and respiratory function, nutrition and quality of life of people affected with congenital myopathy/congenital muscular dystrophy disorders, including their advantages and limitations

Assessment tool	Advantages	Limitations
Manual muscle testing (MRC score)	Routinely assessed during clinic visits.	Subjective, scores will be limited in children with multiple joint contractures
Myasthenia Gravis Composite (MGC) and Myasthenia Gravis Quality of Life (MG-QOL15)	Validated as effective tools to measure disease severity and trajectory for use in paediatric clinical research trials.	Fatiguability would need to be demonstrated at the starting point in order to use this as an outcome measure.
Modified Hammersmith motor function scale (MHMFS)	Suitable for younger, non-ambulant and weaker children. Reliability and validity has been established in children with SMA. Already in use in clinical practice.	Requires availability of physiotherapists.
10-metre walk test (10MWT)	Excellent test-retest reliability in children with neuromuscular disorders and good intercorrelation with gross motor function in children with cerebral palsy.	Patient would need to be ambulant.
6-minute walk test (6MWT)	Objective standardised evaluation of functional exercise. Used to assess function in a wide range of neuromuscular disorders and has been accepted as a clinically significant endpoint in Duchenne muscular dystrophy (DMD)	Patient would need to be ambulant.
Box and block assessment	Measure of gross upper limb manual dexterity. Endurance shuttle box and block assessment has been demonstrated as a validated and sensitive test of fatiguability for proximal arm function in patients with SMA.	

Table 3. Standard tools used in studies and clinical practice to assess muscle and respiratory function, nutrition and quality of life of people affected with congenital myopathy/congenital muscular dystrophy disorders, including their advantages and limitations *(Continued)*

Hand held myometry	Objective and quantitative method to measure muscle strength.	Requires equipment and younger children may have difficulty using dynometer.
Repetitive grip myometry	Using an electric dynometer has been demonstrated to be sensitive in detecting muscular fatigue in adults with chronic fatigue syndrome.	Requires equipment and younger children may have difficulty using dynometer.
Quality of life assessment tools incl. pain questionnaires	Provide additional information on aspects of the disease which may not be readily identified by the clinician.	Subjective, may be more challenging to administer in younger patients.
Respiratory assessments FEV1/FVC	Quantitative measurement of respiratory function.	Requires respiratory technician. Challenging to perform in younger patients.
Feeding/swallowing assessments	Provide more detailed information about patient's feeding/swallowing.	Requires speech therapist. May be more challenging to perform in younger patients.

FEV1: forced expiratory volume in one second; FVC: forced vital capacity; MRC: Medical Research Council; SMA: spinal muscular atrophy