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EMQN Best Practice Guidelines for Genetic Testing and Reporting in *RYR1*-related disorders

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Here, we present EMQN Best Practice Guidelines for Genetic Testing and Reporting in *RYR1*-related disorders. They aim is to aid clinical genetic laboratories in testing, and unequivocal and comprehensive reporting of *RYR1* variants for the benefit of patients and their relatives. These guidelines are supported by experts in the field of anaesthesia, (paediatric) neurology, clinical genetics and clinical laboratory genetics. The ryanodine receptor type 1 is a large calcium channel that regulates calcium release from the sarcoplasmic reticulum resulting in muscle contraction. This receptor is encoded by the *RYR1* gene and expressed predominantly in skeletal muscle. Pathogenic *RYR1* variants are associated with several allelic disorders: malignant hyperthermia, a hypermetabolic reaction to certain anaesthetics in otherwise healthy individuals, exertional rhabdomyolysis and both autosomal dominant and recessive congenital myopathies. In general, *RYR1* gain-of-function variants are associated with malignant hyperthermia susceptibility, whereas dominant-negative and loss-of-function variants are associated with dominant and recessive myopathies, respectively. However, a small subset of *RYR1* variants is associated with a combination of dominant malignant hyperthermia susceptibility with either a dominant or a recessive myopathy or exertional rhabdomyolysis. The apparent discrepancy between molecular mechanisms and different phenotypes is currently poorly understood. As a consequence, the context-dependent interpretation of *RYR1* variants is challenging in diagnostic genetic testing. In particular, it is not trivial to assign a possible associated risk for an allelic disorder for an individual or their relatives, which is especially relevant in family planning.

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INTRODUCTION

Scope of these guidelines

These guidelines are primarily written to aid clinical laboratory geneticists in the reporting of *RYR1* variants in diagnostic settings. *RYR1* variants may be involved in the inherited pharmacogenetic disorder malignant hyperthermia susceptibility, in exertional rhabdomyolysis and in several autosomal dominant and recessive (congenital) myopathies. Some *RYR1* variants may be associated with multiple phenotypes, i.e. dominant myopathy and malignant hyperthermia susceptibility, whereas the majority is involved only in one associated phenotype. Appropriate reporting of *RYR1* variants and assessments of risks to patients and their relatives is thus paramount.

Furthermore, *RYR1* variants are also detected as unsolicited or incidental findings with increased use of exome and genome sequencing techniques. Appropriate interpretation and risk assessments, especially regarding malignant hyperthermia susceptibility, are needed before such variants are disclosed.

Cellular function of RyR1

The ryanodine receptor type 1 (RyR1) is a 2.4 megaDalton homotetrameric membrane-bound protein that forms the calcium release channel of skeletal muscle sarcoplasmic reticulum. Calcium release from intracellular stores in skeletal muscle is regulated by physical communication between RyR1 and the dihydropyridine receptor (DHPR). The DHPR is the voltage sensor and responds to depolarisation of the sarcolemma caused by electrical stimulation. It resides in the T-tubule membrane of the skeletal muscle cell and connects with the RyR1 located in the terminal cisternae of the sarcoplasmic reticulum membrane to trigger Ca^{2+} release from the sarcoplasmic reticulum through RyR1 [1–3]. The result is release of Ca^{2+} from the sarcoplasmic reticulum into the sarcoplasm, triggering muscle contraction and a range of associated metabolic changes including an increase in glycogen breakdown, glycolysis and oxidative metabolism. Ca^{2+} is returned to the sarcoplasmic reticulum by a Ca^{2+}-ATPase (SERCA) at the expense of ATP hydrolysis [4]. RyR1 channels are modulated by numerous

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associated protein regulators, physiological and pharmacological agents as well as post-translational modifications [5]. Protein regulators include calsequestrin, which acts as a calcium buffer in the lumen of the sarcoplasmic reticulum, and FKBP12, which stabilises the closed state of the channel. High concentrations of Ca^{2+} are inhibitory and low concentrations of Ca^{2+} are stimulatory of Ca^{2+} release, respectively. Mg^{2+} inhibits Ca^{2+} release by binding competitively at the Ca^{2+} activation site or to a low affinity Ca^{2+}/Mg^{2+} inhibitory site [6]. Post-translational modifications include phosphorylation, nitrosylation, oxidation, glutathionylation and palmitoylation. Effects can be stimulatory or inhibitory depending on the mechanism of action [7].

Malignant Hyperthermia susceptibility (OMIM #145600)

Malignant hyperthermia (MH) is a potentially lethal hypermetabolic reaction triggered by either depolarising neuromuscular blocking agents such as succinylcholine, or volatile halogenated anaesthetics [8]. It is a pharmacogenetic disorder of skeletal muscle mainly associated with variants in *RYR1*.

The features of MH result from dysregulation of skeletal muscle calcium homoeostasis where larger than normal amounts of Ca^{2+} are released from the sarcoplasmic reticulum through RyR1 into the cytosol where muscle contraction and other Ca^{2+}-dependent metabolic pathways are stimulated resulting in a hypermetabolic state. Metabolic changes include an increase in ATP hydrolysis associated with increased activity of SERCA generating heat and a rise in core body temperature. Other features include muscle rigidity, tachycardia, hypoxaemia and combined respiratory and metabolic acidosis, which, if not promptly recognised and treated, can lead to death [9]. Susceptible individuals may develop MH with their first exposure to the trigger agents, or only during subsequent anaesthetics. Furthermore, while MH can only occur during general anaesthesia, it has now become evident that those at risk of MH also may experience other muscular manifestations, such as muscle cramps, stiffness, myalgias, rhabdomyolysis and weakness, in the absence of anaesthetic exposure [10, 11].

While most MH cases (~3 in 4) are associated with variants in *RYR1*, <2% of cases are associated with variants in *CACNA1S*, encoding the alpha$_{s_1}$ subunit of the DHPR [12]. Suspected MH reactions have also been reported in patients with autosomal recessive Stac3 disorder (formerly known as North American myopathy) and is associated with variants in the *STAC3* gene, encoding an adaptor protein associated with both RyR1 and the DHPR [13]. Although clinically convincing, MH reactions in these patients have not been confirmed by performing an open muscle biopsy with an in-vitro contracture test (IVCT), considered the gold standard for diagnosis of MH susceptibility.

Autosomal dominant and recessive congenital myopathies (OMIM #117000 and #255320)

Variants in *RYR1* have been associated with a wide range of early-onset congenital myopathies, traditionally defined by characteristic structural abnormalities on muscle biopsy, including central core disease (CCD, mainly dominantly inherited) [14] and multi-minicore disease (MmD) [15], centronuclear myopathy (CNM) [16] and congenital fibre type disproportion (CFTD) [17]—all mainly recessively inherited.

Autosomal dominant CCD is usually a relatively mild condition, but there may be intrafamilial variability and more severe cases have been reported in particular in association with de novo occurrence of *RYR1* variants [18]. CCD is characterised by proximal weakness pronounced in the hip girdle with often prominent exertional myalgia. Orthopaedic complications, in particular scoliosis and congenital dislocation of the hips, are common. Despite differences in histopathological appearance, autosomal recessive MmD, CNM and CFTD often share common clinical features characterised by, in contrast to CCD, prominent involvement of the extraocular muscles, a more generalised weakness and a more pronounced bulbar and respiratory impairment. In the occasional CCD case where CK may be elevated, limb-girdle muscular dystrophy also enters the differential diagnosis [19]. All forms of *RYR1*-related congenital myopathy demonstrate a characteristic pattern of selective involvement on muscle MRI that may be diagnostic [20, 21], in particular in the absence of unequivocal histopathological features.

In a recent ENMC workshop, a revised classification was proposed following the evident variability of biopsy findings, the primacy of genetics in the current diagnostic approach to this group of disorders, and the fact that many patients with congenital myopathies may not have had a biopsy. This new nomenclature for congenital myopathies includes: mode of inheritance, related gene and if available the main histopathological phenotype [22]. For example, the nomenclature of CCD is proposed as 'AD RYR1 congenital myopathies with central cores', etcetera.

Whereas CCD has been mainly associated with heterozygous dominant missense variants localising to the RyR1 C-terminal mutational hotspot and altering RyR1 calcium release, the recessive genotypes associated with MmD, CNM and CFTD typically feature at least one truncating variant and are distributed throughout the *RYR1* coding sequence [23].

The differential diagnosis includes other congenital myopathies with cores and central nuclei on muscle biopsy, in particular those due to variants in *TTN* [24] and *MYH7* [25], that often are associated with cardiac involvement, a feature typically absent in *RYR1*-related myopathies.

Finally, an episode of rhabdomyolysis without suspicion of an acquired cause, repetitive episodes of rhabdomyolysis, idiopathic hyperCKaemia or a combination of rhabdomyolysis and hyperCKaemia may be signs of an underlying *RYR1* myopathy [26, 27].

Malignant hyperthermia susceptibility with *RYR1* myopathies

The association between MH and myopathies was noted soon after its original description, when it was recognised that patients with CCD may also be MH susceptible and that MH patients may have cores on muscle biopsy even in the absence of muscle weakness [28]. Another specifically MH-associated myopathy is King-Denborough syndrome (OMIM #619542), characterised by dysmorphic facial features with ptosis, short stature, scoliosis and a tendency to suffer from severe muscle rigidity and hyperthermia even outside the context of general anaesthesia [28, 29]. More recently, *RYR1* variants, including some implicated in the risk of MH under general anaesthesia, have been identified as a common finding in patients with a mild myopathy [30] or (exertional) myalgia and rhabdomyolysis [10, 26]. Furthermore, considering subtle clinical and histopathological manifestations in MH susceptible patients [11, 31, 32] it has become increasingly clear that *RYR1*-related MH susceptibility and dominant myopathies form part of a clinical-pathological continuum.

Unexpectedly, a subgroup of *RYR1* variants that are associated with MH have been found in recessive myopathies, either in homozygosity or compound heterozygosity [33, 34]. As such, the group of MH associated variants is versatile in causing no, dominant or recessive myopathies.

RyR1 gain-of-function versus loss-of-function phenotypes

In MH, RyR1 protein variants are triggered by certain compounds into an overactive state (gain-of-function) (see Fig. 1). Other *RYR1* variants result in altered calcium homoeostasis with a dominant mode of action or are loss-of-function and are typically associated with myopathies such as CCD and MmD, rather than MH per se [34, 35]. Some *RYR1* variants however have been identified both in patients susceptible to MH and those with either autosomal recessive or dominant myopathies [33, 36].

A gain-of function phenotype can be explained by a variant residing in a region of the RyR1 protein thought to be involved

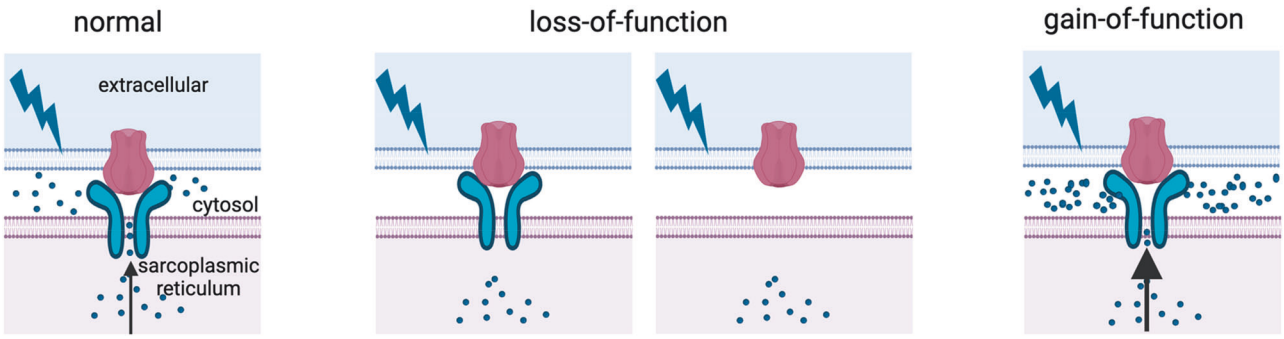

Fig. 1 Schematic representation of RyR1 function in health and disease. The ryanodine receptor type 1, RyR1 (shown in turquoise) is a Ca^{2+} channel. The channel is situated in the sarcoplasmic reticulum (indicated) membrane and the receptor extends into the cytosol (indicated), where it interacts with the dihydropyridine receptor, DHPR (burgundy red). Plasma membrane depolarisation (electrical symbol) results in activation of the DHPR/RyR1 complex and opening of the Ca^{2+} channel. In normal (indicated) conditions, this will result in the release of Ca^{2+} from the sarcoplasmic reticulum into the cytosol and muscle contraction. Loss-of-function (indicated) of RyR1, either due to non-functional RyR1 (second image) or absence of RyR1 (third image), leads to attenuated Ca^{2+} release and decrease in muscle contraction. In contrast, gain-of-function (indicated) of RyR1, as in malignant hyperthermia, results in the trigger-related increase of Ca^{2+} release and a hypermetabolic reaction in muscle. Schematic created using BioRender (Toronto, ON, Canada).

in regulation of channel opening. For example, the FKBP12 protein is thought to stabilise the closed state of the RyR1 channel, therefore a variant that resides in an FKBP binding site may inhibit or ablate FKBP binding, resulting in a channel that is either leaky or prone to remain in the open state. Furthermore, the RyR1 protein itself is composed of a number of domains, some of which form interacting surfaces [37]. Channel opening in response to electrical stimulus is thought to result from a number of conformational changes in interacting domains that are transmitted in an allosteric manner through the cytosolic regulatory region of the RyR1 protein to the membrane-spanning selectivity-filter vestibule and ion-conducting pore. It is therefore feasible that an amino acid change that disrupts a domain interface could render the channel more susceptible to opening, resulting in gain-of-function.

Vice versa, loss-of-function of RyR1 can be explained by an amino acid change in the abovementioned domain interface that renders the RyR1 channel less susceptible to opening. Moreover, the majority of autosomal dominant CCD-associated *RYR1* variants occur in the region of the gene encoding the transmembrane region housing the selectivity-filter vestibule and ion-conduction pore [38]. An amino acid change in this region could block the pore or change the selectivity-filter to exclude the entry of Ca^{2+}. Since these RyR1 variants are expressed, they are likely to form (hetero)tetramers with wildtype RyR1 and decrease overall RyR1 function by competition, steric hindrance within the tetramer, or both.

Complete loss-of-function variants, such as stop-gain or splice site variants, are defined as variants that lead to loss of protein due to nonsense-mediated mRNA decay or protein degradation. Typically, these types of loss-of-function variants are detected in trans with partially functional variants in autosomal recessive MmD, CNM or CFTD [17, 39], while biallelic loss-of-function variants in *RYR1* are not compatible with life and result in lethal multiple pterygium syndrome and antenatal death [40]. Apparently, as parents are healthy, loss of half of the RyR1 protein is dispensable. Thus, haploinsufficiency is not a mechanism in *RYR1* related disease.

The mechanism by which some of the *RYR1* variants can result in both a gain-of-function (MH) and loss-of-function (CCD/MmD) phenotype in the same individual is not fully understood. It has been suggested that *RYR1* variants associated with both MH and CCD cause an uncompensated calcium leak from the sarcoplasmic reticulum, leading to reduced sarcoplasmic reticulum calcium stores under non-triggering conditions [41, 42]. This would be consistent with the CCD phenotype in that less calcium available in general could lead to the typical phenotypic features of CCD. Because the phenotypic effect of MH only occurs after a specific pharmacological stimulus, it is hypothesised that acute marked SR Ca^{2+} release induces store-operated calcium entry through the plasma membrane to sustain muscle contraction and associated metabolic events [43].

METHODS

Based on the ongoing complexities of accurately reporting *RYR1* variants, the creation of formal, consensus-based guidance was deemed necessary. To achieve wide expert agreement, a working group of nine representatives across six centres were invited to share their long-term knowledge and expertise related to genetic testing, clinical experience and research associated with *RYR1*-related disorders. The working group first arranged a virtual meeting on 25th February 2022 where the writing strategy and scope was agreed. Subsequently, the representatives met virtually over regular intervals between February 2022 and December 2022 to discuss the outline and roles for drafting the guidelines. Robinson and Kamsteeg collated the chapters, thoroughly edited and optimised the draft document through e-mail correspondence from January 2023 to March 2025 to finalise a working draft.

Subsequently, the draft document was made available through EMQN to a community of 120 participants in the EMQN-organised external quality assessment (EQA) scheme for *RYR1* related myopathies and malignant hyperthermia susceptibility. The community consultation period was held between the 29th of May and the 29th of June 2025. Feedback from the EMQN community consultation was reviewed and the guidelines were revised accordingly. All nine representatives reviewed and approved the final draft of the guidelines in November 2025.

This endeavour is in synergy with efforts from the 259th European Neuromuscular Centre (ENMC) workshop on Anaesthesia in neuromuscular disorders [44]. Part 3 of that virtual workshop focused on genetic counselling in patients with malignant hyperthermia, rhabdomyolysis and congenital myopathies.

RECOMMENDATIONS

For quick reference, a summary of the major recommendations from this section, stratified by variants and clinical context is provided (box 1).

Criteria for genetic testing of *RYR1*

Suspected clinical diagnosis of malignant hyperthermia. Patients who had features of a hypermetabolic reaction under general

Box 1

Variant type	Clinical Context	Variant Interpr. Framework	Reporting Strategy (variant classification for clinical context)
Missense/In-frame	**MH reaction or MHS**	**EMHG scoring matrix/VCEP criteria**	**P**: Confirms MHS **LP**: Consistent with MHS **VUS**: Inconclusive, assess MHS in family by IVCT, dissuade predictive genetic testing (use IVCT instead) Recommend referral to an MH unit in **all cases**
Missense/In-frame (C-terminal hotspot)	**Myopathy, autosomal dominant**	**ACMG guidelines/EMHG matrix/ VCEP criteria**	**P**: Confirms a dominant myopathy **LP**: Consistent with a dominant myopathy **VUS**: Inconclusive, assess pathogenicity by segregation analyses **Crucial:** assess and report MHS risk (known or unknown) in **all cases** Recommend referral to an MH unit in **all cases**
Loss-of-Function (monoallelic)	**MH reaction or MHS**	**EMHG scoring matrix/VCEP criteria**	Assess MHS in family by IVCT; Explicitly state there is **no correlation** of MH with loss-of-function RYR1 variants Report as **carrier status** for recessive myopathy[a]
Biallelic	**Myopathy, autosomal recessive**	**ACMG guidelines & EMHG matrix/VCEP criteria**	**P (biallelic)**: Confirms a recessive myopathy **LP (biallelic) or P/LP:** Consistent with a recessive myopathy **VUS (biallelic), VUS/P or VUS/LP:** inconclusive **Crucial:** determine phase (cis/trans); assess and report MHS risk (known or unknown) for missense and in-frame variants Recommend referral to an MH unit in **all cases** with missense/in frame variant(s)
MHS-associated variant (P/LP)	**Incidental finding**	**EMHG scoring matrix/VCEP criteria**	Report as an incidental finding with **clinical actionability** Recommend referral to a specialist MH unit

MH malignant hyperthermia, *MHS* malignant hyperthermia susceptibility, *EMHG* European malignant hyperthermia group, *VCEP* variant curation expert panel, *ACMG* American College of Medical Genetics, *P* pathogenic, *LP* likely pathogenic, *VUS* variant of uncertain significance, *IVCT* in vitro contracture test.
[a]local policies in reporting carrier status may vary.

anaesthesia with the depolarising neuromuscular blocking agent succinylcholine or volatile halogenated anaesthetics, are suspected of being MH susceptible. The signs of an MH reaction include unexplained increase in carbon dioxide production, tachycardia, temperature increase, muscle rigidity, rhabdomyolysis, disseminated intravascular coagulation and/or death occurring during anaesthesia or within 1 h of discontinuation of anaesthesia. However, none of these features are specific to MH and it is recommended to refer the case to an MH Investigation Unit for expert review, if possible. If an MH expert anaesthesiologist cannot exclude the possibility of MH on clinical grounds, or such review is not available, a genetic analysis of the *RYR1* gene (and *CACNA1S*) is highly recommended. Similarly, individuals that were diagnosed as MH susceptible based on an open muscle biopsy with in-vitro contracture testing (IVCT or CHCT), e.g. in case of a family history of (suspected) MH, are also eligible for genetic analyses.

Suspected clinical diagnosis of a myopathy. Patients with a (congenital) myopathy, with a suspected genetic cause, should be offered untargeted genetic testing (panel, exome or genome sequencing), which includes *RYR1*, unless there is a high suspicion of a diagnosis that cannot easily be detected using (short-read) sequencing (e.g. repeats as in myotonic dystrophy or facioscapulohumeral dystrophy, or genes with copies such as in spinal muscular atrophy). Targeted *RYR1* testing may be an alternative approach in cases where there is a high suspicion of an *RYR1*-related myopathy, or if untargeted testing is unavailable. A recent ENMC workshop provided guidance on to decide when untargeted genetic testing should be pursued in individuals with exertional rhabdomyolysis, following the RHABDO features [45].

Testing of unaffected family members. Policies, ethical issues and access to genetic testing differs widely between countries. In many countries, the request for testing of unaffected family members is restricted to clinical geneticists. It is good practice to offer cascade testing in families where a relevant *RYR1* variant associated with MH susceptibility has been found in order to guide risk assessments. Similarly, predictive (prenatal) testing of at-risk family members of patients with a dominant or recessive myopathy may be offered. Regarding autosomal recessive myopathies, carrier testing is often performed to determine the cis/trans orientation of variants in a proband.

A less straightforward full-gene carrier test may be requested for unaffected partners of patients with, or carriers of, a recessive myopathy in order to assess the risk of the couple having a child affected by the recessive myopathy. No accurate population carrier frequencies for recessive *RYR1*-related disease are known, but estimates range from 1 in 10,000 in the USA [46] to 1:2000 in Japan [38]. As the population frequency of autosomal recessive *RYR1* variants is, thus, considered low, the risk for any partner to be a carrier may also be low, unless couples are consanguineous or from isolated populations. However, variability due to ascertainment and testing strategies (in the past not the full gene was sequenced), caution not to overinterpret population estimates when counselling individual couples. The downside of a full-gene test in unaffected partners is the inherent risk of detecting variants associated with MH susceptibility (unsolicited findings) and variants of uncertain significance (VUS). The risk assessment and other case-specific considerations for testing lie with the involved genetic counsellor.

Methodology used for testing

The clinically relevant transcript of *RYR1* is transcript 1, with reference sequence NM_000540, having 106 coding exons. The vast majority of *RYR1* variants are single nucleotide variants or small deletions or duplications that can be detected by sequencing. Large deletions or duplications are rare. Despite the proposed hotspot regions in *RYR1*, variants have been detected in almost every exon in many of the *RYR1*-related disorders. Therefore, it is recommended to sequence all 106 exons of *RYR1*, including the intronic splice site regions, regardless of the *RYR1*–related phenotype. Detection of copy number variants is good practice for myopathic phenotypes but not relevant when a suspected MH reaction is the referral indication.

Sequencing strategies can be very diverse, including Sanger sequencing, targeted gene panel analyses using next-generation sequencing, or exome or genome wide sequencing. It is important to understand any technical limitations of the test used, such as coverage or sequence quality (e.g. exon 91 of *RYR1*), as well as limitations in design, e.g. whether intronic variants or copy

Table 1. The role of *RYR1* variant types in allelic RYR1 related disorders.

VARIANT EFFECT	VARIANT TYPE[a]	MHS	DOMINANT MYOPATHY	RECESSIVE MYOPATHY	HOTSPOT
LOF (COMPLETE)	stop-gain frameshift splice site missense in-frame deletion in-frame duplication	-	-	+	no
DOM NEG	missense in-frame deletion in-frame duplication	-	+	-	C-terminal[e]
GOF	missense in-frame deletion in-frame duplication[b]	+	+[c]	+[d]	no

Variants are defined by their cellular effect as loss-of-function (LOF), dominant negative (DOM NEG) or gain-of-function (GOF). Malignant hyperthermia susceptibility (MHS) and myopathies are indicated.
[a]In practice, the cellular effect of a variant is often not evident, but inferred from predictions. Hence, they should be evaluated for their likely effect (e.g. stop-gain variant in the last exon may not be LOF; splice site variants may lead to in-frame exon deletions; etc). The effect of missense variants in particular cannot be predicted with a high likelihood.
[b]Theoretical, not yet described.
[c]*RYR1* variants can be involved in both CCD and MHS; the mechanism of action (i.e. dominant negative in one situation, but GOF in another situation) is not well understood.
[d]*RYR1* variants can be involved in both recessive myopathies and MHS; the mechanism of action (i.e. LOF in one situation, but GOF in another situation) is not well understood.
[e]The C-terminal domain consists of the proposed transmembrane domains and connecting loops.

number variants are detectable with the applied test. It is recommended to describe the technical and design limitations in the clinical report.

In case of (predictive) testing for familial variants it is strongly recommended to test for the presence/absence of the given variant(s) only, to avoid identifying additional *RYR1* variants that may be unsought. The risk of detecting additional variants can be reduced by restricted sequencing of one or multiple exons that bear the variant(s) or by (bio-informatically) restricting the analysis to the genomic location of interest.

Conversely, testing of the full gene (or gene panel) may be indicated for family members that are more severely affected (i.e. a recessive case in a dominant family) or in the case of an individual diagnosed MH susceptible using the IVCT but lacking the familial variant.

Reporting

In general, diagnostic reports should be written to answer the referral question [47], which is even more important for allelic disorders. In the case of *RYR1*, it is essential to know whether the counselee has an MH phenotype, a (congenital) myopathy, or both, or whether it concerns symptomatic or predictive testing. It is also relevant to consider the observed inheritance pattern (i.e. dominant, recessive or sporadic) in relation to the *RYR1* gene testing results. Furthermore, it is also important to make recommendations for further actions (referral to an MH centre and/or genetic counselling), and appropriate follow up for family members, if applicable.

Mechanistic role of RYR1 variants—phenotype dependence. RyR1 variants associated with MH susceptibility prolong the open state upon activation (delayed inactivation of the calcium channel) and are considered gain-of-function variants. In contrast, variants in myopathies are 'loss-of-function' or dominant negative variants (see Table 1). Thus, the relevance of *RYR1* variants is context dependent. For example, a stop-gain variant implicated in the context of a recessive myopathy is not implicated in the risk of MH. Nevertheless, some missense *RYR1* variants are involved in both MH susceptibility and recessive core myopathy, such as p.(Arg401Cys) [48], p.(Thr2206Met) [49, 50] and p.(Val4849Ile) [49, 51], or involved in both dominant central core disease and MH susceptibility, such as p.(Arg163Cys) [12, 52], p.(Tyr522Ser) [42] and p.(Arg4861His) [53]. The molecular mechanism behind these overlapping phenotypes is poorly understood.

Reporting in malignant hyperthermia susceptibility in probands. MH is a hypermetabolic response to potent volatile anaesthetics or succinylcholine in (usually) otherwise healthy subjects. Other conditions not involving anaesthesia, e.g. heat illness, are not considered MH. *RYR1* variants associated with MH are typically missense variants, although in-frame variants may also be involved. Therefore, missense (and in-frame) variants that are classified as VUS or as (likely) pathogenic in the context of MH should be reported. In the case of a (likely) pathogenic missense or in-frame variant, the diagnosis of an increased risk of MH has been confirmed and cascade testing should be recommended. Missense or in-frame VUS should be reported with appropriate caution and be accompanied by the recommendation to perform segregation analysis in conjunction with IVCT (the gold standard to determine MH susceptibility). This is summarised in Fig. 2 and Supplemental Table 1, the latter includes 'recommended wording' for the diagnostic reports.

In the event that a referral for MH susceptibility results in the detection of a likely loss-of-function variant (such as a stop-gain or frameshift variant), the variant should be reported in the appropriate context, i.e. carrier status for a recessive myopathy with no increased risk of MH susceptibility.

Reporting in autosomal dominant myopathy (with or without MH susceptibility) in probands. Autosomal dominant forms of *RYR1*-related disease (e.g. central core disease) are caused mainly by missense variants, though some in-frame deletions have been reported as well [54]. Many of the variants are in the C-terminal region, encoding the transmembrane domains, calcium channel pore region and intersecting loops [55]. The phenotypes may consist of myopathy only, or myopathy with MH susceptibility [56]. In the case of a (likely) pathogenic missense or in-frame variant, the diagnosis of a dominant myopathy has been confirmed, and cascade testing may be recommended. It is recommended to also assess the risk of MH susceptibility in all cases. If the role of a particular variant in MH susceptibility is known (either associated or not), this should be mentioned in the report. Missense or in-frame VUS should be reported with appropriate caution and be accompanied by the recommendation to perform segregation

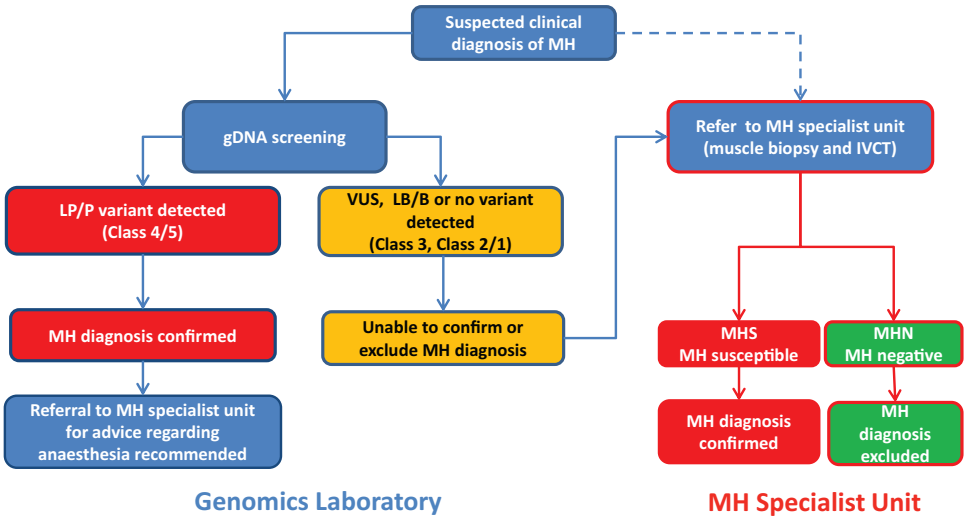

Fig. 2 Diagnostic testing strategy for individuals with a clinical diagnosis of MH. Genetic screening is advocated as the initial route for investigation, however under certain circumstances it may be appropriate to investigate a suspected case using the muscle biopsy test. This would be co-ordinated by an MH specialist unit. LP Likely pathogenic (class 4); P Pathogenic (class 5); VUS Variant of Uncertain Significance (Class 3), LB Likely Benign (Class 2), B Benign (Class 1), IVCT In Vitro Contracture Test, MHS MH susceptible, MHN not susceptible to MH. ACMG modified criteria are available [64] i.e. EMHG specific guidance [66] and ClinGen RYR1 gene specific guidance [65].

analysis in conjunction with IVCT, since the genetic test alone cannot be used to assess the risk for MH susceptibility. In all reports with variants that are associated with MH susceptibility or have an uncertain status for MH susceptibility, a referral to an MH specialist unit is recommended. This is summarised in Fig. 3 and 'recommended wording' for the diagnostic reports can be found in Supplemental Table 2.

Reporting in autosomal recessive myopathy in probands. The presence of two or more variants in trans is in agreement with recessive inheritance (Fig. 3 and Supplemental Table 2). Combinations of a loss-of-function variant and another variant (missense, in-frame deletions/duplication) may be detected. Two complete loss-of-function variants are associated with lethal multiple pterygium syndrome [40, 57], whereas other combinations of variants are associated with (congenital) autosomal recessive phenotypes of central core disease, multi-minicore disease, centronuclear myopathy, or congenital fibre type disproportion. When detecting two or more (likely) pathogenic *RYR1* variants, this will very likely confirm a recessive myopathy, although phase determination is needed for definite confirmation. In the scenario where one or more of the variants is a VUS, a molecular diagnosis is inconclusive. Phase determination is important and can either lead to a less-likely role for *RYR1* (in cis orientation) or to maintaining the inconclusive role (in trans orientation). In some situations, only one heterozygous (likely) pathogenic *RYR1* variant may be detected, making the molecular diagnosis of a recessive myopathy inconclusive. In these cases, and where the suspicion of an RYR1 myopathy is likely, it is good practice to complement poorly covered regions (in WES or WGS) or to perform copy-number variant analysis or RNA sequencing. Alternatively, as not all laboratories have the possibility to perform these additional tests, the limitations of the primary test should be stated.

Unexpectedly, some missense variants in *RYR1* are associated with both MH susceptibility and a recessive myopathy [49, 56]. If such variants are detected, a risk for MH in the clinical report should be mentioned, as well as advice to refer the individual to an MH unit. In the majority of cases, however, the role of missense (or in-frame) variants in MH is unknown. In such cases, it may be recommended to inform the clinicians and patients of the possibility of the IVCT to determine the MH susceptibility risk and refer them to an MH specialist unit.

Overall, in recessive *RYR1*-related conditions it is important to determine the phase to ensure that variants are bi-allelic, to inform physicians and patients of the inheritance mode to prevent recurrence and to inform about a possible risk of MH if appropriate (not in case of LOF variants).

Reporting in sporadic myopathy in probands. When there exists no family history of a myopathy, and one heterozygous *RYR1* variant is detected, a de novo dominant inheritance may be considered. Parental testing is indicated to determine de novo occurrence. Alternatively, two heterozygous or a homozygous variant may cause a sporadic myopathy. Phase determination is important and can either lead to a less-likely role for *RYR1* (*in cis* orientation) or to maintaining the inconclusive role (in trans orientation).

Testing of unaffected family members. In MH families, a (likely) pathogenic *RYR1* variant is considered a major factor contributing to the anaesthetic risk. However, discordance between IVCT phenotype and *RYR1*-genotype was recognised 30 years ago [58, 59] and has been estimated to occur in at least 25% of families [15]. Thus, family members are considered to have an increased risk of MH compared to the general population even when the familial variant is absent, which may be explained by a threshold model of one or more genetic risk factors (known, e.g. *RYR1* and unknown) for the development of MH [12]. In cascade testing, therefore, the MH risk should not be downgraded to the population risk in absence of the familial variant. In such cases, referral to an MH specialist unit is recommended. Supplemental Table 1 provides 'recommended wording' for the diagnostic reports for these scenarios. Furthermore, some MH-associated *RYR1* variants are also detected in recessive myopathies. In such rare cases, individuals with the familial variant are also carriers of a recessive *RYR1*-related myopathy. It is recommended to also mention carrier status in the diagnostic report in such cases.

Similarly, unaffected family members, e.g. parents or siblings, of patients with an autosomal recessive *RYR1*-related myopathy are (at risk of being) carriers of a recessive *RYR1*-related myopathy. Carriers should receive genetic counselling to assess their risk of having children with a recessive *RYR1*-related myopathy.

Testing of spouses. Both patients and carriers of a recessive *RYR1*-related myopathy are at an increased risk of having children with

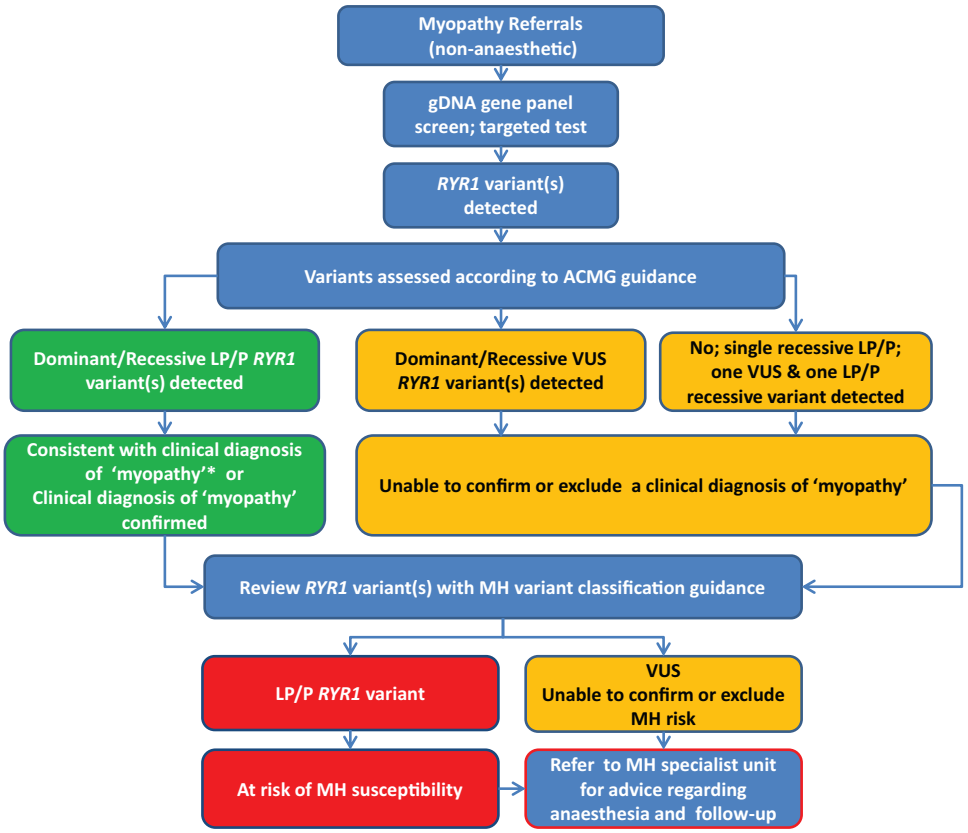

Fig. 3 Diagnostic testing strategy for individuals with a (congenital) myopathy (non-anaesthetic) discovering RYR1 variants. This flow chart is intended to assist in the analysis and interpretation of RYR1 variants identified as part of gene panel screening undertaken for individuals where a suspected clinical diagnosis of MH is not the primary reason for referral. LP Likely pathogenic (Class 4), P Pathogenic (Class 5), VUS Variant of uncertain significance (Class 3), * requires determination of phase where two LP/P variants have been detected to confirm diagnosis, IVCT In Vitro Contracture Test, MHS MH susceptible, MHN not susceptible to MH. For 'myopathy' non-anaesthetic related RYR1 analysis referrals, it is recommended to first assess variants according to ACMG rare disease variant classification guidance [64], or appropriate others. Modified ACMG guidance or gene specific guidance, i.e. EMHG scoring matrix [66] or ClinGen RYR1 guidance [65] should also be used to review pathogenicity in relation to MH risk.

the recessive myopathy. However, the carrier frequencies of autosomal recessive *RYR1*-related myopathy are considered low. Hence, the risk in outbred populations for patients and carriers and their unrelated spouses of having children with a recessive *RYR1*-related myopathy is also low. Whether testing of spouses in such cases is done may depend on local policies (i.e. the national genomics directory of the NHS (UK) uses a carrier frequency of >1:70 to commence testing of partners). However, if testing of spouses is considered, it is highly recommended to only report (likely) pathogenic variants and to mention that the risk of carrier status has been decreased, not excluded. Ideally, this is also mentioned in the pretesting counselling.

Incidental RYR1 findings. Panel-based testing for disorders with locus heterogeneity bears the risk of detecting pathogenic variants not related to a patient's disorder, i.e. incidental findings. Panels for myopathies should contain the *RYR1* gene, since it is a frequent cause of (congenital) myopathies. Hence, (likely) pathogenic *RYR1* variants, in the context of MH, may be detected as incidental findings. Such an incidental finding will have implications for the patient as well as family members. From a legal and ethical aspect, it is good practice to have a system in place to deal with such incidental findings. If reported, it is recommended to suggest referral to an MH specialist unit.

To complicate this issue, some variants can cause a recessive myopathy as well as dominant MHS. As such variants may be part of the molecular cause of a myopathy, the inherent MH risk is not an incidental finding per se. The additional familial risk of developing MH after exposure to triggering agents should however be mentioned in the report, accompanied by the referral to an MH specialist unit.

Criteria for recommending a referral to an MH specialist unit. MH specialist units have the possibility to perform an IVCT on an open muscle biopsy, which is the gold standard to determine whether an individual is susceptible or, perhaps most importantly, not susceptible to MH. These units also perform or have access to genetic testing. More importantly, MH specialist units can advise patients, anesthesiologists, geneticists and neurologists regarding the care and prevention of MH. Any missense, or in-frame, variant in *RYR1* that is not classified as either benign or a risk factor for MH by assessment according to the EMHG or the VCEP ClinGen guidelines, is considered a VUS for MH susceptibility. Therefore, in any setting (suspected MH susceptibility or myopathy) where a VUS in *RYR1* is detected, a referral to an MH specialist unit for advice regarding anaesthesia should be recommended. A comprehensive list of MH specialist units can be found on the website of the European Malignant Hyperthermia Group [60]. It is recognised that MH units are thinly and unevenly spread geographically. This may inhibit a physical referral to an MH unit and unavailability of the IVCT. In such cases, clinicians, patients and relatives of patients should rely on guidelines from the EMHG and take anaesthetic precautions as deemed necessary.

To assess the causality of *RYR1* variants in causing myopathies, the IVCT is not informative.

GENERAL REPORTING GUIDANCE

Internal and external quality control

EMQN recommends that all laboratories offering genetic testing for *RYR1*-related disorders must follow established good laboratory practice, as documented for example in Guidelines for Quality Assurance in Molecular Genetic Testing, published by the Organisation for Economic Co-operation and Development [61]. In addition to following such guidelines, a laboratory should ideally demonstrate that it complies with internationally recognised standards for laboratory testing (e.g. ISO standards 15189:2022) [62], by achieving formal accreditation with a member organisation of the International Laboratory Accreditation Cooperation (ILAC) or equivalent national accreditation body.

All tests should be validated/verified in individual laboratories prior to implementation. External quality assessment (EQA) schemes provide further validation of testing procedures and methods, and it is recommended that laboratories participate in appropriate EQA schemes for testing for *RYR1*-related disorders.

Variant nomenclature

For intra- and interlaboratory comparisons and unequivocal traceability of variants, it is important to use a standard on variant nomenclature. Internationally, the human genome variation society (HGVS) guidelines on the description of sequence variants [63] is considered the gold standard. Thus, it is recommended for *RYR1* variants to use genomic, coding DNA and protein nomenclature according to these HGVS standards. Additionally, it is important to state the zygosity of variants, and, in case of multiple variants in *RYR1*, the phasing. HGVS nomenclature on zygosity and phasing are less intuitive and optional.

Variant interpretation

It is recommended to comply with the guidelines for interpretation of sequence variants developed by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) [64]. In reports, it is also recommended to describe variants as 'pathogenic', 'likely pathogenic' or 'variant of uncertain significance' rather than using numeric classifications. Guidelines for curation of *RYR1* variants in association with MH susceptibility have been independently developed by a ClinGen Variant Curation Expert Panel (VCEP) [65] and by the European Malignant Hyperthermia Group (EMHG) [66]. Each of these groups adapted the generic guidelines for interpretation of sequence variants originally developed by the ACMG/AMP [65] to the pathogenicity of *RYR1* variants in the context of MH susceptibility. The VCEP originally published curation of 84 [67] followed by a further 251 variants [68]. ClinGen guidelines for curation of *RYR1* variants in myopathies are available [69].

General reporting

Diagnostic reports should contain all necessary information without being elaborate and should address the request from the referral. It is recommended to use a logical order in the report and to place the information in appropriate paragraphs with headings. It is strongly recommended not to mention (likely) benign variants in diagnostic reports. The reporting of (subclasses of) variants of uncertain significance (VUS) may be dependent on local regulations or guidelines. If (a subclass of) VUS are not reported, this should be explicitly stated in the diagnostic report. Guidelines on reporting of DNA variants in general are available [47].

REFERENCE TO OTHER GUIDELINES

MH units affiliated with the EMHG [60].

259th ENMC international workshop: Anaesthesia and neuromuscular disorders 11 December, 2020 and 28-29 May, 2021 [44].

Guidelines for curation of *RYR1* variants in association with MHS [67, 68].

ClinGen guidelines for curation of *RYR1* variants in myopathies [69].

CONCLUSION

These guidelines provide an extensive reference to be used in diagnostic reporting of *RYR1* variants in malignant hyperthermia susceptibility, exertional rhabdomyolysis and *RYR1*-related myopathies. Some types of variants may be associated with one of these phenotypes only, whereas others are detected in two or all phenotypes. Therefore, reporting *RYR1* variants can be quite challenging. Flow diagrams and supplemental tables are provided to guide laboratory geneticists through the process of reporting in the correct risk/disease context and with more completeness regarding counselling and recommendations for further testing. Thus, these guidelines will aid in unequivocal and comprehensive reporting of *RYR1* variants for the benefit of patients and their family members.

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AUTHOR CONTRIBUTIONS

WGD, KSh, EJK conceived and designed the work that led to the submission. All contributors drafted and revised the manuscript, approved the final version, agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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ADDITIONAL INFORMATION

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