Supplement to:

Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for the use of potent volatile anesthetic agents and succinylcholine in the context of *RYR1* or *CACNA1S* genotypes

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LITERATURE REVIEW

We searched the PubMed database (1966 to February 2018) for keywords (RYR1 OR ryanodine receptor type 1) AND (malignant hyperthermia) and (CACNA1S OR calcium voltage-gated channel subunit alpha1 S) AND (malignant hyperthermia). Using the specified search criteria, 213 publications for RYR1 and 30 publications for CACNA1S were identified after excluding non-English publications, commentaries, proceedings, or review articles and non-human studies or in-vitro studies without subject information. Inclusion criteria included analyses for the association between malignant hyperthermia susceptibility and the variants on the European Malignant Hyperthermia Group (EMHG) diagnostic MH mutation list (access 08/14/2018). Following application of the inclusion criteria, 101 publications for RYR1 and six publications for CACNA1S were reviewed and included in the evidence table.

AVAILABLE GENETIC TEST OPTIONS

Commercially available genetic testing options change over time. Below is some information that may assist in evaluating options.

Desirable characteristics of pharmacogenetic tests, including naming of alleles and test report contents, have been extensively reviewed by an international group, including CPIC members (1). CPIC recommends that clinical laboratories adhere to these test reporting standards. CPIC gene-specific tables (see Allele Functionality Table and Frequency Table (2)) adhere to these allele nomenclature standards (1). Moreover, the Allele Functionality and Frequency Tables may be used to assemble lists of known functional and actionable pharmacogenetic variants and their population frequencies, which may inform decisions as to whether tests are adequately comprehensive in interrogations of alleles. Some of these general approaches do not align well with the genetics of *RYR1*, *CACNA1S*, and MHS and those issues are highlighted in the accompanying article.

RYR1 is a gene for which most actionable genomic variants are rare (MAF <0.01) in most populations, and thus sequencing-based approaches are recommended.

RYR1 is a gene that is included on the list of actionable secondary findings by the American College of Medical Genetics and Genomics (3, 4).

The Genetic Testing Registry (GTR) provides a central location for voluntary submission of genetic test information by providers and is available at http://www.ncbi.nlm.nih.gov/gtr/.

LEVELS OF EVIDENCE

The evidence summarized in **Supplemental Table S1** is graded using a scale modified slightly from Valdes et al. (5)

> High: Evidence includes consistent results from well-designed, well-conducted studies.
>
> Moderate: Evidence is sufficient to determine effects, but the strength of the evidence is limited by the number, quality, or consistency of the individual studies; generalizability to routine practice; or indirect nature of the evidence.
>
> Weak: Evidence is insufficient to assess the effects on health outcomes because of limited number or power of studies, important flaws in their design or conduct, gaps in the chain of evidence, or lack of information

For this guideline, authors defined the above levels of evidence using the following criteria:

High: Includes evidence of positive IVCT or CHCT results.

Moderate: Includes evidence of an MH reaction BUT not the IVCT or CHCT. May include positive CICR results.

Weak: Includes no evidence of positive contracture test results or MH reaction.

STRENGTH OF RECOMMENDATIONS

CPIC's recommendations are based on weighing the evidence from a combination of preclinical functional and clinical data, as well as on some existing disease-specific consensus guidelines (6). Overall, the therapeutic recommendations are simplified to allow rapid interpretation by clinicians. CPIC uses a slight modification of a transparent

and simple system for just three categories for recommendations adopted from the rating scale for evidence-based recommendations on the use of antiretroviral agents (7):

Strong recommendation for the statement**:** "The evidence is high quality and the desirable effects clearly outweigh the undesirable effects."
Moderate recommendation for the statement: "There is a close or uncertain balance" as to whether the evidence is high quality and the desirable clearly outweigh the undesirable effects.
Optional recommendation for the statement: The desirable effects are closely balanced with undesirable effects, or the evidence is weak or based on extrapolations. There is room for differences in opinion as to the need for the recommended course of action.
No recommendation: There is insufficient evidence, confidence, or agreement to provide a recommendation to guide clinical practice at this time

RESOURCES TO INCORPORATE PHARMACOGENETICS INTO AN ELECTRONIC HEALTH RECORD WITH CLINICAL DECISION SUPPORT

Clinical decision support (CDS) tools integrated within electronic health records (EHRs) can help guide clinical pharmacogenetics at the point of care (8-12). See https://cpicpgx.org/guidelines/ cpic-guideline-for-ryr1-and-cacna1s for resources to support the adoption of CPIC guidelines within an EHR. Based on the capabilities of various EHRs and local preferences, we recognize that approaches may vary across organizations. Our intent is to synthesize foundational knowledge that provides a common starting point for incorporating the use of *RYR1* and/or *CACNAS1* genotype results to guide anesthesia and succinylcholine use.

Effectively incorporating pharmacogenetic information into an EHR to optimize drug therapy should have some key attributes. Pharmacogenetic results, an interpreted phenotype, and a concise interpretation or summary of the result must be documented in the EHR (13, 14). To incorporate a phenotype in the EHR in a standardized manner, genotype test results provided by the laboratory must be consistently translated into an interpreted phenotype (**Table 1, main manuscript**). Because clinicians must be able to

easily find the information, the interpreted phenotype may be documented as a problem list entry or in a patient summary section; these phenotypes are best stored in the EHR at the "person level" rather than at the date-centric "encounter level". Additionally, results should be entered as standardized and discrete terms to facilitate using them to provide point-of-care CDS (8, 15).

Because pharmacogenetic results have lifetime implications and clinical significance, results should be placed into a section of the EHR that is accessible independent of the test result date to allow clinicians to quickly find the result at any time after it is initially placed in the EHR. To facilitate this process, CPIC is providing gene-specific information figures and tables that include full genotype to phenotype tables, diagram(s) that illustrate how *RYR1* and/or *CACNA1S* pharmacogenetic test results could be entered into an EHR, example EHR consultation/genetic test interpretation language and widely used nomenclature systems for genes relevant to the CPIC guideline (see https://www.pharmgkb.org/page/RYR1RefMaterials)(2).

Passive Clinical Decision Support

Given genomic information relating to *RYR1* and *CACNA1S* has high value outside of traditional pharmacy ordering pathways, traditional CDS approaches have limited utility in integrating this genetic information into clinical care.

Point-of-care CDS should be designed to effectively notify clinicians of prescribing implications at any time after the test result is entered into the EHR. CPIC is also providing gene-drug specific tables that provide guidance to achieve these objectives with diagrams that illustrate how point-of-care CDS should be entered into the EHR, example pre- and post-test alert language, and widely used nomenclature systems for drugs relevant to the CPIC guideline **(**see https://cpicpgx.org/guidelines/cpic-guideline-for-ryr1-and-cacna1s).

Additional Implementation Resources

Anesthesia Work Flows

Anesthesia providers are uniquely positioned among medical professionals to order, prepare, and administer medications without the intervention of a pharmacist. This is particularly true of inhaled anesthetics and complicates the implementation of this guideline. We acknowledge that pharmacy support for surgery varies by practice site. An in depth understanding of anesthesia work flows and documentation practices at an institutional level is essential to the implementation of this guideline.

Genetic Counselor Involvement

MHS is an autosomal dominant trait. As such, a positive result for a patient has far reaching familial ramifications beyond those conferred with general pharmacogenomic findings. We recommend that patients identified to have MHS be referred to a genetic counselor for explanation of results and to initiate testing of at-risk relatives.

Additional Professional Resources

The Malignant Hyperthermia Association of the United States (MHAUS) is a non-profit organization focused on increasing patient and provider knowledge of malignant hyperthermia (www.mhaus.org). Through their mission of promoting optimal care and scientific understanding of MHS and related disorders, they have developed healthcare professional facing resources to prepare facilities to manage an MH crisis and have curated recommendations for the treatment of affected patients.

SUPPLEMENTAL TABLE S1. EVIDENCE LINKING *RYR1* AND *CACNA1S* GENOTYPE WITH MALIGNANT HYPERTHERMIA

Type of Study (*in vitro*, *in vivo*, preclinical, or clinical)	Variant rsID cDNA and Predicted Protein Change (*RYR1* NM_000540.2; NP_000531.2, *CACNA1S* NM_000069.2; NP_000060.2)	Major Findings	References	Level of Evidence
Clinical	rs193922747 *RYR1* c.103T>C; p.(Cys35Arg)	*RYR1* variants associated with Malignant Hyperthermia (MH) based on positive IVCTs and MH reaction.	Lynch, et al. (1997) (16) Monnier, et al. (2005) (17) Heytens, et al. (2007) (18) Tammaro, et al. (2011) (19)	High
Clinical	rs193922748 *RYR1* c.130C>T; p.(Arg44Cys)	*RYR1* variants associated with Malignant Hyperthermia (MH) based on positive IVCTs and MH reaction.	Tammaro, et al. (2003) 12709367 Klingler, et al. (2014) (20)	High
Clinical	rs118192161 *RYR1* c.487C>T; p.(Arg163Cys)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs, positive CHCTs, positive CICR, and MH reactions and/or family history of MH.	Quane, et al. (1993) (21) Fagerlund, et al. (1994) (22) Fletcher, et al. (1995) (23) Barone, et al. (1999) (24) Sambuughin, et al. (2001) (25) Rueffert, et al. (2002) (26) Robinson, et al. (2002) (27) Fiege, et al. (2002) (28) Galli, et al. (2002) (29) Monnier, et al. (2002) (30) Sei, et al. (2004) (31) Sambuughin, et al. (2005) (32) Monnier, et al. (2005) (17) Ibarra, et al. (2006) (33) Galli, et al. (2006) (34) Gillies, et al. (2008) (35)	High

			Carpenter, et al. (2009) (36) Kraeva, et al. (2011) (37) Broman, et al. (2011) (38) Brandom, et al. (2013) (39)	
Clinical	rs193922753 *RYR1* c.488G>T; p.(Arg163Leu)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs and MH reaction.	Monnier, et al. (2005) (17) Fiszer, et al. (2015) (40)	High
Clinical	rs1801086 *RYR1* c.742G>A or c.742G>C; p.(Gly248Arg)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs, positive CHCTs, and MH reactions and/or family history of MH reaction.	Gillard, et al. (1992) (41) Sambuughin, et al. (2001) (25) Robinson, et al. (2002) (27) Sei, et al. (2004) (31) Gillies, et al. (2008) (35) Broman, et al. (2009) (42) Carpenter, et al. (2009) Brandom, et al. (2013) (39)	High
Clinical	rs193922762 *RYR1* c.982C>T; p.(Arg328Trp)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive CHCTs and MH reaction in proband.	Loke, et al. (2003) (43)	High
Clinical	rs121918592 *RYR1* c.1021G>C; p.(Gly341Arg)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCT, abnormally enhanced CICR, and family history of MH reaction.	Monnier, et al. (2005) (17) Ibarra, et al. (2006) (33)	High
Clinical	rs121918592 *RYR1* c.1021G>A; p.(Gly341Arg)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs, positive CHCTs, and MH reactions and/or family history of MH.	Quane, et al. (1994) (44) Healy, et al. (1996) (45)# Monsieurs, et al. (1996) (46)# Adeokun, et al. (1997) (47) Barone, et al. (1999) (24)# Rueffert, et al. (2002) (26) Robinson, et al. (2002) (27) Fiege, et al. (2002) (28) Monnier, et al. (2002) (30)#	High

			Sambuughin, et al. (2005) (32) Monnier, et al. (2005) (17) Heytens, et al. (2007) (18)# Gillies, et al. (2008) (35) Carpenter, et al. (2009) (36) Tammaro, et al. (2011) (19) Kraeva, et al. (2011) (37) Brandom, et al. (2013) (39)# Klingler, et al. (2014) (20) Snoeck, et al. (2015) (48) Li, et al. (2017)(49)* *Article described variant as 'p.G341A (c.1021G>A)'. #Note that several citations here specified only the predicted protein change and not the cDNA change.	
Clinical	rs193922764 *RYR1* c.1201C>T; p.(Arg401Cys)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs and MH reactions.	Rueffert, et al. (2002) (26) Monnier, et al. (2005) (17) Gillies, et al. (2008) (35)* Klingler, et al. (2014) (20) Gillies, et al. (2015) (50) **RMH protocol*	High
Clinical	rs118192116 *RYR1* c.1209C>G; p.(Ile403Met)	*RYR1* variant associated with central core disease but has not been associated with Malignant Hyperthermia. This mutation was found in a family with CCD.	Quane, et al. (1993) (21)	Weak
Clinical	rs118192162 *RYR1* c.1565A>C; p.(Tyr522Ser)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs and MH reactions.	Quane, et al. (1994) (51) Girard, et al. (2008) (52)	High

Clinical	rs111888148 *RYR1* c.1589G>A; p.(Arg530His)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs and MH reaction.	Levano, et al. (2009) (53) Zullo, et al. (2009) (54)	High
Clinical	rs193922768 *RYR1* c.1597C>T; p.(Arg533Cys)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs.	Tammaro, et al. (2003) (55)	High
Clinical	rs144336148 *RYR1* c.1598G>A; p.(Arg533His)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive CICR and MH reaction.	Ibarra, et al. (2006) (33)* **p.R533H found together with p.P1592L*	Weak
Clinical	rs193922770 *RYR1* c.1654C>T; p.(Arg552Trp)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs and MH reactions.	Keating, et al. (1997) (56) Yeh, et al. (2005) (57) Fiszer, et al. (2015) (40)	High
Clinical	rs118192172 *RYR1* c.1840C>T; p.(Arg614Cys)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs, positive CHCTs, and MH reactions and/or family history of MH.	Gillard, et al. (1991) (58) Hogan, et al. (1992) (59) Fletcher, et al. (1995) (23) Fagerlund, et al. (1995) (60) Steinfath, et al. (1995) (61) Moroni, et al. (1995) (62) Deufel, et al. (1995) (63) Serfas, et al. (1996) (64) Quane, et al. (1997) (65) Fagerlund, et al. (1997) (66) Barone, et al. (1999) (24) Fortunato, et al. (1999) (67) Brandt, et al. (1999) (68) Tobin, et al. (2001) (69) Rueffert, et al. (2001) (70) Rueffert, et al. (2001) (71) Sambuughin, et al. (2001) (25) Girard, et al. (2001) (72)	High

			Rueffert, et al. (2002) (26) Robinson, et al. (2002) (27) Fiege, et al. (2002) (28) Steinfath, et al. (2002) (73) Monnier, et al. (2002) (30) Muniz, et al. (2003) (74) Shepherd, et al. (2004) (75) Sei, et al. (2004) (31) Monnier, et al. (2005) (17) Galli, et al. (2006) (34) Broman, et al. (2007) (76) Newmark, et al. (2007) (77) Heytens, et al. (2007) (18) Gillies, et al. (2008) (35) Carpenter, et al. (2009) (36) Kraeva, et al. (2011) (37) Brandom, et al. (2013) (39) Riazi, et al. (2014) (78) Klingler, et al. (2014) (20) Fiszer, et al. (2015) (40) Gillies, et al. (2015) (50) Snoeck, et al. (2015) (48) Bamaga, et al. (2016) (79) Butala, et al. (2017) (80)	
Clinical	rs193922772 *RYR1* c.1841G>T; p.(Arg614Leu)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs and MH reactions.	Quane, et al. (1997) (65) Barone, et al. (1999) (24) Brandt, et al. (1999) (68) Monnier, et al. (2005) (17) Heytens, et al. (2007) (18) Broman, et al. (2009) (42) Tammaro, et al. (2011) (19) Klingler, et al. (2014) (20)	High
Clinical	rs118192175 *RYR1* c.6487C>T; p.(Arg2163Cys)	*RYR1* variant associated with Malignant Hyperthermia (MH)	Manning, et al. (1998) (81) Brandt, et al. (1999) (68) Robinson, et al. (2002) (27)	High

		based on positive IVCTs and positive CHCTs.	Sei, et al. (2004) (31)	
Clinical	rs118192163 *RYR1* c.6488G>A; p.(Arg2163His)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs, positive CHCTs, and MH reactions and/or family history of MH.	Manning, et al. (1998) (81) Robinson, et al. (2002) (27) Galli, et al. (2002) (29) Sei, et al. (2002) (82) Sambuughin, et al. (2005) (32) Ibarra, et al. (2006) (33) Galli, et al. (2006) (34) Carpenter, et al. (2009) (36) Kraevak et al. (2011) (37) Brandom, et al. (2013) (39) Bamaga, et al. (2016) (79)	High
Clinical	rs118192176 *RYR1* c.6502G>A; p.(Val2168Met)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs, positive CHCT, and MH reactions and/or family history of MH.	Manning, et al. (1998) (81) Brandt, et al. (1999) (68) Sambuughin, et al. (2001) (25) Girad, et al. (2001) (72) Rueffert, et al. (2002) (26) Sei, et al. (2002) (82) Tammaro, et al. (2003) (55) Sei, et al. (2004) (31) Monnier, et al. (2005) (17) Yeh, et al. (2005) (57) Carpenter, et al. (2009) (36) Tammaro, et al. (2011) (19) Brandom, et al. (2013) (39) Klingler, et al. (2014) (20) Forrest, et al. (2015) (83) Gillies, et al. (2015) (50)	High
Clinical	rs118192177 *RYR1* c.6617C>G; p.(Thr2206Arg) or c.6617C>T; p.(Thr2206Met)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs, positive CHCTs, MH reactions and/or family history of MH.	Manning, et al. (1998) (81) Brandt, et al. (1999) (68) Sambuughin, et al. (2001) (25) Rueffert, et al. (2002) (26) Galli, et al. (2002) (29) Wehner, et al. (2002) (84)	High

			Sei, et al. (2004) (31) Sambuughin, et al. (2005) (32) Monnier, et al. (2005) (17) Yeh, et al. (2005) (57) Galli, et al. (2006) (34) Broman, et al. (2007) (76) Gillies, et al. (2008) (35) Carpenter, et al. (2009) (36) Rueffert, et al. (2009) (85) Brandom, et al. (2013) (39) Klingler, et al. (2014) (20) Gillies, et al. (2015) (50) Snoeck, et al. (2015) (48)	
Clinical	rs112563513 *RYR1* c.7007G>A; p.(Arg2336His)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs, positive CHCT, and MH reactions and/or family history of MH.	Levano, et al. (2009) (53) Carpenter, et al. (2009) (36) Brandom, et al. (2013) (39) Freiermuth, et al. (2013) (86) Klingler, et al. (2014) (20)	High
Clinical	rs121918596 *RYR1* c.7042_7044delGAG; p.(Glu2348del) In some sources the variant is described as c.7042_7044delGAG, while in others c.7039_7041delGAG (rs121918596)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs, positive CHCT* and MH reactions or family history of MH*.	Sambuughin, et al. (2001) (87) Brandom, et al. (2013) (39)* Stephens, et al. (2016) (88) **variant listed as delE 2347*	High
Clinical	rs193922802 *RYR1* c.7048G>A; p.(Ala2350Thr)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs, positive CHCT, and MH reactions and/or family history of MH.	Sambuughin, et al. (2001) (89) Wehner, et al. (2004) (90) Sei, et al. (2004) (31) Sambuughin, et al. (2005) (32) Monnier, et al. (2005) (17) Carpenter, et al. (2009) (36) Klingler, et al. (2014) (20)	High

			Snoeck, et al. (2015) (48) Bamaga, et al. (2016) (79)	
Clinical	rs193922803 *RYR1* c.7063C>T; p.(Arg2355Trp)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs, positive CHCTs, and MH reactions.	Wehner, et al. (2004) (90) Carpenter, et al. (2009) (36) Kravea, et al. (2013) (91) Brandom, et al. (2013) (39) Schiemann, et al. (2014) (92) Broman, et al. (2015) (93)	High
Clinical	rs193922807 *RYR1* c.7124G>C; p.(Gly2375Ala)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs and MH reactions.	Wehner, et al. (2003) (94) Wehner, et al. (2004) (90) Klingler, et al. (2014) (20)	High
Clinical	rs193922809 *RYR1* c.7282G>A; p.(Ala2428Thr)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs.	Rueffert, et al. (2002) (26) Monnier, et al. (2005) (17)	High
Clinical	rs121918593 *RYR1* c.7300G>A; p.(Gly2434Arg)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs, positive CHCTs, and MH reactions and/or family history of MH.	Brandt, et al. (1999) (68) Brinkmeier, et al. (1999) (95)* Sambuughin, et al. (2001) (25) Girard, et al. (2001) (72) Rueffert, et al. (2002) (26) Robinson, et al. (2002) (27) Fiege, et al. (2002) (28) Galli, et al. (2002) (29) Sei, et al. (2002) (82) Sei, et al. (2004) (31) Sambuughin, et al. (2005) (32) Monnier, et al. (2005) (17) Girard, et al. (2006) (96) Galli, et al. (2006) (34) Broman, et al. (2007) (76) Gillies, et al. (2008) (35) Carpenter, et al. (2009) (36) Tammaro, et al. (2011) (19)	High

			Kraeva, et al. (2011) (37) Broman, et al. (2011) (38) Brandom, et al. (2013) (39) Dlamini, et al. (2013) (97) Riazi, et al. (2014) (78) Klingler, et al. (2014) (20) Gillies, et al. (2015) (50) Snoeck, et al. (2015) (48) Roux-Buisson, et al. (2016) (98) Butala, et al. (2017) (80) **Article lists G2435R but references an article for variant detection method that is for G2434R.*	
Clinical	rs28933396 *RYR1* c.7304G>A; p.(Arg2435His)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs, positive CICRs, and MH reactions and/or family history of MH.	Rueffert, et al. (2002) (26) Robinson, et al. (2002) (27) Rueffert, et al. (2004) (99) Monnier, et al. (2005) (17) Ibarra, et al. (2006) (33) Galli, et al. (2006) (34) Broman, et al. (2007) (76) Heytens, et al. (2007) (18) Carpenter, et al. (2009) (36) Tammaro, et al. (2011) (19) Broman, et al. (2011) (38) Riazi, et al. (2014) (78) Broman, et al. (2015) (93)	High
Clinical	rs118192124 *RYR1* c.7354C>T; p.(Arg2452Trp)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs and MH reactions.	Chamley, et al. (2000) (100) Rueffert, et al. (2002) (26) Shepherd, et al. (2004) (75) Klingler, et al. (2014) (20) Roesl, et al. (2014) (101)	High
Clinical	rs193922816 *RYR1* c.7360C>T; p.(Arg2454Cys)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs, and	Brandt, et al. (1999) (68) Gencik, et al. (2000) (102) Monnier, et al. (2002) (30)	High

		positive CHCTs, and MH reactions.	Monnier, et al. (2005) (17) Tammaro, et al. (2011) (19) Klingler, et al. (2014) (20) Potts, et al. (2014) (103)	
Clinical	rs118192122 *RYR1* c.7361G>A; p.(Arg2454His)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs, positive CHCTs, and MH reactions and/or family history of MH.	Barone, et al. (1999) (24) Brandt, et al. (1999) (68) Sambuughin, et al. (2001) (25) Rueffert, et al. (2002) (26) Tammaro, et al. (2003) (55) Sei, et al. (2004) (31) Monnier, et al. (2005) (17) Broman, et al. (2007) (76) Carpenter, et al. (2009) (36) Tammaro, et al. (2011) (55) Kraeva, et al. (2011) (37) Brandom, et al. (2013) (39) Klingler, et al. (2014) (20) Snoeck, et al. (2015) (48) Bamaga, et al. (2016) (79)	High
Clinical	rs28933397 *RYR1* c.7372C>T; p.(Arg2458Cys)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs, and MH reaction.	Manning, et al. (1998) (104) Barone, et al. (1999) (24) Girard, et al. (2001) (72) Galli, et al. (2002) (29) Sambuughin, et al. (2005) (32) Monnier, et al. (2005) (17) Galli, et al. (2006) (34) Klingler, et al. (2014) (20)	High
Clinical	rs121918594 *RYR1* c.7373G>A; p.(Arg2458His)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs, positive CICR, and MH reactions.	Manning, et al. (1998) (104) Ibarra, et al. (2006) (33) Gillies, et al. (2008) (35) Carpenter, et al. (2009) (36) Li, et al. (2017) (105)	High
Clinical	rs193922818	*RYR1* variant associated with Malignant Hyperthermia (MH)	Wu, et al. (2006) (106) Ibarra, et al. (2006) (33)	High

	RYR1 c.7523G>A; p.(Arg2508His)	based on positive CICRs, positive IVCTs, and MH reactions and/or family history of MH.	Galli, et al. (2006) (34) Brandom, et al. (2013) (39) Snoeck, et al. (2015) (48)	
Clinical	rs118192178 *RYR1* c.7522C>T; p.(Arg2508Cys)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive CICRs and MH reactions.	Wu, et al. (2006) (106) Ibarra, et al. (2006) (33) Migita, et al. (2009) (107) Joseph, et al. (2017) (108)	Moderate
Clinical	rs118192178 *RYR1* c.7522C>G; p.(Arg2508Gly)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCT and CICRs in CCD patients.	Wu, et al. (2006) (106) Broman, et al. (2009) (42)	Moderate
Clinical	rs193922832 *RYR1* c.9310G>A; p.(Glu3104Lys)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs.	Carpenter, et al. (2009) (36)	High
Clinical	rs193922843 *RYR1* c.11969G>T; p.(Gly3990Val)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs and MH reaction.	Carpenter, et al. (2009) (36)	High
Clinical	rs118192167 *RYR1* c.14387A>G; p.(Tyr4796Cys)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCT.	Monnier, et al. (2000) (109)	High
Clinical	*rs121918595* *RYR1* c.14477C>T; p.(Thr4826Ile)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs, positive CHCT, and MH reactions.	Brown, et al. (2000) (110) Monnier, et al. (2005) (17) Gillies, et al. (2008) (35) Carpenter, et al. (2009) (36) Snoeck, et al. (2015) (48)	High
Clinical	rs193922876 *RYR1* c.14497C>T; p.(His4833Tyr)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs and MH reactions.	Anderson, et al. (2008) (111) Grievink, et al. (2010) (112)	High

Clinical	rs193922878 *RYR1* c.14512C>G; p.(Leu4838Val)	RYR1 variant associated with Malignant Hyperthermia (MH) based on positive IVCTs, positive CICRs, and MH reaction.	Oyamada, et al. (2002) (113) Ibarra, et al. (2006) (33) Tanabe, et al. (2008) (114) Levano, et al. (2009) (53)	High
Clinical	rs118192168 *RYR1* c.14545G>A; p.(Val4849Ile)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs, positive CHCTs, and MH reactions and/or family history of MH.	Sambuughin, et al. (2005) (32) Monnier, et al. (2005) (17) Broman, et al. (2009) (42) Carpenter, et al. (2009) (36) Kraeva, et al. (2011) (37) Broman, et al. (2011) (38) Brandom, et al. (2013) (39) Klingler, et al. (2014) (20) Kraeva, et al. (2015) (115)* Snoeck, et al. (2015) (48) ** found in compound heterozygous state with other RYR1 variants*	High
Clinical	rs63749869 *RYR1* c.14582G>A; p.(Arg4861His)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs in CCD patients, positive CHCT, and possible family history of MH reaction.	Davis, et al. (2003) (116) Broman, et al. (2007) (76) Brandom, et al. (2013) (39)	High
Clinical	rs118192170 *RYR1* c.14693T>C; p.(Ile4898Thr)	*RYR1* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs* in CCD patients. *positive IVCT results were not strong	Lynch, et al. (1999) (117) Broman, et al. (2007) (76)	Weak
Clinical	rs772226819 *CACNA1S* c.520C>T; p.(Arg174Trp)	*CACNA1S* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs and MH reaction.	Carpenter, et al. (2009) (118) Levano, et al. (2017) (119)	High

Clinical	rs1800559 *CACNA1S* c.3257G>A; p.(Arg1086His)	*CACNA1S* variant associated with Malignant Hyperthermia (MH) based on positive IVCTs and MH reaction and/or family history of MH.	Monnier, et al. (1997) (120) Stewart, et al. (2001) (121) Monnier, et al. (2002) (30) Monnier, et al. (2005) (17)	High

Abbreviations:
MH – Malignant Hyperthermia
IVCT - in vitro contracture test (biopsied muscle strips)
CHCT - caffeine-halothane contracture test (biopsied muscle strips)
CICR - calcium-induced calcium release test (chemically skinned muscle fibers)
Level of Evidence description:
High: Includes evidence of positive IVCT or CHCT results.
Moderate: Includes evidence of an MH reaction BUT not the IVCT or CHCT. May include positive CICR results
Weak: Includes no evidence of positive contracture test results or MH reaction.

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