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PharmGKB Summary: Succinylcholine Pathway, Pharmacokinetics/Pharmacodynamics

Maria L. Alvarellos¹, Ellen M. McDonagh³, Sephalie Patel⁴, Howard L. McLeod⁵, Russ B. Altman^{1,2}, and Teri E. Klein¹

¹Department of Genetics, Stanford University, Stanford, California, USA

²Department of Bioengineering, Stanford University, Stanford, California, USA

³Genomics England, Queen Mary University of London, UK

⁴Department of Anesthesiology, Moffitt Cancer Center, Tampa, Florida, USA

⁵DeBartolo Family Personalized Medicine Institute, Moffitt Cancer Center, Tampa, Florida, USA

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Introduction

The application of surgical treatments is greatly enhanced by the availability of anesthetic agents, such as neuromuscular blockers. Succinylcholine chloride (2,2'-[(1,4-dioxo-1, 4-butanediyl) bis (oxy)] bis [*N, N, N*-trimethylethanaminium] dichloride), also known as suxamethonium chloride, is a depolarizing neuromuscular blocking agent (NMBA) on the World Health Organization's List of Essential Medicines. Because of its rapid onset of action and short half-life, it is commonly used in medical procedures that require short-term skeletal muscle paralysis, including rapid intubation in emergency medical situations. The clinical application of succinylcholine (SCH) is tempered by the occurrence of rare, but dramatic adverse reactions and some were the earliest known examples of pharmacogenetics. In many cases, patients with functionally characterized single nucleotide polymorphisms (SNPs) in specific genes in either the pharmacokinetic (PK) or pharmacodynamic (PD) pathways of SCH are at increased risk of these adverse reactions. The ubiquity of SCH in medical procedures makes understanding the pharmacogenomics of SCH critical for identifying susceptible patients such that suitable interventions and alternatives may be utilized. Figure 1 illustrates the PD and PK pathways of SCH and a fully interactive version of these pathways can be accessed at PharmGKB (<https://www.pharmgkb.org/pathway/PA166122732>).

Corresponding Author: Dr. Teri E. Klein, Shriram Center for Bioengineering and Chemical Engineering, 443 Via Ortega, Room 213, MC: 4245 Stanford, CA 94305, Phone: (650)-725-0659, FAX: (650)-725-3863, feedback@pharmgkb.org.

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Pharmacodynamics

Structurally, SCH consists of two acetylcholine (ACh) molecules linked end to end by their acetyl groups [1, 2]. ACh is the endogenous agonist of the nicotinic acetylcholine receptor (nAChR), a ligand-gated, non-specific cation channel that is formed by five sub-units organized around a central pore. There are two $\alpha 1$ subunits, and one $\beta 1$, δ , and ε sub-units. Each sub-unit of the nAChR is encoded by one of four genes ($\alpha 1$ is encoded by *CHRNA1*, $\beta 1$ is encoded by *CHRNB1*, δ is encoded by *CHRND*, and ε is encoded by *CHRNE*) (Figure 1). The nAChR is located on the motor end plate of neuromuscular junction (NMJ) of the skeletal muscle membrane, also known as the sarcolemma. Binding of an agonist, such as ACh or SCH, promotes the open state of the channel. When the nAChR opens, sodium ions rush into the cell and potassium ions rush out resulting in membrane depolarization and generation of an action potential. In myocytes, depolarization stimulates muscle contraction [3-5].

L-type voltage gated calcium channels, also known as dihydropyridine receptors (DHPR), are located on invaginations of the sarcolemma called the transverse tubules (T-tubules). The DHPR is a complex of four sub-units (α_1 , $\alpha_2\delta$, β , γ) and a distinct gene encodes each sub-unit. *CACNA1S* encodes the α_1 sub-unit (also called $\text{Ca}_v1.1$), the primary sub-unit of the channel that contains the voltage sensor, gating apparatus and channel pore of DHPR [6]. The DHPR is mechanically coupled to the ryanodine receptor (RYR1), a homotetrameric voltage gated calcium channel that is located on the sarcoplasmic reticulum (SR) and encoded by the *RYR1* gene [7] (Figure 1). When skeletal muscles are at rest, the troponin complex allosterically inhibits the formation of a cross-bridge between myosin and actin. When calcium is released into the myoplasm, it binds the troponin complex and allows myosin to bind to actin to initiate muscle contraction and continues for as long as ATP is freely available [8]. Upon depolarization of the sarcolemma, the DHPR undergoes a conformational change and transmits a signal to RYR1, which opens to release SR calcium stores into the myoplasm to initiate muscle contraction. Muscle relaxation occurs when calcium ATPases on the sarcoplasmic reticulum (SERCA) remove calcium from the myoplasm and pump it back into the SR [9].

Because it is a depolarizing NMBA, SCH first induces muscle fasciculations followed by flaccid muscle paralysis. SCH takes effect within 60 seconds of intravenous administration and paralysis lasts between 4-6 minutes during which time patients are monitored with an electric nerve stimulator [1]. Because of its short half-life SCH is indicated for medical procedures requiring short-term muscle paralysis, such as endotracheal intubation, neuromuscular surgery, and electroconvulsive therapy. Because SCH paralyzes the respiratory muscles, patients require mechanical ventilation and close monitoring for the duration of paralysis. It has no direct effect on smooth or cardiac muscle contraction. SCH is often administered in combination with other anesthetics, analgesics and narcotics because although it blocks muscle contraction it has no effect on pain perception [1, 2, 10].

Pharmacokinetics

SCH is rapidly hydrolyzed by butyrylcholinesterase (BCHE; also known as plasma cholinesterase and pseudocholinesterase), which is synthesized in the liver and present in plasma. BCHE hydrolyzes succinylcholine to succinylmonocholine, succinic acid and choline (Figure 1) [11]. The duration of the neuromuscular block caused by SCH is determined both by its rate of dissociation from the nAChR as well as the rate of metabolism by BCHE in the plasma. ACh is rapidly metabolized by acetylcholinesterase but unlike acetylcholinesterase, BCHE is not present in the synaptic cleft of the NMJ. Consequently SCH is hydrolyzed more slowly and remains bound to the nAChR for longer than ACh. For as long as SCH is bound to the nAChR, it maintains the membrane potential above the threshold for repolarization and ACh cannot trigger depolarization of the sarcolemma until it has been repolarized [1].

Pharmacogenomics

Prolonged apnea

The adoption of SCH as a muscle relaxant was rapid and widespread after it was introduced in the early 1950s, but doctors sometimes encountered patients in whom the paralyzing effects of SCH lasted considerably longer than normal, putting the patients at risk of asphyxiation due to prolonged incapacitation of their respiratory muscles (prolonged apnea). In 1956, Dr. Werner Kalow and colleagues reported that the prolonged apnea was caused by the presence of an “atypical” variant (termed the A-variant) of BCHE that impairs its ability to bind and hydrolyze SCH as compared to the “usual” variant (termed the U-variant). They also devised a phenotyping assay, still used today, called the “dibucaine number” (DN) test to detect presence of the A-variant [11-13].

BCHE

The *BCHE* gene is located on chromosome 3q26.1-26.2 and is approximately 64 Kb long [14, 15]. Since the initial discovery of the A-variant of *BCHE*, over 60 polymorphisms in the *BCHE* gene have been reported [15]. Genetic variants that impair BCHE enzyme activity can be broadly classified as being either “qualitative” or “quantitative” variants. The qualitative variants affect BCHE enzyme substrate affinity whereas the quantitative variants affect the quantity of enzyme that is produced without affecting BCHE substrate affinity. Both types of variants increase a patient's risk of prolonged apnea in the presence of SCH, but the duration of the apnea depends on the type and number of variants present [11, 15, 16]. A list of the most common genetic variants of *BCHE* can be found in Table 1.

The A-variant (NP_000046.1:p.Asp98Gly; rs1799807 T>C) is the most common qualitative variant with an incidence of 1:3500 in Caucasians [11]. The A-variant results in a BCHE enzyme that is resistant to inhibition by dibucaine and can be detected by the DN test. The DN number indicates the percentage of BCHE enzyme activity remaining after inhibition by dibucaine. Patients with a very low DN are characterized as being homozygous for the A-variant (AA) [11, 15, 17]. A common quantitative variant (NP_000046.1:p.Ala567Thr; rs1803274 C>T) is the Kalow variant (termed the K-variant). The K-variant decreases BCHE enzyme levels by 30% and is often in linkage disequilibrium with the A-variant [15,

18]. Less common variants of *BCHE* include three fluoride resistant variants (rs28933389, rs28933390 and rs121918557) and many silent variants (termed “S-variants”). Some S-variants lead to drastic reductions of BCHE (Table 1). Multiple mutations, including point mutations, deletions, insertions and combinations of mutations may result the S-variant form of BCHE, including some that have only been described in specific populations as private variants [14, 19].

While DN indicates whether a patient carries an A-variant, other tests are required to indicate whether a patient is a carrier of an F-variant or an S-variant (tests of fluoride resistance, and tests of BCHE enzyme activity, respectively). BCHE is highly polymorphic and compound homozygosity and heterozygosity have been observed in patients with BCHE deficiency [14]. An Australian study reported that in 44% of the BCHE deficient patients that were genotyped, the most common BCHE variant was the compound homozygous A-variant and homozygous K-variant [20]. Because the condition is rare, and it can only indicate whether an individual is a carrier of the atypical variant (the A-variant) of *BCHE*, the DN test is not routinely used to check for presence of BCHE deficiencies. According to Miller's Anesthesia (8th Edition), the DN test is indicated for individuals suspected of carrying the A-variant. This is important since this condition can also be caused by environmental factors such as exposure to organophosphate pesticides, liver diseases, and pregnancy [11]. In cases of suspected BCHE deficiency caused by environmental factors, enzymatic activity testing, rather than genetic testing, could be useful before administration of succinylcholine [21].

Malignant hyperthermia

SCH can also trigger malignant hyperthermia (MH) in some patients. MH was first described in Australia in 1960. A patient expressed concern about being under general anesthesia for his surgery because ten of his family members had died while under anesthesia or shortly afterward. The doctor, assuming that ether had been the cause of death, administered halothane instead but shortly after being administered halothane the patient experienced a severe drop in blood pressure and a rapid rise in body temperature. By packing the patient with ice, the doctor stabilized the patient's body temperature and saved his life. The patient was referred to the geneticist Dr. Michael Denborough. Dr. Denborough concluded that the patient was a carrier of an autosomal dominant mutation of incomplete penetrance that made him and members of his immediate family susceptible to hyperthermia in the presence of general anesthesia [22]. Other case studies emerged of patients who experienced masseter spasm and jaw rigidity in addition to hyperthermia after administration of SCH [23].

MHS is inherited in an autosomal dominant manner that manifests in a subset of patients who are administered depolarizing muscle relaxants, such as SCH, or potent inhalational anesthetics such as sevoflurane, isoflurane, or desflurane. Epidemiological reports give the occurrence of MH as ranging anywhere from 1 in 5000 to 1 in 100,000 patients; however, since it only reveals itself in the presence of potent inhalational anesthetics and depolarizing muscle relaxants, the genetic prevalence of MH susceptibility (MHS) has been estimated to be as high as 1 in 2000 [24-27]. Given that the overall incidence of MH in the general

population is low it is likely that sporadic causative mutations are rare, [28]. Most MHS-causative mutations are inherited in an autosomal dominant manner, which explains why MHS heritability is so high [68].

Multiple epidemiological studies also show that males are overrepresented among surgical patients diagnosed with MH. For example, in two retrospective studies of patients who had experienced MH episodes during anesthesia between 64.8 and 70% were male [26, 27, 29]. This could be due to bias for the types of surgeries requiring general anesthesia that may be more commonly performed in males. However, one study found that males were more likely to be diagnosed as MHS than females on the basis of in vitro contracture test (IVCT, see below) even when there was no statistically significant difference in numbers of males and females in the samples [30].

Early symptoms of MH include metabolic (elevated CO₂ production and O₂ consumption), cardiovascular (tachycardia and arrhythmia), and muscular abnormalities (masseter spasm and muscle rigidity). Later and more severe symptoms can include a rapid increase in body temperature (hyperthermia), rhabdomyolysis, hyperkalemia, severe metabolic and respiratory acidosis, and cardiac arrest [1, 25, 31]. A clinical grading scale (CGS) is a scoring system used to estimate the likelihood that a patient administered anesthesia has experienced an MH episode based on multiple indicators, such as the aforementioned symptoms [26]. The mortality rate for patients who experience an MH crisis has dropped significantly to between 5-10% with the introduction of dantrolene, a RYR1 inhibitor; prior to dantrolene, the mortality rate was as high as 70% [25].

MH is characterized by a rapid and uncontrolled rise of myoplasmic calcium levels in skeletal muscle in response to depolarizing muscle relaxants or potent inhalational anesthetics followed by a hypermetabolic state caused in part by the ATP-intensive removal of excess calcium by SERCA and other calcium pumps [7]. Two mechanisms of extracellular calcium entry have been proposed to explain how an MH crisis can be maintained if SR calcium stores are limited. Store-operated calcium entry (SOCE) promotes extracellular entry of calcium into cells when SR calcium stores are depleted and excitation contraction coupled entry (ECCE) promotes extracellular calcium entry independent of SR calcium stores but is triggered by prolonged depolarization of the sarcolemma [32, 33]. There is evidence that SOCE as well as ECCE are induced in muscles with *RYR1* mutations but not in wild-type muscles in the presence of low levels halothane. The identities of all of the calcium channels responsible for extracellular calcium entry are still being discovered [34, 35].

The role of SCH as a trigger in MHS individuals is still unclear because although it has been reported to trigger MH when administered alone, the majority of MH crises have occurred when SCH was co-administered with a potent inhalational anesthetic [23, 25, 26]. One retrospective analysis reported that the relative risk of MH is greater when potent inhalational anesthetics and SCH are co-administered rather than when either is administered alone [36]. A more recent analysis concluded that there was no significant difference in the severity of MH when comparing between those who were administered potent inhalational anesthetics alone and those who were co-administered SCH. However,

the study did report that the onset of a MH crisis was significantly more rapid in patients who were co-administered SCH and potent inhalational anesthetics as compared to either alone [26]. A third study reported that SCH was associated with greater elevations of serum creatine kinase levels in MHS patients as compared to when SCH was not administered [37].

Genetics of malignant hyperthermia

The most commonly reported mutations in MHS individuals are in *RYR1*. As of October 2013 there were at least 397 reported SNPs in *RYR1* but only 33 have been functionally characterized and designated as “MHS causative” by the European Malignant Hyperthermia Group (EMHG), a research consortium specializing in MH [69]. There are also polymorphisms in *CACNA1S*, associated with MHS but 30-50% of patients who are diagnosed with MHS have no known MHS-causative mutations in *CACNA1S* or *RYR1* [25, 26, 27, 38]. Studies *in vitro*, in mouse models, as well as in humans have demonstrated an association between mutations in *RYR1* and *CACNA1S*, MHS and elevated basal levels of myoplasmic calcium in skeletal muscle. Why some mutations in *RYR1* or *CACNA1S* could lead to elevated basal calcium is not yet known although elevated calcium has been attributed to increased sensitivity of *RYR1* to agonists due to increased passive leaks from the SR [35, 39-42]. The American College of Medical Genetics' (ACMG) 2013 report for recommendations on reporting incidental findings in clinical exome and genome sequencing also includes *RYR1* and *CACNA1S* in their list of genes to report [43].

RYR1

RYR1, the primary locus of MHS, is located on chromosome 19q13.1-13.2. *RYR1* is found complexed with other proteins, but because most pharmacogenetic studies of MHS are on *RYR1*, they will not be discussed here. Early efforts to sequence *RYR1* were hampered due to the large size of the gene; *RYR1* is approximately 16 Kb and contains 106 exons [38]. There are three recognized “hot-spots” in *RYR1* in which SNPs are often found: the N-terminus, C-terminus and a central region that includes exons 39, 40 and 44-46 [44]. The location of individual mutations in *RYR1* or *CACNA1S* is likely to determine the specific mechanism responsible for producing elevations in resting calcium levels, but with so many different MH causative mutations (particularly in *RYR1*), a single mechanism is not likely to be found. For example, a study in one *RYR1* knock-in mouse (Tyr522Ser) reported that the mutation was associated with increased *RYR1* sensitivity to pharmacologic agonists as well as to stimulation by depolarization, but it had no effect on basal calcium levels [45]. A second study in a different *RYR1* knock-in mouse (Arg163Cys) reported that the mutation was associated with elevated basal calcium levels as well as increased sensitivity to *RYR1* agonists when compared to the wild-type mouse [46].

Several mutations in *RYR1* are also associated with various neuromuscular diseases including central core disease (CCD) and multi-minicore disease (MmD). The clinical presentation of these diseases is variable and they may be distinguished histologically [38, 47]. Some individuals may experience hypotonia and delayed motor development from infancy, while others may be unaware that they have a neuromuscular disease until they are diagnosed for muscle weakness or tested for MHS well into adulthood [7, 48]. Although SCH and potent inhalational anesthetics are contraindicated in people with CCD, the disease

does not always segregate with MHS, nor do MHS causative *RYR1* mutations always segregate with CCD, further complicating the relationship between *RYR1* and MHS [47, 49].

The currently accepted “gold standard” for diagnosis is the IVCT. An almost identical procedure called the caffeine-halothane contracture test (CHCT) is used in the United States [50]. The IVCT is an invasive test that requires freshly dissected muscle fibers. Muscle fibers are exposed to caffeine and halothane separately, and the force of contracture is measured using a myo-electrical transducer. An MHS positive IVCT is when the force of the contracture is greater than 2mN (milli-Newtons) in the presence of 2mmol/L or less of caffeine and 0.44 mmol/L or less of halothane [26, 27]. Patients can be diagnosed as MHS (sensitive to both halothane and caffeine) or MH negative (sensitive to neither). Patients that are sensitive to either halothane or caffeine, but not both, are MHEc for caffeine and MHEh for halothane [26]. The EMHG has recently revised its guidelines regarding the use of genetic testing to diagnose MHS. Now, IVCT is no longer the primary diagnostic test for MHS and anyone in whom an MHS-causative mutation is detected is now automatically diagnosed as MHS. The EMHG still recommends that MHS individuals and relatives in whom an MHS-causative mutation is not detected should still be tested with IVCT because of the possibility of false negative diagnosis, due in part to the possible presence of novel MHS-causative mutations in *RYR1* [67]. The EMHG has established guidelines for how to recognize and treat patients during a fulminant or abortive MH crisis, guidelines for molecular genetic testing and a protocol for IVCT testing. In the case of a positive MH diagnosis, positive IVCT, or MHS positive genetic test the EMHG strongly recommends that IVCT and genetic counseling be offered to first-degree relatives of the proband [31, 51]. The Malignant Hyperthermia Association of the United States (MHAUS) explains that *RYR1* hot spots are sequenced first in an effort to detect the presence of known causative mutations (33 as of the writing of this manuscript) and the remaining exons are sequenced if no causative mutations are found in those hot-spots [52]. So while genetic testing has the benefit of being faster and less invasive than IVCT, the EMHG strongly recommends that relatives of patients that carry MHS causative mutations undergo IVCT even after genetic testing due to instances of discordance between genetic test results and IVCT results. Discordance between IVCT phenotype and *RYR1* genotype may possibly be because of the heterogeneity of the *RYR1* gene and complexity of the MHS phenotype [38, 53]. A list of the EMHG's MHS causative mutations in *RYR1* can be found in Table 3.

CACNA1S

CACNA1S, the second gene implicated in MHS, is 93.5 Kb long and is located on chromosome 1q.32. Currently there are two missense SNPs in *CACNA1S* that are designated to be MHS causative by the EMHG (Table 4) [39, 54]. Monnier *et al.* (1997) performed linkage analysis in a large French family after the death of a proband following a suspected MH crisis shortly after he was administered SCH and isoflurane. Ten out of eighteen members of his family were subsequently diagnosed as MHS and three were diagnosed as MHEh by IVCT [55]. After determining the locus that segregated with MHS, the authors performed sequence analyses and discovered the causative mutation in *CACNA1S*. The mutation (NP_000060.2: p.Arg1086His; rs1800559 C>T) causes an arginine residue to be substituted by a histidine at position 1086. This polymorphism was also found in a large

North American case-control study in two patients diagnosed as MHS by IVCT [56]. The second SNP (NP_000060.2: Arg174Trp c.520 C>T; rs772226819) was discovered in a study that sequenced *CACNA1S* cDNA in 50 MHS positive individuals in the UK. The mutation was found to segregate with MHS in one family, and was not found in any of the samples from MHN patients [66]. Polymorphisms at additional loci, including the gene encoding the $\alpha_2\delta$ subunit of the DHPR, have been proposed as being MHS causative but no strong associations between those polymorphisms and MHS have emerged [57-59].

Hyperkalemia

Hyperkalemia, a serum concentration of potassium exceeding 5.5mmol/L, is a dangerous condition that can lead to arrhythmia and cardiac arrest [60]. The FDA-approved drug label for SCH contraindicates it for patients in the acute phase of injury as well as for patients with genetic mutations that cause Duchene and Becker's muscular dystrophies, because they are at increased risk of hyperkalemia [1, 61]. In skeletal muscle, the nAChR is normally only present at the NMJ but nerve damage and denervation stimulates nAChR gene expression throughout the muscle membrane. Denervation also stimulates expression of a second nAChR isoform called α_7 AChR. α_7 AChR is a homomeric non-specific cation channel that responds equally well to succinylcholine and choline [61]. The simultaneous up-regulation of both nAChR isoforms increases a patient's risk of excessive potassium release and hyperkalemia when triggered by SCH. Patients with Duchene and Becker's muscular dystrophies suffer significant denervation of their muscles and run a high risk of experiencing hyperkalemia if they are administered SCH [62, 63].

Conclusions and Future Directions

The FDA drug label for SCH cautions that patients homozygous for the "Atypical-variant" of *BCHE* should be closely monitored, but a more complete list of *BCHE* genetic variants, (including rsID numbers) known to increase a patient's risk of prolonged apnea could help to improve anesthesia and testing decisions in patients. Critical interventions, such as the use of dantrolene and core temperature monitoring have reduced the likelihood of death in MHS patients administered SCH but continued awareness of the adverse events associated with specific genetic variants in *RYR1* and *CACNA1S* is important to ensure that the appropriate interventions are taken and that alternative anesthetics and non-depolarizing muscle relaxants be considered. Our understanding of the etiology of MH as well as advancements in sequencing technologies have made genetic testing for MHS a viable alternative to the IVCT, provided that a diagnostic mutation is detected. To that end, clinicians and patients would benefit from FDA-approved drug labels with expanded information about the genetics of MH, as well as recommendations for genetic testing and IVCT testing for suspected MHS patients. Finally, as several studies have shown, MHS is closely associated with disorders of calcium homeostasis in the skeletal muscle. The desire to further reduce use of the IVCT will necessitate increased investigation into the complex etiology of MHS. More recent studies have used targeted sequencing and exome sequencing of *RYR1* and *CACNA1S*, and have looked to other genes involved in calcium homeostasis in an attempt to uncover additional genetic contributions to MHS outside of those two genes [64, 65].

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Figure 1.

Stylized cells depicting the metabolism and mechanism of action of succinylcholine. Note: the star symbol on the DHPR indicates that it has been activated by depolarization of the t-tubules. A fully interactive version is available at PharmGKB <http://www.pharmgkb.org/pathway/PA166122732>.

Table 1

BCHE polymorphisms associated with prolonged apnea

	rsID	Amino Acid Translation (NP_000046.1)	Phenotype	cDNA Change/Alleles (NM_000055.2)	Alternate Names
1	rs289333389	Thr271Met	Resistant to inhibition by sodium fluoride; decreases BCHE enzyme activity	c.812G>A	F- Variant; BCHE*243M; Thr243Met
2	rs289333390	Gly418Val	Resistant to inhibition by sodium fluoride; decreases BCHE enzyme activity	c.1253C>A	F- Variant; BCHE*390V; Gly390Val
3	rs1799807	Asp98Gly	Resistant to inhibition by dibucaine; decreases the affinity of BCHE for SCH	c.293T>C	A- Variant; Che*70G Asp139Gly; Asp70Gly;
4	rs121918558	Tyr156Cys	Significant decrease in BCHE levels	c.467T>C	Tyr128Cys
5	rs104893684	Leu335Pro	BCHE is absent from plasma; found only in the Vysya of India	c.1004A>G	S- Variant; Leu335Pro
6	rs121918557	Leu358Ile	Resistant to inhibition by sodium fluoride and dibucaine; decreases BCHE enzyme activity; found only in Japan	c.1072A>T	F- Variant; Leu330Ile
8	rs121918556	Glu525Val	Causes a 66% reduction in circulating enzyme and decrease in BCHE activity	c.1574T>A	J-variant; Glu497Val
9	rs1803274	Ala567Thr	30% reduction in BCHE enzyme activity	c.1699C>T	K-variant; CHE*539T Ala539Thr;
10	N/A	No change	No change in BCHE activity	None	U- Variant

Abbreviations: butyrylcholinesterase (BCHE), succinylcholine (SCH). All alleles have been complemented to the (+) chromosomal strand. Source: [15, 16]

Table 3
***RYR1* polymorphisms that are associated with MHS**

	rsID	Amino Acid Translation (NP_000531.2)	Phenotype	cDNA Change/Alleles (NM_000540.2)
1	rs193922747	Cys35Arg	MHS Causative	c.103T>C
2	rs118192161	Arg163Cys	MHS Causative; CCD associated	c.487C>T
3	rs193922753	Arg163Leu	MHS Causative	c.488G>T
4	rs1801086	Gly248Arg	MHS Causative	c.742G>A c.742G>C
5	rs121918592	Gly341Arg	MHS Causative	c.1021G>A
6	rs118192116	Ile403Met	MHS Causative	c.1209C>G
7	rs118192162	Tyr522Ser	MHS Causative; CCD associated	c.1565A>C
9	Rs111888148	Arg530His	MHS Causative	c.1589G>A
10	rs193922770	Arg552Trp	MHS Causative	c.1654C>T
11	rs193922772	Arg614Leu	MHS Causative	c.1841G>T
12	rs118192172	Arg614Cys	MHS Causative	c.1840C>T
13	rs118192175	Arg2163Cys	MHS Causative; CCD associated	c.6487C>T
14	rs118192163	Arg2163His	MHS Causative; CCD associated	c.6488G>A
15	rs118192176	Val2168Met	MHS Causative	c.6502G>A
16	rs118192177	Thr2206Arg	MHS Causative	c.6617C>G
17	rs118192177	Thr2206Met	MHS Causative	c.6617C>T
18	rs112563513	Arg2336His	MHS Causative	c.7007G>A
19	rs193922802	Ala2350Thr	MHS Causative	c.7048G>A
20	rs193922807	Gly2375Ala	MHS Causative	c.7124G>C
21	rs193922809	Ala2428Thr	MHS Causative	c.7282G>A
22	rs121918593	Gly2434Arg	MHS Causative	c.7300G>A
23	rs28933396	Arg2435His	MHS Causative; CCD associated	c.7304G>A
24	rs193922816	Arg2454Cys	MHS Causative	c.7360C>T
25	rs118192122	Arg2454His	MHS Causative; CCD associated	c.7361G>A
26	rs28933397	Arg2458Cys	MHS Causative	c.7372C>T
27	rs121918594	Arg2458His	MHS Causative	c.7373G>A
28	rs118192178	Arg2508Cys	MHS Causative	c.7522C>T
29	rs118192167	Tyr4796Cys	MHS Causative; CCD associated	c.14387A>G
29	rs121918595	Thr4826Ile	MHS Causative	c.14477C>T
30	rs193922876	His4833Tyr	MHS Causative	c.14497C>T
31	rs193922878	Leu4838Val	MHS Causative	c.14512C>G
32	rs63749869	Arg4861His	MHS Causative; CCD associated	c.14582G>A
33	rs118192170	Ile4898Thr	MHS Causative; CCD associated	c.14693T>C

Abbreviations: ryanodine receptor 1 (RYR1), malignant hyperthermia susceptible (MHS), central core disease (CCD). Source: <http://www.emhg.org/genetics> (accessed March 10, 2015) [24]

Table 4
***CACNA1S* polymorphisms that are associated with MHS**

	rsID	Amino Acid Translation (NP_000060.2)	Phenotype	cDNA Change/Alleles (NM_000069.2)
1	rs772226819	Arg174Trp	MHS Causative	c.520G>A
2	rs1800559	Arg1086His	MHS Causative	c.3257C>T

Abbreviations: malignant hyperthermia susceptible (MHS). Alleles have been complemented to the (+) chromosomal strand. Source: <http://www.emhg.org/genetics> (accessed July 2, 2015) [24]