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PharmGKB summary: very important pharmacogene information for CACNA1S

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Overview

The *CACNA1S* gene encodes the α1s subunit of the dihydropyridine receptor (DHPR), a voltage-gated calcium channel and voltage sensor for Ca^{2+} release in skeletal muscle. Variants in the *CACNA1S* gene have been linked to the pharmacogenetic disorder known as malignant hyperthermia susceptibility (MHS) and hypokalemic periodic paralysis (hypoPP). Two variants in *CACNA1S* are verified by the European Malignant Hyperthermia Group (EMHG) to be associated with MHS [1]. Although the occurrence of MHS during anesthesia is relatively rare, the genetic prevalence of MHS-causative mutations is estimated to be between 1 in 400 [2] to 1 in 2000–3000 [3]. The American College of Medical Genetics (ACMG) "Guidelines for Reporting Incidental Findings in Clinical Exome and Genome Sequencing" includes "known pathogenic" and "likely pathogenic" variants in *CACNA1S* related to MHS in its list of genetic variants to report as incidental findings [4, 5]. In addition, 9 *CACNA1S* variants are linked to hypoPP [6]. Finally, several *CACNA1S* variants and polymorphisms are proposed to be associated with thyrotoxic periodic paralysis (TPP) [7], hyperCKemia [8] and statin-associated myopathy [9]. Thus, the *CACNA1S* gene is associated with an eclectic array of muscle disorders with clinical manifestations ranging from subclinical myopathies, episodic paralysis, and life-threatening response to anesthesia.

Keywords

CACNA1S; malignant hyperthermia susceptibility; hypokalemic periodic paralysis; thyrotoxic periodic paralysis; statin-associated myopathy; succinylcholine; volatile inhalational anesthetics

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1.0 Protein Structure, Physiologic Role and Molecular Genetics of *CACNA1S*

1.1 Protein structure

Calcium channels are named according to the transported ion (Ca^{2+}), primary physiological regulator (voltage or V), gene subfamily (gene family 1) and the number denoting the order of discovery of the α subunit within the subfamily; hence, CaV1.1 denotes the α1s subunit (encoded by *CACNA1S*) of the L-type calcium channel, which is highly expressed in skeletal muscle. Other members of the CaV1 family are found in various tissues including cardiac/smooth muscle (α1c subunit or CaV1.2 encoded *CACNA1C*), neuroendocrine/cochlea (α1D subunit or CaV1.3 encoded by *CACNA1D*) and retina (α1F subunit or CaV1.4 encoded by *CACNA1F*). Additionally, accessory subunits of the CaV1.1 DHPR include the β1a (encoded by *CACNB1*) α2-δ (encoded by *CACNA2D1*), and γ (encoded by *CACNG1*) subunits [10, 11]. All multimeric CaV1 family members bind dihydropyridines, an important class of clinical agents, and thus, are collectively referred to as dihydropyridine receptors (DHPRs).

α1s is the largest subunit of the skeletal muscle DHPR (~170 kDa [10, 11]) and it contains the ion conduction pore, voltage sensor, and gating apparatus. The α1 subunits of Cav1 channels bind a large class of clinically important drugs called dihydropyridines, also known as calcium channel blockers, used to treat hypertension and cardiovascular disease (hence, CaV1 channels are collectively referred to as dihydropyridine receptors or DHPRs). The α1s subunit is organized into four homologous domains (I-IV) with each domain containing six transmembrane segments. The N- and C-termini, as well as the linker connecting each of the four domains are intracellular. The 4th transmembrane segment (S4) of each domain contains positively charged amino acids every third residue, which senses changes in the membrane electric field to initiate conformational changes in the protein that both trigger intracellular Ca^{2+} release and promote pore opening. The intracellular “loop” connecting the second and third domains (“II-III loop”) contains a critical region of amino acids (620–764) that is required to mechanically couple changes in membrane voltage to intracellular Ca^{2+} release by type 1 ryanodine receptor (RYR1) Ca^{2+} release channels located in the terminal cisternae of the sarcoplasmic reticulum (orthograde signaling) [12, 13]. The critical domain of the II-III loop also promotes a signal from RYR1 channels that augment DHPR channel activity (retrograde signaling) [12, 13].

1.2 Excitation-Contraction Coupling

Excitation-contraction coupling (ECC) is the process that links depolarization of the muscle membrane (sarcolemma) to calcium release from the sarcoplasmic reticulum (SR), ultimately resulting in calcium-mediated muscle contraction. ECC begins with the release of acetylcholine (ACh) from the innervating motoneuron into the synaptic cleft at the skeletal muscle neuromuscular junction [14, 15]. The binding of ACh to the nicotinic acetylcholine receptor (nAChR) results in depolarization of the post-synaptic endplate to a level sufficient to bring volatage-gated sodium channels to threshold to initiate an action potential in the sarcolemma. The action potential rapidly propagates along the sarcolemma and down transverse tubules (t-tubules), invaginations of the sarcolemma that propagate action

potentials transversely along the muscle fiber to the ECC apparatus. Depolarization of the t-tubule membrane activates CaV1.1 voltage sensors (or DHPRs), which are arranged in groups of four ("tetrads") and mechanically coupled to every other homotetramer of RYR1 Ca^{2+} release channels in the SR. Thus, depolarization-induced activation of the DHPR triggers the opening of RYR1 channels to open and release calcium from the terminal cisternae of the SR into the myoplasm to drive myofilament shortening and muscle contraction (orthograde signaling). In addition to the α1s subunit of the DHPR and RYR1, stac3 and the β1a subunit of the DHPR also play essential roles in the ECC process. During retrograde signaling, RYR1 and stac3 promote the calcium channel properties of the DHPR [12, 16]. The ECC coupling apparatus in skeletal muscle also includes several additional regulatory proteins including FKBP12, triadin, junctin, calsequestrin, junctophilin, and JP45 [15, 17].

1.3 Molecular Genetics

The α1s subunit of the DHPR is encoded by the gene *CACNA1S* (previously referred to as *CACNL1A3* [18]). *CACNA1S* is located on the negative chromosomal strand, contains 44 exons (an embryonic splice variant lacks exon 29) and is ~90 kb long [19]. *CACNA1S* cDNA is ~ 6 kb [http://www.ncbi.nlm.nih.gov/gene/779 Accessed April 7 2019] and the gene product is 1873 amino acids long [19]. In mice, a non-functional *CACNA1S* gene is embryonic lethal [20]. *CACNA1S* is also polymorphic: a study of 870 healthy volunteers from ClinSeq in 2013 [21] uncovered 48 missense variants, one frameshift deletion, one splicing, and one non–frameshift insertion/deletion (indel) in the gene [22].

2.0 Monogenetic Disorders Linked to Mutations in *CACNA1S*

2.1 Malignant Hyperthermia Susceptibility (MHS)

While mutations in the *RYR1* gene are observed in the majority (up to 70%) of MHS individuals, *CACNA1S* mutations are thought to account for ~1% of all MHS cases [2, 23, 24].

2.1.1 Pharmacogenetics and Clinical Presentation of MH—MHS is an autosomal dominant pharmacogenetic disorder in which susceptible individuals are at risk for developing a life-threatening hypermetabolic response during exposure to certain volatile anesthetics and succinylcholine. The incidence of an MH event during anesthesia is estimated to be anywhere between 1 in 10,000 −250,000 anesthetics, in spite of the fact that the genetic prevalence of MHS is between 1 in 400 [2] and 1 in 2000–3000 [3]. While 50–70% of individuals diagnosed as MHS are heterozygous for variants in the *RYR1* gene, approximately 1% are heterozygous for a variant in the *CACNA1S* gene [25].

Early manifestations of an MH event include tachycardia, elevation in end-tidal carbon dioxide and muscle rigidity (masseter spasm if succinylcholine is used). These manifestations are typically accompanied by a rise in core body temperature at a rate of 1–2°C every five minutes. Elevations of core body temperature can further increase oxygen consumption and CO_2 production (acidosis). If not recognized and treated quickly, widespread vital organ failure and death can occur. Treatment includes immediate cessation

of the triggering agent(s), cooling of the body with ice packs (or other means) and administration of the RYR1 inhibitor dantrolene. At the cellular level, MH events are believed to result from an uncontrollable rise in myoplasmic calcium levels in skeletal muscle [2, 26]. In addition, resting Ca^{2+} levels are significantly increased in muscle fibers from MHS individuals even in the absence of triggering agents [27].

2.1.2 MHS Triggering Drugs—*In-vitro* studies have established that known MHS-causative variants in *RYR1* increase the sensitivity of RYR1 channels to activation by volatile inhalational anesthetics [28]. Known pharmacological triggers of MH in susceptible individuals include volatile inhalational anesthetics (isoflurane, desflurane, sevoflurane, enflurane, methoxyflurane and halothane) and succinylcholine. The Malignant Hyperthermia Association of the United States (MHAUS) considers all inhalational anesthetics, ether and succinylcholine as MH triggers and classifies these agents as being unsafe for use in MHS individuals http://www.mhaus.org/healthcare-professionals/be-prepared/safe-and-unsafe-anesthetics/ (accessed July 9, 2019).

Succinylcholine is a depolarizing muscle relaxant that binds to the nicotinic acetylcholine receptor (nAChR) located in the post-synaptic muscle membrane of the neuromuscular junction. When succinylcholine binds the receptor, it induces membrane depolarization, calcium release and muscle contraction through ECC (for a graphical representation see the Succinylcholine Pathway on PharmGKB, https://www.pharmgkb.org/pathway/PA166122732) [14]. Sustained binding of succinylcholine to the nAchR inhibits repolarization of the muscle membrane potential, and thus, results in sustained depolarization, sodium channel inactivation and short-term paralysis. The time course of the effect of succinylcholine on muscle excitability is determined by the relatively slow rate at which it is metabolized by butyrlcholinesterase (BCHE) [14].

2.1.3 Confirmation of MHS—According to the EMHG, referrals for MHS testing are based on a patient's medical history, including whether an MH reaction was suspected, family history and likelihood that MHS cannot be excluded. The current gold-standard for testing in Europe is the *in-vitro* contracture test (IVCT); an analogous caffeine-halothane contracture test (CHCT) is used in North America. The test is performed under very specific conditions and is highly-sensitive for detecting MHS. The test consists of separately exposing freshly biopsied muscle to increasing concentrations of caffeine and halothane while measuring the force of contracture under each condition. Production of a sustained increase of at least 2mN (or 0.2g) from the baseline with a caffeine concentration of 2 mmol/liter or less or 0.44 mmol/liter or less of halothane results in a diagnosis of MHShc. Sensitivity to only halothane classifies a patient as MHSh and sensitivity to only caffeine as MHSc. Patients whose muscle samples do not reach a contracture of at least 2mN to these threshold concentrations of either halothane or caffeine are classified as MHS negative (MHN) [1].

The EMHG recommends DNA screening as a viable alternative to test for MHS in specific cases. For example, DNA screening is recommended in family members of confirmed MHS individuals found to possess a well-characterized MHS-causative variant. However, in cases in which genetic testing is performed for diagnostic purposes, the EMHG recommends that a

"clear clinical indication is a prerequisite for genetic testing (e.g. a patient either has a positive IVCT or a clinically suspected MH episode)." If a causative variant is identified, clinicians may then proceed with predictive family testing [1], (https://www.emhg.org/testing-for-mh-1 (accessed July 9, 2019). The EMHG maintains guidelines for investigating MHS and a list of MHS diagnostic variants in *RYR1* (48 variants) and *CACNA1S* (2 variants) that is updated periodically.

The EMHG currently designates two variants in *CACNA1S* as being causative for MHS: p.Arg1086His (rs1800559 C>T) and p.Arg174Trp (rs772226819 G>A) [1] https://www.emhg.org/diagnostic-mutations (accessed July 9, 2019). The Clinical Pharmacogenetics Consortium (CPIC) has published a clinical practice guideline for the use of potent volatile anesthetic agents and succinylcholine in the context of *RYR1* or *CACNA1S* genotypes [29]. The CPIC mission is to provide information to allow interpretation of selected genotypes, and to provide clinical recommendation guidance, when specific genotypes are available [30, 31]. The two *CACNA1S* variants designated as MHS-causative by the EMHG are included with the recommendation that halogenated volatile anesthetics or the depolarizing muscle relaxants succinylcholine are contraindicated in persons with MHS and that these agents should not be used, except in extraordinary circumstances in which the benefits outweigh the risks [29].

2.1.4 CACNA1S Mutations Linked to MHS—The EMHG currently recognizes two *CACNA1S* mutations as being causative for MHS (summarized in Table 1).

2.1.4.1 p.Arg1086His (rs1800559 C>T): A single nucleotide polymorphism (SNP) that changes an arginine at position 1086 to a histidine residue (p.Arg1086His, NC_000001.11:g.201060815C>T, rs1800559) in the α1s subunit of the DHPR was first reported in a large French family [32]. The young, male proband suffered a fulminant and fatal episode of MH after being administered succinylcholine and isoflurane during surgery. IVCT and genetic sequencing after linkage analysis identified 10 additional family members as MHS (MHShc) and 3 as MHEh (MHSh) [32, 33]. This variant was subsequently identified in several additional families [34–36].

An *in-vitro* study using α1s-null (dysgenic) myotubes transfected with either normal or R1086H mutated α1s subunits showed that the R1086H mutation resulted in a significant increase in RYR1 sensitivity to activation by caffeine and voltage [37]. Specifically, calcium release in R1086H-expressing myotubes occurred at lower concentrations of caffeine and more negative voltages as compared to myotubes expressing normal α1s. R1086H-expressing myotubes also exhibited higher levels of resting calcium, leading the authors to speculate that elevated resting calcium may contribute to the observed enhanced sensitivity, and that under normal conditions CACNA1S acts as a negative regulator of RYR1 and that this role is disrupted by the R1086H mutation in α1s [37]. Although the EMHG only includes the arginine-to-histidine substitution at this position in its list of MHS-causative *CACNA1S* variants, a different SNP resulting in a serine substitution at the same position (p.Arg1086Ser, NC_000001.11:g.201060816 G>T, rs80338782) was reported in two different studies [38, 39]. The SNP was found in a homozygous state in one individual who appeared to experience an MH reaction during anesthesia with sevoflurane but the patient

rejected the muscle biopsy required for an *in-vitro* contractor test. Using the MH clinical grading scale [40], which ranks the likelihood that an adverse anesthetic event represents MH, a score of 63 was calculated based on the patient's clinical scenario [38]. The score of 63 falls in the raw score range of 50+ (the highest rank), which translates to a MH rank of 6 and a designation for the risk with which MH could occur to "almost certain".

2.1.4.2 p.Arg174Trp (rs772226819 G>A): In a cohort study of 50 MHS patients that lacked *RYR1* variants and the *CACNA1S* p.Arg1086His variant, an arginine to tryptophan substitution at position 174 (p.Arg174Trp, NC_000001.11:g.201091993G>A, rs772226819) was identified in one MHS patient that was concordant with disease within the family (i.e. also present in the proband's mother with a positive IVCT, but not in a sibling with a normal IVCT) and was not detected in 100 MHN control samples [41]. The R174W variant was subsequently identified in several additional MHS individuals [39, 42]. Finally, an *in-vitro* study compared dysgenic myotubes expressing either normal or R174W mutated α1s subunits found that the R174W substitution abolished α1s-mediated L-type calcium current without altering depolarization-induced Ca^{2+} release and sensitized the DHPR-RYR1 complex to activation by caffeine, halothane and isoflurane [43] In addition, compared to myotubes expressing normal α1s, R174W-expressing myotubes also exhibited elevated resting myoplasmic calcium levels and reduced SR calcium stores. The authors concluded that the R174W mutation disrupts both α1s-mediated calcium channel activity and suppression of RyR1-mediated SR calcium leak.

2.1.4.3 Additional CACNA1S Variants Associated with MHS: Many studies that sequence *CACNA1S* in MHS cohorts report finding multiple, common variants, sometimes in the same person, as well as identifying several additional *CACNA1S* variants. The findings from these studies are summarized in Table 2 [22, 39, 41, 42, 44–51].

2.2 Hypokalemic Periodic Paralysis (HypoPP)

HypoPP is caused by mutations in both *CACNA1S* (HypoPP1) and *SCN4A* (HypoPP2), the gene that encodes the voltage dependent sodium channel in skeletal muscle [52].

2.2.1 Clinical Manifestation and Genetics of HypoPP—HypoPP is a relatively rare (1:100,000 people) autosomal dominantly inherited skeletal muscle disorder that is characterized by episodes of generalized flaccid muscle weakness, more marked proximally than distally with normal to decreased deep tendon reflexes, that are associated with a reduction in serum potassium (<3.5 mEq/L) [52]. Paralytic episodes can occur repeatedly at daily, weekly or monthly intervals, typically develop over minutes to hours and can last anywhere between a few minutes to several days. Attacks can occur spontaneously or be triggered by carbohydrate-rich evening meals, cold, stress, alcohol, salt intake, rest after strenuous exercise, and anesthetic procedures [53]. Some individuals experience a myopathy independent of paralytic attacks that predominantly affects proximal muscles of the lower limbs. Treatment options are limited and include avoidance of triggers, potassium supplementation to increase serum potassium levels, and the use of carbonic anhydrase inhibitors (e.g. acetazolamide and dichlorphenamide) [52, 53].

Pathogenic variants in the *CACNA1S* gene (40–60%; HypoPP1), which encodes the α1s subunit of the DHPR (CaV1.1), or the *SCN4A* gene (7–14%; HypoPP2), which encodes the α1 subunit of the voltage-gated sodium channel of skeletal muscle (NaV1.4), are observed in ~70% of HypoPP individuals [52]. The remaining 30% of HypoPP cases are either sporadic and/or of currently unknown genetic origin. The clinical presentation and treatment of HypoPP1 and HypoPP2 are essentially identical with a similar high penetrance (>90%) for both subtypes, though a higher penetrance is observed in males compared to females.

Currently, 9 pathogenic variants in CaV1.1 (R528C, R528G, R528H, V876E, R897S, R900G, R900S, R1239G and R1239H) (see section 2.2.3 and Table 1) [54] and 12 pathogenic variants in NaV1.4 (R222G, R222W, R669H, R669G, R672C, R672G, R672H, R672S, R1132G, R1132Q, R1135C, and R1135H) are linked HypoPP1 and HypoPP2, respectively [52]. All but one of these pathogenic variants (V876E in CaV1.1) involve neutralization of a positively charged arginine residue in a voltage-sensor S4 transmembrane domain in either repeats II-IV of CaV1.1 or repeats I-III of NaV1.4.

2.2.2 Pathophysiology of HypoPP—The common etiology for the muscle weakness observed in all HypoPP individuals is that a reduction in serum potassium results in an anomalous depolarizing inward current in muscle fibers that produces a paradoxical depolarization of the resting membrane potential [55]. The paradoxical depolarization results in sodium channel inactivation and fiber inexcitability. The observation that this depolarization is not prevented by blockers of either calcium current through CaV1.1 channels (e.g. nitrendipine) or sodium current through NaV1.1 channels (tetrodotoxin) suggested that the anomalous inward current must involve a pathway other than that through the CaV1.1 [55] or NaV1.4 channel pores [56].

The breakthrough in solving this puzzle was the demonstration that pathogenic HypoPP variants of specific arginine residues within the S4 voltage sensor result in voltage-dependent accessibility of a cation translocation pathway within a cleft of the channel where the S4 voltage sensor moves during gating. As a result, this small anomalous inward current mediated by the S4 gating pathway is referred to as a “gating pore current,” which explains why the current is not blocked by inhibitors of the conventional channel pore or permeation pathway. For HypoPP variants in CaV1.1 and NaV1.4, the altered S4 domain supports a gating pore current when the voltage sensor is in the resting (“downward”) position during hyperpolarization, which is promoted by low potassium. As low potassium also shifts the rectification of inward rectifier potassium channels to more negative voltages, outward current though these channels to counteract the anomalous inward gating pore current become limiting. Quantitative simulation modeling shows that under these conditions the anomalous inward gating pore current is sufficient to drive membrane potential depolarization to potentials that inactivate NaV1.4 channels, and thus, reduce muscle excitability [57].

2.2.3 CACNA1S Mutations Linked to HypoPP—Nine different variants in *CACNA1S* (R528C, R528G, R528H, V876E, R897S, R900G, R900S, R1239G and R1239H) situated in transmembrane segments of CaV1.1. are associated with HypoPP type 1 [54]. Among these, p.Arg528His and p.Arg1239His are the most prevalent (see Table 1).

2.2.3.1 p.Arg528His (rs80338777C>T): The arginine to histidine substitution at position 528 (p.Arg528His, NC_000001.11:g.201077915C>T, rs80338777) was reported in multiple case and family studies [6, 58–71] and also as *de-novo* mutation [61]. Although, HypoPP1 is inherited as an autosomal dominant trait, penetrance in males is much higher than in females. Elbaz *et al.* reported incomplete penetrance analyzing of the clinical characteristics in females for the R528H variant compared to R1239H variant [58].

Several potential reasons for why females might not have a phenotypic manifestation were discussed: 1) hormonal control of the tubular system where the dihydropyridine receptor is located, or 2) other subunits of the receptor modulating the alpha1 subunit function, or 3) interaction with other proteins such as RYR1 [58]. While Ke *et al.* suggested that estrogen and progesterone may play a role in reducing and preventing HypoPP attacks and also that androgens might confer increased penetrance [68], Kawamura *et al.* argued against a direct relation with female hormones [62].

A mouse model for HypoPP showed that heterozygous male Cacna1s R528H mice exhibited a more severe HyoPP phenotype than heterozygous female Cacna1s R528H mice, although the mechanistic cause was not further discussed [72]. Homozygous mice of both genders have similar HypoPP phenotypes.

2.2.3.2 p.Arg528Gly (rs80338778G>C): While the histidine substitution at position 528 is the most prevalent HypoPP1 variant, Wang *et al.* identified a change to glycine (p.Arg528Gly, NC_000001.11:g.201077916G>C, rs80338778) linked to HypoPP1 in a four-generation family with 43 living members and 19 affected individuals. The variant co-segregated with all affected individuals in the family and additionally was not detected in 200 matched normal controls [73]. Similar to p.Arg528His, the penetrance was complete in male 528Gly carriers, but reduced in female carriers [73]. The authors highlight the gender difference for the penetrance of HypoPP and state that the underlying molecular mechanism is unknown [73]. The p.Arg528Gly change was also detected in another HypoPP pedigree by screening 3 HypoPP families for variants in *CACNA1S* and *SCN4A* [74].

2.2.3.3 p.Arg528Cys (rs80338778G>A): Sequencing *CACNA1S* and *SCN4A* genes resulted in the detection of a p.Arg528Cys (NC_000001.11:g.201077916G>A, rs80338778) substitution in a male patient that presented with severe HypoPP [75]. Furthermore, p.Arg528Cys was detected in three other adult males with HypoPP and in two asymptomatic females within the proband's family [75].

2.2.3.4 p.Val876Glu (rs267606698A>T): Different from all the other CACNA1S variants associated with HypoPP1, the p.Val876Glu variant does not affect an arginine residue within an S4 domain, but rather involves a substitution of a valine residue at position 876 with glutamate (p.Val876Glu, NC_000001.11:g.201066917A>T, rs267606698), which is located in the S3 segment of domain III [76]. A family with 12 living individuals in four generations was characterized and 6 members were diagnosed with HypoPP1. The onset of the symptoms in these members ranged from 1 to 9 years of age with all affected members possessing the p.Val876Glu variant. This substitution was not present in the other individuals of this family or in 160 controls [76]. The mutation was also identified in a

female patient with severe HypoPP while none of her family members had a history of similar episodes or other significant illness [77].

2.2.3.5 p.Arg897Ser (rs80338779C>A): Chabrier *et al.* identified a p.Arg897Ser (NC_000001.11:g.201066283C>A, rs80338779) substitution as a *de novo* mutation in a child with early onset HypoPP1as early as 1 year of age [78]. Utilizing whole-exome sequencing (WES), p.Arg897Ser was also identified in a child with previously considered diagnoses of paroxysmal dyskinesia, paroxysmal dystonia, and HypoPP1 [79].

2.2.3.6 p.Arg900Gly: An arginine to glycine substitution at position 900 (p.Arg900Gly, NC_000001.11: g.201066276T>C) was identified in a male patient who experienced periodic episodes of paralysis since the age of 21 years. Sequence analysis revealed a heterozygous A to G transition, resulting in an arginine to glycine substitution. Two other members of the family were found to be heterozygous carriers of this variant [80].

2.2.3.7 p.Arg900Ser: An extensive sequence analysis of all voltage sensors in *SCN4A* and *CACNA1S* in 83 clinically confirmed HypoPP cases detected p.Arg528Gly/His in 25 cases and p.Arg1239Gly/His in 39 cases. A p.Arg900Ser (NC_000001.11:g.201066274C>A) variant was identified in one of the remaining 19 cases [81]. This variant was also identified in a separate study that screened the *CACNA1S* and *SCN4A* genes in patients clinically diagnosed with HypoPP [82]. In a family with history of HypoPP, the sequencing of “hot spots” in *CACNA1S* and *SCN4A* failed to identify a pathogenic variant. Performing WES on two affected individuals showed that both carried the p.Arg900Ser variant, which was subsequently detected in 3 males and 3 females in this pedigree. All male carriers experienced such HypoPP attacks, while all female carriers were asymptomatic [83].

2.2.3.8 p.Arg1239Gly (rs28930069G>C): An arginine to glycine substitution at position 1239 (p.Arg1239Gly, NC_000001.11:g.201053539G>C, rs28930069) was identified in a boy diagnosed with HypoPP from a family in which 6 members in three generations exhibited HypoPP symptoms [84]. In a different family, this variant was identified in a case for which myopathy had developed over two years before attacks of HypoPP first occurred [85].

2.2.3.9 p.Arg1239His, rs28930068C>T: At the same position, the change of the arginine to a histidine residue (NC_000001.11:g.201053538C>T, rs28930068) was found as a de-novo variant in a family where the parents of the affected man tested negative for the variant while the proband transmitted the disorder to his son, but not his daughter [58]. This variant was subsequently further reported in several other cohorts [66, 68, 71, 82, 86–88].

2.2.3.10 Additional CACNA1S variants identified in HypoPP patients: Besides the nine variants described above, Li *et al.* described a p.His916Gln (NC_000001.11:g. 201065943G>C, rs229702) substitution in a male HypoPP patient in a family that encompassed five generations with 10 affected individuals. All *CACNA1S* exons were sequenced and no other changes were found besides the p.His916Gln variant, which co-segregated with the disease phenotype in all affected individuals except the three female carriers [89]. Compared to the S4 variants described above, the p.His916Gln variant is

located in the intracellular S4-S5 linker. A cohort of patients that received a molecular diagnosis of skeletal muscle channelopathy, which included individuals diagnosed for HypoPP, were analyzed for variants in *SCN4A* and *CACNA1S* among other genes. Besides the p.Arg528His and p.Arg1239His mutations, a p.Arg489His (NC_000001.11:g. 201078032C>T, rs553739117) variant in *CACNA1S* was detected in a HypoPP pedigree, but was not further discussed [71].

3.0 Disorders Associated with *CACNA1S* Variants and Polymorphisms

In addition to MHS and HypoPP, several *CACNA1S* variants have been proposed to contribute to hyperCKemia and exercise-induced rhabdomyolysis, thyrotoxic periodic paralysis (TPP), and statin-associated myopathy.

3.1 HyperCKemia and Exercise-induced Rhabdomyolysis

Several studies interrogated *CACNA1S* for variants in individuals with hyperCKemia and exercise-induced rhabdomyolysis. However, the link between identified *CACNA1S* variants with these phenotypes requires further studies in order to establish a more definitive connection with these disease phenotypes. A marked rapid elevation of serum creatine kinase (CK) levels, ten times more than normal, followed by a return to normal levels is indicative of acute rhabdomyolysis that can occur after intense physical exercise, muscle damage and for certain types of neuromuscular disorders. Persistent elevations in serum CK, defined by at least a 1.5-fold increase over normal levels, over two measurements at 30-day intervals, is defined as hyperCKemia [8]. A WES analysis found a p.Ala560Ser (NC_000001.11:g.201077069C>A, rs763794604) variant in *CACNA1S* in a subject who presented with episodes of hyperCKemia and rhabdomyolysis that were preceded by an episode of extreme exercise [90]. In a different study, an individual with hyperCKemia and exercise-induced rhabdomyolysis was found to carry a SNP in *CACNA1S* that results in an arginine to a leucine substitution at amino acid position 528 (NC_000001.11:g. 201077915C>A, p.Arg528Leu, rs80338777) [91].

3.2 Thyrotoxic Periodic Paralysis (TPP)

TPP is a complication of thyrotoxicosis found throughout the world, but most commonly observed in East Asian males [7]. The potentially lethal attacks associated with this disorder are acute and vary in presentation – from mild muscle weakness to total muscle paralysis with full recovery after 72 hours. TPP symptoms are typically observed several hours after consuming a heavy meal (excess carbohydrates, salt, or alcohol) or strenuous exercise. The symptoms are similar to hypokalemic periodic paralysis except that they are secondary to hyperthyroidism and are usually resolved once hyperthyroidism is diagnosed and treated. While *CACNA1S* variants have been reported in some TPP patients [63, 92], the majority of cases of TPP involve mutations in other voltage-gated ion channels [93–95] or lack variants in *CACNA1S* that could explain the clinical presentation [96, 97].

3.3 Statin-associated Myopathy

A recent study evaluated the potential role of the *RYR1* and *CACNA1S* genes as potential loci for statin-associated muscle symptoms [9]. This study included 76 subjects with severe

statin-associated muscle symptoms and 50 statin-tolerant controls whose genomic DNA was analyzed by WES or whole genome sequencing. The findings focused on probable pathogenic variants in *RYR1* or *CACNA1S*, as well as additional variants of unknown significance in both genes that are too common in the general population to be considered individually pathogenic. The study reported five *CACNA1S* variants (summarized in Table 3) observed together with additional variants in other genes that were not present in statin-tolerant controls [9].

4.0 Conclusions

Several genetic variants in *CACNA1S* are causative to MHS and HypoPP1, while other *CACNA1S* variants have been indirectly linked to a diverse array of myopathic conditions including hyperCKemia, exercise-induced rhabdomyolysis, TPP, and statin-associated myopathy. While significant advances have recently been made in elucidating the molecular mechanisms by which *CACNA1S* mutations lead to MHS and HypoPP1, considerable work is needed to determine the pathomechanisms by which *CACNA1S* variants contribute to these other muscle disorders. In any event, clinicians and patients would benefit greatly from both inclusion of the *CACNA1S* locus in routine genetic screens and updated information regarding the potential pathogenic nature of any identified *CACNA1S* variants.

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6.0 References

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Table 1.

CACNA1S variants causative for Malignant Hyperthermia Susceptibility or Hypokalemic Periodic Paralysis

rsID	Protein Change	Genomic Change	PMID	Notes
rs772226819	p.Arg174Trp	NC_000001.11: g.201091993G>A	19825159, 28259615; 30236257	MHS
rs1800559	p.Arg1086His	NC_000001.11: g.201060815C>T	9199552, 11260227, 12411788, 16163667	MHS
rs80338777	p.Arg528His	NC_000001.11: g.201077915C>T	7847370, 7897626, 10881598, 11328898, 15098604, 15711422, 17587224, 18162704, 21841462, 22253645, 23019082, 25088161, 28972032, 12548523, 29606556	HypoPP1
rs80338778	p.Arg528Cys	NC_000001.11: g.201077916G>A	25430699	HypoPP1
rs80338778	p.Arg528Gly	NC_000001.11: g.201077916G>C	15726306, 26252573	HypoPP1
rs267606698	p.Val876Glu	NC_000001.11: g.201066917A>T	19779499, 23948435	HypoPP1
rs80338779	p.Arg897Ser	NC_000001.11: g.201066283C>A	18835861, 22901280	HypoPP1
N/A	p.Arg900Gly	NC_000001.11: g.201066276T>C	21855088	HypoPP1
N/A	p.Arg900Ser	NC_000001.11: g.201066274C>A	19118277, 25213595, 26433613	HypoPP1
rs28930069	p.Arg1239Gly	NC_000001.11: g.201053539G>C	15716625, 18229654	HypoPP1
rs28930068	p.Arg1239His	NC_000001.11: g.201053538C>T	7847370, 11555352, 17418573, 21841462, 23019082, 25213595, 29606556, 30090141	HypoPP1

Abb.: MHS: malignant hyperthermia susceptible, HypoPP1: Hypokalemic periodic paralysis

Table 2.

Additional *CACNA1S* variants found in MHS subjects/cohorts

rsID	Protein Change	Genomic Change	Cohort/Group Studied	PMID	Notes
rs12406479	p.Ala69Gly	NC_000001.11: g. 201110216G>C	UK MHS Cohort	25658027	Found in several MH samples in combination with different *RYR1* and/or *CACNA1S* variants (also found in MHN and MHS Exertional Heat Illness cohort samples)
				19825159	In 4 MHS subjects but also detected in 7 from 100 MHN samples
rs35534614	p.Gly258Asp	NC_000001.11: g. 201089385C>T	UK MHS Cohort	19825159	Not detected in 100 MHN controls but observed to be frequently discordant with MH status in families (observed in a total of 3 MHN samples, 2 MHS and 3 MHE samples)
rs12742169	p.Leu458His	NC_000001.11: g. 201083182A>T	UK MHS Cohort	25658027	Found in several MH samples by itself or in combination with different *RYR1* and/or *CACNA1S* variants (also found in MHN and MHS Exertional Heat Illness cohort samples)
			Italian MHS Cohort	20681998	Only mentioned in table 3 of referenced article
			Swiss MHS Cohort	28259615	Detected by capillary sequencing of 56 MHS samples
N/A	p.Thr476Pro	NC_000001.11: g. 201078072T>G	UK MHS Cohort	30236257	Found in 1 family (might potentially have also pathogenic RYR1 variant)
rs150590855	p.Arg498Leu	NC_000001.11: g. 201078005C>A	Australian cohort	25735680	IVCTc Max, IVCTh Max, Family history
rs140662085	p.Ser516Leu	NC_000001.11: g. 201077951G>A	Swiss MHS Cohort	28259615	Detected by capillary sequencing of 56 MHS samples
rs142356235	p.Ser606Asn	NC_000001.11: g. 201076930C>T	Swiss MHS Cohort	28259615	Detected by capillary sequencing of 56 MHS samples
			UK MHS Cohort	25658027	Found in MH samples together with rs12742169 (*CACNA1S*) or rs375626634 (*RYR1*) and rs35364374 (*RYR1*) (also found in MHS Exertional Heat Illness cohort samples)
				19825159	Not detected in 100 MHN controls but observed to be frequently discordant with MH status in families (MHE and MHN siblings)
rs1047863274	p.Thr617Ala	NC_000001.11: g. 201075594T>C	UK MHS Cohort	30236257	Found in 1 family (might potentially have also pathogenic RYR1 variant)
rs759887262	p.Pro758Leu	NC_000001.11: g. 201070359G>A	UK MHS Cohort	30236257	Found in 1 family (might potentially have also pathogenic RYR1 variant)
rs777247285	p.Arg759Cys	NC_000001.11: g. 201070357G>A	Australian cohort	25735680	IVCTc Min, IVCTh Mod, CGS 60
rs760674518	p.Arg794His	NC_000001.11: g. 201069581C>T	Italian MHS Cohort	20681998	Identified in MHS proband but did not co-segregate with MHS in the family
rs200334886	p.Thr852Met	NC_000001.11: g. 201066989G>A	Canadian MHS patients with ER	28326467	Subject with positive CHCT, non-anesthetic rhabdomyolysis triggered by strenuous exercise, no MH family history or event
N/A	p.Val875Met	NC_000001.11: g. 201066921G>A	North American MH Registry	25268394; 23558838	Found with p.Arg3283Gln (RYR1)

rsID	Protein Change	Genomic Change	Cohort/Group Studied	PMID	Notes
N/A	p.Leu885Pro	NC_000001.11: g. 201066890A>G	UK MHS Cohort	30236257	Found in 1 family (might potentially have also pathogenic RYR1 variant)
N/A	p.Arg900Ser	NC_000001.11: g. 201066274C>A	UK MHS Cohort	30236257	Found in 1 family (might potentially have also pathogenic RYR1 variant)
rs139935847	p.Asn909Ser	NC_000001.11: g. 201066248T>C	UK MHS Cohort	30236257	Found in 1 family (might potentially have also pathogenic RYR1 variant)
rs571902899	p.Val923Met	NC_000001.11: g. 201065924C>T	Australian cohort	25735680	IVCTc Mod, IVCTh Mod, Family history
rs527520015	p.His992Asp	NC_000001.11: g. 201062023G>C	UK MHS Cohort	30236257	Found in 1 family (might potentially have also pathogenic RYR1 variant)
rs200224590	p.Thr1009Lys	NC_000001.11: g. 201061971G>T	UK MHS Cohort	24013571	Variant found in 6 IVCT-positive + 1 IVCT-negative, seven IVCT-negative did not carry variant
				25658027	Found in MH sample with rs12742169 (*CACNA1S*) and did not segregate with phenotype
				30236257	Found in 2 families (might potentially have also pathogenic RYR1 variant)
rs777328046	p.Val1031Met	NC_000001.11: g. 201061431C>T	Australian cohort	25735680	IVCTc Mod, IVCTh Mod, CGS 25, reference reports as c.3091G>A, p.Val1032Met
rs80338782	p.Arg1086Ser	NC_000001.11: g. 201060816G>T	Case study	20431982	Individual developed fulminant MH under sevoflurane anesthesia.
			UK MHS Cohort	30236257	Found in 2 families (might potentially have also pathogenic RYR1 variant)
rs148870919	p.Gly1210Arg	NC_000001.11: g. 201054543C>T	UK MHS Cohort	30236257	Found in 1 family (might potentially have also pathogenic RYR1 variant)
rs145910245	p.Thr1354Ser	NC_000001.11: g. 201051037T>A	Italian family	20861472	Segregated with MHS in one family: studies show it increased RYR1 sensitivity *in-vitro*. However, the minor allele frequency of this variant in gnomAD (0.26%) is much higher than the incidence of MHS in the general population.
			ClinSeq (unselected for MHS)	24195946	Identified in 9 of 870 ClinSeq exomes; concluded that it should be scored “unknown pathogenicity”; likely in LD with pathogenic variant
			Australian cohort	25735680	Subject U: IVCTc Max, IVCTh Mod, Family history (together with RYR1 Lys1393Arg); subject AW: IVCTc Mod, IVCTh Mod, CGS 15
rs3850625	p.Arg1539Cys	NC_000001.11: g. 201047168G>A	UK MHS Cohort	25658027	Found in MH samples by itself or together with rs193922753 (*RYR1*), rs193922770 (*RYR1*) or rs12406479 (*CACNA1S*) (also found in MHN and MHS Exertional Heat Illness cohort samples)
			Swiss MHS Cohort	28259615	Detected by capillary sequencing of 56 MHS samples
rs13374149	p.Arg1658His	NC_000001.11:g. 201043356C>T	Swiss MHS Cohort	28259615	Detected by capillary sequencing of 56 MHS samples
rs367577681	p.Thr1696Met	NC_000001.11: g. 201041551G>A	UK MHS Cohort	30236257	Found in 1 family (might potentially have also pathogenic RYR1 variant)
rs12139527	p.Leu1800Ser	NC_000001.11: g. 201040054A>G	UK MHS Cohort	25658027	Found in MH sample together with rs35364374 (*RYR1*) (also found in MHN

rsID	Protein Change	Genomic Change	Cohort/Group Studied	PMID	Notes
					and MHS Exertional Heat Illness cohort samples)
			Italian MHS Cohort	20681998	Only mentioned in table 3 of referenced article
			Swiss MHS Cohort	28259615	Detected by capillary sequencing of 56 MHS samples

Abb.: CHCT: caffeine-halothane contracture test, CGS: Clinical Grading Score ER: exertional rhabdomyolysis, IVCT: *in-vitro* contracture test, IVCTc: caffeine, IVCTh: halothane, MH: malignant hyperthermia, MHS: malignant hyperthermia susceptible, NHN: malignant hyperthermia normal, MHE: malignant hyperthermia equivocal, MIN: minimal, MOD: moderate, MAX: maximal.

Table 3.

CACNA1S variants identified in patients with severe statin myopathy [PMID: 30325262]

rsID	Protein Change	Genomic Change	Statin	Additional variant information based on subject report and tables
rs1204964501	p.His1139Gln	NC_000001.11: g.201059297G>C	atorvastatin (20 mg)	*COL6A3* p.Arg1064Trp
rs377474103	p.Arg1447Gln	NC_000001.11: g.201048683C>T	simvastatin (40 mg) changed to pravastatin (40 mg)	*ENO3* p.Lys193Asn; *RYR1* p.Pro4495Leu
rs1225367412	p.Glu697del	NC_000001.11: g. 201073615_201073617delCTC	simvastatin (40 mg)	*RYR1* p.Gly893Ser; *AGRN* p.Gly719Ser
rs768445692	p.Gly628Ser	NC_000001.11:g.201075561C>T	rosuvastatin (20 mg)	No additional variants mentioned
rs529038948	p.Arg364Trp	NC_000001.11: g.201085496G>A	atorvastatin (10 mgs)	No additional variants mentioned

Abb.: *AGRN*: agrin, *CACNA1S*: calcium voltage-gated channel subunit alpha1 S, *COL6A3*: collagen type VI alpha 3 chain, *ENO3*: enolase 3, *RYR1*: ryanodine receptor