INVITED REVIEW

ABSTRACT: Malignant hyperthermia (MH) is a potentially life-threatening event in response to anesthetic triggering agents, with symptoms of sustained uncontrolled skeletal muscle calcium homeostasis resulting in organ and systemic failure. Susceptibility to MH, an autosomal dominant trait, may be associated with congenital myopathies, but in the majority of the cases, no clinical signs of disease are visible outside of anesthesia. For diagnosis, a functional test on skeletal muscle biopsy, the in vitro contracture test (IVCT), is performed. Over 50% of the families show linkage of the IVCT phenotype to the gene encoding the skeletal muscle ryanodine receptor and over 20 mutations therein have been described. At least five other loci have been defined implicating greater genetic heterogeneity than previously assumed, but so far only one further gene encoding the main subunit of the voltage-gated dihydropyridine receptor has a confirmed role in MH. As a result of extensive research on the mechanisms of excitation-contraction coupling and recent functional characterization of several disease-causing mutations in heterologous expression systems, much is known today about the molecular etiology of MH.

 Muscle Nerve **23:** 4–17, 2000

GENETICS AND PATHOGENESIS OF MALIGNANT HYPERTHERMIA

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Accepted 16 August 1999

At the start of this century, anesthetic deaths by so-called late ether convulsions were observed occasionally. But it was after the introduction of halothane and succinylcholine combinations into routine general anesthesias in the mid-1960s that this syndrome, characterized by muscle contractures and high temperature, was distinguished as a new pharmaco-induced entity with an autosomal dominant mode of transmission.[27] Apparently, symptoms could be triggered by both nonhalogenated anesthetics such as ether, and halogenated anesthetics such as halothane, and were additionally enhanced by the administration of depolarizing muscle relaxants such as succinylcholine.[55]

Clarification of the pathogenesis of the condition denoted as malignant hyperthermia (MH) involved multiple steps made possible by studies on the physiology of the contraction process and muscle metabolism. In these, calcium plays an important role, regulating both actin-myosin interaction[31] and glycolysis,[14] disturbances of which account for the observed clinical signs of muscle contractures and hypermetabolism.[35,73] These studies indicate that the underlying pathogenesis is an uncontrollably high myoplasmic free calcium, as later shown directly.[67]

Another important step to understanding disease pathogenesis was the identification of the major underlying defect, mutations in the ryanodine receptor RYR1, the calcium release channel of skeletal muscle. This required the use of a phenotypically similar animal model, swine suffering from both stress-induced and halothane-triggered typical MH

Abbreviations: ATP, adenosine triphosphate; ATPase, adenosine triphosphatase; CCD, central core disease; CICR, calcium-induced calcium release; CK, creatine kinase; DHPR, dihydropyridine receptor; EC, excitation-contraction; EMHG, European Malignant Hyperthermia Group; HEK, human embryonic kidney; HypoPP, hypokalemic periodic paralysis; IVCT, in vitro contracture test; L-type, long-lasting; MH, malignant hyperthermia; MHE, MH-equivocal; MHN, not susceptible to MH; MHS, MH-susceptible; NAMHG, North American Malignant Hyperthermia Group; pS, piko Siemens; PSE meat, pale soft exudative pork; RYR1, ryanodine receptor type 1 (human); RYR3, ryanodine receptor type 3 (human); ryr1, porcine ryanodine receptor type 1; SR, sarcoplasmic reticulum; S1-S6, transmembrane protein segments 1–6

Key words: malignant hyperthermia; ryanodine receptor; dihydropyridine receptor; in vitro contracture test; excitation contraction coupling; central core disease

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CCC 0148-639X/00/010004-14

episodes.[45,153] Here, we briefly give an introductory overview on the genetics and pathogenesis of MH. More detailed reviews are available for the interested reader.[51,91,109,124]

CLINICAL FEATURES

Malignant hyperthermia is an autosomal dominantly transmitted predisposition of clinically inconspicuous individuals to respond with uncontrollable skeletal muscle hypermetabolism upon exposure to volatile anesthetics or depolarizing muscle relaxants.[26] The triggering substances lead to an increase in the concentration of free myoplasmic calcium which is released from the sarcoplasmic reticulum calcium stores via the muscle ryanodine receptor, the calcium release channel. During an MH reaction, increased myoplasmic calcium induces contractures of the (often first-noticed) masseter and potentially other skeletal muscles, and activates glycogenolysis and cell metabolism resulting in heat and excess lactate production. Activation of the oxidative cycle leads to high oxygen consumption and carbon dioxide production followed by muscular ATP depletion and systemic changes such as acidosis, hypercapnia, and hypoxemia. Tachycardia is a frequent, however unspecific, early symptom, whereas hyperthermia may be a late sign. During the course of the crisis, rhabdomyolysis occurs with subsequent creatine kinase elevation, hyperkalemia potentially leading to cardiac arrhythmia or even arrest, and myoglobinuria with the possibility of renal failure. If an episode is survived, normalization of edematous muscle and creatine kinase (CK) levels occur within 10–15 days (for clinical overview, see Table 1).

Severity of the MH reaction is defined clinically by the Larach score, which takes several parameters of the metabolic disturbance and the rate of progression into account.[85] Without immediate treatment, up to 70% of patients may die from ventricular fibrillation, pulmonary edema, intravascular coagulopathy, cerebral hypoxic damage, cerebral edema, or renal failure. For patients known or suspected of being susceptible, potential triggers must be avoided and alternative methods of anesthesia applied, such as with propofol and xenon.[8,29,57,58,83,99] During a crisis, treatment consists of early administration of dantrolene.[82] This lipid-soluble hydantoin derivative acts by inhibiting the release of calcium from the sarcoplasmic reticulum, therefore inhibiting muscle contractures induced by triggering agents and normalizing myoplasmic calcium concentration.[34] Recently, a specific protein receptor for dantrolene located in the skeletal muscle triad has been described.[129] Early administration of this drug has successfully aborted numerous fulminant crises [82] and reduced the mortality rate from about 70% to the present 10%[125] A further reduction in deaths may be achieved with earlier recognition of the disorder by anesthetists, and perhaps in the future by better prevention.

Table 1. Clinical features of malignant hyperthermia.

Pathogenetic mechanism	Resulting symptoms
Elevated myoplasmic Ca^{2+}	Masseter muscle spasm
	Generalized muscle spasms
	Heat production
	Hypermetabolism
	Rhabdomyolysis
Hypermetabolism	Tachycardia
	Acidosis
	ATP depletion
	Hypercapnia
	Hypoxemia
	Heat production
Rhabdomyolysis	CK and K^+ elevation
	Cardiac arrhythmia
	Myoglobinuria
	Renal failure

Incidence of MH crises during general anesthesia varies age-dependently from 1:15,000 in children to 1:50,000 in adults.[6,13,118,124] As the triggering substances elicit an event only in a fraction of anesthesias,[30] the true prevalence of MH susceptibility may be higher[51] than the very low clinical penetrance.[72] In accordance with the varying severity of the clinical picture, nonanesthetic MH-like episodes triggered by overheating,[24,65] body exertion,[2,12] and infections[28] have been described. Evidence for a relation to the sudden infant death syndrome[27,131] is rather weak.[33] MH-like crises have also been observed in patients with myopathies such as myotonia fluctuans,[141,168] Duchenne and Becker dystrophy,[15,78,120] myotonia congenita,[62] and myotonic dystrophy. It seems very likely that the molecular mechanisms underlying these MH-like events differ from those of true MH susceptibility, i.e., increased sensitivity of skeletal muscle to the triggering agents by an increased resting calcium level[158] or increased myotonic reactions to anesthetic agents.[87] This, of course, does not obviate the need for caution when considering general anesthesia in these disorders.

The existence of accessory symptoms to the trigger-induced anesthetic episodes is a matter of debate. Strazis and Fox[154] reviewed 503 cases from the literature and found a significantly higher incidence of musculoskeletal defects such as cleft palate, club foot, scoliosis, ptosis, strabismus, cryptorchism, or congenital hernias in the group of MH individuals

compared to the general surgical population. This difference was not only visible in the group of pediatric patients, but also in the adult group (classified as over age 15). In contrast, another study involving 155 Scandinavian patients found no difference in clinical examination of MH patients compared to controls, the only potential abnormality being core-targetoid fibers.[138]

ASSOCIATED MYOPATHIES

Predisposition to true MH has been established only for three defined myopathies: Evans myopathy, King Denborough syndrome, and central core disease. Of these, the first presents with proximal muscle wasting of the vasti laterales, adductores, and glutei with hypertrophy of the sternocleidomastoids, peronei, and thigh muscles. Partial ptosis, lumbar lordosis, elevated serum CK, and varying myopathic histologic patterns indicated an autosomal dominant pattern of inheritance in Denborough's original family, in which about 57 persons were known to be affected.[26,81]

Another, although very rare, MH-associated myopathy is King Denborough syndrome[80] characterized by short stature, cryptorchism, lumbar lordosis, thoracic kyphosis, pes cavus, pectus carinatum, dislocating shoulders and patellas, and high-arched palate. The children additionally have an unusual face with low-set ears, micrognathia, ptosis, strabismus, and down-slanting palpebral fissures.[50] Even though there seems to be considerable overlap with the Noonan syndrome, no patient with King Denborough syndrome has been reported yet with the Noonan combination of hypertelorism, epicanthic folds, lymphedema, bleeding diathesis, and characteristic heart defects.

Finally, central core disease (CCD[152]) has also been associated with MH episodes.[151] This myopathy is characterized by muscle hypotonia (floppy infant syndrome), delayed motor development, proximal symmetrical weakness, and CK elevation. It is the only known myopathy for which exercise is beneficial.[54] The name of the syndrome derives from the typical histological central cores along the whole length of type 1 muscle fibers expressing the slow-twitch Ca^{2+}-ATPase pump. These cores consist of unstructured myofibrils and areas lacking mitochondria and thus oxidative enzymes enabling histochemical detection. The absence of type 2 fibers expressing the fast-twitch and slow-twitch Ca^{2+}-ATPase pumps is as yet unexplained. Electron microscopic investigations of CCD muscle tissue have demonstrated: (a) amorphous central areas (cores) in type 1 fibers with a relative lack, if not complete absence, of mitochondria in core regions; (b) less numerous glycogen granules; (c) less well-defined myofibrils and loss of myofibril alignment in adjacent sarcomeres; (d) contracted sarcomeres; (e) Z disc streaming; and (f) pathological changes in the sarcoplasmic reticulum (SR) and transverse tubules in both core and noncore regions.[60]

Pathogenetically in CCD, myoplasmic calcium overload is thought to cause mitochondrial damage and thus decreased metabolic activity. Additionally, compensatory genes are likely to be activated, resulting in proliferation of the sarcoplasmic reticulum and transverse tubules. Both processes can lead to muscle weakness and loss of muscle fibers. Apparently, the absence of mitochondria per se in central core regions may not contribute significantly to muscle weakness since there does not appear to be a direct relationship between the extent of the central cores and the clinical severity of the disease. Inheritance is autosomal dominant, and not all family members may develop this myopathy but instead may only have the MH trait.[70]

DIAGNOSTIC TESTING

Due to lack of clinical symptoms under normal conditions, an MH in-vitro contracture test (IVCT) for biopsied muscle bundles was developed by the European[37] and North American[84] malignant hyperthermia groups (EMHG, NAMHG). This test requires a large fresh muscle biopsy and is therefore invasive in nature and not easily performed on children. It is based on the tendency of MH muscle to be abnormally sensitive to stimuli that induce SR calcium release. The underlying procedure in both EMHG and NAMHG test protocols is the measurement of contractures upon flooding or gradually increasing concentrations of halothane (0.5, 1, 2, 3, and 4% for EMHG, and 3% for NAMHG) or caffeine (0.5, 1, 2, 3, 4, 32 mmol/l for EMHG, and 0.5, 1, 2, 4, 8, 32 mmol/l for NAMHG). A positive reaction to a triggering agent is dependent on contracture force at concentrations below predefined thresholds for each substance. For EMHG, contractures of at least 2 mN at maximally 2% halothane or 2 mmol/l caffeine are considered pathologic, and for NAMHG, a minimal contracture of 5 g at 3% halothane or 2 g at 2 mmol/l caffeine. Three categories result by each test according to the European protocol: contracture under or at the thresholds of both substances is considered to be MH-susceptible (MHS), one pathologic and one normal result is classified as equivocal (MHE), and two normal reactions to both agents means not susceptible (MHN).

In general, correlation between the results of

these two tests is quite good,[42] and the test shows a high sensitivity (true positives, 99% for EMHG, and 92–97% for NAMHG) and specificity (true negatives, 93.6% for EMHG, and 53–78% for NAMHG.[3,125] Discrepancies, both false-positive as well as false-negative results compared to genetic data, have occurred (for review, see elsewhere[100]). This is because the concentration threshold values and minimal contracture values considered as pathologic are (arbitrarily) standardized in a way so that sensitivity approaches 100%, ensuring identification of as many patients at risk as possible. Alternative approaches to improve evaluation such as normalizing the contracture force to the cross-sectional area or studying time course of relaxation have been employed in single studies, but significant data is only available for a raising of the cut-off contracture value from 0.5 to 0.7g of tension at 3% halothane in the NAMHG protocol, which increases specificity from 78 to 81% but decreases sensitivity from 97 to 88%. Therefore, altered IVCT cut-off points in some cases have permitted investigators to link MH families to the RYR1 gene that were previously unlinked when conventional cut-off points were applied.[61,97,148]

Remarkably in the IVCT as outlined above, contracture threshold concentration and tension values correlate well for caffeine but not for halothane. This suggests halothane reacts on an all-or-nothing basis with a higher rate of nonspecific effects (MHE results) than caffeine, which produces results linear to the severity of the mutations[104] even though halothane is considered to bind specifically to the calcium release channel while caffeine initiates several additional pathways. In an effort to employ more specific activators of the skeletal muscle calcium release channel than caffeine, IVCT using ryanodine[59,66,159,169] and 4-chloro-m-cresol[9,49,63,126,163] have been tested and show promising results, but are not yet widely enough adopted to be employed for routine diagnostic purposes.

In contrast to the IVCT test protocols primarily aimed at determining the clinical risk of anesthesia-related events, diagnostic testing in Japan is performed by a functional test based on the quantification of calcium-induced calcium release (CICR) in saponized muscle fibers.[36,75] The precision of this method and correlation to the other protocols is unknown.

ANIMAL MODELS

So-called stress-susceptible pigs[57,111,153] have more muscle mass and their meat is less fat than other swine. Boars were therefore selectively bred despite higher losses during transport and reduced meat quality of stressed animals (PSE meat = pale, soft, exudative pork[32,110]). Upon triggers such as stress or halothane, susceptible swine may either have acute localized muscle necrosis of the longissimus dorsi muscle or generalized attacks of malignant hyperthermia with features as in human MH, i.e., changes in vital signs, metabolism, acid-base balance, temperature, and muscle rigidity.[96,139] As over the last decades meat quality has been considered increasingly important, only pigs negative in the in-vivo halothane test have been taken for breeding. Nowadays with the availability of molecular testing, sows not carrying the mutation are covered by boars that are homozygous carriers of the mutation, thus generating only heterozygous litters. The heterozygous animals are characterized by large muscle mass similar to susceptible pigs, and stress resistance like MH-negative swine. Nevertheless, muscle heterozygous animals react abnormally when exposed to high concentrations of various agents.[46,147,170]

It was in this animal model that an important clue to which chromosomal region might bear the most common human MHS locus was provided. Soon after linkage of the porcine stress syndrome to the so-called halothane locus on chromosome 6,[4,5,23] the corresponding cluster of genes was linked to MHS in several human families and localized to chromosome 19q11.2-13.2.[98,105] Likewise, soon after the mapping of a gene cluster including the calcium release channel of skeletal muscle, the ryanodine receptor, ryr1, to porcine chromosome 6q12,[21,56] this gene was found to be linked to the MH locus on human chromosome 19q.[98,101] Even the mutation detected in the animal model, the first ryr1 mutation, R615C,[45,127] is homologous to one of the most frequent human mutations, R614C [48,64] but shows a founder effect in contrast to humans.

A mechanism by which the porcine ryr1 mutation gives rise to lean, heavily-muscled swine has been proposed: ryr1 hypersensitivity could stimulate spontaneous muscle contractions leading to muscle hypertrophy and consequently improved energy utilization limiting fat deposition,[102] but the close association of a "lean meat gene" to ryr1 cannot be excluded.

Next to swine, alterations of ryr1 have been assumed in chicken dystrophy, an autosomal-recessive myogenic disease that primarily affects fast-twitch fibers. While in normal muscle SR preparations the density of ryr1 proteins decreased in the first days after birth, such decrease did not occur in dystrophic muscle even though the density of low-affinity binding sites increased progressively.[130] Additionally, high-affinity ryanodine binding showed an increased

sensitivity to stimulation by caffeine and to inhibition by high calcium concentrations.

MAJOR GENETIC BACKGROUND

In over 50% of the MH families, linkage of the autosomal dominant MHS trait to the so-called MHS-1 locus, i.e., the gene encoding the human skeletal muscle ryanodine receptor RYR1, can be found.[6] When reflecting the decreasing expressivity with age, the low estimated clinical penetrance, and thus the limited predictive value of the IVCT for milder cases, a higher percentage of MH families linked to RYR1 would be expected by lowering the standardized thresholds in the IVCT.[97] Further linkage concerning the MH-associated CCD confirmed the suspected allelism of the two disorders,[53,74,114,146] showing mutations in RYR1 in CCD (Table 2).

RYR1, named after a plant alkaloid binding specifically to the channel, is one of the largest known proteins, with 2,200 kDa corresponding to 5,000 amino acids encoded by 106 exons.[132] This complexity of the gene makes the detection of new mutations very time-consuming. Even so, mutations have been reported for both MH and CCD, clustering in two main regions ranging from amino acid residues 35 to 614 (exons 2 to 17) and a mid-region ranging from residues 2163 to 2458 (exons 39 to 46), both situated in the long N terminus of the protein, the so-called foot of the channel complex (Table 2 and Fig. 1). Additionally, the recent discovery of the I4898T mutation for CCD suggests that the C-terminal region of the RYR1 may represent a third mutation hot spot,[95] all three of which are highly conserved across all known ryanodine receptor isoforms. Other regions of the gene have not yet been studied to the full extent but may yield additional hot spots.

The most frequent RYR1 mutations in MH are R614C, the porcine homologue, G2434R, and G341R, the three together making up over 10% of the cases. Regional differences occur, with G341R being very frequent in Ireland, England, and Scandinavia,[104,39,136] and R614C appears more often on the mainland of Europe.[11] Only in approximately 20% of all affected families can one of the known mutations be identified, suggesting that genetic screening cannot replace IVCT phenotyping at this time. General testing is further complicated by heterogenity with the possibility of false-negative results

Table 2. Mutations in proteins of the excitation-contraction coupling complex of skeletal muscle: dihydropyridine receptor α1 subunit (DHPR) and ryanodine receptor (RYR1).

Nucleotide	Exon	Substitution	Disorder	Frequency	First report (ref. no.)
DHPR: hypokalemic periodic paralysis (HypoPP) and malignant hyperthermia (MH)					
G1583A	11	R528H	HypoPP	40%	71
C3256T	26	R1086C	MH	1 family	145
G3257A	26	R1086H	MH	1 family	113
C3715G	30	R1239G	HypoPP	3%	133
G3716A	30	R1239H	HypoPP	40%	133
RYR1: malignant hyperthermia (MH) and/or central core disease (CCD)					
T103C	2	Cys-35-Arg	MH	1 family	94
C487T	6	Arg-163-Cys	MH;CCD	2%	134
G742A	9	Gly-248-Arg	MH	2%	47
G1021A	11	Gly-341-Arg	MH	6%	136
C1209G	12	Ile-403-Met	CCD	1 family	134
A1565C	14	Tyr-522-Ser	MH;CCD	1 family	135
C1654T	15	Arg-552-Trp	MH	1 family	76
C1840T	17	Arg-614-Cys	MH	4%	48
G1841T	17	Arg-614-Leu	MH	2%	137
C6487T	39	Arg-2163-Cys	MH	4%	104
G6488A	39	Arg-2163-His	MH;CCD	1 family	104
G6502A	39	Val-2168-Met	MH	7%	104
C6617T	40	Thr-2206-Met	MH	1 family	104
C6617G	40	Thr-2206-Arg	MH	1 family	11
G7303A	45	Gly-2434-Arg	MH	4%	77
G7307A	45	Arg-2435-His	MH;CCD	1 family	172
G7307T	45	Arg-2435-Leu	MH	1 family	7
G7361A	46	Arg-2454-His	MH	1 family	7
C7360T	46	Arg-2454-Cys	MH	1 family	11
C7372T	46	Arg-2458-Cys	MH	4%	103
G7373A	46	Arg-2458-His	MH	4%	103
T14693C	102	Ile-4898-Thr	CCD	1 family	95

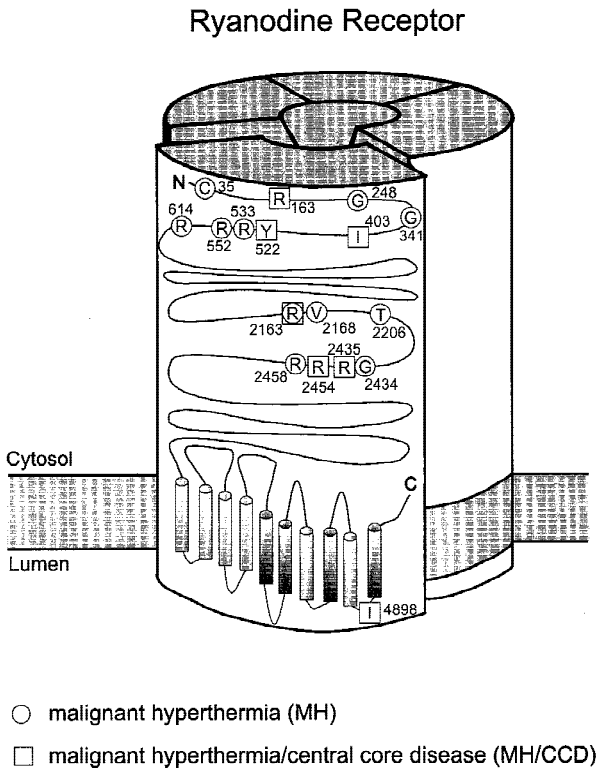

FIGURE 1. The homotetrameric ryanodine receptor, the calcium release channel situated in the membrane of the sarcoplasmic reticulum (SR). The cytosolic part of the protein complex, the so-called foot, bridges the gap between the transverse tubular system and the SR. Mutations have been described for the skeletal muscle ryanodine receptor (RYR1), which cause susceptibility to malignant hyperthermia and central core disease. Conventional abbreviations are used for the replaced amino acids whose positions are given by the respective numbers of the human RYR1.

in families in which MH-causing mutations segregate independently.[30] Of the over 20 mutations causing MH, four are also associated with CCD.

Whether the King Denborough syndrome too is associated with RYR1 mutations has not been clarified, but the so-called crooked neck dwarf mutation of embryonic chicken, a potential animal model, may indicate that this is so. In skeletal muscle of this chicken, normal ryr1 isoform could not be detected, and the extremely low levels of ryr1 immunoreactivity found showed atypical distribution and pointed to the presence of abnormal protein.[1]

SECONDARY LOCI

In the past, a number of families not linked to chromosome 19 linked have been reported, pointing to considerable genetic heterogeneity (reviewed elsewhere[102]). In North America, the first alternative locus to chromosome 19q was assigned to chromosome 17q11.2-q24,[90] the MHS-2 locus, suggesting the voltage-dependent sodium channel of skeletal muscle membrane as a candidate gene.[112,123,168] The findings could not be confirmed in Europe[69,156] perhaps pointing to a regional type of MH or to the fact that myotonic syndromes caused by sodium channel mutations predispose to MH-like events.[141] Suggestions of further loci have been made not only for MH,[142] but also for CCD.[38]

The candidate gene approach was applied to screen loci containing genes encoding proteins involved in excitation-contraction (EC) coupling, such as the pentameric dihydropyridine receptor (DHPR) which acts as the voltage sensor for RYR1. By this method, one European family was found to be linked to a locus on chromosome 7q21.1 containing the gene encoding the α2/δ DHPR subunit with a lod score of 2.91 (MHS-3 locus[68]). No mutation in this gene, however, has been identified yet.

By a systematic genome screening on several large, apparently non-chromosome 19 linked European MH families, the MHS-4 locus on chromosome 3q13.1 was identified in a single family generating a lod score of 3.22.[157] Sequencing of candidate genes at this location is currently in progress in this pedigree. Further studies identified one new MHS locus on chromosome 1q32 (MHS-5) and a tentative locus on chromosome 5p (MHS-6[143]), each in a single pedigree. For the MHS-5 locus, two disease-causing mutations were identified in the candidate gene of the region encoding the DHPR α1 subunit (Table 2 and Fig. 2[113,145]). The mutations affecting the same amino acid residue are located in an intracellular loop of the protein whose functional significance for EC coupling is unknown, but whose functional link to RYR1 has been demonstrated recently.[89] This could indicate that a possible pathogenetic mechanism for MH might be disturbance of DHPR activation. Interestingly, mutations linked to hypokalemic periodic paralysis have been reported in the same gene,[71,133] although the disorder is not thought to be associated to MH susceptibility.[87] For the MHS-6 locus, no causative gene has been identified to date.

In contrast to the MHS-1 locus, the other MHS loci have been described only in one or two pedigrees each, a fact that does not point to a second major locus explaining the MH families not linked to RYR1, but perhaps implying false exclusion of RYR1 locus. A recent linkage analysis study of 20 large, well-defined MHS families confirms this, demonstrating linkage to chromosome 19q in 9 families, a single recombinant perhaps pointing to misdiagnosis by IVCT phenotyping in 8 families, and definite exclusion of MHS-1 by multiple recombinants in 3

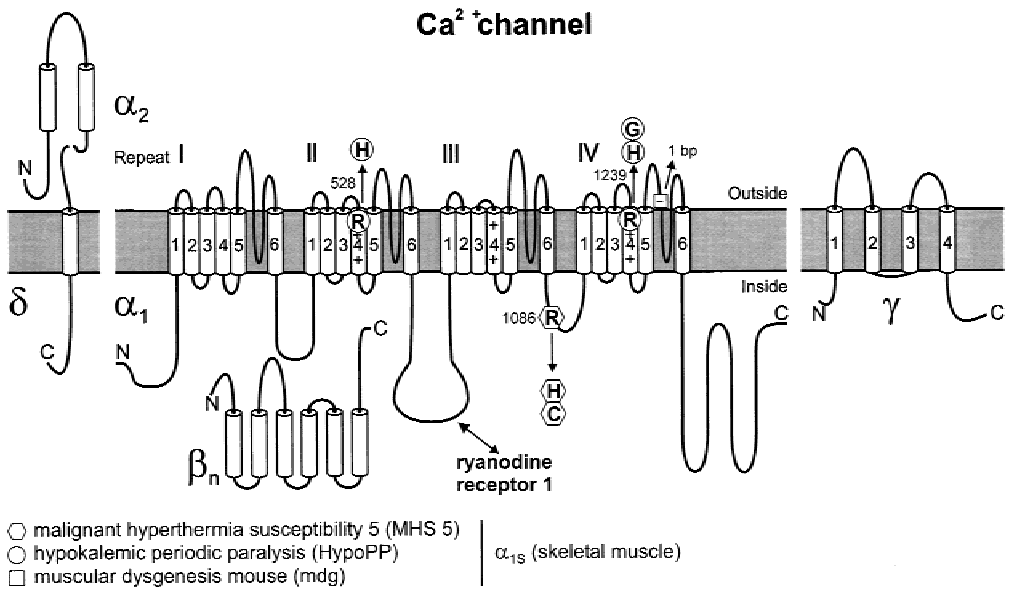

FIGURE 2. Subunits of the voltage-gated calcium channel. The α subunit consists of four highly homologous domains (repeats I–IV) containing six transmembrane segments each (S1–S6). The S5–S6 loops form the ion selective pore, and the S4 segments contain positively charged residues conferring voltage dependence to the protein. The repeats are connected by intracellular loops; the II–III and III–IV interlinker interacting with the ryanodine receptor to mediate excitation-contraction coupling. α2/δ, β1 to β4, and γ are auxilliary subunits. Mutations in the α1S subunit of the skeletal muscle L-type calcium channel (= dihydropyridine receptor, DHPR) have been described for humans (HypoPP, MHS5). Conventional abbreviations are used for the replaced amino acids whose positions are given by the respective numbers of the α1S subunit. The symbols used for the point mutations indicate the resulting diseases as explained at the bottom of the left-hand side.

families.[142] If the 8 familes with one recombinant were indeed linked to MHS-1, this study would suggest that more than 85% of the families are linked to RYR1. Examples of families with false IVCT results have been reported[61,97,148] and as mutational screening progresses, judgment of quality of the IVCT based on genetic data will be enabled.

EXCITATION-CONTRACTION COUPLING

The DHPR and RYR1 are situated in the triadic junctions of the T-tubular system and the SR respectively (Fig. 3). The latter has a quatrefoil structure with the hydrophobic parts of the four subunits forming an SR-membrane spanning baseplate and the hydrophilic segments forming a cytoplasmic domain, the foot, which bridges the gap between T-tubular and SR membrane. This cytosolic part of RYR1 contains binding sites for various activating ligands like calcium (μM), ATP, calmodulin (which binds in the absence of calcium), caffeine and ryanodine (nM), and inactivating ligands like calcium (>100 μM) and magnesium in mM concentrations.[22,106] RYR1 activity is modulated by exogenous ligands including ryanodine, caffeine, and dantrolene sodium.[173] Ryanodine modulates RYR1 in a biphasic manner with nM concentrations activating and >100 μM concentrations inhibiting the channel,[16] caffeine activates RYR1 by increasing the affinity of the calcium activation site,[10,107] and dantrolene sodium inhibits RYR1 by limiting channel activation by calmodulin and calcium.[44]

Despite the huge size of over 5,000 amino acids, RYR1 channels can be functionally expressed by transient transfection. In Chinese hamster ovary cells, immunoreactivity, ligand binding[160] and single channel recordings[19] resembled native channel properties, but there were some differences in gating modes and subconductance states. In human embryonic kidney (HEK-293) cells, however, even calcium conductance of 116 pS in 50 mM luminal calcium and an open time constant of 0.22 ms were identical to that of the native protein.[20]

Functional significance was studied by a RYR1 knockout mouse.[159] Skeletal muscle from these mice did not show any contractile response to electrical stimulation and calcium release induced by caffeine, ryanodine, and adenine nucleotides was attributable to the residual expression of another isoform, RYR3.[161] At the subcellular level, the cytoplasmic foot domain of triads was missing, although junctions between SR cisternae and T-tubules were still formed. This led to the term "dyspedic mice."

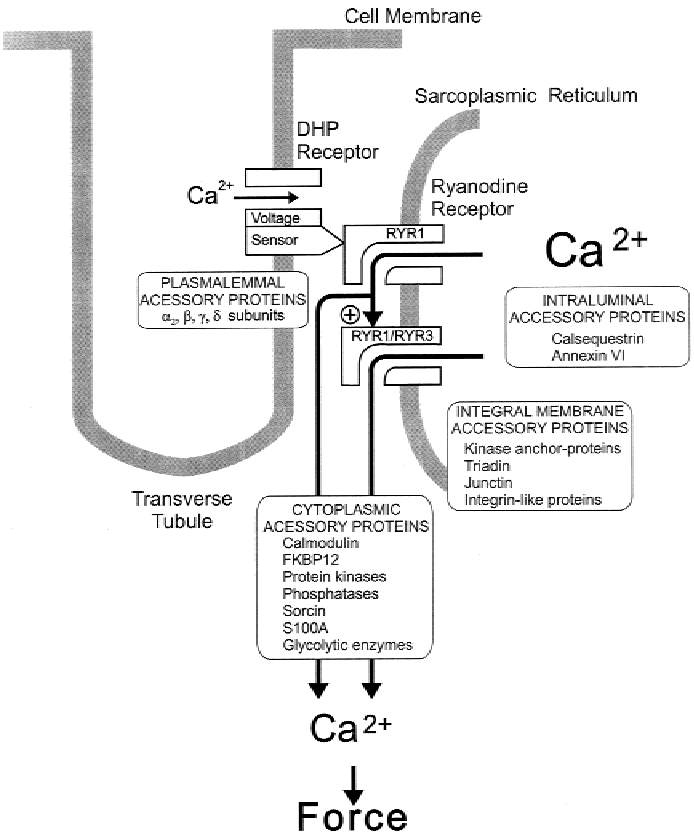

FIGURE 3. The triadic junction between a transverse tubule and the sarcoplasmic reticulum is the place of interaction; of the two calcium channels of skeletal muscle, the L-type calcium channel, also called dihydropyridine receptor (DHPR), and the calcium release channel, also called ryanodine receptor (RYR1). The coupling between the two channels is not fully elucidated, although several contributing mechanisms are known: (i) at the level of T-tubular membrane; are the additional subunits of the pentameric DHPR (ii) various cytoplasmic accessory proteins influence the activity especially of RYR1, i.e., kinases and calmodulin at micromolar calcium concentrations decrease RYR1 activity while phosphatases, S100 and calmodulin at nanomolar calcium concentrations increase the activity; sorcin binding to the DHPR helps to mediate the interaction with RYR1; and the FK506 binding proteins (FKPB) are involved in protein folding and association of RYR1; (iii) integral membrane proteins like triadin and junctin form a quaternary complex together with RYR1 and calsequestrin whereby triadin also interacts directly with the DHPR; additional anchoring proteins support the vicinity of the regulatory kinases; and (iv) intraluminal proteins like calreticulin and calsequestrin influence luminal calcium binding and thus rate of calcium release, or increase RYR1 activity like annexin VI.

The DHPR is a calcium channel with a long-lasting (L-type) current sensitive to dihydropyridines and acts as the voltage sensor for RYR1. The α1 subunit of the α1-α2-δ-β1-γ pentameric DHPR complex determines main characteristics of the complex conveying ion selectivity and containing the ion conducting pore, voltage sensors, gates for the different opened and closed channel states, and important binding sites for endogenous and exogenous ligands (reviewed elsewhere[88]). It consists of four domains of internal homology each containing six transmembrane α-helical segments, numbered S1–S6, connected by both intracellular and extracellular loops, the interlinkers (Fig. 2). Voltage sensitivity is associated with the S4 segments that contain a density of positively charged amino acid residues, and the interlinkers S5–S6 are thought to contribute to the lining of the pore.

The α1 DHPR subunit has been shown to interact with RYR1 by the interlinker between domains II and III, residues 666–690 and 724–760, for mediating EC coupling.[52,93,144,162] Corresponding regions of RYR1 binding to the DHPR are residues 1303–1406 whose deletion preserved the function of the channel but led to loss of EC-coupling.[171] Recently, inspired by the detection of the MH-causing mutations in the intracellular loop between domains III and IV,[113,145] both this part of the protein and the II–III loop have been shown to also bind to RYR1 residues 954–1112.[89]

RYR1 not only receives an activating signal from DHPR, but also gives a retrograde signal enhancing

DHPR activity, which is mediated by the RYR1 residues 2659–3720.[115]

DISEASE PATHOGENESIS

The diagnostically important increased sensitivity of MHS muscle to caffeine has been related to altered RYR1 function.[108] Functional tests on isolated SR vesicles have shown that calcium regulation is disturbed: lower calcium concentrations activate the channel to a higher than normal level, and higher than normal calcium concentrations are required to inhibit the channel (reviewed elsewhere[109]). Investigations of reconstituted RYR1 in lipid bilayers, designed to find the reason for the increased calcium and caffeine sensitivity of MH muscle, led to controversial results: electrophysiological single-channel measurements on RYR1 did not show increased sensitivity (porcine R615C[17,40,79,149,150]), whereas other studies showed both increased affinity (porcine R615C[63]) and sensitivity (human G2434R,[140] human and porcine MH[122,167]). The inhibiting properties of calmodulin were found unaltered in mutant RYR1, but its activating properties in the absence of calcium were drastically increased (porcine R615C[119]). Due to lack of calcium inactivation, the open probability of RYR1 was higher in mutants than in wildtype at low pH (porcine R615C[92,150]) or under halothane stimulation (human MH[117]). Recently, reduced inhibition of calcium release by magnesium was reported as a possible pathogenetic mechanism (porcine R615C).[86]

In skinned fibers obtained from MH patients and swine, an increased rate of SR calcium release[36,75] and increased sensitivity to caffeine were observed (porcine R615C[116,121]), whereas the threshold for calcium-induced calcium release was unchanged (human and porcine MH[41,43]).

Functional characterization by calcium photometry of mutations in the N-terminus and the central part of the RYR1 foot in heterologous expression systems revealed comparable results, i.e., increased sensitivity of the mutant RYR1 to activating concentrations of calcium and exogenous and diagnostically used ligands such as caffeine, halothane, and 4-chloro-m-cresol, whereas there was no difference to wildtype in the resting intracellular calcium level (porcine R615C mutant in myotube C2C12 cell line,[128] porcine R615C in COS-1 cells,[166] human R163C in human primary myotubes[18]). In HEK-293 cells, increased sensitivity to halothane and caffeine was demonstrated for 15 MH-causing RYR1 mutants, whereby the photometric responses correlated with the IVCT for caffeine but not for halothane and indicated the IVCT to be the less precise examination system.[165] In the same cell line, expression of RYR1 MH mutants revealed higher resting calcium, smaller endoplasmic reticulum calcium stores, and reduced maximal calcium release compared to wildtype; CCD-causing mutants showed the strongest effects of all.[164,95] Complex studies on excised native muscle fibers from MH susceptible individuals revealed a threefold higher maximal peak rate of calcium release than for normal muscle, whereas neither kinetics nor voltage dependence of the release were affected.[155]

PERSPECTIVES

Since the description of MH almost 40 years ago, a good understanding of the physiological and genetic basis of MH in humans and the porcine animal model has been achieved. For the future, the identification of all genes associated with MH susceptibility and all of the mutations therein is one of the main goals. The mechanism of pathogenesis will then have to be elucidated more precisely in order to identify important regulatory domains in EC coupling proteins and determine modulating factors that might influence the occurrence of crises in susceptible individuals. A third goal will be to clarify the relationship of MH to other myopathies and accessory symptoms and provide a reliable method for both diagnosing the predisposition and performing precise differential diagnosis. In order to achieve these goals, basic scientists, anesthesiologists, neurologists, and geneticists will have to cooperate in making MH an interdisciplinary field of research.

The authors thank the Muscular Dystrophy Association (MDA) of the USA, the Interdisziplinäres Zentrum für Klinische Forschung (IZKF) of the University of Ulm sponsored by the Bundesministerium für Bildung und Forschung (BMBF), and the European Community, TMR Programme on Excitation-Contraction Coupling, for their support.

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