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Malignant Hyperthermia

David H. MacLennan* and Michael S. Phillips

In humans genetically predisposed to malignant hyperthermia, anesthesia can induce skeletal muscle rigidity, hypermetabolism, and high fever, which, if not immediately reversed, can lead to tissue damage or death. The corresponding condition in swine leads to stress-induced deaths and devalued meat products. Abnormalities in the Ca^{2+} release channel of skeletal muscle sarcoplasmic reticulum (the ryanodine receptor) have been implicated in the cause of both the porcine and human syndromes by physiological and biochemical studies and genetic linkage analysis. In swine, a single founder mutation in the ryanodine receptor gene (*RYR*1) can account for all cases of malignant hyperthermia in all breeds, but a series of different *RYR*1 mutations are likely to be uncovered in human families with MH. Moreover, lack of linkage between malignant hyperthermia and *RYR*1 in some families indicates a heterogenous genetic basis for the human syndrome.

Although anesthesia provides little cause for concern for most humans, exposure to a combination of potent inhalational anesthetics and depolarizing skeletal muscle relaxants presents a hazard to those genetically predisposed to malignant hyperthermia (MH) (*1*). The commonly used combination of halothane and succinylcholine can trigger skeletal muscle rigidity, accompanied by hypermetabolism, high fever, and cellular ion imbalances in susceptible individuals. If therapy is not initiated immediately, the patient may die within minutes from ventricular fibrillation, within hours from pulmonary edema or coagulopathy, or within days from neurological damage or obstructive renal failure. The practice of monitoring for the early symptoms of an MH episode and responding to such symptoms by terminating the anesthetic process and infusing the clinical antidote, dantrolene, has lowered the death rate for such episodes from over 80% to less than 7% in recent years. Neurological or kidney damage, however, still contributes to the morbidity resulting from MH episodes.

MH also occurs in domestic animals such as swine and therefore has worldwide economic consequences (*2*). Swine are seldom exposed to anesthesia, but animals homozygous for the abnormality respond to stress in the same way that heterozygous humans respond to anesthetics—with muscle rigidity, hypermetabolism, and high fever. The stress-induced death of such animals (porcine stress syndrome or PSS) is but one aspect of economic loss due to the syndrome. An equally serious problem is that the same reaction can be triggered when a hog experiences acute stress prior to slaughter, resulting in pale, soft, exudative (PSE) pork in large segments of the carcasses of susceptible animals.

An abnormality in the Ca^{2+} release channel of skeletal muscle sarcoplasmic reticulum [the ryanodine receptor (*3*)] may account for the disorder because, in skeletal muscle, both contraction and metabolism are regulated by the concentration of intracellular Ca^{2+} (*1*, *2*). The deduced amino acid sequences derived from sequences of cDNAs encoding the Ca^{2+} release channels from an MH and a normal pig differ at a single amino acid; in the MH pig Cys is substituted for Arg^{615} (*4*). This mutation was linked to MH with a lod score (the logarithm of the odds that genetic linkage exists) of 102 for a recombination fraction of 0.0 in studies of 338 informative meioses (*5*). This discovery has made it possible to develop a diagnostic test for normal, heterozygous (carrier), and MH-susceptible animals in all breeds of swine. The availability of a diagnostic test for the mutation provides breeders with the opportunity to eliminate the MH gene from their herds, thereby eliminating the major cause of stress-induced death and PSE. On the other hand, the MH gene may contribute to leanness and heavy muscling in swine (*6*) and breeders might find it advantageous to retain the gene and benefit from possible uniform gains in dressed carcass weight if heterozygous market animals can be produced economically.

Linkage between polymorphisms in and near the skeletal muscle ryanodine receptor (*RYR*1) gene and MH has also been established in studies of inheritance in human families (*7*, *8*). Moreover, substitution of Cys for Arg^{614}, corresponding to the porcine MH mutation, was found in 1 of 35 human families studied, and this mutation also cosegregated with MH (*9*). The combination of a very high lod score for linkage between the porcine *RYR*1 mutation and MH, with the existence of the corresponding mutation in humans, indicates that this mutation in *RYR*1 is the cause of at least

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some forms of MH. Nevertheless, linkage to polymorphisms in *RYR1* has not been observed in some human MH families (*10, 11*), indicating that abnormalities in proteins other than the Ca^{2+} release channel may disrupt Ca^{2+} regulation within skeletal muscle cells and lead to the MH syndrome.

Human Malignant Hyperthermia

Although descriptions of clinical manifestations of human malignant hyperthermia date from the early 1900's, autosomal dominant genetic predisposition to the disorder was first reported in 1960 (*12*). Statistical data, collected prior to concerted efforts to predict and circumvent anesthetic-induced MH reactions, indicated that the incidence of MH episodes was about 1 in 15,000 anesthetics in children's hospitals and about 1 in 50,000 in adult hospitals (*1*). These figures underestimate the true genetic predisposition to the condition, however, because many full-blown episodes occur for the first time in patients who have previously undergone uneventful anesthesia.

Because MH does not pose a serious threat to susceptible individuals in their daily lives or in any way incapacitate most of them, a major goal of MH research has been to identify MH-susceptible individuals prior to administration of anesthetics. If MH susceptibility is known, use of specific alternate anesthetics and nondepolarizing muscle relaxants can virtually eliminate triggering of MH episodes (*1*). To achieve this end, in vitro diagnostic tests for MH susceptibility were developed (*13*).

In the in vitro caffeine-halothane contracture test, a fiber from a muscle biopsy is attached to a force displacement transducer and then exposed to single or incremental doses of caffeine, to single or incremental doses of halothane, or to incremental doses of caffeine in the presence of halothane. Muscle fibers from normal and MH-susceptible individuals differ in their limits of induced tension, or in their sensitivity to caffeine or halothane. North American (*14*) and European (*15*) diagnostic tests have been developed on the basis of these principles, but they differ in detail and in interpretation. In the European test, individuals whose fibers undergo contractures exceeding threshold with both halothane and caffeine are considered to be MH-susceptible, but those whose fibers undergo contractures exceeding threshold with either caffeine or halothane, but not both, are diagnosed as MH-equivocal. The North American test accepts positive results from either test as defining MH susceptibility. In both cases, the test is invasive and expensive and, because of the potential danger of false negative diagnosis, may err on the side of false positive diagnosis (*16*). Diagnostic testing over two decades has confirmed the autosomal dominance of inheritance of the MH gene.

Porcine Malignant Hyperthermia

The incidence of MH (PSS) in swine varies from breed to breed and from country to country. On the basis of a selected study in Ontario, over 10% of commercial animals are estimated to be heterozygous carriers for the syndrome, while about 1.5% are homozygous (*17*). Up to 12% of homozygotes die of PSS and up to 50% of the carcasses of homozygotes are devalued through PSE. Why then have efforts to rid swine stocks of this apparently deleterious gene not been successful? The first reason is that the gene may have both beneficial and deleterious effects. The beneficial effects of the MH gene are associated with leanness and with muscle hypertrophy, which appears to add 2 to 3% to lean, dressed carcass weight (*6*). In selecting breeding stock for such characteristics as large ham conformation, large loin eye area, and excessive leanness, selection is inadvertently being made for the MH gene. Although it is not known in which breed the MH gene arose, its advantage was sufficiently obvious to assure its dissemination among breeds throughout the world by overt or covert interbreeding.

The deleterious effects of the gene were first reported in 1953 (*18*) and provided the stimulus for attempts to eliminate the MH gene from breeding stock. These efforts have been frustrated, however, by the continued selection for desirable meat characteristics and by the second reason for preservation of the gene–that, until now, it has not been possible to detect heterozygous carriers with the accuracy required to eliminate the gene within acceptable limits of cost. As a result, the incidence of the MH gene has stabilized in most lean, heavily muscled breeds of swine.

Physiological Basis of Malignant Hyperthermia

The underlying cause of MH became apparent as understanding emerged that the primary biochemical abnormalities associated with the syndrome occur in skeletal muscle (*1, 2*) and that contraction, relaxation, and energy metabolism in muscle are regulated by Ca^{2+}. The sarcoplasmic reticulum is the major regulator of Ca^{2+} concentrations in skeletal muscle (*19*) (Fig. 1). Ca^{2+} is pumped into the sarcoplasmic reticulum by a Ca^{2+} pump to initiate relaxation, stored in the junctional terminal cisternae in association with calsequestrin, and released through a Ca^{2+} re-

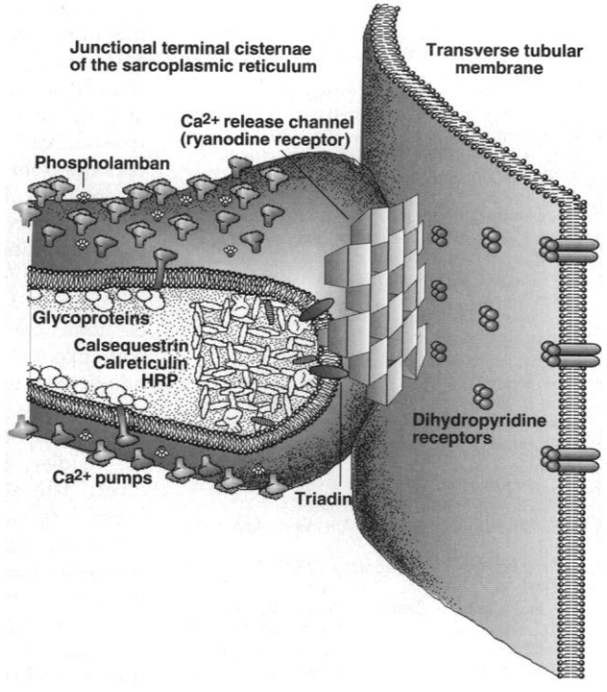

Fig. 1. Proposed arrangement of proteins in the sarcoplasmic reticulum and transverse (T) tubule membranes. A functional terminal cistern and its contiguous longitudinal sarcoplasmic reticulum are shown abutting the T tubule membrane. Dihydropyridine receptors in the T tubule membrane are shown associated as tetrad complexes, physically apposed to every other calcium release channel (ryanodine receptor). The ryanodine receptor is visualized as a square pyramidal structure in the junctional face of the terminal cistern. An aggregate of elongated calsequestrin molecules is shown within the lumen of the terminal cistern, anchored to transmembrane proteins in the junctional face membrane, such as triadin. The Ca^{2+}-ATPase is the major protein within the longitudinal membrane of sarcoplasmic reticulum. A pentamer of phospholamban molecules is shown extending from the cytoplasmic face of the longitudinal sarcoplasmic reticulum. Also present in the lumen are calreticulin (a shaded oblong), HRP (shown as a more globular bilobed membrane-associated structure) and the two most abundant glycoproteins, gp53 and gp160 (sarcalumenin), which are shown attached to the membrane and also to each other [adapted from (*19*)].

lease channel. The Ca^{2+} then binds to troponin in the thin filament and initiates muscle contraction. Ca^{2+} also binds to phosphorylase kinase and activates glycolytic pathways and the resynthesis of ATP to replenish that used during contraction.

The Ca^{2+} release channel of skeletal muscle sarcoplasmic reticulum has been characterized in situ in fibers (*20*) and in "heavy" sarcoplasmic reticulum vesicles (*21*) and as a single channel after its incorporation into planar lipid bilayers (*22*). The protein was identified and subsequently isolated (*23*) through its high affinity binding to a plant alkaloid, ryanodine, which modulates channel opening. The name ryanodine receptor has been applied to the two isoforms of the Ca^{2+} release channel, which appear to be the only cellular sites of ryanodine binding. Full-length cDNAs encoding the rabbit (*24, 25*), human (*25*), and porcine (*4*) skeletal muscle isoforms (*RYR1*) and the rabbit (*26*) cardiac and brain isoform (*RYR2*) were cloned soon after isolation of these Ca^{2+} release channel proteins.

The Ca^{2+} release channel is a homotetrameric complex constructed from a 565-kD subunit (*3*). Transmembrane sequences are located in the COOH-terminal fifth of each subunit, and the remainder of the subunit is cytoplasmic, bridging the gap between the sarcoplasmic reticulum and the transverse tubule. Transmembrane sequences in the tetramer probably combine to form the transmembrane portion of the Ca^{2+} release channel, and cytoplasmic sequences from each subunit appear to interact to enclose four extended channels which radiate from the central transmembrane channel and exit in peripheral vestibules (*27*). Single channel measurements in planar bilayers have shown that Ca^{2+} release is mediated by a ligand gated channel with a conductance greater than 100 pS in 50 mM Ca^{2+} (*22*). Regulatory sequences may exist near the transmembrane sequences (*24*) or in the cytoplasmic domain (*25, 26*). Although it is not clear what signals open the channel in the muscle cell, Ca^{2+} and ATP act synergistically to open the channel in isolated vesicles, and Mg^{2+} and calmodulin inhibit channel opening (Fig. 2A). Dantrolene, the clinical antidote for MH reactions (*1*), inhibits halothane-induced (*28*) and Ca^{2+}-induced (*29*) Ca^{2+} release from sarcoplasmic reticulum preparations. A direct interaction of dantrolene with the Ca^{2+} release channel has not been demonstrated, however.

The release of Ca^{2+} is the end result of a cascade of events including depolarization of nerve, muscle, and transverse tubular membranes, charge movement associated with the slow Ca^{2+} channel of the transverse tubular membrane (the dihydropyridine receptor) (*30*), and opening of the

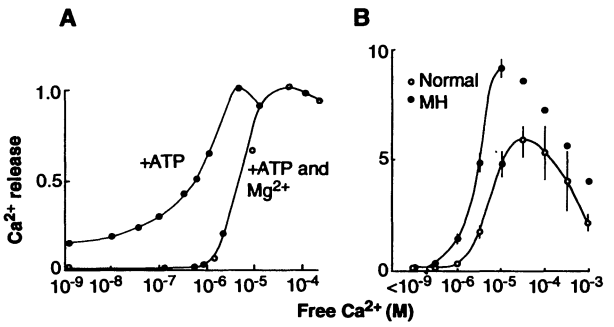

Fig. 2. Regulation of Ca^{2+}-induced Ca^{2+} release. (**A**) Regulation of Ca^{2+}-induced Ca^{2+} release from heavy sarcoplasmic reticulum from normal rabbit skeletal muscle by ATP, Mg^{2+}, and Ca^{2+}. The relative rate of Ca^{2+} release of 1 corresponds to the maximal Ca^{2+} release rate [adapted from (*21*)]. (**B**) Ca^{2+}-induced Ca^{2+} release from human skeletal muscle fibers from normal or MH individuals. The rate of Ca^{2+} release is expressed in units such that all Ca^{2+} would be released from the sarcoplasmic reticulum in 1 min if the release rate were 1 [adapted from (*33*)].

Ca^{2+} release channel. Many of the proteins involved in this cascade are well characterized (*19*), but others undoubtedly are yet to be identified. Ca^{2+} pumps and exchangers in the plasma membrane and carriers in the mitochondrial membrane are also regulated by Ca^{2+} and contribute to Ca^{2+} regulation within muscle cells (*31*). An abnormality in regulation of Ca^{2+} within skeletal muscle could account for all of the symptoms of malignant hyperthermia (Fig. 3). In particular, contracture may result from the continued presence of Ca^{2+} within the cell, and enhanced glycolytic and aerobic metabolism might deplete ATP, glucose, and oxygen; produce excess CO_2, lactic acid, and heat; and upset cellular and extracellular ion balances.

Abnormalities in regulation of the intracellular concentrations of Ca^{2+} that lead to MH might result from mutations in genes encoding the Ca^{2+} pump, the Ca^{2+} release channel, or other proteins that participate in the cascade of excitation-contraction coupling. Abnormalities in the Ca^{2+} pump were ruled out in biochemical studies (*32*), but higher rates of Ca^{2+}-induced Ca^{2+} release, particularly at low concentrations of Ca^{2+}, have been observed in preparations from both human (*33*) and porcine (*28, 34*) muscle (Fig. 2B). Closing of single porcine MH channels at high Ca^{2+} concentrations was inhibited (*35*). In comparable studies of humans, Ca^{2+} release channels with abnormally increased caffeine sensitivity were detected in MH individuals (*36*). In sarcoplasmic reticulum from swine with MH, ryanodine binding, which is dependent on the open state of the Ca^{2+} release channel, is enhanced (*37*), and digestion with trypsin revealed an alteration in the amino acid sequence of the Ca^{2+} release channel in MH animals (*38*).

Ca^{2+} Release Channel (*RYR*) Genes

Two genes encode Ca^{2+} release channels of the sarcoplasmic reticulum: *RYR1* encodes the Ca^{2+} release channel of both slow- and fast-twitch skeletal muscle; *RYR2* encodes a second Ca^{2+} release channel that is expressed in cardiac muscle and brain (*26*). *RYR1* is located on human chromosome 19q13.1 (*39*) and *RYR2* is located on human chromosome 1 (*26*). The identification of a series of restriction fragment length polymorphisms (RFLPs) in *RYR1* permitted a study of linkage between inheritance of one or more of these RFLPs and inheritance of MH, as defined by caffeine-halothane contracture tests. Cosegregation was found in 23 meioses in 9 families. The lod score of 4.2 favored linkage for a recombination fraction of 0.0 (*7*).

In studies of swine, linkage was demonstrated between inheritance of MH (the *HAL* gene) and polymorphisms in the enzyme glucose phosphate isomerase (*GPI*) (*40*). A linkage group consisting of *HAL*, *GPI*, 6-phosphogluconate dehydrogenase (*PGD*), and alpha 1 B glycoprotein (*A1BG*) genes, the H blood group locus, and loci controlling the expression of the (A-O) blood groups was localized near the centromere of pig chromosome 6 (*41*). A similar region was identified on the long arm of human chromosome 19 (*42*) where a number of human markers, including *GPI*, were located. These human markers were used to link MH to the region of human chromosome 19q13.1-13.2 where *RYR1* was localized (*8*).

Genetic Basis of Porcine Malignant Hyperthermia

A single deduced amino acid sequence difference was observed in a comparison of *RYR1* cDNA sequences from normal (Yorkshire) and MH (Pietrain) pigs (*4*). The substitution of T for C1843 in the nucleotide sequence leads to the substitution of Cys for Arg^{615} in the amino acid sequence. The nucleotide substitution leads to loss of a Hin PI restriction endonuclease site and to the gain of a Hgi AI site, thus providing a straightforward procedure for detection of

the mutation in genomic DNA (Fig. 4) amplified by the polymerase chain reaction (PCR) (*4*, *43*). Linkage of the mutation to phenotypic porcine MH was not straightforward, however, because the commonly used halothane challenge test (*44*) is only about 95% effective in detection of MH homozygotes and also picks up a small percentage of heterozygotes. To establish linkage, crosses were made between homozygotes and heterozygotes from long-established lines with defined *GPI* and *PGD* haplotypes (*5*). For each animal in the study, halothane challenge testing and *GPI* and *PGD* haplotyping were done, and DNA was amplified and tested for mutations. The combination of halothane challenge with haplotyping provided precise evaluation of MH status, and analysis of nucleotide 1843 determined the presence or absence of the mutation. In a study of 376 animals, including 338 informative meioses, complete linkage was observed between the presence of the nucleotide 1843 mutation and MH diagnosis, leading to a lod score favoring linkage of 102 for a recombination fraction of 0.0.

Because the same mutation was found in five breeds of pigs, it might have originated in a founder animal. Analysis of three polymorphic sites across about 150 kb within the *RYR1* gene provided evidence for a common *RYR1* haplotype in every MH animal tested, consistent with its origin in a founder animal (*4*). Leanness and heavy muscling may be manifestations of the gene (*6*) and these traits are readily selected by astute swine breeders. There is a physiological rationale for the contributions of the gene to leanness and heavy muscling. An abnormal Ca^{2+} release channel could stimulate spontaneous muscle contraction in these sedentary animals. The continued toning of such muscles would result in muscle hypertrophy and, because of greater energy utilization, in the limitation of fat deposition. The analogy of an office worker doing isometric exercises to build muscle is apt. An alternate rationale is that the haplotype inherited in these animals includes very closely linked genes responsible for desirable carcass traits. The *APOE*, *LIPE* or *TGFβ-1* genes, known to be linked close to *RYR1* on the same chromosome, could be candidates (*5*).

Genetic Basis of Human Malignant Hyperthermia

Although studies of a large number of MH families have demonstrated linkage between MH and *RYR1* (*7*, *8*), and two mutations in *RYR1* have been associated with MH in additional families (*9*, *45*), it has not been possible to demonstrate linkage in studies of several other families (*10*, *11*). There is evidence that individuals with central core disease, King-Denborough syndrome, Duchenne muscular dystrophy, and other myopathies (*46*, *47*) are at risk for anesthetic-induced MH episodes. Abnormalities in cellular Ca^{2+} regulation are probably secondary events in such myopathies, perhaps resulting from fragility of the sarcolemma in muscular dystrophy (X-linked) and manifested in myotonic dystrophy [separated from *RYR1* on chromosome 19 (*39*)] by myotonia, the inability to relax. If these abnormalities were exacerbated pharmacologically to the point where excess Ca^{2+} remained in the muscle, MH episodes could result. Central core disease and MH are strongly associated and genetic linkage studies have provided lod scores as high as 11.8 for a recombination fraction of 0.0 for cosegregation of *RYR1* and CCO (*48*). Thus the central regions with low metabolic function that are observed in muscle fibers of patients with central core disease may represent a second manifestation of an abnormality in *RYR1*.

In the MH families in which *RYR1* was not linked to MH (*10*, *11*), no alternate

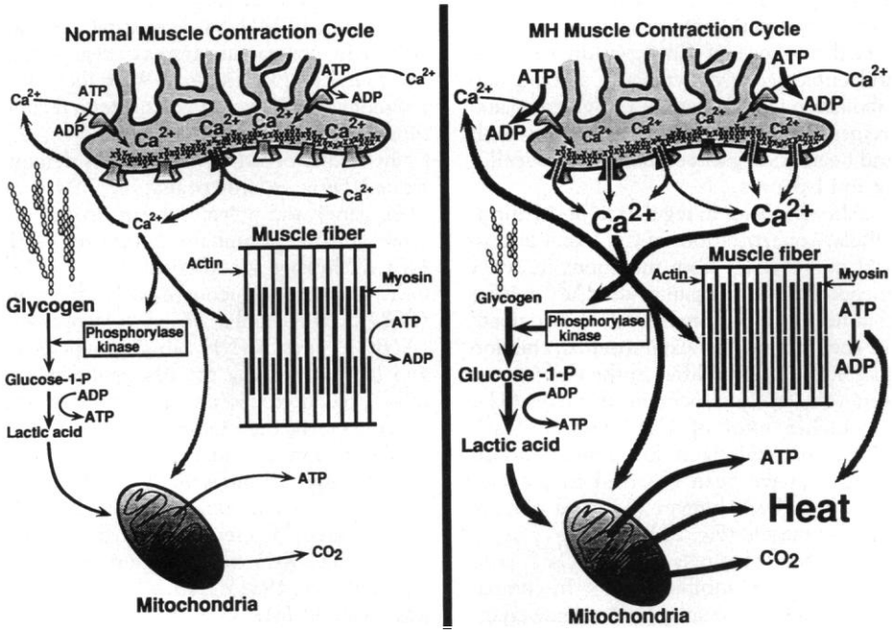

Fig. 3. A proposed mechanism for induction of malignant hyperthermia caused by abnormalities in the Ca^{2+} release channel of skeletal muscle sarcoplasmic reticulum. Muscle contraction, glycolysis and mitochondrial function are regulated by cytoplasmic Ca^{2+} concentrations. In a normal relaxation-contraction cycle (left), Ca^{2+} is pumped into the sarcoplasmic reticulum by a Ca^{2+} ATPase to initiate relaxation, stored within the lumen in association with calsequestrin, and released through a Ca^{2+} release channel to initiate contraction. Glycolytic and aerobic metabolism proceed only rapidly enough to maintain the energy balance of the cell. The Ca^{2+} release channel can be regulated by Ca^{2+} itself, ATP, Mg^{2+}, and calmodulin and, even when stimulated, has a relatively short open time. The abnormal malignant hyperthermia Ca^{2+} release channel (right) is sensitive to lower concentrations of stimulators of opening, releases Ca^{2+} at enhanced rates, and does not close readily. The abnormal channel floods the cell with Ca^{2+} and overpowers the Ca^{2+} pump that ordinarily lowers cytoplasmic Ca^{2+}. Sustained muscle contraction accounts for rigidity, and sustained glycolytic and aerobic metabolism account for the generation of lactic acid, CO_2, and heat and enhanced oxygen uptake. Damage to cell membranes and imbalances of ion transport can account for the life threatening systemic problems that appear during a malignant hyperthermia episode.

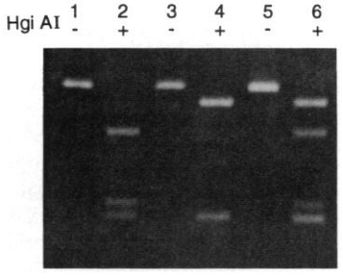

Fig. 4. Diagnosis of porcine MH status. PCR-amplified DNAs from individual swine (lanes 1, 3, and 5) were digested with Hgi A1 endonuclease (*43*). Complete digestion of both constant and mutant restriction endonuclease sites (lane 2) allowed identification of an MH homozygote; DNA from a normal animal was digested only at the constant site in both alleles (lane 4); DNA from a heterozygote was digested at the constant site in both alleles and at the mutant site in one of two alleles (lane 6).

myopathy was associated with the syndrome and, with the caveat that diagnosis may have been faulty, the conclusion must be reached that genes other than *RYR1* or the known myopathic genes can cause MH. Because excitation-contraction coupling requires the activity of several proteins, abnormalities in such proteins could also result in MH episodes. Alterations in signaling systems that generate fatty acids (*49*) and inositol 1,4,5-trisphosphate (IP_3) (*50*) have also been proposed as potential causes of MH.

In one case where lack of linkage between MH and *RYR1* was reported (*51*), MH status may, indeed, have been falsely diagnosed. In a study of a large French Canadian kindred, all individuals but one were classified as MH susceptible by standard criteria for caffeine-halothane contracture tests. Two clear groupings could be discerned within those tested, however, with one of them correlating precisely with the inheritance of an *RYR1* RFLP. If diagnostic limits for the various tests were altered in accordance with the different test groupings and with the genetic grouping, then linkage between MH and *RYR1* polymorphisms was complete, and the lod score of 3.84 favored linkage for a recombination fraction of 0.0. The accuracy of MH diagnosis by the halothane-caffeine contracture test is and will remain a serious problem in determining genetic heterogeneity in MH. Progress is being made in standardizing this test through the efforts of the North American Malignant Hyperthermia Registry (*14, 16*) and the European Malignant Hyperthermia Group (*15*), and in improving its predictive value through the application of rigorous definitions of the MH clinical syndrome.

Future Directions

Because MH is, at worst, a subclinical myopathy for heterozygous human carriers of abnormal genes, there is little urgency to effect a cure. The critical research goal, however, is to be able to detect in advance of anesthesia the presence of any genetic abnormality that might predispose an individual to an MH episode. Accordingly, identification of all MH mutations and development of diagnostic tests for them is the highest priority. To date, only the Arg^{614} to Cys mutation can be used for positive diagnosis of MH susceptibility. In addition, diagnosis of MH susceptibility is possible through analysis of inheritance of closely linked markers in families in which a large number of individuals have been diagnosed previously by the in vitro contracture test (*52*).

The *RYR1* gene is at least 240 kb in length and has approximately 100 exons (*53*). Exon-intron boundaries and flanking intron sequences have been obtained for 85 of these exons. Moreover, a variety of polymorphisms not associated with MH have been identified in human *RYR1* cDNAs (*7, 45*). Current research is concerned with the identification of sequence alterations in *RYR1* genomic DNA and cDNA that might be associated with MH. Such studies may yield a variety of candidate MH mutations in human *RYR1*. Attempts are also being made to link MH to abnormalities in other genes.

Genetic testing may never provide preoperative diagnosis of all individuals susceptible to MH; the apparent heterogeneity in the cause of the disease suggests that many causal mutations will be found. However, DNA-based testing of all relatives of any MH proband in which a linked genetic abnormality is found is likely to be incorporated into any comprehensive health care program, because the test is noninvasive, accurate and relatively inexpensive, properties not shared by current halothane-caffeine contracture tests for MH. Such a diagnosis would assure that MH susceptible individuals would receive safe anesthetics, whereas normal family members could safely utilize conventional, inexpensive anesthetic routines. Such tests would be cost effective because they would eliminate the need for altering anesthetic procedures for normal family members who are frequently treated as potentially MH susceptible.

For the swine industry, application of MH diagnostic testing will be of great practical importance. The test, which is accurate, noninvasive, and relatively inexpensive, is designed to detect the single mutation that has been found in all breeds. Swine breeders, however, must soon make a choice between two alternative breeding programs. If they choose to eliminate all carriers of the PSS gene from apex breeding stocks, the entire porcine population could be free of PSS within 5 years, resulting in substantial savings to the industry by elimination of PSS deaths and reduction of PSE. Such a drastic step would, however, carry the potential of eliminating associated beneficial genes in particular lines. Alternatively, breeders might decide to take advantage of the apparent beneficial effects of the gene by, for example, establishing homozygous MH boar lines and normal sow lines for the purpose of producing heterozygous market animals. Before embarking on such a course of action, however, it is essential for breeders to know whether the cost of carrying homozygous MH boar lines and the cost of low levels of potential PSS and PSE in heterozygotes would offset the 1 to 3% advantage in lean, dressed carcass weight that is currently attributed to the MH gene.

The probability that human MH is a heterogeneous condition raises the possibility that discovery of linkage of other genes to MH might provide insight into the roles of new proteins in excitation-contraction coupling. Moreover, studies of MH may contribute to the understanding of structure-function relationships in the Ca^{2+} release channel protein. The observations (*4*) that alteration of Arg^{615} leads to abnormal regulation of the channel by its ligands and that the region of the channel in which Arg^{615} is located is homologous to the IP_3 binding region of an analogous Ca^{2+} release channel from the endoplasmic reticulum, the IP_3 receptor (*54*), has already focused attention on the regulatory function of this region of the molecule. New functional mutations providing insights into muscle physiology, are as eagerly awaited by basic scientists as they are by agricultural and medical geneticists for practical application.

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Gaucher Disease: New Molecular Approaches to Diagnosis and Treatment

Ernest Beutler

Gaucher disease is characterized by the accumulation of glucocerebroside, leading to enlargement of the liver and spleen and lesions in the bones. It is caused by an inherited deficiency of the enzyme glucocerebrosidase. Many mutations exist, but four of these account for over 97% of the mutations in Ashkenazi Jews, the population group in which Gaucher disease is the most common. Although there is a strong relation between the mutations and disease manifestations, genetic counseling is made difficult by the fact that within each genotype there is considerable variability in the severity of the disease. Intravenous infusion of glucocerebrosidase is an effective treatment, but the availability of enzyme replacement therapy is limited by its high cost. Marrow transplantation is also effective in treating the disease, but is rarely performed because of the risks involved. In the future gene transfer may become the treatment of choice.

The breakdown of endogenous cellular components and foreign substances by the body is an exquisitely orchestrated function that is largely accomplished within the lysosome. In this organelle highly specific acid hydrolases sequentially separate the building blocks of macromolecules. Deficiencies in any of these enzymes may prevent the breakdown of its substrate and result in its accumulation. The accumulation of these trapped intermediates of the catabolism of complex molecules results in a storage disease, each with its own clinical characteristics. The most common of these disorders is Gaucher disease, which is characterized by a deficiency of glucocerebrosidase and hence accumulation of the glycolipid glucocerebroside. Less common glycolipid storage diseases include Tay-Sachs disease, in which GM_2 ganglioside is stored, Fabry disease, in which ceramide trihexoside accumulates, and Niemann-Pick disease, in which the storage compound is sphingomyelin.

Although glucocerebrosidase deficiency exists in all body cells of patients with Gaucher disease, the disease phenotype is expressed only in the macrophages except in the very rare neuronopathic forms of the disorder. As a consequence, patients with Gaucher disease are burdened by an enlarged liver and spleen and often by painful bone lesions. Macrophages are derived from bone marrow stem cells and are in constant contact with the blood stream. Gaucher disease is therefore ideally suited for the study of a variety of interventional strategies. These have included enzyme replacement, bone marrow transplantation, and gene transfer.

Gaucher disease was first described in 1882. The composition of the storage material was correctly identified in 1934, and the enzyme deficiency was demonstrated in 1965 (*1*). Yet only in the last decade has identification of lesions that cause this disease and implementation of successful therapeutic approaches been possible with the application of modern techniques of cellular and molecular biology. Recent findings have raised a host of biologic, economic, and ethical questions that did not exist previously.

The Enzyme Deficiency

The cause of Gaucher disease is the inability to catabolize glucocerebroside, which is normally hydrolyzed to ceramide and glucose by the β-glucosidase glucocerebrosidase. In the vast majority of cases the disease results from a deficiency of the enzyme itself (*2*). Glucocerebrosidase is a glycoprotein with a molecular size that ranges from 58 to 66 kD, after glycosylation and removal of a leader sequence. Some enzyme protein, generally with decreased catalytic activity, can be detected in the cells of patients with Gaucher disease (*3*). Some of the mutant forms of glucocerebrosidase are unstable (*4*), and kinetic abnormalities of the residual enzyme include abnormalities in activation by saposin C and phospholipids, and in inhibition by the active site inhibitors conduritol B epoxide and glucosphingosine (*5*).

Because glucocerebrosidase is a β-glucosidase, water soluble fluorogenic β-glucosides have been useful as enzyme substrates for both the diagnosis of the disease and purification of the enzyme (*6*). Examination of the bone marrow or other tissues, once the mainstay of diagnosis, has become an anachronism; the modern method of diagnosis is the estimation of the leukocyte acid β-glucosidase activity. Although the average enzyme activity of the leukocytes of heterozygotes is about one-half that of normal individuals, the distribution is so broad that the usefulness of the enzyme assay in the detection of heterozygotes is limited (*7*).

The Glucocerebrosidase Gene

Complementary DNAs (cDNAs) encoding glucocerebrosidase were cloned independently by two groups with the use of expres-

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