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Review article

Molecular basis of malignant hyperthermia

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Abstract. Malignant hyperthermia (MH) is a clinical syndrome in which genetically susceptible individuals respond to the administration of potent inhalation anaesthetics and depolarization skeletal muscle relaxants with skeletal rigidity, unstable blood pressure, tachycardia, arrhythmias, hyperventilation, hypoxia, lactic and respiratory acidosis and high fever. In studies of the genetic basis of MH, a mutation was identified in the porcine (C1843T) and human (C1840T) skeletal muscle ryanodine receptor (*RYR1*) gene. This gene is mapped on human chromosome 19q13.1. The *RYR1* gene contains 106 exons, of which two are alternatively spliced.

Key words: malignant hyperthermia, polymorphism, *RYR1* gene, RYR1 receptor.

History

The first publication describing malignant hyperthermia (MH) concerned a young person who had a compound fracture of the right leg. He was less concerned about his leg than a risk of general anaesthesia, for, since 1922, ten of his relatives had died as a direct consequence of anaesthesia (GRONERT 1980). He subsequently survived an episode of malignant hyperthermia, and Denborough and Lovells brief report of accelerated metabolism during anaesthesia culminated in a world-wide awareness of the risk of genetic susceptibility

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to certain drugs and stress (DENBOROUGH, LOVELL 1960). Between 1955 and 1958 Locher became involved with the anaesthetic care of a family in which 30 members had died in conjunction with general anaesthesia. Other investigators also contributed to the growing recognition of intraoperative hyperthermia and its possible etiology (CAPIZZI et al. 1969, WANG et. al. 1969, RYAN, PAPPER 1970). Evaluation of susceptibility was aided by the recognition of abnormal creatine phosphokinase (CPK) levels (ISAACS, BARLOW 1970), and the identification of low-threshold skeletal muscle contracture responses by KALOW et al. (1970).

The porcine model of malignant hyperthermia evolved from a report describing pork that was unsuitable for making sausage (HERTER, WILSDORF 1914). Later Ludvigsen described in 1953, a muscular degeneration in pigs, and subsequently demonstrated its genetic relationship (LUDVIGSEN 1958). This entity became important to swine breeders because stress related to slaughter resulted in accelerated metabolism and deterioration of muscle of susceptible pigs, with the resulting production of pale soft exudative (PSE) pork (BRISKEY 1964, EIKELENBOOM, MINKEMA 1974). Unsuitable pork was seldom obtained from normal swine because the time required for slaughter, cooling and processing of the animal was not long, nor was the metabolism sufficient, to produce the marked acidosis and higher temperature peculiar to the susceptible animals (BRISKEY 1964, NELSON et al. 1974). The porcine models of the malignant hyperthermia have provided an ideal means for investigation of the pathophysiology and identification of susceptible individuals (HARRISON et al. 1969).

The syndrome of malignant hyperthermia

Malignant hyperthermia is a rare myopathy with low frequency in humans 1 : 12,000 - 1 : 40,000. It is observed in patients during anaesthesia when rapid high temperature, muscle rigidity, and consequently death, appear. Simultaneously neurological, liver, and kidney damage might occur. Malignant hyperthermia occurs in swine, and, like in humans, leads to deaths during anaesthesia. Any stress to which susceptible pigs are subjected, e.g., transport, high environment temperature, separation, shipping, weaning, fighting, coitus, parturition, or slaughter might lead to increased metabolism, acidosis, rapid rise in body temperature, gross musculature rigidity, blotchy cyanosis, and death. In Malignant Hyperthermia Susceptible (MHS) animals the level of reproduction performance is observed, generally in amount of piglets per litter and amount of piglets on the 21st day (CARDEN et al. 1985).

Molecular basis of malignant hyperthermia

In MHS pigs, the homeostasis of Ca^{2+} in the cell is disturbed, and is linked with a rapid dissolution of glycogen to lactic acid in muscles, with a decrease of ATP and creatine phosphokinase (CPK) levels. The ryanodine receptor (RYR1), which is responsible for the release of Ca^{2+} from intracellular stores (sarcoplasmic reticulum) and, thus, for the increases in the concentration of cytosolic Ca^{2+}, in MHS animals gives evidence of abnormal function. The functional calcium release channel in Malignant Hyperthermia Normal (MHN) pigs is thought to have a tetrameric structure. The three ryanodine receptors from skeletal, cardiac muscles, and brain have been purified and biochemically characterized. These receptors are named RYR1, RYR2, and RYR3, respectively. They are named ryanodine receptors since they were first isolated based on their ability to bind ryanodine, a plant alkaloid. Ryanodine receptors have mainly been studied in muscle fibres, where they are abundantly expressed. The receptors are the actual ion channels that activate muscle contraction, releasing Ca^{2+} from sarcoplasmic reticulum (SR) in response to either transverse tubule depolarization (in skeletal muscle), or increased $[Ca^{2+}]_i$ (in the heart).

While a schematic model of ten transmembrane domains (TMs) of the ryanodine receptor was prepared by SORRENTINO and VOLPE (1993), a four domain model was proposed by TAKESHIMA et al. (1989) and HAKAMATA et al. (1992). Each monomer (~560 kDa) contains several potential transmembrane domains in the terminal part of the molecule (SORRENTINO, VOLPE 1993). Ryanodine receptors appear to have a fourfold symmetry compatible with the tetrameric structure of the channel. The transmembrane domains of each monomer may then combine to form the pore of the homotetrameric Ca^{2+} channel (SORRENTINO, VOLPE 1993). SORRENTINO and VOLPE (1993) suggested that the large N-terminal domain (~4000 amino acids) of the molecule protrude in the cytoplasm. The transmembrane segments that anchor the molecule to the membrane of the Ca^{2+} stores are localized in the last fifth of the molecule; the C-terminal of the molecule is also located in the cytoplasm (SORRENTINO, VOLPE 1993). Thus, only a relatively small part of the large molecule is intralumenal. The isolated Ca^{2+} release channel is large enough so that its overall structure can be resolved by electron microscopy (INUI et al. 1987, LAI et al. 1989, WAGENKNECHT et al. 1989, RADERMACHER et al. 1994).

The sarcoplasmic reticulum is an intracellular membrane network that regulates skeletal muscle contraction and relaxation through the regulation of

myoplasmic free Ca^{2+} concentrations. Concentration is initiated by the release of Ca^{2+} through a Ca^{2+} release channel located in the terminal cisternae of the SR, and relaxation is achieved by the rapid reuptake of Ca^{2+} by the Ca^{2+} pump (FLEISHER, INUI 1989).

Research within the area of molecular biology has provided the structure of the *RYR* genes in different species (NAKAI et al. 1990, ZORZATO at al. 1990, SORRENTINO, VOLPE 1993). These genes (*RYR1*, *RYR2*, *RYR3*) have been cloned (TAKESHIMA et al. 1989, NAKAI et al. 1990, ZORZATO et al. 1990) and localized to human chromosomes. The *RYR1* gene was mapped to chromosome 19 in the q13.1 band (MACLENNAN et al. 1990, MCCARTHY et al. 1990, MACKANZIE et al. 1990, BELGE et al. 1995), *RYR2* was mapped to chromosome 1 (OTSU et al. 1991), and *RYR3* was localized to position 15q14-q15 (SORRENTINO et al. 1993). Expression of *RYR2* mRNA is observed in the heart, brain and stomach, whereas the *RYR1* gene is expressed only in fast- and slow-twitch muscles (NAKAI et al. 1990, OTSU et al. 1990). The *RYR3* gene appears to be widely expressed. The products of the *RYR3* gene were found in several tissues, including the spleen, lungs, kidneys, skeletal muscle, stomach, ileum and jejunum (GIANNINI et al. 1992). *RYR3* is also coexpressed with *RYR2* in the brain.

The cDNAs were reported to be over 15,000 bp and to encode proteins about 5,035 amino acids with the mass of about 564,000 kDa. The tetrameric ryanodine receptor is one of the largest known proteins. The *RYR1* gene is composed of 106 exons, of which 2 are alternatively spliced (PHILLIPS et al. 1996), making it slightly less complex than the human type VII collagen gene (COL7A1), which has 118 exons (CHRISTIANO et al. 1994). The *RYR1* gene is 158 kb, making it larger than the 31-kb COL7A1 gene (CHRISTIANO et al. 1994), but much smaller than the 2400-kb human dystrophin gene. The dystrophin gene is the third most complex gene characterized, having 79 exons (ROBERTS et al. 1993).

The amino acid composition deduced from the nucleotide sequence of the mink cDNA as well as from the rabbit amino acid sequence (HAKAMATA et al. 1992), indicates that the *RYR3* shares an overall homology of about 70% with *RYR1* and *RYR2*. It is not clear if mutations in *RYR2* and *RYR3* are responsible for any diseases. In the pig genome, the *RYR1* gene was mapped to chromosome 6 in locus 6q11-q12, and 18 polymorphic sites were found (FUJII et al. 1991). In studies of the genetic basis of MH, a mutation was identified in the porcine skeletal muscle ryanodine receptor (RYR1) gene, Arg^{615} to Cys (FUJII et al. 1991), that was linked to MH with a lod score of 102 for θ max. = 0.0 (OTSU et al. 1991). The corresponding human *RYR1*

mutation, Arg^{614} to Cys, was found to be expressed in about 2% of MH families (GILLARD et al. 1991, HOGAN et al. 1992, DEUFEL et al. 1995, MORONI et al. 1995). Another *RYR1* mutation, Gly^{2433} to Arg, has been linked to 4% of the MH families in Europe and in Canada (KEATING et al. 1994, PHILLIPS et al. 1994). The mutation of Gly^{341} to Arg has been linked to 10% of MH families in Europe (QUANE et al. 1994).

Central Core Disease (CCD)

Central core disease (CCD) is rare myopathy linked to MHS (SHY, MAGEE 1956, DENBOROUGH et al. 1973), in which susceptibility to malignant hyperthermia, proximal muscle weakness, and a lack of metabolic activity in the central cores of skeletal muscle fibers are common features. CCD has been linked to four *RYR1* mutations: Arg^{2434} to His (ZHANG et al. 1993); Tyr^{522} to Ser (QUANE et al. 1994); Arg^{163} to Cys (QUANE et al. 1993); and Ile^{403} to Met (QUANE et al. 1993). Since these known *RYR1* mutations account for MH and CCD in only a fraction of human MH and CCD families, extensive research will be necessary to find all the additional MH and CCD mutations in the *RYR1* gene or in potential alternate loci (ILES et al. 1994).

Phenotypic effects

The pleiotropic effect of the *RYR1* locus on stress susceptibility, lean content and porcine meat quality is now well known. Thus, the mutant T allele, the normal one being C, was identified as responsible for the liability to sudden death syndrome, PSE meat condition and muscular hypertrophy. Regarding the dominance situation, T is generally assumed to be completely recessive for porcine stress syndrome (PSS) and PSE syndrome (MINKEMA et al. 1977, ARCHIBALD, IMLAH 1985), whereas the gene effect at the *RYR1* locus is additive for carcass lean content.

REFERENCES

ARCHIBALD A.L., IMLAH P. (1985). The halothane sensitivity locus and its linkage relationships. Anim. Blood Groups Biochem. Genet. 16: 253-263.

BELGE G., GARCIA E., DE JONG P., BARNITZKE S., BULLERDIEK J. (1995). FISH analyses of a newly established thyroid tumour cell line showing a t(1;19)(p35 or p36.1;q13) reveal that the breakpoint lies between 19q13.3-13.4 and 19q13.4. Cytogenet. Cell Genet. 69: 220-222.

BRISKEY E.J. (1964). Etiologic status and associated studies of pale, soft, exudative porcine musculature. Adv. Food Res. 13: 89-178.

CAPIZZI L.S., PHILLIPS O.C., HARRIS L.C., Jr. (1969). Malignant hyperthermia during anesthesia. Anesthesiology 31: 97-99.

CARDEN A.E., HILL W.G., WEBB A.J. (1985). The effects of halothane susceptibility on some economically important traits in pigs. Anim. Prod. 40: 351-358.

CHRISTIANO A.M., HOFFMAN G.G., GUNG-HONET L.C., LEE S., CHENG W., UITTO J., GREENSPAN D.S. (1994). Structural organization of the human type VII collagen gene (COL7A1), composed of more exons than any previously characterized gene. Genomics 21: 169-179.

DENBOROUGH M.A., LOVELL R.R.H. (1960). Anaesthetic deaths in a family. Lancet 2: 45.

DENBOROUGH M.A., DENNETT X., ANDERSON R.M. (1973). Central core disease and malignant hyperthermia. Br. Med. J. 1: 272-276.

DEUFEL T., SUDBRAK R., FEIST Y., RUBSAM B., DU CHESNE I., SCHAFER K.L., ROEWER N., GRIMM T., LEHMANN-HORN F., HARTUNG E.J., MULLER C.R. (1995). Discordance, in a malignant hyperthermia pedigree, between in vitro contracture-test phenotypes and haplotypes for the MHSI region on chromosome 19q12-13.2, comprising the C1840T transition in the RYRI gene. Am. J. Hum. Genet. 56: 1334-1343.

EIKELENBOOM G., MINKEMA D. (1974). Prediction of pale, soft, exudative muscle with a non-lethal test for the halothane-induced porcine malignant hyperthermia syndrome. Neth. J. Vet. Sci. 99: 421-426.

FLEISHER S., INUI M. (1989). Biochemistry and biophysics of excitation-contraction coupling. Annu. Rev. Biophys. Chem. 18: 333-364.

FUJII J., OTSU K., ZORZATO F., De LEON S., KHANNA V.K., WEILER J.E., OBRIEN P.J., MACLENNAN D.H. (1991). Identification of a point mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science 253: 448-451.

GIANNINI G., CLEMENTI E., CECI R., MARZIALI G., SORRENTINO V. (1992). Expression of a ryanodine receptor-Ca^{2+} channel that is regulated by TGF-β. Science 257: 91-94.

GILLARD E.F., OTSU K., FUJII J., KHANNA V.K., De LEON S., DERDEMIZI J., BRITT B.A., DUFF C.L., WORTON R.G., MACLENNAN D.H. (1991). A substitution of Cysteine for Arginine 614 in the ryanodine receptors is potentially causative of human malignant hyperthermia. Genomics 11: 751-755.

GRONERT G.A. (1980). Malignant hyperthermia. Anesthesiology 53: 395-423.

HAKAMATA Y., NAKAI J., TAKESHIMA H., IMOTO K. (1992). Primary structure and distribution of a novel ryanodine receptor/calcium release channel from rabbit brain. FEBS Lett. 312: 229-235.

HARRISON G.G., SAUNDERS S.J., BIEBUYCK J.F. (1969). Anaesthetic-induced malignant hyperpyrexia and a method for its prediction. Br. J. Anaesth. 41: 844-855.

HERTER M., WILSDORF G. (1914). Die Bedeutung des Schweines fur die Fleischversorgung. Berlin, Arbeiten der Deutscher Landwirtschaft-Gesellschaft, Heft 270.

HOGAN K., COUCH F., POWERS P.A., GREGG R.G. (1992). A cysteine-for-arginine substitution (R614C) in the human skeletal muscle calcium release channel cosegregates with malignant hyperthermia. Anesth. Analg. 75: 441-448.

ILES D.E., LEHMANN-HORN F., SCHERER S.W., TSUI L.C., OLDE WEGHUIS D., SUIJKERBUIJK R.F., HEYTENS L., MIKALA G., SCHWARTZ A., ELLIS F.R. (1994). Localization of the gene encoding the alpha 2/delta-subunits of the L-type voltage-dependent calcium channel chromosome 7q and analysis of the segregation of flanking markers in malignant hyperthermia susceptible families. Hum. Mol. Genet. 3: 969-975.

INUI M., SAITO A., FLEISCHER S. (1987). Purification of the ryanodine receptor and identity with feet structures of junctional terminal cisternae of sarcoplasmic reticulum from fast skeletal muscle. J. Biol. Chem. 262: 1740-1747.

ISAACS H., BARLOW M.B. (1970). Malignant hyperpyrexia during anaesthesia: possible correlation with subclinical myopathy. Br. Med. J. 1: 275-277.

KALOW W., BRITT B.A., TERREAU M.E. (1970). Metabolic error of muscle metabolism after recovery from malignant hyperthermia. Lancet 2: 895-898.

KEATING E.K., QUANTE K.A., MANNING M.B., LEHANE M., HARTUNG E., CENSIER K., URWYLER A., KLAUSNITZER M., MULLER C.R., HEFRON J.J.A., MCCARTHY T.V. (1994). Detection of a novel RYR1 mutation in four malignant hyperthermia pedigrees. Hum. Mol. Genet. 3: 1855-1858.

LAI F.A., MISRA M., XU L., SMITH H.A., MEISNNER G. (1989). The ryanodine receptor Ca^{2+} release channel complex of skeletal muscle sarcoplasmic reticulum: Evidence for co-operatively coupled, negatively charged homotetramer. J. Biol. Chem. 264: 16776-16785.

LUDVIGSEN J. (1958). Den genetiske og den ernaeringsetingede muskeldegeneration. Ugeskrift for Landmaend, No. 47 and 48.

MACKANZIE A.E., KORNELUK R.G., ZORZATO F., FUJII J., PHILLIPS M., ILES D., WIERINGA B., LEBLOND S., BAILLY J., WILLARD H.F., DUFF C., WORTON R.G., MACLENNAN D.H. (1990). The human ryanodine receptor gene: Its mapping to 19q13.1, placement in a chromosome 19 linkage group, and exclusion as the gene causing myotonic dystrophy. Am. J. Hum. Genet. 46: 1082-1089.

MACLENNAN D.H., DUFF C., ZORZATO F., FUJII J., PHILLIPS M., KORNELUK R.G., FRODIS W. (1990). Ryanodine receptor gene is a candidate for predisposition to a malignant hyperthermia. Nature 343: 559-561.

MCCARTHY T.V., HEALY J.M.S., HEFRON J.J.A., LEHANE M., DEUFEL T., LEHMANN-HORN F., FARRAL M., JOHNSON K. (1990). Localization of the malignant hyperthermia susceptibility locus to human chromosome 19q12-13.2. Nature 343: 562-564.

MINKEMA D., EIKELENBOOM G., Van ELDIK P. (1977). Inheritance of MHS-susceptibility in pigs. Proc. Third Int. Conference on Production Disease in Farm Animals, Wageningen, Netherlands, Pudoc.: 203-220.

MORONI I., GONANO E.F., COMI G.P., TEGAZZIN V., PRELLE A., BORDONI A., BRESOLIN N., SCARLATO G. (1995). Ryanodine receptor gene point mutation and malignant hyperthermia susceptibility. J. Neurol. 242: 127-133.

NAKAI J., IMAGAWA T., HAKAMATA Y., SHIGEKAWA M., TAKESHIMA H., NUMA S. (1990). Primary structure and functional expression from cDNA of the cardiac ryanodine receptor/calcium release channel. FEBS Lett. 271: 169-177.

NELSON T.E., JONES E.W., HENDRICKSON R.L. (1974). Porcine malignant hyperthermia: observations on the occurrence of pale, soft, exudative musculature among susceptible pigs. Am. J. Vet. Res. 35: 347-350.

OTSU K., KHANNA V.K., ARCHIBALD A.L., MACLENNAN D.H. (1991). Cosegregation of porcine malignant hyperthermia and a probable causal mutation in the skeletal muscle ryanodine receptor gene in backcross families. Genomics 11: 744-750.

OTSU K., WILLARD H., KHANNA V.K, ZORZATO F., GREEN M., MACLENNAN D.H. (1990). Molecular cloning of cDNA encoding the Ca^{2+} release channel (ryanodine receptor) of rabbit cardiac muscle sarcoplasmic reticulum. J. Biol. Chem. 265: 13472-13483.

PHILLIPS M.S., FUJII J., KHANNA V.K., De LEON S., YOKOBATA K., De JONG P.J., MACLENNAN D.H. (1996). The structural organization of the human skeletal muscle ryanodine receptor (RYR1) gene. Genomics 34: 24-41.

PHILLIPS M.S., KHANNA V.K., De LEON S., FRODIS W., BRITT B.A., MACLENNAN D.H. (1994). The substitution of Arg for Gly2433 in the human skeletal muscle ryanodine receptors is associated with malignant hyperthermia. Hum. Mol. Genet. 3: 2181-2186.

QUANE K.A., HEALY J.M.S., KEATING K.E., MANNING B.M., COUCH F.J., PALMUCCI L.M., DORIGUZZI C., FAGERLUND T.H., BERG K., ORDING H., BENDIXEN D., MORTIER W., LINZ U., MULLER C., MCCARTHY T.V. (1993). Mutations in the ryanodine receptor gene in central core disease and malignant hyperthermia. Nature Genet. 5: 51-55.

QUANE K.A., HEALY J.M.S., KEATING K.E., MANNING B.M., COUCH F.J., PALMUCCI L.M., DORIGUZZI C., FAGERLUND T.H., BERG K., ORDING H., BENDIXEN D., MORTIER W., LINZ U., MULLER C., MCCARTHY T.V. (1994). Mutation screening of the RYR1 gene malignant hyperthermia: detection of the novel Tyr to Ser mutation in a pedigree with associated central cores. Genomic 23: 236-239.

RADERMECHER M., RAO V., GRASSUCCI R., FRANK J., TIMERMAN A.P., FLEISCHER S., WAGENKNECHT T. (1994). Cryo-electron microscopy and the three-dimensional reconstruction of the calcium release channel/ryanodine receptor from skeletal muscle. J. Cell Biol. 127: 411-423.

ROBERTS R.G., COFFEY A.J., BOBROW M., BENTLEY D.R. (1993). Exon structure of the human dystrophin gene. Genomics 16: 536-538.

RYAN J.F., PAPPER E.M. (1970). Malignant fever during and following anesthesia. Anesthesiology 32: 196-201.

SHY G.M., MAGEE K.R. (1956). A new congenital non-progressive myopathy. Brain 79: 610-621.

SORRENTINO V., VOLPE P. (1993). Ryanodine receptors: How many, where and why? Trends Pharmocol. Sci. 14: 98-103.

SORRENTINO V., GIANNINI G., MALZAC P., MATTEI M.G. (1993). Localization of a novel ryanodine receptor gene (RYR3) to human chromosome 15q14-q15 by *in situ* hybridization. Genomics 18: 163-164.

TAKESHIMA H. NISHIMURA S., MATSUMOTO T., ISHIDA H., ANGAWA K., MINAMINO N., MATSUO H., UEDA M., HANAOKA M., HIROSE T., NUMA S. (1989). Primary structure and expression from complementary DNA of skeletal muscle ryanodine receptor. Nature 339: 439-445.

WAGENKNECHT T., GRASSUCCI R., FRANK J., SAITO A., INUI M., FLEISCHER S. (1989). Three dimensional architecture of the calcium channel/foot structure of sarcoplasmic reticulum. Nature 338: 167-170.

WANG J.K., MOFFITT E.A., ROSEVEAR J.W. (1969). Oxidative phosphorylation in acute hyperthermia. Anesthesiology 30: 439-442.

ZHANG Y., CHEN H.S., KHANNA V.K., De LEON S., PHILLIPS M.S., SCAPPERT K., BROTT B.A., BROWNELL A.K.W., MACLENNAN D.H. (1993). A mutation in the human ryanodine receptor gene associated with central core disease. Nature Genet 5: 46-50.

ZORZATO F., FUJII J., OTSU K., PHILIPS M., GREEN N.N., LAI F.A., MEISSNER G., MACLENNAN D.H. (1990). Molecular cloning of cDNA encoding human and rabbit forms of the Ca^{2+} release channel (ryanodine receptor) of skeletal muscle sarcoplasmic reticulum. J. Biol. Chem. 265: 2244-2256.