

REVIEW ARTICLE

The genetics of malignant hyperthermia

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Abstract

Malignant hyperthermia susceptibility remains the commonest cause of death owing to general anaesthesia. This is despite the availability of presymptomatic testing, admittedly by a highly invasive method, and a recognised treatment for implementation immediately a patient shows signs of developing a crisis. Recently the finding of linkage to markers from chromosome 19q13.1-13.2 and the identification of mutations in a candidate gene held out hope of genetic diagnosis being available. However, it is likely that only about 50% of families have a mutation of the skeletal muscle calcium release channel gene. With this degree of genetic heterogeneity, presymptomatic testing based on DNA markers can only be offered at present to a limited number of families where linkage to markers from 19q13.1-13.2 has been clearly shown.

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Malignant hyperthermia (MH) is an inherited pharmacogenetic disorder characterised by accelerated metabolism and rigidity in skeletal muscle. MH occurs in susceptible subjects after exposure to triggering agents such as inhalational anaesthetics and depolarising muscle relaxants.¹ The incidence of MH has been estimated at 1 in 50 000 to 75 000 anaesthetised patients in the UK (unpublished data from the UK Malignant Hyperthermia Investigation Unit, Leeds). Other studies indicate a higher incidence than this of 1 in 10 000, especially in childhood, which may reflect the reduced penetrance of MHS with increasing age.²⁻⁴

Clinical malignant hyperthermia

A family in which 10 deaths occurred as a result of general anaesthesia for minor surgery has been described.⁵ In three of these cases raised body temperature was recorded after the administration of the anaesthetic. The pattern of inheritance of this sensitivity to anaesthetic agents was deduced to be autosomal dominant with incomplete penetrance.

An MH episode, or crisis, is characterised by muscle rigidity, accelerated metabolism, and skeletal muscle breakdown, leading to raised body temperature, tachycardia, and raised arterial pCO₂ levels resulting in hyper-

ventilation and raised blood potassium. An untreated crisis results rapidly in death from cardiac arrest. Patients who survive an MH crisis may experience widespread muscle damage with accompanying myoglobinuria which can give rise to acute renal failure and short term neurological disturbances. Sodium dantrolene has been used for a decade to treat MH crises effectively.⁶ The use of dantrolene has led to a significant decline in the mortality associated with MH, but it remains the commonest cause of death from general anaesthesia in otherwise healthy patients.

Presymptomatic diagnosis

The development of *in vitro* contracture testing has permitted the identification of the MH susceptible (MHS) relatives of patients presenting with clinical MH.^{7,8} MH susceptibility is determined by the contracture response of living muscle tissues to *in vitro* exposure to halothane and to caffeine. A standardised European protocol has been adopted by the European Malignant Hyperthermia Group (EMHG) since 1984. In this test, intact muscle bundles are suspended in Ringer's solution at 37°C with one end immobilised and the other end attached to a transducer. The tension in the fibre is monitored as it is exposed to specified concentrations of halothane or caffeine. Patients are classified into three categories by this test. If they show abnormal responses to both halothane and caffeine they are MHS, if they are abnormal with either halothane or caffeine they are MH equivocal (MHE), and if normal with both MHN.

In North America a different testing protocol has been adopted called the caffeine-halothane contracture (CHC) test. The main difference between this protocol and that of the EMHG is that an abnormal response to a combined challenge with halothane and caffeine is taken to indicate MH susceptibility. This test is not performed in the EMHG protocol as it has been shown to result in false positives.^{9,10}

The *in vitro* contracture test in Europe has confirmed the autosomal dominant mode of inheritance.^{4,11} However, the test itself is highly invasive and requires a specialised laboratory set up to obtain reproducible results. It is not practicable to screen all patients before general anaesthesia using this test. As a result only contactable relatives of patients who have suffered an MH crisis can be

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screened, and there is a need for a reliable, cheap, and non-invasive test.¹²

The pig model

A disorder that may be similar to human MH has been described in certain breeds of pig, where it has major implications for commercial breeders. Porcine stress syndrome (PSS) is considered to be homologous to human MHS.¹³ It results in shortness of breath, rapid increase in body temperature, patches of blanching and flushing of the skin, collapse, and rapid death followed by almost immediate rigor mortis. PSS is precipitated by a number of stimuli, such as separation, weaning, fighting, coitus, and slaughter. The onset of the condition renders the carcass worthless as it results in a deterioration of the meat quality, described as pale soft exudative pork.¹⁴

PSS was considered to be transmitted by a single autosomal recessive gene, Hal.¹⁵ Halothane sensitivity is determined by a 'barnyard' test where the pigs are challenged by increasing concentrations delivered by a face mask. Animals fall into two groups, those that are halothane positive and exhibit limb rigidity when challenged, or those that are halothane negative. This test only detects animals as positive if they are homozygous for the Hal mutation. If the EMHG in vitro contracture test is applied to the pigs, or if they are challenged simultaneously with halothane (1%) and succinylmethonium (1 mg/kg), the heterozygote animals are positive.¹⁶ These results mean that the original apparent difference in mode of inheritance of the human and porcine phenotypes is almost certainly the result of the different sensitivities of the tests used in each case.

Physiology of MH

PSS has been studied extensively as an animal model to investigate experimentally the pathophysiology of human MH, to identify potential triggering agents, and in the development of therapeutic agents, such as dantrolene.

The rapid increase in body temperature in MH reflects a sudden, massive increase in cell metabolism causing increases in heat production, blood potassium, carbon dioxide, and lactate.¹⁷ Early in the development of an MH reaction, abnormalities in the metabolism of skeletal muscle are detected. Since skeletal muscle comprises about 40% of the body mass these changes have a profound effect upon the whole body, inducing a severe metabolic acidosis. This in turn produces a higher oxygen demand adding to the heat increase. The hyperpyrexia and acidosis reflect the hypermetabolic state experienced early in an acute MH episode, but the molecular mechanisms underlying these changes are still the subject of research.

The central role of calcium in producing the hypermetabolic state in skeletal muscle has been shown by several studies. Calcium is the predominant regulator of contraction and

metabolism in muscle. The strength of muscular contraction is proportional to the concentration of calcium and sustained muscular contraction would explain the muscle rigidity and the excess heat generation seen in MH. Thus, the primary defect in MH is likely to result in an alteration of calcium homeostasis.

A number of physiological studies comparing halothane positive pigs with halothane negative ones of different breeds have detected an abnormality in calcium release from the sarcoplasmic reticulum (SR) as the primary cause of MH.^{18–21} However, these changes could just reflect interbreed differences.

The calcium pump activity appears to be normal judged by the Ca-ATPase activity which is identical in halothane positive and negative animals. The calcium release channel (CRC) of SR binds the plant alkaloid ryanodine and is often referred to as the ryanodine receptor (RYR). The CRC activity resides in a homotetrameric protein (subunits MW > 500 K) which forms the junctional 'feet' structures seen under EM in the terminal cisternae of the SR.^{22–26} The CRC of halothane positive pigs has a higher affinity for binding [³H]-ryanodine than those of negative pigs and the threshold level of binding of ryanodine is lower in positive animals.²⁷ These observations have implicated the CRC as the product of the Hal locus in pigs.²⁸

Gene mapping in pigs and humans

Genetic mapping in pigs has shown that the Hal locus is tightly linked to the glucose phosphate isomerase (GPI) and H blood group loci on chromosome 6.^{15,29} This region of the pig chromosome is homologous with human chromosome 19q and provided the first clue as to the likely location of the MHS locus in humans.

A genetic study of markers from human chromosome 19q12–13.2 in three large Irish families segregating for malignant hyperthermia showed linkage between markers in the GPI region and MHS.¹¹ The cloning of a cDNA encoding the human CRC (*RYR1*)³⁰ enabled this locus to be mapped in somatic cell hybrids to 19q13.1–13.2.³¹ These data clearly support the proposal that a defect in the CRC underlies both human and porcine MHS. This hypothesis was further strengthened when polymorphisms detected by the CRC (*RYR1*) cDNA were shown to be linked to MHS in nine small Canadian families, with no recombination between the marker and MHS.³² In these families the diagnosis of MHS depends on positive CHC test results in at least two subjects who could, from subsequent data, be false positives. A second candidate gene that encodes hormone sensitive lipase (LIPE) has also been mapped to the region of chromosome 19q13.2.³³ However, recombination between this marker and MHS has been detected in a large Irish pedigree in which the CRC is tightly linked with no recombination (T McCarthy, personal communication). This would indicate that the CRC is more likely to

be the site of the mutation in that family than the LIPE gene.

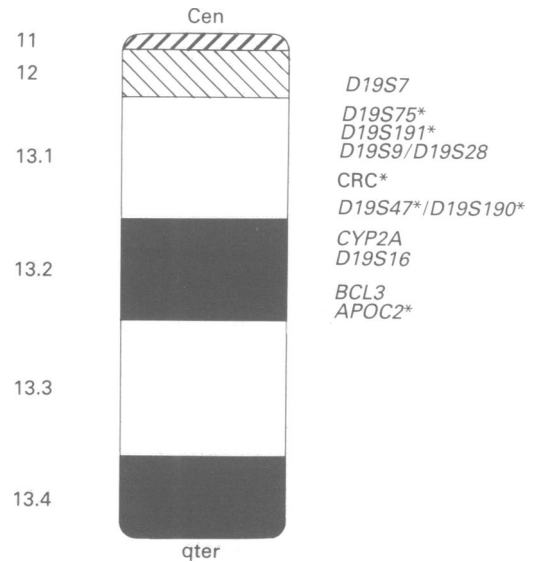
Linkage studies in the pig have also shown tight linkage between polymorphisms detected by the CRC (*RYR1*) cDNA and *PSS*, with no recombinants detected.³⁴⁻³⁶ The sequence of the CRC cDNA in halothane positive and negative animals was compared and these studies detected 18 base substitutions, only one of which resulted in an altered amino acid.³⁷ This mutation (C-T at nucleotide 1843) leads to Arg⁶¹⁵ being substituted for Cys and was found in all of more than 450 positive animals from six *PSS* susceptible breeds.

The same mutation at the DNA level has been detected in one Canadian MH family out of over 50 tested.³⁸ This clearly reflects the differences between the highly inbred pig population, where a single mutation has become fixed by selective breeding, and the human population where locus heterogeneity is expected. Indeed, it has been suggested that the mutation predisposing to MHS also predisposed towards increased muscle mass.¹³ As a result the Hal mutation may have been subjected to powerful selection by pig breeders and transmitted to all six pig breeds in which it has been found from a single ancestral mutation. Indeed the large size of the CRC gene (> 250 kb) makes it likely that many different mutations in the CRC gene could give rise to the MHS phenotype.

Heterogeneity in MH

While there are clearly linkage and mutation data supporting the skeletal muscle CRC as the site of the primary defect in some MHS subjects, a growing number of non-chromosome 19q13.1 linked families have been reported.³⁹⁻⁴² In these studies a number of large pedigrees in which MHS is segregating have been shown by genetic recombination to exclude linkage to the region of the CRC gene on 19q. At a meeting of the EMHG Genetics Section in January 1992 a survey showed that eight out of 15 large families from within the group showed exclusion with markers from 19q13.1-13.2. Therefore, for approximately 50% of European families genetic diagnosis with markers from this region is contraindicated. However, where family structure permits the clear demonstration of linkage to the CRC gene, or closely flanking markers, with a lod score greater than 3 within that family, genetic diagnosis can and has been performed.⁴³ A summary of the most informative markers used in all of these studies is shown in the figure.

It is well documented that the MHS phenotype, as detected by the *in vitro* contracture test, can arise secondarily in patients with other neuromuscular diseases. These include myotonic dystrophy (DM),⁴⁴ myotonia congenita,⁴⁵ Duchenne and Becker muscular dystrophy (DMD and BMD),⁴⁶ and central core disease (CCD).⁴⁷ These conditions are clinically and genetically distinct from one another although the loci for DM and CCD also map to chromosome 19q. It is well established that



The genetic map of the markers used by the European Malignant Hyperpyrexia Group, Genetics Section for linkage analysis. Those marked with * are typed by PCR. The distance between D19S75 and D19S47 in the combined sex map is 10 to 12 cM. The order of the markers is as determined genetically and physically.

DM and MHS map to distinct loci on 19q,^{11 30} but possible that CCD and MHS may be allelic from the common coexistence of these two conditions and the linkage data.⁴⁸⁻⁵¹ The possibility of another inherited myopathy being the primary cause of the MHS in individual subjects needs to be excluded before investigation with markers from the CRC region. However, where MHS is found without a coexisting myopathy, genetic heterogeneity must be borne in mind at all times. It is only feasible to offer genetic counselling and pre-symptomatic diagnosis with 19q13.1-13.2 markers in large families where definitive linkage to this region can be shown. In all other cases at present a muscle biopsy and *in vitro* contracture testing are absolutely essential.

To determine the extent of heterogeneity and to try to determine phenotypic differences between the chromosome 19 linked and the non-linked forms will require studies of large numbers of extended pedigrees. It is also possible that the study of other candidate gene loci may identify the gene products in which mutations occur in families not linked to chromosome 19 markers. These candidate genes are the Ca²⁺ pump and the dihydropyridine (DHP) sensitive Ca²⁺ channel. The latter has tissue specific forms; the skeletal muscle form is a pentameric complex of five different subunits. There are also cardiac and brain specific forms that are pharmacologically and electrophysiologically distinct. The locus encoding the α -1 subunit of the cardiac DHP sensitive Ca²⁺ channel was recently mapped to chromosome 12p.⁵² Alterations in levels of expression or activity of gene products which regulate fatty acid and inositol triphosphate (IP₃) levels have also been proposed as possible mechanisms by which the pathophysiological changes occur.^{53 54}

There are very few families that are sufficiently large and in which most members have been tested by the IVCT to perform this

search for other mutations causing MHS. For this reason, and because of the pre-existence of the EMHG in which all members use a standardised diagnostic procedure, it was decided to tackle this problem as a European collaboration. The Genetics Section of the EMHG has been active for nearly a year now and has begun identifying suitable families with this aim in mind. The group has now reached critical mass and eight suitable families have already been identified and are under study.

There may be a common mutation in the CRC gene in those families linked to chromosome 19 that may be detectable by looking for linkage disequilibrium with polymorphisms within the RYR gene itself. The fact that many polymorphisms have recently been described within the CRC (*RYR1*) gene should facilitate these studies.⁵⁵ The detection of a common mutation would enable many of the nuclear families, currently unanalysable because of the heterogeneity, to be investigated and add valuable information to the genetic analysis of this important clinical trait.

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