The Genetic Basis of Malignant Hyperthermia

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Key words: malignant hyperthermia, ryanodine receptor, skeletal muscle, anesthesia, *in vitro* muscle contracture test, dominant inheritance, genotype-phenotype correlation, mutational hot spot

IMAJ 2007;9:39–41

Malignant hyperthermia is a potentially lethal disorder of skeletal muscle calcium homeostasis [1], first described in 1962 by Denborough and Lovell [2] in a young male who suffered from a fractured tibia. The patient who was scheduled to undergo an operation was much more concerned from the general anesthesia than from his injury since 10 of his relatives died during or following general anesthesia. Indeed, following exposure to halothane he himself developed hyperthermia, tachycardia and tachypnea and was ultimately saved by aggressive cooling. Denborough found that the deaths of the patient's relatives followed an autosomal dominant pattern, thus concluding the existence of a dominantly inherited disorder associated with susceptibility to life-threatening hyperthermia upon exposure to general anesthetic agents [2]. Subsequent reports suggested that underlying skeletal muscle pathology confers the susceptibility to this disorder [3]. According to the classical terminology, MH episodes are triggered by exposure to certain potent volatile inhaled anesthetics like halothane, ether or isoflurane and/or by depolarizing muscle relaxants, such as succinylcholine. Nevertheless, in rare cases MH crisis may evolve after alcohol intake or viral infections [4]. MH has an estimated frequency varying from 1:15,000 anesthetic administrations in children to 1:50,000 anesthetics in adults. The actual frequency is difficult to assess since many susceptible individuals will never be exposed to the anesthetic triggers [5]. MH is considered an important cause of anesthesia-induced death and has also been associated with sudden infant death syndrome [6,7] as well as with three other myopathies: Evans [8], King Denborough [9], and central core disease [10].

Clinical symptoms and diagnosis

Clinically, MH episodes are characterized by tachycardia, accelerated muscle metabolism (manifested by elevation of exhaled CO_2, elevation of serum creatine phosphophokinase, phosphate and potassium levels), contractures, a rapid rise of body temperature, metabolic acidosis, rhabdomylosis and renal failure, and up to a 70% mortality rate in the absence of immediate treatment [5]. The syndrome is caused by an abnormal increased release of calcium from the sarcoplasmic reticulum into the sarcoplasm, resulting in increased oxygen consumption, anaerobic metabolism, and increased muscle rigidity leading to the symptoms described above [11]. Early intravenous administration of dantrolene helps abort MH crises and reduce mortality to less than 10% [5]. Dantrolene is a selective modulator of skeletal muscle excitation-contraction coupling and an inhibitor of Ca^{++} release from sarcoplasmic reticulum [12].

In 1974 Moulds and Denborough [13] first introduced the *in vitro* muscle contracture test for the diagnosis of MH susceptibility. IVCT measures the contracture responses of a muscle specimen (usually taken from the thigh) to caffeine and halothane. The IVCT is offered to patients who suffered a suspected MH event and to their family members. Patients with normal response are defined as MH normal, those with abnormal response to either one of the agents as MH equivocal, and those with an abnormal response to both as MH susceptible [14]. Mould and Denborough's test lacked the appropriate sensitivity and specificity and therefore independent sets of guidelines were established by the American and European MH Groups [15,16]. A study from the North American MH centers reported a sensitivity and specificity of 97% and 78%, respectively, while the European MH group reported a sensitivity of 99% and specificity of 93.6%. The high cost and the necessity of skilled personnel to conduct this test have made IVCT available to only a limited number of patients and family members. Anetseder et al. [17] proposed as an alternative a less invasive open muscle biopsy in which intramuscular injections of caffeine lead to an increase in carbon dioxide pressure only in MH-susceptible subjects.

Genetic basis

During the 1970s and early 1980s several studies on extensive kinships supported Denborough's initial observation suggesting autosomal dominant inheritance [18], although others offered a multifactorial mode of inheritance [19]. The evolution of an

MH = malignant hyperthermia

IVCT = *in vitro* muscle contracture test

Table 1. Loci and genes involved in MH

Locus	Chromosomallocation	Gene
MHS1	19q13.1	*RYR1*
MHS2	17q11.3-q24	Unidentified
MHS3	7q21-q22	Unidentified
MHS4	3q13.1	Unidentified
MHS5	1q32	*CACNA1S*
MHS6	5p	Unidentified

animal model for MH and the phylogenetic similarity between the swine and human ryanodine receptor form 1 (*RYR1*) led to the discovery of mutations in MH-susceptible individuals and established a major role for this gene in MH. The *RYR1* gene is located on chromosome 19 and encodes for the ryanodine receptor of skeletal muscles. The receptor is located in the sarcoplasmic reticulum and functions as an intracellular Ca^{++} release channel [20]. *RYR1* contains 106 exons and encodes a 5038 amino acid protein. To date, 47 missense mutations have been found to segregate with the MH-susceptibility trait, the majority of which appear to be clustered within three regions between residues 35 and 614 (N-terminus), 2163 and 2458 (within the sarcoplasmic reticulum membrane), and 4214 and 4806 (C-terminal transmembrane region) [21,22]. Nevertheless, ongoing research on MH families has also revealed mutations outside these three clusters [23]. The mutations are thought to alter Ca^{++} homeostasis by two possible pathways: a) interference in the E-C coupling mechanism, and b) increasing the passive Ca^{++} leak during unstimulated conditions of the *RYR1* channel [24].

In addition to MH, mutations in *RYR1* have been implicated in two myopathies: central core disease and multi-minicore disease with external ophthalmoplegia. Central core disease is characterized by general hypotonia, muscle weakness and the presence of central cores on muscle biopsy [25]. The inheritance is usually autosomal dominant, and heterozygous mutations in *RYR1* have been described in about 90% of patients [26].

Minicore myopathy is a heterogeneous group of disorders manifested by severe hypotonia and generalized weakness, with predominance in axial and proximal limb muscles, respiratory muscle involvement and external ophthalmoplegia. Muscle biopsy reveals multiple areas of loss of oxidative staining consistent with loss of oxidative activity [27,28]. In contrast to central core disease, the inheritance is autosomal recessive and in one form of the disease the underlying genetic basis involves compound heterozygote or homozygote mutations in *RYR1*. Some of the *RYR1* mutations described in minicore myopathy have been associated with susceptibility to MH [29].

Soon after the discovery of *RYR1* involvement in MH it became clear that the picture is far from complete. *RYR1* mutations were found in 30–86% of families with MH susceptibility [22,23,30], but in some families linkage to this locus was excluded. Indeed, association studies based on the extended disequilibrium test in 131 MH families displayed the genetic heterogeneity of this disorder with evidence for involvement of additional loci beside *RYR1* [31].

In the last 14 years, another five MH susceptibility genes have been mapped to specific chromosome intervals, but only one additional gene has been identified [3]. The nomenclature of the *MHS* loci followed the order of their appearance [Table 1]. *MHS2* was located to chromosome 17q11.3-q24 in North American and South African MH families [32], a region that contains a very appealing candidate, *SCN4A*. This gene encodes for the adult voltage-dependent sodium channel alpha-subunit of the skeletal muscle membrane, however no mutations were identified in the linked families. A single German MH family showed linkage to a locus on chromosome 3 (*MHS4*) [33] and *MHS6* was mapped to chromosome 5p in a Belgian family [34]. To date, the *CACNA1S* gene that encodes the α1S subunit of the dihydropyridine receptor (*MHS5*) located on chromosome 1q32 is the second gene in which mutations have been identified [35]. The dihydropyridine receptor acts as a T-tubule bound voltage sensor for *RYR1* and is estimated to account for 1% of all MH cases. *MHS3*, assigned to chromosome 7q21-q22, contains *CACNL2*A, a gene that encodes another part of the dihydropyridine receptor, the α_2/δ subunit. Despite playing a tentative role in the MH pathway, no mutations in this gene were detected in MH subjects [36]. MH has also been associated with myopathies such as Duchenne and Becker muscular dystrophies, hypokalemic periodic paralysis, and exercise-induced rhabdomyolysis [3,37,38].

Evidence for an interaction between the different genes was provided by Robinson and colleagues [39], who found co-involvement of the *RYR1* gene with loci on chromosomes 5 and 7, whereas the 1q locus segregated with the disorder alone or in interaction with the loci on chromosomes 7 and 9.

Malignant hyperthermia is a life-threatening disorder induced by anesthetic agents in prone individuals. The in vitro muscle contraction test can identify the vast majority of individuals at risk

Due to the extended locus heterogeneity, exclusion of MH risk on the basis of genetic testing for *RYR1* mutations has a low positive predictive value. Nevertheless, mutation analysis on a clinical basis has recently become available in the United States [40], and screening for the 17 most common *RYR1* mutations is offered to IVCT-positive patients and their relatives, as well as to patients who suffered from suspected MH episodes and their relatives [3]. The assay yields positive results in up to 25% of patients with MH susceptibility. Despite the low detection rate, considering the inconvenience and high cost of the IVCT, mutation screening for MH susceptibility is on the rise.

A more practical clinical approach, and one gaining increas-

E-C = excitation contraction

MHS = MH susceptible

ing popularity in MH-suspected individuals, involves the use of "non-triggering" anesthesial agents, thus bypassing the need for an extensive preoperative workup.

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