Malignant Hyperthermia and *Ryanodine Receptor Type 1* Gene (*RyR1*) Mutation in a Family in Singapore

Daphne WY Li, 1MBBS, Poh San Lai, 2MD, Delice W Lee, 1MBBS, MMed (Anaes), Rita YY Yong, 3MD, Tat Leang Lee, 1,4MBBS, MMed (Anaes), FANZCA

Abstract

**Introduction:** Sporadic clinical episodes of malignant hyperthermia (MH) that develop during general anaesthesia (GA) have been reported in Singapore. However, there is no published local report of a confirmed case of MH susceptibility (MHS) by skeletal muscle contracture tests and/or molecular tests. **Materials and Methods:** We report 2 patients from an extended family who developed signs of clinical MH while under GA. The MH episodes were successfully treated with intravenous dantrolene sodium. Sequence analysis of the entire *Ryanodine Receptor Type 1* (*RyR1*) coding gene was carried out in an index patient. **Results:** The index patient was found to carry a c.7373G>A (p.Arg2458His) mutation in exon 46. This particular mutation satisfies the criteria for a MHS causative mutation. Hence, the index patient was considered to be MHS and did not need to undergo further muscle contracture testing. The same mutation was also found in 3 other members of his extended family. **Conclusion:** This is the first report of a Singaporean family with at least 4 members carrying a MH-causative mutation in *RyR1* gene. This report serves to highlight the existence of the putative gene for MH in Singapore, and the need for clinical vigilance during anaesthesia involving the use of triggering agents.

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Malignant hyperthermia susceptibility (MHS) is a pharmacogenetic disorder, which usually develops during or immediately after the application of general anaesthesia (GA) involving volatile agents (e.g. halothane, isoflurane, sevoflurane, desflurane, and enfurane) and/or the depolarising muscle relaxant suxamethonium. The classic malignant hyperthermia (MH) crisis consists of a hyper-metabolic state caused primarily by continued activation of the skeletal muscles, which leads to massive carbon dioxide (CO₂) production, skeletal muscle rigidity, tachyarrhythmias, unstable haemodynamics, respiratory acidosis, cyanosis, hyperkalaemia, lactic acidosis, fever, and eventual (if untreated) death. 1 The estimated incidence of MHS ranges from 1 in 3000 anaesthetics to 1 in 50,000 anaesthetics, with most estimating an incidence in children of about 1 in 10,000 anaesthetics and in adults of 1 in 50,000 anaesthetics. 2 The incidence of MHS varies depending on the routine use of triggering anaesthetics, as well as the prevalence of susceptibility mutations in the population. For example, Monnier et al and Ibarra et al’s analyses of individuals with homozygous or compound heterozygous mutations yielded an estimated MHS prevalence as high as 1 in 2000 to 3000 in the French and Japanese populations, respectively. 3,4 The incidence of MHS in Singapore is unknown, although sporadic clinical MH cases have been reported. 5,6 The standard diagnostic test for MHS has been the in vitro measurement of contracture response of freshly biopsied muscle to graded concentrations of caffeine and the anaesthetic halothane. The test is referred to as the caffeine/halothane contracture test (CHCT) in North America 7 and the in vitro contracture test (IVCT) in Europe. 8 The test is currently not available in Singapore.
MHS is an autosomal dominant myopathy; its pathophysiology has been attributed to calcium (Ca\(^{2+}\)) dysregulation within the myofilaments. To date, mutation in two genes predisposing to MHS have been identified; the **ryanodine receptor type 1** gene (**RyR1**), encoding the skeletal muscle isoform of the Ca\(^{2+}\) release channel of the sarcoplasmic reticulum;\(^8\) and the **voltage-gated calcium channel subunit alpha1 S** (**CACNA1S**), encoding the α1 subunit of the L-type Ca\(^{2+}\) channel isoform of the sarcolemna (dihydropyridine receptor).\(^9\) Pathogenic variants in **RyR1** have been identified in up to 70%-80% of individuals with confirmed MHS,\(^1,10\) while pathogenic variants in **CACNA1S** account for only 1% of all MHS.\(^11\) Although more than 200 **RyR1** variants have been associated with MHS, only 35 have been shown to have functional effects consistent with MH pathogenicity.\(^12\)

We report a Singaporean family with 4 members carrying a MH-causative mutation in **RyR1**; 2 of them had developed a clinical MH episode each. Prior to clinical assessment and genetic testing, written informed consent was obtained from the index patient (proband), and members of his family.

**Case Report 1**

Our index patient is a 20-year-old healthy Chinese male (American Society of Anesthesiologists physical status I) who was a full-time army Service personnel. He was admitted for open reduction and internal fixation of a fracture of his left lower radius following a motorcycle accident. Preoperative assessment revealed no physical abnormalities and he was serving combat duty in his army unit. The index patient was unaware of any significant medical history among his family members, and he opted for general anaesthesia (GA). General anaesthetics consisting of intravenous (IV) propofol and fentanyl for induction, followed by desflurane in an oxygen-air mixture were administered via a laryngeal mask airway (LMA) (Proseal®) for maintenance. The patient’s lungs were mechanically ventilated to keep the end-tidal carbon dioxide (CO\(_2\)) between 35 to 45 mmHg. The patient also received 2 grams of IV cefazolin for prophylaxis prior to the start of surgery.

Monitoring during GA included electrocardiogram (lead II), non-invasive blood pressure monitor, pulse oximetry, end-tidal CO\(_2\) (ETCO\(_2\)), and an esophageal temperature probe. The patient’s ETCO\(_2\) was noted to be gradually increasing about 40 minutes into the surgical procedure (from 36 to 90 mmHg). The set respiratory rate of the mechanical ventilator was increased, and the breathing circuit (circle system) and the CO\(_2\) absorber were checked to exclude machine malfunction or exhaustion of the CO\(_2\) absorber. There was no increase in the temperature (36.5°C) and he was haemodynamically stable. The warm air administrating device was switched off. An arterial blood gas was obtained and this confirmed hypercapnia and respiratory acidosis (pH 7.14, PaCO\(_2\) 87.8 mmHg, BE-3, bicarbonate 28.9 mmol/L). A provisional diagnosis of clinical MH was made and the surgeon was informed to abort the surgical procedure, and to complete the wound closure as soon as possible.

MH treatment protocol was activated. This included asking for help and the MH cart. One person was designated to dilute dantrolene sodium (Dantrium®, DSM Pharmaceuticals), and arrangements were made to bring in a stand-by anaesthesia machine and to commence surface cooling with ice packs. The ETCO\(_2\) and PaCO\(_2\) decreased within 10 to 15 minutes after the administration of IV dantrolene (2.5 mg/kg). Blood samples were dispatched for electrolytes, creatine kinase (CK), and thyroid screening panel. The bladder was catheterised to monitor urine output and a urine sample was also sent for myoglobin. All the blood results were within normal limits including repeated CK levels over the next 2 days (293--317--291 [normal range 30-350 U/L]).

The patient was monitored in the surgical intensive care unit (ICU) for the first 24 hours and subsequently in the high dependency unit for the next 24 hours before being discharged to the general ward. He was discharged on the fifth postoperative day and was reviewed by one of the authors (LTL) a week later. The patient was well apart from complaining of a feeling of general weakness as well as a heavy feeling in his head.

The patient was readmitted 4 weeks later to complete the surgery under a brachial plexus block. He made an uneventful recovery from the anaesthesia and surgery—no dantrolene prophylaxis was given for the second operation. Arrangements were made for the patient to be registered with the ‘Medik Awas’ programme (a local medical alert registry) with the Singapore Medical Association. A letter was issued to the patient and the Ministry of Defence was also notified.

**Case Report 2**

While the patient was being monitored in the ICU, he was visited by his mother who told us that the patient’s paternal grandmother had an “anaesthetic reaction” some years ago while undergoing surgery and anaesthesia in another hospital. We interviewed the patient’s grandmother and obtained written informed consent to trace her medical records.

The patient’s grandmother had an uneventful coronary bypass surgery (CABG) performed for triple vessel coronary artery disease in 2009. Isoflurane was administered as part of the GA for about 1 hour (end-tidal [ET] concentration...
ranged from 0.3%-1.15%) before the patient was put on cardiopulmonary bypass (CPB) for about 30 minutes. After the CPB, the patient’s ET concentration ranged from 0.4%-0.45%. Her postoperative recovery following the CABG procedure was uneventful. The patient subsequently underwent an elective surgery for left eye strabismus under GA in 2011. Sevoflurane in an oxygen-air mixture was administered via an endotracheal tube and the patient’s ETCO$_2$ was noted to rise about 30 minutes after induction of GA from 40 to 90 mmHg. There was no mention of temperature monitoring in the chart. The operation was aborted and the patient was also given IV dantrolene sodium (3 mg/kg). She made an uneventful recovery. She was given a letter by the Department of Anaesthesia to alert her healthcare providers to avoid giving her volatile agents or depolarising muscle relaxant. Her blood CK levels done during the clinical MH episode were mildly raised (212→221→253 U/L [normal 40-200 U/L]), and myoglobin was detected in her urine (113→73.4→47.1→49 μg/L [normal <21 μg/L]). A repeat CK level performed 5 months after the MH episode was reported as 400 U/L.

**Identification of RyR1 Mutation**

As muscle contracture tests are not available in Singapore, and the patient was unwilling to undergo the test in an overseas centre at his own expense, we proceeded to perform RyR1 full gene sequencing after obtaining written informed consent from our index patient. The genetic test was carried out at the Emory Genetic Laboratory in Georgia, United States of America (USA). Sequence analysis of the entire RyR1 coding gene identified a c.7373G>A (p.Arg2458His) mutation in exon 46. This mutation has been previously reported in patients with MHS.15-18 This missense mutation, involving a substitution of arginine to histidine, is predicted to be deleterious or possibly damaging to protein function by computational tools (SIFT score 0 and PolyPhen2 score 0.987). This gain-of-function mutation (c.7373G>A p.Arg2458His), as well as other mutations located in the similar hotspot Region II of the RyR1 gene, have been shown to cause aberrations in RyR1 channel function (such as hyper-activation of the channel by, and hyper-sensitisation of the channel to, various physiological and pharmacological agonists, resulting in a leaky Ca$^{2+}$ channel).19-20 After obtaining written informed consents, blood tests for the same RyR1 mutation were carried out for his father and paternal grandmother by the same laboratory, which also turned out to be positive. The grandmother’s daughter and siblings were interviewed by 2 of the authors (DWYL and LTL), and the same mutation (c.7373G>A [p.Arg2458His]) was also found in one of the proband’s paternal grand aunt (Fig.1). The rest of the family members were not willing to undergo the genetic testing. Tests for the specific RyR1 mutation for 3 family members were carried out at the Human Genetics Laboratory of the Department of Paediatrics at the National University of Singapore.

**Discussion**

This is the first reported case of MH with a causative RyR1 mutation, and the first report of multiple members within the same family carrying the same mutated RyR1 in Singapore.

MHS is a potentially fatal pharmacogenetic disorder; therefore confirmation of the diagnosis is important to prevent the patient and any affected family members from any future exposure to triggering agents. A confirmed diagnosis will also facilitate genetic counselling and may also affect career choices. For example, the military service...
of the USA does not enlist individuals with MHS.\textsuperscript{21} Cases of “awake” MH episodes during exertion, heat challenge, and febrile illness in the absence of triggering drugs have been reported.\textsuperscript{22-24} These reports show that non-anaesthetic awake episodes induced by heat stress and MH share some clinical (muscle hypermetabolism) and biological (positive in vitro contracture test) features, however, the underlying molecular cause of non-anaesthetic awake MH is not fully established, although novel mutations in the \textit{RyR1} have recently been identified in 2 unrelated children who experienced fatal, non-anaesthetic awake episodes associated heat stress, and in 13\% of a military cohort who had a well documented exertional heat stroke crisis.\textsuperscript{25,26} Our index patient (Case 1) was still serving his national service commitment in the army when the MH event occurred. However, he had been coping well with his military training.

Although MHS is considered a form of myopathy, except for a few congenital myopathies with a strong association with MH (e.g. King-Denborough syndrome, and central core disease), the majority of the MHS patients display no symptoms/signs of myopathy until they are exposed to triggering agents (Cases 1 and 2). MHS is a pharmacogenetic disorder of autosomal dominant inheritance (as illustrated in this family, Fig. 1) with incomplete penetrance and variable expressivity (e.g. Case 2). The variability in clinical MH responses can be attributed to some as yet unidentified environmental factors, and the different \textit{RyR1} variants present in the affected individual.\textsuperscript{16} The moderate hypothermia (28°C) used as part of the CPB procedure during the CABG surgery could have masked/aborted the MH reaction in Case 2. It is worth noting that our index patient is a young male and therefore has a bigger muscle mass compared to his grandmother. However, his CK levels were not elevated during and after the MH episode, while his grandmother’s CK levels were only mildly elevated. The lack of a significant rise in CK levels could be attributed to the early detection and early administration of dantrolene. In addition, it has been shown that different \textit{RyR1} variants are associated with quantitative differences in MH phenotype including the CK levels.\textsuperscript{16} It is fortunate that both cases were identified at an early stage when the ETCO\textsubscript{2} levels were rapidly rising due to hyper-metabolism, and were successfully treated with adequate doses of dantrolene. It has been shown that the likelihood of any complication increased 2.9 times per 2°C increase in maximum temperature and 1.6 times per 30-minute delay in dantrolene use.\textsuperscript{27}

In addition to exposure to GA, situations in which a MHS patient is likely to receive a MH triggering agent would include the emergency room or ICU, where clinicians may use the depolarising muscle relaxant suxamethonium to facilitate tracheal intubation during emergent airway management. Suxamethonium should be avoided in favour of a non-depolarising muscle relaxant such as rocuronium in a MHS patient. Clinicians who use suxamethonium in their clinical practice should be familiar with the early signs of MH, MH crisis management protocol, and ensure the availability of dantrolene to treat MH crisis. Current literature does not support the use of dantrolene prophylaxis to prevent MH crisis.

As many early signs of MH episodes present in a variable manner, a clinical grading scale (CGS) has been developed to address concerns for objectivity evaluation of the clinical MH episode.\textsuperscript{28} However, this scale lacks sensitivity because incomplete recording of necessary data or early termination of the crisis (e.g. Cases 1 and 2) would not yield scores indicative of MH, even in the presence of a true MH episode. The CGS was not intended to be a clinical guide in the operating room but to assist MH researchers in classifying adverse events and help to identify those subjects with the most convincing episodes of MH for subsequent evaluation of the sensitivity of the diagnostic tests.\textsuperscript{28} The CGS calculated retrospectively for our index patient (Case 1) was 20 points, based on an inappropriate rise in the ETCO\textsubscript{2} (15 points), and a rapid reversal of the respiratory acidosis with IV dantrolene (5 points). A raw score of 20 points will yield a MH rank of 4 (i.e. the qualitative likelihood of MH is somewhat greater than likely).

In vitro muscle contracture test is currently considered the gold standard to establish the diagnosis of MHS. A sensitivity of 97\% and specificity of 78\% are reported for the North America Malignant Hyperthermia Group (NAMHG) protocol while figures of 99\% sensitivity and 94\% specificity are obtained with the European Malignant Hyperthermia Group (EMHG) protocol.\textsuperscript{7,8} In addition to cost, the individual must be physically present at an overseas MH diagnostic centre as the test involves a muscle biopsy usually from the vastus muscle group, and has to be carried out within 5 hours of harvesting. It is usually carried out 3 to 6 months after the clinical MH episode depending on the degree of rhabdomyolysis, and hence may not be suitable for someone who is waiting for an urgent surgery (e.g. fracture).

Since the identification of the 2 MH causative genes (\textit{RyR1} and \textit{CACNA1S}),\textsuperscript{9,10} deoxyribonucleic acid (DNA) analysis offers an alternative to the muscle contracture test. Both the NAMHG and EMHG currently consider DNA screening to be a viable alternative primary diagnostic approach to the muscle contracture test, although limited to a positive diagnosis and to those few mutations that have been functionally characterised.\textsuperscript{29,30} It is important to note that because of the heterogeneity of the disorder, as well as discordance within families, a negative DNA result cannot be used to rule out MHS and a muscle contracture test is recommended where a DNA test is negative for a familial mutation.\textsuperscript{29,30} The test requires only a blood specimen, which
can be sent to an accredited diagnostic laboratory, and it can be performed on patients of all age groups. When a causative mutation is found on RyR1 sequence analysis, first-degree relatives can be tested for the identified mutation with reduced cost. Our index patient was found to have a causative RyR1 mutation (c.7373G>A [p.Arg2458His]). As this particular mutation (1 of the 35 causative mutations published to-date) satisfies the criteria for a MHS causative mutation, our index patient is considered to be MHS and he did not need to undergo any further muscle contracture testing. The same causative RyR1 mutation was subsequently found in 3 other members of his extended family (Fig. 1), therefore, these 3 family members are also considered MHS without any need for IVCT/CHCT.

We counselled the family members to encourage their first-degree relatives to test for the familial causative RyR1 variant. If an individual does not have the familial RyR1 variant, the entire RyR1 should be examined and IVCT/CHCT performed in an overseas MH diagnostic biopsy centre to rule out MHS. This is because more than one gene is associated with MHS, and not all are known. Furthermore, the IVCT/CHCT is the only test that can produce a true negative diagnosis. MH diagnostic biopsy centres will also provide professional genetic counsellors to better advise patients on their test results. In the meantime, relatives who have tested negative for the familial variant or who are unwilling to be tested are advised to inform their doctors and anaesthetists that their MHS status is unknown, and it would be best to avoid administering triggering agents to these patients.

The incidence of MHS in Singapore is unknown. An e-mail survey conducted by one of the authors (LTL) with Heads of Department/Clinical Directors of all the Department of Anaesthesia in Singapore (excluding private practice) gave an estimate of approximately 1 clinical MH episode per 100,000 general anaesthetics, which is lower than what is generally quoted in anaesthetic literature. Practice (2010) gave an estimate of approximately 1 clinical MH episode per 100,000 general anaesthetics, which is lower than what is generally quoted in anaesthetic literature. The low incidence will hamper the setting up of a local MH registry and MH diagnostic centre, however, we can explore the possibility of establishing an Asian MH referral centre in the future. At the moment, suspected clinical MH cases will need to be referred to an overseas MH diagnostic centre for the muscle contracture test. The low incidence of the condition could partly be due to under-diagnosis arising from lack of awareness and lack of availability of local testing facilities. Although genetic testing is less invasive than a muscle contracture test, clinical testing currently has to be sent overseas due to a lack of accredited local laboratories offering such services. Diagnostic testing for this condition using DNA screening also has its limitations—bearing in mind that the sensitivity of detecting a RyR1 causative mutation is only about 40% in someone without a prior positive muscle contracture test. This report serves to remind clinicians of the existence of this uncommon and potentially fatal condition within our community. Anaesthetists should remain vigilant and be trained to recognise this clinical condition early and to treat the condition in a timely manner.

REFERENCES


