Malignant hyperthermia in infancy and identification of novel RYR1 mutation

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Malignant hyperthermia (MH) has been reported as non-existent in children less than 1 yr old, although several unconfirmed reports have been published. A case report of MH in a 6-month-old child is presented, with confirmation of MH susceptibility by in vitro contracture testing of quadriceps muscle at 13 yr old. Genetic analysis revealed a novel RYR1 mutation that substitutes arginine 2452 for tryptophan in a region of the calcium channel mutated in several other MH pedigrees.

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Malignant hyperthermia (MH) is an inherited hypermetabolic disorder of skeletal muscle triggered by depolarizing muscle relaxants and potent inhalation anaesthetic agents. The incidence has recently been reported as 1:50 000 anaesthetics administered in adults and 1:15 000 in children.1 MH in infancy is considered rare2 or even non-existent.3 There have been several reports indicating MH reactions in infants4–8 (Allen, personal communication), but confirmation by in vitro contracture testing (IVCT) was not demonstrated in the proband or in immediate relatives. A case report of an MH reaction in a 6-month-old child is presented, and also evidence of a novel RYR1 mutation associated with MH in this family.

Case report

A 6-month-old male born at term (in 1984) following an uneventful pregnancy presented for repair of complete cleft of the right primary and secondary palate, and incomplete cleft of the left primary palate. A first stage repair using a modified Manchester technique was planned. There was no family or medical history of note, he weighed 7.4 kg and physical examination was normal, other than his congenital cleft palate.

He was premedicated with atropine 0.2 mg and droperidol 1 mg orally 90 min before induction. An uneventful gaseous induction of anaesthesia was carried out using halothane, and the trachea was intubated with a size 4.0 Oxford tube. The child breathed spontaneously a mixture of halothane, oxygen and nitrous oxide through a humidified T piece circuit, and was monitored using an ECG, digital plethysmograph, precordial stethoscope and rectal temperature probe (capnography, agent analysis and pulse oximetry were not in general use at this time). Fluids were given through a 24-gauge cannula in the foot, and the patient was well draped to prevent heat loss.

Anaesthesia was uneventful for the first 90 min with a heart rate (HR) of between 155 and 165, temperature increasing from the initial recording of 36.4°C to 37.3°C. During the last 10 min of the case (110–120 min from the start) HR increased from 160 to 190, and his temperature continued to increase, reaching 39°C. At the end of the procedure (120 min from the start) the surgeon noted unexpected masseter muscle rigidity when removing the mouth gag, and on removing the drapes the patient was noted to be very flushed and sweaty. Anaesthetic agents were discontinued, respiration was thought to be adequate, the trachea was extubated and the child taken to recovery where his temperature was found to be 39.5°C. Tachypnoea was then noted, blood-gas analysis showed a pH of 7.195, Pco2 3.8 kPa, Po2 13.4 kPa.
with a base deficit of 13.4 mmol litre\(^{-1}\) and a diagnosis of malignant hyperthermia was made. Dantrolene 15 mg (2 mg kg\(^{-1}\)) was administered, cooling was commenced using a fan over a wet sheet, and an arterial line and urinary catheter inserted. Sodium bicarbonate, 7.5 mmol was given. Serum potassium was measured at 5.6 mmol litre\(^{-1}\). The maximum temperature reached was 39.5\(^\circ\)C 20 min after diagnosis, and 140 min following induction. The temperature returned to normal 80 min after diagnosis, 200 min from the start. No myoglobin was present in the urine and creatine kinase (CK) was initially 290 i.u., rising to 1845 i.u. (normal 0–300 i.u.) 2 h post-anaesthetic. No further therapy was required and an uneventful recovery followed.

Investigation of the family at the time using CK showed his mother to have an elevated level of 623 i.u., while father’s and siblings’ CK were normal. The CK of the patient measured several months after the reaction was 243 i.u.

At 13 yr old the patient underwent lateral quadriceps muscle biopsy. The test was performed in accordance with the European MH Group protocol.\(^9\) This demonstrated a strongly positive contracture (MHS) to both halothane and caffeine (6.4 g tension to 2% halothane and 5.1 g tension to caffeine 2 mmol litre\(^{-1}\) – n<0.2 g tension). The mother of the patient had a 4.3 g response to 2% halothane and a 3.7 g response to caffeine 2 mmol litre\(^{-1}\), and was also diagnosed MHS. Histology of the muscle was normal. There was no evidence of myositis or recognizable dystrophy.

The proband was screened for published MH RYR1 mutations in the central mutation region of the ryanodine receptor (RYR1) gene by PCR-amplification of cDNA and automatic sequencing. None of the nine published mutations\(^{10-15}\) in this region was detected. However, a novel C7354T nucleotide transition that substitutes arginine 2452 for tryptophan was identified. The mother of the proband was also positive for the Arg2452Trp mutation. The mutation was present in the DNA of one of two siblings examined, but these individuals are yet to be examined by IVCT. Segregation of the Arg2452Trp mutation in this small family (family 1) is shown in Figure 1A.

The method of ‘single stranded conformational polymorphism analysis’ (SSCP)\(^{16}\) was used to screen for the Arg2452Trp mutation in exon 46 amplified from genomic DNA (Fig. 2). The mutation was not found in 100 normal chromosomes indicating that it is not likely to be a common polymorphism. DNA samples from MHS probands from other NZ MH families were also screened for the mutation by SSCP analysis

The Arg2452Trp mutation was not detected in the DNA from 33 MHS probands examined. However, a new conformational variant was detected in exon 46 in one unrelated MHS patient as shown in Fig. 2. Sequence analysis of this second variant revealed a G7361A mutation that changes arginine 2454 to histidine. This second mutation was identified in an unrelated young male who developed a fulminant MH reaction with masseter muscle spasm, significant hypercapnia, tachycardia and rhabdomyolysis during an emergency orthopaedic procedure. IVCT was strongly positive. The mother of this patient had a normal IVCT (MHN), and both mother and sister were negative with respect to the mutation. The results of a screen for the Arg2454Trp mutation in members of family 2 are presented in Figure 1B.

**Discussion**

Pyrexia, increasing tachycardia, rigidity, tachypnoea, and significant metabolic acidosis as well as flushing and
sweating provided clinical evidence for MH in this child. The MH clinical grading scale\textsuperscript{17} ranks the likelihood that an adverse anesthetic event represents MH. Points are assigned to abnormal signs and laboratory findings (clinical indicators), the points are then summed to produce a raw score which is translated to a qualitative MH rank indicating with which likelihood MH could occur − from 1 (almost never) to 6 (almost certain). This infant ranked 6 on this clinical grading scale. Confirmation of an MH reaction was demonstrated by positive IVCT, the only reliable screening test for MH susceptibility.\textsuperscript{18}

Jaw rigidity was first noted by the surgeon. The anaesthetist at the time (DC) was not aware of rigidity in other muscle groups. In the course of an MH reaction rigidity of the jaw usually presents as masseter muscle spasm at induction of anaesthesia following the use of suxamethonium. In patients not given suxamethonium, muscles of the chest, abdomen and extremities may be involved initially, although eventually rigidity may be generalized.\textsuperscript{19} Isolated jaw rigidity in the absence of suxamethonium, 120 min after induction, may represent an alternative presentation of MH in an infant.

Management of the reaction proceeded with few problems. Dantrolene was administered in a dose of 2 mg kg\textsuperscript{-1} which is lower than the recommended starting dose of 2.4 mg kg\textsuperscript{-1}.\textsuperscript{20} Lerman found normal metabolism of dantrolene in young children and a dose of 2.4 mg kg\textsuperscript{-1} produces safe and predictable blood concentrations. Reconstituted dantrolene, pH 9–10, is irritant to veins and in an infant should be injected into a fast-running intravenous infusion or large central vein.\textsuperscript{21} Intraosseous administration provides an alternative route if venous access is problematic.

Reactions in infants younger than 6 months have not been previously proven. There have been several reports of MH reactions in the newborn associated with the stress of labour, but alternative aetiologies for these reactions could be proposed.\textsuperscript{21–23} At our institution MH suspect newborn are monitored for 4 h with pulse, ventilatory frequency and temperature, and no significant abnormalities have been reported in 66 infants born to MHS or mothers related to MHS individuals (Langton, unpublished observations). Reactions associated with farrowing have not been reported in susceptible piglets. There may in fact be resistance to the development of MH in the neonatal period and early infancy. Fay and Gallant\textsuperscript{24} found that MHS piglets had delayed reactions when exposed to triggering agents, and \textit{in vitro} responses were not as pronounced as the contractures usually observed in muscle from older MHS pigs. These findings were confirmed by Wedel.\textsuperscript{2}

As indicated earlier, several case reports in infants younger than 6 months have been published in the anaesthetic literature, but with the exception of Faust and co-workers,\textsuperscript{25} IVCT was not performed. In this latter case triggering agents were not administered, the reaction ranked 3 or ‘somewhat less than likely’ on the MH clinical grading scale and IVCT was performed from sterno-mastoid muscle and not quadriceps, and therefore the diagnosis could be questioned.

IVCT in this case was undertaken in early teenage years, and in general we follow the policy of Ellis and co-workers\textsuperscript{8} who established a lower age limit of 10 yr due to inconsistency in muscle responses in young children, possibly related to immature muscle tissue. The size of the muscle sample to be removed and scarring are other considerations. However, Gronert\textsuperscript{26} has found normal contracture responses in small numbers of children and other units will test younger children.\textsuperscript{27} If the proband is less than 10 yr old, some units will test the parents and if they are normal the child is considered normal.\textsuperscript{9,28} However, in our unit there has been parental resistance to this procedure and a reaction could also result from a \textit{de novo} mutation in the proband.

The primary event triggering MH is the abnormal release of calcium from the sarcoplasmic reticulum (SR) that initiates sustained contractile and metabolic activity in skeletal muscle.\textsuperscript{29} The SR calcium release channel was identified as the ryanodine receptor, a large tetramer, observed as the ‘foot’ structure spanning the junctional SR and surface membranes.\textsuperscript{30} Abnormalities in the regulation of calcium release from the ryanodine receptor were revealed in studies of skeletal muscle from MHS pigs and humans.\textsuperscript{31} In 1990, McCarthy and co-workers\textsuperscript{32} identified the ryanodine receptor gene (RYR1) as a likely candidate for the MHS locus, and in the subsequent 9 yr the role of RYR1 has been confirmed with the discovery of numerous RYR1 mutations in MH families.\textsuperscript{13} The Arg2452Trp mutation described in this report takes the number of published MH/central core disease mutations in the RYR1 gene to 20. RYR1 mutations may underlie only 50% of cases of MH susceptibility,\textsuperscript{33} and defects in any number of proteins involved in myoplasmic calcium homeostasis may be commonly expressed as the MH syndrome. In fact, genetic heterogeneity in MH is now firmly established with the description of five additional MH loci on chromosomes 17q, 7q, 3q, 1q and 5p.\textsuperscript{34–38} The emerging complexity of MH genetics excludes the development of a general genetic test to replace the IVCT in the near future. There are several reports of MHS diagnoses in individuals who are negative for a particular mutation segregating in their family\textsuperscript{11,39–41} suggestive of either false positive IVCT or the occurrence of additional MH genes. Consequently, genetic testing cannot safely replace the IVCT to establish MHN status.

We have searched for the published mutations in the central RYR1 region by automatic sequencing of complementary DNA (cDNA), using skeletal muscle tissue excised for IVCT. RNA was extracted from frozen muscle tissue and used as a template to synthesize the cDNA. Because the cDNA comprises only the coding region of a gene, this approach allows efficient simultaneous screening for clustered RYR1 mutations, as well as enabling detection of any novel mutations. A novel arginine 2452 to tryptophan RYR1 mutation was identified in this patient. The mutation
was also detected in the proband’s mother, who tested MHS, and a sibling who is still too young to undergo IVCT examination. The MH status of the father has not yet been examined by IVCT. An investigation of the co-segregation of the mutation with the MH disorder was restricted by the small number of patients investigated by IVCT in this family. Therefore, it is possible that the mutation represents a polymorphism that is not causative of MH. However, the mutation is highly unlikely to be a coincidental polymorphism as it was not detected in the normal population.

While screening the DNA of unrelated MHS probands for the novel Arg2452Trp mutation, a second mutation that changes arginine 2454 to histidine was found in an unrelated adult MHS proband. The Arg2454His substitution identified in this second proband (family 2) occurs only 2 amino acids downstream of the Arg2452Trp mutation that was identified in the first proband (family 1). The Arg2454His mutation was recently described in an Italian family. The independent identification of the same mutation in an unrelated NZ proband supports a causative role for the Arg2454His substitution in MH.

The two arginine substitutions detected in this study (Arg2452Trp and Arg2454His) occur in close proximity to two previously described MH mutations at the arginine 2458 residue. The clustering of four MH mutations within a 7 amino acid region, and the conservation of the three affected arginine residues across species in all RYR1, RYR2 and RYR3 sequences implies a critical regulatory role for the positively charged amino acids in this domain of the ryanodine receptor. Interestingly, nine out of 11 mutations reported in the central region of the ryanodine receptor involve arginine residues.

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