Genotype and phenotype relationships for mutations in the ryanodine receptor in patients referred for diagnosis of malignant hyperthermia

J. E. FLETCHER, L. TRIPOLITIS, M. HUBERT, G. M. VITA, R. C. LEVITT AND H. ROSENBERG

Summary

Anaesthesia-induced malignant hyperthermia (MH) may be caused by specific gene defects in the skeletal muscle ryanodine receptor. We have studied the frequency of occurrence of the C1840T mutation, analogous to the porcine mutation, and three mutations associated both with MH and central core disease (G7301A, C487 T and C1209G). We investigated skeletal muscle specimens from up to 137 patients testing negative and 101 patients testing positive for MH susceptibility by the North American MH Group protocol. The presence or absence of the mutations was determined by polymerase chain reaction and restriction enzyme digestion. The frequencies of occurrence of the C1840T and C487T mutations were 2% and 1%, respectively, in MH-positive subjects and were the only two mutations identified. One subject with central core disease did not have any of the three mutations examined associated with this disorder. Therefore, the porcine and central core disease-associated mutations examined in the ryanodine receptor account for a small proportion (approximately 3%) of MH-positive diagnoses. The mutations examined did not occur in any of the MH-negative patients, supporting an association between defects in the ryanodine receptor and a positive diagnosis for MH.

The relatively weak response to triggering anaesthetics (only masseter muscle rigidity) in two subjects with the C1840T ryr1 mutation, including 18 uneventful anaesthetics (only masseter muscle rigidity) in two subjects with the C1840T mutation, including 18 uneventful anaesthetics (only masseter muscle rigidity) in two subjects with the C1840T mutation, including 18 uneventful anaesthetics (only masseter muscle rigidity) in two subjects with the C1840T mutation, including 18 uneventful anaesthetics (only masseter muscle rigidity) in two subjects with the C1840T mutation, including 18 uneventful anaesthetics (only masseter muscle rigidity), suggests that additional modulating factors must contribute to the syndrome or the presence of the C1840T mutation is not a causative factor in MH. (Br. J. Anaesth. 1995; 75: 307–310)

Key words


Linkage analyses have revealed that approximately 25–50% of susceptible families demonstrate MH cosegregating in the region of chromosome 19q13.1 encoding ryr1 [1]. Several mutations in ryr1 may be associated with human MH [2–5]. There is widespread agreement that MH is a heterogeneous disorder that may be caused by mutations in genes on chromosomes other than 19q [1, 6]. A mutation in the dihydropyridine receptor α1β-subunit on chromosome 7q [7] has been one suggested alternative to ryr1, and anaesthetic deaths have occurred in families in which MH co-segregated with genetic markers on chromosome 17 in the vicinity of the Na+ channel [8, 9]. It does seem clear that at least one MH gene must lie in or very close to the gene encoding ryr1 and that defects in ryr1 may at least exacerbate the response to anaesthetics, if they are not causative of MH.

In some families MH susceptibility has been strongly correlated with central core disease (CCD); that is, it has been suggested that these two skeletal muscle disorders may result from the same mutation and thus could be co-inherited and may comprise a specific subtype of MH [3, 4, 10]. However, the relationship between these two disorders is much more complex than was first assumed [3].

Patients and methods

Patients were referred for diagnosis primarily because of a family history of MH or signs of MH during previous anaesthesia. About 3 g of vastus lateralis was biopsied under local anaesthesia [11]. Fibre bundles were dissected for mounting in a tissue bath for an in vitro diagnostic contracture testing, as described previously [12]. Patients were diagnosed as MH susceptible (MH+) by the North American MH Group protocol [13] if any one of three strips exposed to 3% halothane in the gas phase exhibited a contracture > 0.7 g or if any one of three strips exhibited a cumulative contracture ≥ 0.3 g with caffeine 2 mmol litre−1 during construction

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of a dose–response curve. Patients not meeting the criteria were considered normal (MH−). We have evaluated these criteria in swine and estimated 96 % sensitivity and 95 % specificity for MH susceptibility [14]. The sensitivity and specificity of these criteria in humans have been estimated at approximately 92 % and 74 %, respectively [15]. Of the 101 MH susceptible subjects, 90 were unrelated.

DNA was extracted from frozen specimens of skeletal muscle, as described previously [14]. The regions of DNA containing the described single base nucleotide substitution mutations were amplified by polymerase chain reaction (PCR) using the primers and conditions suggested in [2–4] and in table 1. Restriction enzyme digests of PCR fragments were resolved on agarose (1.5 %) gels mixed with Resolow (50 %; Integration Separation Systems; Natick, MA, USA) and observed with ethidium bromide and ultraviolet light. For those subjects with incomplete restriction enzyme digestion in a minimum of three separate incubations, the sequence of the PCR fragment was verified to contain the mutation using solid phase techniques [16, 17].

Results
The C1840T mutation was detected in two unrelated subjects (table 2) in our MH+ population, in agreement with earlier estimates, based primarily on studies in families, of about a 2 % frequency [2]. The region amplified by PCR was sequenced to verify the presence of the reported mutation.

The maximum responses to 3 % halothane and caffeine 2 mmol litre−1 are indicated in table 3. Subject No. 1 had masseter muscle rigidity with muscle pains for several days after anaesthesia. Subject No. 2 had been exposed to 18 uneventful anaesthetics before referral for diagnosis. At least 16 anaesthetics were with known triggering agents (halogenated volatile general anaesthetics) and four of the 16 also included suxamethonium. In three of the four anaesthetics (including suxamethonium) masseter muscle rigidity was observed, sometimes with bigeminy. Neither of the subjects with C1840T mutations had signs of acidosis. Of the three CCD-associated mutations, only the C487T was observed (table 2), suggesting that the CCD mutations examined account for about 1 % of MH in patients referred to our laboratory for diagnosis. The C487T mutation had been found previously in an MH susceptible family without CCD [3].

Subject No. 3 had no signs of acidosis or evidence of CCD on histological examination (table 3). The maximum halothane and caffeine contractures for subject No. 3 are indicated in table 3. One subject with CCD tested positive for MH (data not shown), supporting the association between these two disorders. This subject did not have any of the three

<table>
<thead>
<tr>
<th>Nucleotide mutation</th>
<th>Amino acid substitution</th>
<th>Associated disorders</th>
<th>PCR fragment size (bp)</th>
<th>Restriction enzyme</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1840T</td>
<td>Arg614Cys</td>
<td>Porcine MH</td>
<td>74</td>
<td>RsaI</td>
<td>[2]</td>
</tr>
<tr>
<td>G7301A</td>
<td>Arg2434His</td>
<td>CCD/MH</td>
<td>210</td>
<td>HpaI</td>
<td>[4]</td>
</tr>
<tr>
<td>C487T</td>
<td>Arg163Cys</td>
<td>CCD/MH</td>
<td>76</td>
<td>BstUI</td>
<td>[3]</td>
</tr>
<tr>
<td>C1209G</td>
<td>Ile403Met</td>
<td>CCD/MH</td>
<td>92</td>
<td>MboI</td>
<td>[3]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mutation/subject No.</th>
<th>Max. response to 3 % halothane</th>
<th>Max. response to caffeine 2 mmol litre−1</th>
<th>Clinical signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1840T</td>
<td>2.6 g</td>
<td>1.1 g</td>
<td>Masseter muscle rigidity without acidosis. Muscle pain for several days. Normal histology.</td>
</tr>
<tr>
<td></td>
<td>3.4 g</td>
<td>0.1 g</td>
<td>Masseter muscle rigidity without fever or acidosis. 18 previous uneventful anaesthetics. Normal histology.</td>
</tr>
<tr>
<td>C487T</td>
<td>4.3 g</td>
<td>1.4 g</td>
<td>Referred for family history (sister was pyrexial to 41.7 °C). Subject had history of muscle cramps. Normal histology.</td>
</tr>
</tbody>
</table>

Table 1 Methods used to screen for the nucleotide and amino acid mutations in ryr1 associated with porcine MH or central core disease and MH

Table 2 Ryanodine receptor mutation frequency in MH positive (MH+) and negative (MH−) subjects

Table 3 In vitro contracture test results and clinical signs in the three subjects with identified mutations in the ryanodine receptor
Genotype and phenotype in MH

CCD-associated mutations examined. None of the four mutations examined was identified in our MH–population (table 2).

Discussion

The four mutations in ryrl examined in the present study account for a small proportion (approximately 3 %) of defects in MH+ subjects referred to our laboratory. There are problems in explaining human MH invoking the porcine mutation as the direct causative factor. Humans can be heterozygous for the mutation; however, heterozygous swine do not show signs of MH [14] and they do not have positive contracture tests by the North American MH Group testing protocol [14]. The two heterozygous patients with the porcine mutation did not have signs of a true MH episode; however, they did have positive contracture tests. A positive contracture test for MH occurred in swine homozygous for the porcine ryrl mutation, even though at the time of biopsy the pigs did not exhibit the MH syndrome when exposed to triggering anaesthetics [14]. Dissociation between the outcome of the diagnostic test and the clinical expression of the syndrome suggests that the pathophysiology of MH should be re-examined.

Some defects in the α-subunit of the sodium channel can cause myotonia fluctuans (a mild form of myotonia [18]), masseter muscle rigidity and a positive contracture response for MH [17], the latter two of which were the only signs observed in the two subjects with the C1840T mutation. Although masseter muscle rigidity, the only clinical sign in subject Nos 1 and 2, is associated with MH [19], it is a poor predictor of MH susceptibility in the absence of other signs [20]. Therefore, in these individuals the porcine mutation is not sufficient to cause MH, if we define the syndrome as a hypermetabolic response of skeletal muscle to triggering anaesthetics.

It is possible that those mutations that have been identified in ryrl might not be the causative MH defects per se. These polymorphisms may be in linkage disequilibrium with the actual mutation, which must be extremely close on the genome. However, the ryrl mutations may still serve as gene products that somehow play a role in enhancing the abnormal response of skeletal muscle to anaesthetics. A recent study has demonstrated that seven of 30 swine homozygous for the normal ryrl allele were halothane-sensitive [21], suggesting that a gene product other than ryrl may be causative, even in porcine MH. Alternatively, these mutations may cause MH, but genetic or environmental factors may regulate the penetrance of the mutation. For example, fatty acids are produced in excess in MH muscle [22, 23], the up-regulation of fatty acid production parallels the age-related onset of susceptibility in swine [24] and fatty acids markedly enhance halothane-induced Ca2+ release in MH– or MH+ skeletal muscle [25]. Until the causal factors are defined, we concur strongly with Hopkins, Halsall and Ellis [26] that extreme caution must now be used in considering the use of the currently described ryrl mutations for diagnosis of MH susceptibility.

The present study illustrates two main points that may be particularly important. First, patient No. 2 with the C1840T mutation would possibly have been diagnosed as equivocal by the European MH Group protocol, based on the weak outcome of the caffeine test and assuming that the halothane test would be positive by the European MH Group protocol. This finding, assuming that the C1840T mutation is causative of MH, supports the cautious interpretation of the equivocal classification adopted by the European MH Group. Second, one of the patients with the C1840T mutation had undergone 16 triggering anaesthetics without problem. The only problem experienced before referral had been masseter muscle rigidity, and defects in the α-subunit of the sodium channel can also produce these signs [17]. Therefore, it is clear that the link between ryrl mutations (if they do cause MH susceptibility), the MH syndrome and masseter muscle rigidity requires intervening modulators.

Acknowledgements

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References

9. Olckers A, Meyers DA, Meyers S, Taylor EW, Fletcher JE,


