Exclusion of defects in the skeletal muscle specific regions of the DHPR α1 subunit as frequent causes of malignant hyperthermia

Robert O O'Brien, Nichole L Taske, Philip M Hansbro, Klaus I Matthaei, Simon P Hogan, Michael A Denborough, Paul S Foster

Abstract
The molecular defect predisposing to the majority of malignant hyperthermia (MH) cases is unknown, although various point mutations in the ryanodine receptor gene (RYR1) have been associated with susceptibility in a small proportion of cases. We report here that one of these, the Arg163Cys substitution, does not cosegregate with MH susceptibility. Comparison of cDNA sequences encoding the skeletal muscle specific components of the dihydropyridine receptor α1 subunit between MH susceptible (MHS) and MH non-susceptible (MHN) patients was made in subjects without the reported MH linked RYR1 mutations. There were no differences within the sequence encoding the II-III loop or the IS3/IS3-IS4 segment, excluding defects in these functional segments of the α1 subunit as frequent causes of MH.

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Malignant hyperthermia (MH) presents clinically as a life threatening complication of anaesthesia and occurs in subjects with an underlying skeletal muscle disorder which is usually inherited as an autosomal dominant defect.1 Biochemical studies indicate that the primary defect in MH muscle lies in the regulation of calcium release from the sarcoplasmic reticulum (SR) membrane. The dihydropyridine receptor complex (DHPR) of the transverse tubule membrane and the ryanodine receptor (RYR1) of the SR membrane have been identified as key proteins involved in the regulation of SR Ca2+ release.2 The molecular defect underlying the majority of human MH cases is unknown, although various point mutations in the RYR1 gene, which can account for at most 20% of MH cases, have been associated with the disease.3–9 In this investigation we have extended the molecular characterisation of MH by sequencing the cDNA segments which encode the cytoplasmic II-III loop, the transmembrane segment (IS3), and the IS3-IS4 linking peptide (IS3/IS3-S4) of the human skeletal muscle DHPR α1 subunit. The α1 subunit acts as a voltage sensor for E-C coupling10; these components are highly specialised and critical for E-C coupling11 and an abnormality in either of these regions might alter pharmacological sensitivities and gating properties of the SR Ca2+ release channel. The cDNA sequences encoding these regions of the DHPR are relatively short and we were able to screen rapidly for mutations in MHS patients by direct sequencing. Furthermore, sequencing of the candidate gene segments from only a small sample of unrelated patients, each representing an affected family, was required to disclose polymorphisms of high frequency.

Genomic DNA (gDNA) was isolated from peripheral blood,12 and total RNA was isolated13 from frozen biopsy samples of human quadriceps. First strand cDNA was synthesised from total RNA in the presence of oligo dT, primer (25 µg/ml), dNTPs (1 mmol/l), approximately 2 µg of total RNA, and 200 units of M-MLV reverse transcriptase (Promega). Target cDNA segments were PCR amplified in an FTS-1 capillary thermal cycler (Corbett Research) in a volume of 20 µl using 5 µl of a 1 in 10 water dilution of the cDNA synthesis reaction mix as template. Primer sequences were based on the published cDNA sequences encoding the corresponding regions of the rabbit DHPR14 and are as follows: II-III loop Forward-5’GCTGAATTCTTTTCGTCGATCGTCCG3’, Reverse-5’GAAGAGCGAAGATGAAGTGGAAGTGAACCC3’; IS3/IS3-IS4 Forward-5’TCAAGCCGCAATGAACTCCATG3’, Reverse-5’TTTAGGACCCATCTGAAAGG3’. The reaction was carried out in 10 mmol/l Tris-HCl, (pH 9.0), 50 mmol/l KCl, 0-01% (w/v) gelatin, 1.5 mmol/l MgCl2, 0-1% (v/v) Triton X-100, with 0-25 mmol/dNTPs, 5 pmol of each primer, and 0-10 U Supertaq™ DNA polymerase (PH Stehelin et Cie AG). Amplification was performed for one cycle at 94°C for five minutes, 62°C for 20 seconds, 72°C for one minute; followed by five cycles at 94°C for 15 seconds, 62°C for 20 seconds, 72°C for one minute; followed by 31 cycles at 94°C for five seconds, 60°C for 20 seconds, 72°C for one minute; and finally for one cycle at 94°C for five seconds, 62°C for 20 seconds, 72°C for 15 minutes. Following PCR amplification, enzyme and unincorporated dNTPs were removed with the Wizard™ PCR Preps Purification System (Promega). Purified amplified fragments were then sequenced directly in both directions using the fmol™ DNA Sequencing System (Promega).

MH susceptibility of tested subjects was identified by using the established isolated contracture test.15 Patients were from families which had histories of MH episodes. No other neuromuscular disorders were present in the...
Diagnosis of MH susceptibility in the C/D family using the isolated muscle contracture test. The results are expressed as grams of tension produced by muscle fibre bundles. Subjects are diagnosed as MHS if the response to 3% halothane exceeds 0.5 g and if there is a response to a caffeine concentration of 2 mmol/l. Patient IV-5 was diagnosed in 1972 and muscle responses to caffeine at concentrations lower than 4 mmol/l were not obtained. Contracture responses to 3% halothane, however, were rated as very strong on a comparative score.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>3% halothane (g)</th>
<th>2 mmol/l caffeine (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV-2</td>
<td>MHS</td>
<td>1-90</td>
<td>0-40</td>
</tr>
<tr>
<td>IV-5</td>
<td>MHS</td>
<td>+ + +</td>
<td>Not determined</td>
</tr>
<tr>
<td>V-1</td>
<td>MHS</td>
<td>1-75</td>
<td>0-35</td>
</tr>
<tr>
<td>V-2</td>
<td>MHN</td>
<td>0-00</td>
<td>0-00</td>
</tr>
<tr>
<td>V-3</td>
<td>MHN</td>
<td>0-15</td>
<td>0-00</td>
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<tr>
<td>V-4</td>
<td>MHS</td>
<td>0-75</td>
<td>0-25</td>
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<td>MHS</td>
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<td>0-05</td>
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<tr>
<td>V-6</td>
<td>MHS</td>
<td>7-30</td>
<td>2-75</td>
</tr>
</tbody>
</table>

C/D family pedigrees. Family members heterozygous for the Arg163Cys mutation (+/-) and those homozygous for the absence of the Arg163Cys mutation (-/-) are indicated. Family members are numbered left to right through each generation I-V.

was no evidence of polymorphism in the 11 subjects examined, establishing the sequence of this segment of the human gene (GenBank Accession Number U14413). The cDNA sequence of the IS3/IS3-S4 linker segment (GenBank Accession Number U18986) in five unrelated MHS patients and in four MHN controls also showed no differences between subjects. The sizes of these samples ensure, with an 85% and 90% probability respectively, that all polymorphisms with a frequency above 10% in these cDNA segments would have been shown by our analysis. The absence of differences in the molecular structure of the II-III loop and the IS3/IS3-IS4 linker segments between MHS and MHN patients excludes a defect in this important functional region of the voltage sensor molecule as being a frequent cause of MH. The gene encoding the DHPR α1 subunit was also shown recently not to be linked to MH susceptibility in three families not linked to the RYR1 gene on chromosome 19. This finding supports the conclusion from our investigation that a defect in the DHPR α1 subunit is unlikely to be a major cause of MH.

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