Exclusion of defects in the skeletal muscle specific regions of the DHPR α_1 subunit as frequent causes of malignant hyperthermia

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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 co-segregate with MH susceptibility. Comparison of cDNA sequences encoding the skeletal muscle specific components of the dihydropyridine receptor α_1 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 α_1 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 Ca^{2+} release.^{2,3} 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.⁴⁻⁹ 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 α_1 subunit. The α_1 subunit acts as a voltage sensor for E-C coupling³; these components are highly specialised and critical for E-C coupling^{10,11} and an abnormality in either of these regions might alter pharmacological sensitivities and gating

properties of the SR Ca^{2+} 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 dT₁₅ 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'GCTGAATGTCTTCCTG-GCCATCGCCG3', Reverse-5'GAAGAGCAGGATGAAGTTGGTGAACCC3'; IS3/IS3-IS4 Forward-5'TCGAAGCCGCCATGAAGATCATCG3', Reverse-5'GTTGAGGACCACCT-GCAAAGTAGG3'. 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 MgCl_2 , 0.1% (v/v) Triton X-100, with 0.25 mmol/l dNTPs, 5 pmol of each primer, and 0.10 U SupertaqTM DNA polymerase (P H 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 WizardTM PCR Preps Purification System (Promega). Purified amplified fragments were then sequenced directly in both directions using the fmoITM 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

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C/D family pedigree. 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.

people examined. The MHS patients selected for comparative sequence analysis were screened to exclude mutations in the RYR1 gene previously reported to cosegregate with MH.⁴⁻⁹ Two MHS patients (IV-5 and V-6) were found to be heterozygous for the Arg163Cys mutation (figure). Other members of this family also diagnosed as MHS (V-1, V-4) by means of the in vitro contracture test (table) were shown not to possess this mutation (figure), suggesting that the Arg163Cys mutation is not linked with the MH phenotype in all members of this family. Although unlikely, it is also possible that there are two causative mutations segregating in the family. Recently, evidence has also been presented of families in which the MH phenotype did not cosegregate with the Gly341Arg nor the Arg614Cys substitutions, suggesting that these polymorphisms alone may not cause MH.¹⁶

The 378 bp cDNA sequence encoding the DHPR II-III loop was compared between six MHS patients and five MHN controls. There

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-S4 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 α_1 subunit was also shown recently not to be linked to MH susceptibility in three families not linked to the RYR1 gene on chromosome 19.¹⁷ This finding supports the conclusion from our investigation that a defect in the DHPR α_1 subunit is unlikely to be a major cause of MH.

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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.

Patient	Diagnosis	3% halothane (g)	2 mmol/l caffeine (g)
IV-2	MHS	1.90	0.40
IV-5	MHS	++ +	Not determined
V-1	MHS	1.75	0.35
V-2	MHN	0.00	0.00
V-3	MHN	0.15	0.00
V-4	MHS	0.75	0.25
V-5	MHN	0.25	0.05
V-6	MHS	7.30	2.75

1 Foster PS. Malignant hyperpyrexia. *Int J Biochem* 1989;22: 1217-22.

2 Catterall WA. Functional subunit structure of voltage-gated calcium channels. *Science* 1991;253:1499-500.

3 Miller RJ. Voltage-sensitive Ca^{2+} channels. *J Biol Chem* 1992;267:1403-6.

4 Gillard EF, Otsu K, Fujii J, *et al*. A substitution of cysteine for arginine 614 in the ryanodine receptor is potentially causative of human malignant hyperthermia. *Genomics* 1991;11:751-5.

5 Quane KA, Keating KE, Manning BM, *et al*. Detection of a novel common mutation in the ryanodine receptor gene in malignant hyperthermia: implications for diagnosis and heterogeneity studies. *Hum Molec Genet* 1994;3:471-6.

6 Quane KA, Healy JMS, Keating KE, *et al*. Mutations in the ryanodine receptor gene in central core disease and malignant hyperthermia. *Nature Genet* 1993;5:51-5.

7 Phillips MS, Khanna VK, DeLeon S, Frodis W, Britt BA, MacLennan DH. The substitution of Arg for Gly⁴³³ in the human skeletal muscle ryanodine receptor is associated with malignant hyperthermia. *Hum Molec Genet* 1994;3: 2181-6.

8 Gillard EF, Otsu K, Fujii J, *et al*. Polymorphisms and deduced amino acid substitutions in the coding sequence of the ryanodine receptor (RYR1) gene in individuals with malignant hyperthermia. *Genomics* 1992;13:1247-54.

9 Zhang Y, Chen HS, Khanna VK, *et al*. A mutation in the human ryanodine receptor gene associated with central core disease. *Nature Genet* 1993;5:46-50.

10 Nakai J, Adams BA, Imoto K, Beam K. Critical roles of the S3 segment and S3-S4 linker of repeat I in activation of L-type calcium channels. *Proc Natl Acad Sci USA* 1994; 91:1014-18.

11 Zhang JF, Ellinor PT, Aldrich RW, Tsien RW. Molecular determinants of voltage-dependent inactivation in calcium channels. *Nature* 1994;372:97-100.

12 Kendall TL, Byerley DJ, Dean R. Isolation of DNA from blood. *Anal Biochem* 1991;192:74-6.

13 Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987;162:156-9.

14 Tanabe T, Takeshima H, Mikami A, *et al*. Primary structure of the receptor for calcium channel blockers from skeletal muscle. *Nature* 1987;328:313-18.

15 Moulds RFW, Denborough MA. Identification of susceptibility to malignant hyperpyrexia. *BMJ* 1974;ii:245-7.

16 Olckers A, Vita GM, Rosenberg H, Fletcher J, Isaacs H, Levitt RC. Exclusion of the reported Arg 614 and Gly 341 ryanodine receptor mutations as being responsible for MHS in two families that map to chromosome 19q13.1. *Proceedings of the 11th International Symposium on Malignant Hyperthermia, Hiroshima, Japan, APP-4*, 1994.

17 Sudbrak R, Golla A, Hogan K, *et al*. Exclusion of malignant hyperthermia susceptibility (MHS) from a putative MHS2 locus on chromosome 17q and of the α_1 , β_1 and γ subunits of the dihydropyridine receptor calcium channel as candidates for the molecular defect. *Hum Molec Genet* 1993; 2:857-62.