SHORT REPORT

Identification of a novel mutation in the ryanodine receptor gene (*RYR1*) in a malignant hyperthermia Italian family

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Malignant hyperthermia (MH) is an inherited autosomal dominant pharmacogenetic disorder and is one of the main causes of death subsequent to anaesthesia. Around 50% of affected families are linked to the ryanodine receptor (*RYR1*) gene. To date, 19 mutations have been identified in the coding region of this gene and appear to be associated with the MH-susceptible phenotype. Here we report the identification by two independent methods of a novel mutation associated with the MH-susceptible phenotype in the *RYR1* gene: the 6488G- > C transversion, resulting in the replacement of the Arg2163 with a proline residue. *European Journal of Human Genetics* (2000) **8**, 149–152.

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Introduction

Malignant hyperthermia (MH) is a pharmacogenetic disease, triggered by widely used anaesthetics and depolarising muscle relaxants. Clinical symptoms of an MH attack are hyperthermia, accelerated muscle metabolism, muscle contractions, metabolic acidosis and tachycardia and, unless immediately recognised and treated, it is often fatal.[1] Malignant hyperthermia susceptibility (MHS) can be diagnosed by an *in vitro* test, based on the differential contractile response of normal (MHN) and MHS muscles to caffeine and halothane. A standardized European *in vitro* contracture test (IVCT) was established in 1984.[2] Human MHS is inherited as an autosomal dominant trait. A considerable genetic heterogeneity has been reported for MH. Six MH loci have been identified on human chromosomes 19q13.1 (MHS1, MIM 180901), 17q11.2–q24 (MHS2, MIM 154275), 7q21–q22 (MHS3, MIM 154276), 3q13.1 (MHS4, MIM 600467), 1q32 (MHS5, MIM 601887) and 5p (MHS6, MIM 601888), although significant lod score values have been found only for the linkage to the MHS1, MHS4 and MHS5 loci. About 50% of affected families are linked to the MHS1 locus where the gene encoding the skeletal muscle calcium release channel of the sarcoplasmic reticulum, commonly known as ryanodine receptor (RYR1), maps.[1] To date, 19 mutations associated with the MHS phenotype have been identified in the coding region of the *RYR1* gene (reviewed by Manning *et al*,[3] and Barone *et al.*[4]); they are clustered in two regions, the aminoterminal (amino acid residues 35-614) and central (amino acid residues 2163-2458) regions, and 15 of them have been investigated and have shown a functional effect on the calcium channel behaviour of *RYR1*.[5] Here we report a novel mutation located in the central portion of the *RYR1* gene in an Italian pedigree.

Materials and methods

The proband, individual 8 (Figure 1, A), during induction of anaesthesia for plastic hernia developed masseter spasm, and a temperature of 38°C, and the anaesthetic was stopped. Before surgery, the creatine kinase level was 98 U/L (normal range 0–190 U/L) and increased to 10000 U/L 24 h later. Seven members of the family underwent the standardised European IVCT.[2] A force increase of at least 0.2 g following either 2% halothane or 2 mM caffeine exposure is required to score a sample as MHS. Contracture thresholds at higher concentrations than these were considered normal.

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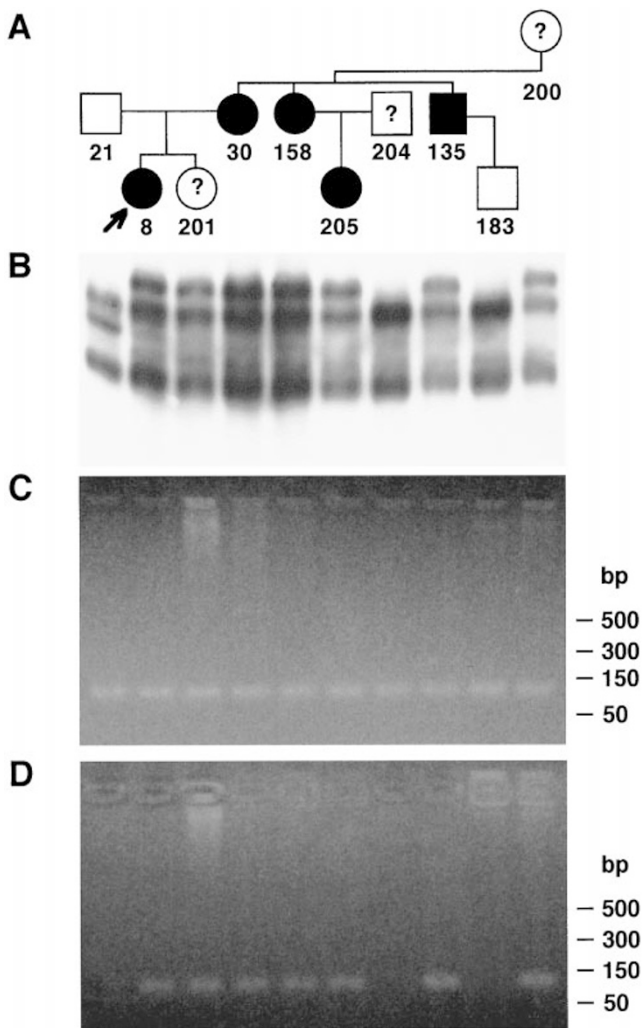
Figure 1 Pedigree of the NA-3 family; solid symbols: MHS individuals identified by IVCT; open symbols: IVCT-typed MHN individuals; ?: untested members of the pedigree. The arrow indicates the index case who experienced an MH episode. B: Autoradiograph of the SSCP analysis of exon 39 of the RYR1 gene in the family NA-3. C and D: ARMS detection of the 6488G->C transversion; gel electrophoresis of the amplification products obtained using as forward primers the wild type sequence (F39 wild type oligonucleotide, see Methods) (C), or the mutated sequence (F39 mut oligonucleotide, see Methods) (D). The patterns of panels B, C, and D are vertically aligned so that they correspond to the members of pedigree in panel A.

DNA was extracted from 10 ml of whole blood samples. All the samples were screened for the Arg163Cys, Gly248Arg, Gly341Arg, Arg614Cys, Arg614Leu, Arg2163Cys, Arg2163His and Val2168Met, Gly2435Arg, Arg2458His and Arg2458Cys mutations, as previously described.[3,6–12]

A 253-bp fragment of RYR1 (exon 39), coding for amino acid residues 2110-2183, was amplified using the primers F39 (5'-ACTTCGTGCAGAGCCCCAGC) and R39 (5'-TGCAAG-TAAGGGGAGGGCGG) under standard polymerase chain reaction (PCR) conditions. The thermal profile was 35 cycles at 94°C, 63°C and 72°C. For the single-strand conformation polymorphism (SSCP) analysis, the samples were run on 5% polyacrylamide (acrylamide:N,N'-methylene-bis-acrylamide, 99:1) gel with 5% glycerol in 0.5 × TBE at 30 mA and at 4°C. The DNA was sequenced by fluorescent dye-terminator cycle sequencing on an automated sequencer (ABI 373A, Applied Division, Perkin Elmer, Foster City, USA).

For the amplification refractory mutation system (ARMS) technique, both forward (F) primers contained an additional mismatch three bases from the 3' end to increase the specificity. The oligonucleotide primers were: F39 wild type, 5'-CGAGTGCCTCGGCCAGATTCG, F39 mut, 5'-CGAGTG CCTCGGCCAGATTCC, and R39. The PCR reactions were carried out with 0.05 μmol/l of each primer, 0.025 mmol/l dNTPs and 1 mmol/l MgCl$_2$l; the annealing temperature was 66°C. The amplification products (111 bp) were analysed by 2% agarose gel electrophoresis.

The pedigree was typed with polymorphic microsatellite markers for loci D19S191, D19S220, D19S422 and D19S223 as described elsewhere.[13]

Results

Genomic DNA samples from 31 unrelated MHS patients were screened for 11 of the most frequent RYR1 mutations (see Materials and methods), accounting for approximately 40% of European MH cases.[3] Only one of the investigated mutations, the Arg614Cys, was found and only in one subject. Mutations in exon 39 were searched for using the SSCP method. An aberrant SSCP pattern was detected only in one MHS individual (subject 8 of Figure 1, A). This unique pattern segregates in the family (NA-3 pedigree) with the MHS phenotype (Figure 1, A and B). Direct DNA sequencing on both directions of amplified exon 39 from all the MHS individuals of the NA-3 pedigree identified the presence at the heterozygous status of a 6488G->C transversion, resulting in the replacement of Arg2163 with a proline residue. SSCP analysis revealed that the candidate mutation was absent from 110 normal chromosomes (data not shown). The segregation of the G6488->C transversion in the family was confirmed by the ARMS technique (Figure 1, C and D): all the DNA samples that showed an aberrant SSCP pattern were amplified with the mutated primer (F39 mut) (Figure 1, D). The pedigree was also typed with polymorphic microsatellite markers of chromosome 19 (data not shown). A specific haplotype cosegregated with the MHS phenotype and with the 6488G->C substitution.

The IVCT results on muscle samples from seven members of the NA-3 pedigree are shown in Table 1.

Discussion

The novel mutation described here occurs at the same codon as two previously identified mutations: the Arg2163Cys

Table 1 IVCT results of NA-3 family

| Individuals | Halothane | | | Caffeine | | | Diagnosis |
	Threshold* %	Contracture (g) at threshold	at 2%	Threshold* mmole/l	Contracture (g) at threshold	at 2 mmole/l	
21	n.d.	n.d.	–0.6	>4	n.d.	–0.4	MHN
30	1.5	0.3	0.4	2	0.2	0.2	MHS
158	0.5	0.3	1.7	1.5	0.2	0.5	MHS
135	0.5	0.2	2.1	0.5	0.45	1.6	MHS
8	1	0.2	0.3	2	0.2	0.2	MHS
205	0.5	0.3	2.1	1	0.3	1.5	MHS
183	n.d.	n.d.	–0.3	4	1.25	–0.5	MHN

*at which a contraction ≥ 0.2 g in muscle fibre was obtained   n.d. = not determined

replacement, reported in approximately 4% of MH-susceptible individuals, and the Arg2163His replacement reported in one family.[3] The novel mutation was not present in 110 chromosomes from MHN-tested individuals; in addition, Manning et al.[3] reported the absence of mutations at codon 2163 in 200 normal chromosomes. Taken together, these observations indicate that the replacement of Arg2163 with a proline residue is very unlikely to be a polymorphism. The Arg2163Pro mutation has been identified so far only in one family (out of 31 MHS unrelated subjects) and strictly segregates with the MHS phenotype and with a specific chromosome 19 haplotype. Moreover, no other RYR1 mutations, among 11 of the most frequent mutations analysed, were detected in this pedigree. On the basis of these arguments, the novel mutation described fulfils the criteria for an MH causative mutation. Therefore, subjects 200 and 201, although not typed by IVCT, should be regarded as MHS for clinical purposes, since they bear the Arg2163Pro substitution with a high-risk familial haplotype.

In a recent statistical analysis of IVCT data for 11 known RYR1 mutations a significant correlation has been reported for each mutation between IVCT threshold values and tension values for the caffeine test but not for the halothane test.[3] Even though all the MHS subjects of pedigree NA-3 bear the same mutation, IVCT responses to caffeine and halothane varied widely. Nevertheless, if the caffeine threshold values ($\bar{x}$ = 1.4 mM, SEM = 0.29) and tension at 2 mM caffeine values ($\bar{x}$ = 0.8 g, SEM = 0.31) of the five MHS-typed individuals of NA-3 family are positioned within the plot devised by Manning et al. (see Figure 2, A of Manning et al.),[3] the novel mutation fits with the data plotted therein and shows an IVCT phenotype intermediate between those generated by the Val2168Met and the Arg614Cys mutations and milder than that produced by the Arg2163Cys mutation.

MH causative mutations, located in the central part of RYR1 (amino acid residues 2163-2458), account for approximately 23% of affected subjects. Functional domains have been identified in the proximity of this region, ie a phosphorylation site at Ser2843 (Ser 2844, new numbering)[14] and calmodulin binding sites.[15] Furthermore, it has been demon-strated that the RYR1 regions encompassing amino acid residues 1641-2437[16] and 1635-2636[17] are essential for the conduction properties of the calcium release channel. Therefore, the novel mutation described here lies in a portion of the molecule crucial for its function.

The identification of novel mutations in the RYR1 gene associated with the MHS phenotype can contribute to the diagnosis. Although the IVCT is the gold standard test to establish the risk of MH susceptibility, if an individual bears one of the known MH causative mutations, he can be considered MH-susceptible even without an IVCT result. Furthermore, even though the diagnostic parameters of the IVCT were established to privilege sensitivity (99%) rather than specificity (93.6%), the genetic analysis, considering the high risk of an MH attack, can be of significance to identify and evaluate the few cases of discordance between genotype, characterised by the presence of a causative mutation, and MH-normal typed phenotype.[13,18]

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