Homozygous and heterozygous Arg614Cys mutations (1840C→T) in the ryanodine receptor gene co-segregate with malignant hyperthermia susceptibility in a German family

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The determination of susceptibility to malignant hyperthermia (MH) by genetic investigation is a controversial issue because of the genetic heterogeneity of this disorder. The requirement for such an approach in MH diagnosis is a strong correlation between MH-associated genetic abnormalities and phenotypic findings in the in vitro contracture test (IVCT). After a severe clinical MH crisis during general anaesthesia a patient was diagnosed by the IVCT in which susceptibility to MH was confirmed. Genetic screening for MH-related mutations in the RYR1 gene revealed the presence of a homozygous 1840C→T base exchange (Arg614Cys substitution) in this patient. A specific search for this defect in 20 relatives led to the identification of a total of 11 Arg614Cys mutations. Of these, 10 were heterozygous (including both parents) and one was homozygous (sister). Further IVCTs were subsequently performed on the parents of the index patient, the homozygous sister and all relatives who did not carry the Arg614Cys in order to determine the genotype/phenotype correlation. After analysing these data, and because of the strong correlation between clinical, phenotypic, and genetic results in the index patient, we assigned the diagnosis ‘MHS’ to all the remaining Arg614Cys mutation carriers of that family without performing the IVCT.

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Malignant hyperthermia (MH) is an inherited autosomal-dominant disorder in which halogenated inhalational anaesthetics and depolarizing neuromuscular blocking drugs may trigger a life-threatening increase of muscle metabolism in susceptible individuals.1 MH may occur in any age group. The typical severe MH crisis is characterized by masseter spasm, generalized muscle rigidity, tachycardia, hypercapnia, cyanosis, high fever, and mixed acidosis. The clinical signs of this hypermetabolic syndrome are caused by an increased concentration of free myoplasmic calcium. However, an MH episode may also be associated with slight, non-specific, ambiguous symptoms which can make the diagnosis difficult and possibly delay therapeutic intervention.2–4 To complicate matters, MH symptoms may also occur after repeated trouble-free general anaesthesia with triggering agents. Nevertheless, the outcome for the patient depends on the severity of the clinical presentation of MH, and this in turn depends on early diagnosis and initiation of treatment.

The MH disposition in suspected individuals or families may be determined by in vitro contracture testing (IVCT) of a skeletal muscle biopsy; this is at present felt to be the most reliable diagnostic method. The procedures for IVCT were developed by the European and North American malignant hyperthermia groups (EMHG, NAMHG). They show a high sensitivity (99% for EMHG, 92–97% for NAMHG) and specificity (94% for EMHG, 53–78% for NAMHG).5–7 Besides determining MH disposition, the IVCT is the basic requirement for interpreting genetic MH data.

An important step towards understanding the pathogenesis of the disease was the identification of MH-associated genetic mutations. The ryanodine receptor (RYR1), which represents the calcium release channel of skeletal muscle sarcoplasmic reticulum, was considered to
be the primary locus for MH susceptibility. In approximately 50% of MH families, a link with the coding region of the RYR1 gene (MHS1 locus on chromosome 19q12–13.1) can be detected. Over 20 point mutations in the RYR1 gene have so far been found to segregate with the MHS phenotype. For most of these missense mutations it has been possible to identify functional effects on the calcium channel reflecting an increased sensitivity of calcium release to triggering substances. Because of this established genotype/phenotype correlation, carriers of a known MH-related mutation should be regarded as having a high risk of MH. A genetic MH analysis may, therefore, be useful for family screening. Guidelines for molecular genetic work have recently been compiled by the EMHG. Using a family with a predisposition to MH as an example, we shall now discuss the diagnostic approaches currently available for determining the MH disposition.

Patients and methods

Patients

The MH family was identified through a 43-yr-old index patient who developed typical symptoms of a severe MH crisis during general anaesthesia for an appendectomy. Anaesthesia was induced with thiopental, in a dose sufficient to abolish the eyelash reflex (400 mg i.v.). Alfentanil (1.5 mg i.v.) and succinylcholine (100 mg i.v.) were given to facilitate orotracheal intubation with a cuffed tube. Anaesthesia was maintained with 0.6–1.0% halothane and 60% nitrous oxide in oxygen. Five minutes after the induction of anaesthesia, the patient developed a tachycardia of 160 beats min⁻¹; the end-tidal concentration of carbon dioxide subsequently rose rapidly to 9.33 kPa and the body temperature to 39.6 °C so that the patient was immediately suspected of developing MH. Standard treatment for an acute MH reaction, including the use of dantrolene, was successful. In the post-operative period the serum creatine kinase concentration reached 22 000 IU litre⁻¹.

Three months after this incident the patient underwent the IVCT.

In vitro contracture test

The IVCT was performed according to the criteria of the standard procedure of the European MH group. The test determines the threshold concentrations of halothane and caffeine that produce a contracture force ≥2 mN in an isolated muscle specimen. Depending on the results, the tested individual is classified as MH susceptible (MHS). MHS: contracture force ≥2 mN at a caffeine concentration of 2.0 mmol litre⁻¹ or less and a halothane concentration of 0.44 mmol litre⁻¹ or less; MH negative (MHN): contracture force ≥2 mN at a caffeine concentration of 3.0 mmol litre⁻¹ or more and a halothane concentration greater than 0.44 mmol litre⁻¹; or MH equivocal (MHE): contracture force ≥2 mN at a caffeine concentration of 2.0 mmol litre⁻¹ or less (MHEc) or a halothane concentration of 0.44 mmol litre⁻¹ or less (MHEh).

Molecular genetic investigation

Direct sequencing

Genomic DNA was prepared from whole blood. The index patient was screened for published MH-related mutations in the amino terminal and central region of the RYR1 gene by PCR amplification and direct sequencing. Purified PCR products were directly sequenced by the ABI Prism DNA Sequencing Kit (Perkin Elmer, USA). The samples were then loaded on 5.25% PAGE-PLUS gel (Amresco), electrophoresed in the ABI PRISM 377 Sequencer (Perkin Elmer) and aligned with Sequence Navigator Software (Perkin Elmer). Both strands were sequenced and compared to permit identification of ambiguities.

Restriction enzyme analysis

A 918-bp PCR product of RYR1 exon 17 was digested with the restriction enzyme RsaI. Standard PCR conditions were used for amplification: denaturation at 95°C for 1 min, annealing at 62°C for 1 min, extension at 72°C for 2 min, 30 cycles; forward primer 5'-TTG CCA CAT CTT ATC CCG ATG CGC; reverse primer 5'-GAA CCT GTC CAG AGA

TGC AGT CCA TC. After purification, the PCR product was mixed with 10 U of the restriction enzyme RsaI and incubated for 8 h at 37°C. According to GenBank (Accession no.: U48557) the following fragments were to be expected (OMIGA 1.1, Oxford Molecular Ltd.): normal type 166, 553, 12, 185, 2 bp; heterozygous C1840T-mutation 719, 185, 12, 2 bp; homozygous C1840T-mutation 719, 185, 12, 2 bp. A 719-bp fragment occurred because of the loss of one restriction site (553+166 bp).

Results

When the index patient’s symptoms were rated on the clinical grading scale for MH the likelihood of MH was found to be very high indeed (raw score range: 50+; MH rank: 6). The clinical MH diagnosis was confirmed by the results of the IVCT in which the patient showed low threshold concentrations and high contracture forces for halothane and caffeine (Table 1, II:3). The molecular genetic search for the most frequent MH-related mutations by the direct sequencing technique revealed the presence of a base exchange from C to T at position 1840 of the RYR1 gene (replacement of Arg614 by Cys), surprisingly in a homozygous state.

All the relatives were then screened specifically for the Arg614Cys mutation by restriction enzyme analysis (Fig. 1). As expected, both parents (I:1, I:2) were heterozygous carriers of the mutation. The mutation was also detected in
all the siblings of the index patient. A further homozygous base exchange was found in one sister (II:9), and her children were necessarily affected by this mutation too (III:7, III:8). The molecular genetic results for the whole family are shown in Figure 1. Out of 21 individuals examined genetically, the Arg614Cys mutation was detected in 12; of these, 10 were heterozygous and two were homozygous. The presence of two homozygous Arg614Cys mutations within one family is an extremely rare finding. According to the EMHG guidelines, those family members who did not carry the mutation observed in the pedigree had to undergo the IVCT investigation. As Table 1 shows, it was possible to exclude a positive MH disposition with certainty for these individuals because of the MHN diagnosis in the IVCT. Moreover, no discordance between the IVCT and the genetic investigation was observed in either MHS or MHN individuals.

An additional IVCT investigation was carried out on the homozygous sister and both heterozygous parents of the index patient as it seemed useful to compare threshold concentrations and contracture forces of these different genotypes. As expected, the homozygous individuals showed significantly lower threshold concentrations and noticeably higher contracture forces for the two triggering substances halothane and caffeine (Table 1).

### Discussion

The role of the RYR1 gene in MH has been confirmed by the discovery of numerous RYR1 missense mutations in MH families. More than 20 mutations have so far been identified as segregating with the MHS trait; most mutation carriers are heterozygous. MHS homozygotes are considered rare in the affected MH population. Only two MHS homozygotes have been detected for the Cys35Arg and one for the Arg614Cys mutation. The Arg614Cys mutation was the first human mutation found to be associated with MH. The majority of patients

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**Table 1** Results of genotyping (search for the MH-related mutation Arg614Cys) and phenotyping (in vitro contracture test) of individuals for whom both diagnostic methods were used

<table>
<thead>
<tr>
<th>Individual</th>
<th>Threshold concentration</th>
<th>Contracture force (mN)</th>
<th>Diagnosis</th>
<th>Arg614Cys mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Halothane mmol litre⁻¹</td>
<td>Caffeine mmol litre⁻¹</td>
<td>Halothane 2 vol%</td>
<td>Caffeine mmol litre⁻¹</td>
</tr>
<tr>
<td>I:1</td>
<td>0.44</td>
<td>2.0</td>
<td>5.5</td>
<td>5</td>
</tr>
<tr>
<td>I:2</td>
<td>0.22</td>
<td>2.0</td>
<td>15</td>
<td>3.5</td>
</tr>
<tr>
<td>II:3</td>
<td>0.11</td>
<td>0.5</td>
<td>23.5</td>
<td>23</td>
</tr>
<tr>
<td>II:9</td>
<td>0.11</td>
<td>0.5</td>
<td>21</td>
<td>19</td>
</tr>
<tr>
<td>II:10</td>
<td>0.66</td>
<td>3.0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>II:12</td>
<td>&gt;0.66</td>
<td>&gt;4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>III:1</td>
<td>&gt;0.66</td>
<td>&gt;4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>III:2</td>
<td>&gt;0.66</td>
<td>&gt;4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>III:4</td>
<td>&gt;0.66</td>
<td>&gt;4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>III:5</td>
<td>&gt;0.66</td>
<td>&gt;4</td>
<td>0</td>
<td>0.5</td>
</tr>
</tbody>
</table>

**Fig 1** Restriction enzyme analysis. The identification of the Arg614Cys mutation is represented by the appearance of the 719-bp fragment in the gel electrophoresis. The index patient (II:3) and his sister (II:9) are homozygous for this mutation (loss of the 166-bp fragment). The parents and the remaining siblings are heterozygous carriers. Band A indicates the normal type, band B the undigested 918-bp sequence of the RYR1 gene. The 12 bp and 2 bp fragments are not visible.
carrying this mutation were classified as MH-susceptible in the IVCT. However, some cases of discordance between the genotype and the phenotype were described for the Arg614Cys mutation, and this raised doubts as to whether the mutation is really the cause.20 21 Most doubts concerned discrepancies between MHS results in the IVCT and the absence of the mutation. Attempts were made to explain these findings by implicating further independent unknown mutations, which might cause MH; therefore, suggesting that the single-gene model underlying MH may be incorrect. This interpretation was reinforced by the identification of five additional MH loci on chromosomes 17q, 7q, 3q, 1q, and 5p22-25.

On the other hand, an explanation for the occurrence of genotype/phenotype discordance may lie in the IVCT, which does not guarantee total phenotypic accuracy. According to the EMHG procedure the IVCT has a sensitivity of 99% and a specificity of 94%.6 Moreover, a recent study shows a considerable between-centre variability of the IVCT results for the same patient.26

The study described here is an excellent example of the effects of the Arg614Cys mutation on the MH phenotype.

Fig 2 Sequencing of the gene segment for the ryanodine receptor with the suspected Arg614Cys (1840C→T) mutation. A complete C to T transition in both alleles (homozygous inheritance) was identified in the index patient; the parents carried the mutation in a heterozygous state.

Fig 3 Prevalence of the RYR1 mutation Arg614Cys in the MH family investigated. The figure shows the results of molecular genetic screening and IVCT. Filled symbols denote persons tested with the IVCT and typed as MHS, opened symbols denote persons tested and typed as MHN, open symbols with question mark denote untested family members. Genotype: ++, homozygous mutation carrier; +/−, heterozygous mutation carrier; −/−, absence of the mutation. Individual II:3 (arrow) is the index patient who had a MH event.
A strong correlation between clinical, IVCT and molecular genetic findings was first established for the homozygous index patient who had developed an MH crisis. Furthermore, both the homozygous sister (II:9) and the heterozygous parents (I:1, I:2) were diagnosed as MHS in the IVCT. The two homozygous individuals showed much greater contracture responses to both halothane and caffeine than the heterozygous subjects. The absence of the Arg614Cys mutation in all MHN individuals also supports the strong association of the mutations with susceptibility. No genotype/phenotype discordance was observed for MHS or MHN individuals.

These results support previous assumptions that inheritance of homozygous-dominant alleles is associated with a more severe phenotype possibly arising from a more deleterious change to the channel structure. On the other hand, both the homozygous and the heterozygous individuals were phenotypically healthy and there were no clinical signs indicating the presence of the MH disposition.27 28

Our findings suggest that the Arg614Cys mutation is the cause of the MH phenotype in this family. The mutation was inherited over three generations and was only present in MHS individuals. Our results are supported by functional expression studies in which Arg614Cys mutant channels showed an increased sensitivity of intracellular calcium release to halothane and caffeine13 or in which the calcium release was activated at lower depolarizing potentials.29

A second objective of this study was to assign MH susceptibility from identification of the familial mutation.16 On the basis of the genotype/phenotype correlation described above we assigned the MHS diagnosis to eight further mutation carriers in this family without performing the invasive IVCT. These individuals, especially, profited from the identification of the familial RYR1 mutation and did not have to undergo the muscle biopsy; this was reflected in a much greater compliance on their part concerning the MH investigation. Individuals who do not carry the familial mutation should still undergo the IVCT because of the genetic complexity of MH.16

After establishing the MH disposition in the index patient, a commonly used testing strategy is to perform an IVCT on one parent in the first instance. If this produced a clear MHS diagnosis it would be possible to assume that the other parent is MHN and does not need to undergo the invasive IVCT because the likelihood of the other parent also being MH-susceptible is very small (1:10 000 to 1:60 000 where volatile anaesthetics are used30 31). With our family, this strategy could have had disastrous consequences for the second parent. Our results support the suggestion that both parents of a MHS subject should always be investigated.32

Our study has demonstrated that genetic data for MH status can in some cases provide additional diagnostic information and prove a valuable supplement to diagnostic testing for MH.

References

11 Ball SP, Johnson KJ. The genetics of malignant hyperthermia. J Med Genet 1993; 30: 89–93
13 Tong J, Ouyum H, Demaureux N, Grinstein S, McCarthy TV, MacLennan DH. Caffeine and halothane sensitivity of intracellular Ca2+ release is altered by 15 calcium release channel (ryanodine receptor) mutations associated with malignant hyperthermia and or central core disease. J Biol Chem 1997; 272: 26332–9
14 Larach MG, Mac Lennan DH. How carefully can we phenotype patients suspected of malignant hyperthermia susceptibility? Anesthesiology 1999; 90: 645–8
18 Larach MG, Localio AR, Allen GC, et al. A clinical grading scale to


