

## COMMENTARY

## Guidelines for molecular genetic detection of susceptibility to malignant hyperthermia<sup>†</sup>

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Malignant hyperthermia (MH) is a potentially fatal pharmacogenetic disease triggered by several anaesthetic agents. The *in vitro* muscle contracture test (IVCT) is the standard test to establish an individual's risk of susceptibility to MH. Clinical practitioners and geneticists of the European MH Group have agreed on the present guidelines for the detection of MH susceptibility using molecular genetic techniques and/or IVCT to predict the risk of MH.

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Malignant hyperthermia (MH) is a potentially fatal pharmacogenetic disease triggered by commonly used potent inhalation anaesthetics and/or succinylcholine. The *in vitro* muscle contracture test (IVCT) is the standard test to establish an individual's risk of susceptibility to MH.<sup>1</sup> The European MH Group has developed a standardized protocol for the IVCT and has initiated and fostered international collaborative molecular genetic studies to investigate the molecular basis of MH. Data from these studies demonstrate that MH displays a high level of locus heterogeneity. Thus, it is not feasible to diagnose MH susceptibility, and, more specifically, to exclude MH risk, on the basis of a simple genetic test alone. However, it is of utmost importance to avoid false MH-negative (MHN) diagnoses because of the potential risk of MH during general anaesthesia for these patients and their offspring. These general obstacles notwithstanding, there may be specific situations where genetic data provide additional diagnostic information or contribute information independent of IVCT. It is the purpose of this document to outline recommended procedures for the potential diagnostic use of such genetic findings depending on the different clinical situations that may arise.

### Referrals

The usual route of entry for individuals into MH investigations follows a suspected MH crisis and referral of the patient to an MH Investigation Unit, where diagnostic

procedures and genetic counselling should be performed according to Figure 1.

### IVCT

An IVCT is performed on the patient or, if the patient is too young or has not survived the anaesthetic event, his or her parents. If MH susceptibility status is confirmed by IVCT, then there is a clinical responsibility to offer the IVCT to the relatives of the index case, assuming autosomal dominant inheritance and starting with first-degree relatives.

### Genetic investigations

#### *Mutation analysis*

At this stage, molecular genetic testing for causative mutations in the ryanodine receptor gene (*RYR1*) of the index case could lead to quicker results for the rest of the kinship. An up-to-date list of mutations that have been shown to directly alter *RYR1* caffeine or halothane sensitivity is shown in Table 1.

Genetic analysis should be performed in, or only after consultation with an MH Investigation Unit. Once a causative mutation has been detected in the proband or index patient, it can be used to test relatives who have not

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yet been tested by the IVCT. Mutation carriers should consequently be regarded as susceptible to MH. However, family members who do not carry the mutation observed in the pedigree should still undergo IVCT investigation. The reason for such caution is the observation in several pedigrees investigated by members of the European MH

Group of discordance between genetic and IVCT results, implicating a second MH susceptibility gene segregating in the kinship.<sup>2,3</sup>

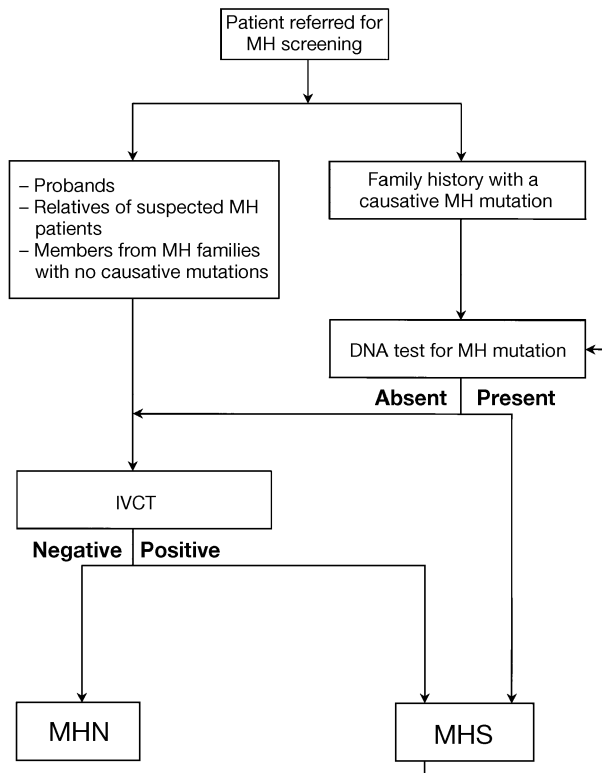
### Segregation analysis

Once the MH status of the extended pedigree (e.g. 10 informative meioses) has been determined by the IVCT, it may be possible to undertake genetic segregation analysis with markers close to known MH susceptibility loci. An up-to-date list of recommended markers and details of genetic modelling compiled by the European Malignant Hyperthermia Group, Genetics Section, is available on the internet (<http://www.emhg.org>).

Rarely, a single pedigree may be sufficiently large to establish linkage to a candidate locus with a high probability (lod score >3.0). In such a situation the question arises as to whether or not haplotype analysis can be used to assign MH status. Under these circumstances, individuals carrying the high-risk haplotype should be regarded as susceptible to MH even without confirmation by a positive IVCT. The converse is not true, that is, identification of the low-risk haplotype does not equate with MHN status and such individuals should have IVCT determination of their MH status.

In families where linkage to a candidate gene, *RYR1* or another locus, is suggested but not firmly established (i.e. lod score <3.0) haplotype analysis for predictive testing is not appropriate due to the high level of locus heterogeneity in MH. In such families, however, it is desirable to search for unknown mutations in the suggested candidate gene for research purposes.

Failure to reach a lod score of +3.0 in a single family due to the occurrence of a single individual in whom there is recombination between the haplotype and IVCT-deter-



**Fig 1** Suggested route for MH susceptibility testing. IVCT=*in vitro* muscle contracture test; MHN=malignant hyperthermia negative; MHS=malignant hyperthermia susceptible.

**Table 1** List of *RYR1* mutations potentially causative for MH susceptibility (MHS) and central core disease (CCD). Residue numbering within the *RYR1* nucleotide and amino acid sequence corresponds to the human *RYR1* sequence according to Zorzato and colleagues<sup>18</sup> (accession number J05200.1), updated according to Zhang and colleagues<sup>16</sup> and Phillips and colleagues.<sup>19</sup> Functional characterization of the *RYR1* mutations on *RYR1* channel activity have been performed by calcium photometry on myotubes and/or COS-7 or HEK cells transfected with *RYR1* genes bearing the mutations<sup>20–24</sup>

Exon	Mutation position codon change	RYR1 amino acid change	Functional comparison with wild-type RYR1		Phenotype	Estimated incidence	Reference
			Caffeine sensitivity	Halothane sensitivity			
2	103TGC→CGC	Cys35→Arg	no difference	increased	MHS	one family	4
6	487CGC→TGC	Arg163→Cys	increased	increased	MHS and/or CCD	2%	5
9	742GGG→AGG	Gly248→Arg	increased	increased	MHS	one family	6
11	1021GGG→AGG	Gly341→Arg	increased	increased	MHS	6 – 10%	7
12	1209ATC→ATG	Ile403→Met	increased	increased	CCD; MHS unknown	one family	5
14	1565TAT→TCT	Tyr522→Ser	increased	increased	MHS and/or CCD	one family	8
15	1654CGG→TGG	Arg552→Trp	increased	increased	MHS	one family	9
17	1840CGC→TGC	Arg614→Cys	increased	increased	MHS	4 – 9%	10
17	1841CGC→CTC	Arg614→Leu	increased	increased	MHS	2%	11
39	6487CGC→TGC	Arg2163→Cys	increased	increased	MHS	4%	12
39	6488CGC→CAC	Arg2163→His	increased	increased	MHS and/or CCD	one family	12
45	7300GGA→AGA	Gly2434→Arg	increased	increased	MHS	4–10%	13–15
45	7304CGC→CAC	Arg2435→His	increased	increased	MHS and/or CCD	one family	16
46	7372CGC→TGC	Arg2458→Cys	increased	increased	MHS	4%	17
46	7373CGC→CAC	Arg2458→His	increased	increased	MHS	4%	17

mined MH status will require closer scrutiny and possible reassessment of the genetic and bioassay results to attempt to resolve the basis of the discordance. For predictive diagnosis in such families, the more conservative estimation, i.e. the higher risk outcome (either the MH susceptibility test result from the IVCT or the high-risk haplotype) should be the basis for the clinical decision.

## Appendix

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