Criteria for gene mutations to be used in genetic testing of Malignant Hyperthermia susceptibility

Clemens R. Müller for the EMHG

On its 22nd Annual Meeting in Brunnen, Switzerland, the European Malignant Hyperthermia Group (EMHG) has discussed criteria which any genetic mutation should fulfil prior to its use in predictive genetic testing. The following criteria have been adopted. At present, they have been defined for mutations in the RYR1 gene but should be applicable analogously to mutations in other genes involved on MH.

1. Genetic characterisation.
   Each mutation should be fully characterised at the genetic level, including:
   - A full description at the DNA and protein level, considering aspects of evolutionary conservation and change in charge, polarity or structure introduced by the amino acid replacement,
   - Co-segregation of the mutation with the disease in at least 2 pedigrees,
   - Absence of the sequence change from 100 control samples for exclusion of polymorphisms.

2. Functional characterisation.
   The effect of each mutation on RYR1 function should be assayed by one or more of the following test systems:
   - Recombinant in vitro expression on a defined genetic background.
     The standard system, introduced by D.H. MacLennan’s group, uses the expression of a rabbit RYR1 cDNA construct (with appropriate mutations) in HEK293 cells. Calcium release is measured fluorimetrically in response to trigger agents (Tong et al 1997, Lynch et al. 1999, Tong et al. 1999, Monnier et al. 2000, Sambuughin et al. 2001, Oyamada et al. 2002). Although this is a non-muscle cell type, the advantage of the system is the defined cDNA and the standardised genetic background of the recipient cell line. This allows for direct comparison between mutations and eliminates the potential influence of mutations in other genes which could modify RYR1 function in cells taken from patients.
     Alternatively, myotubes of the dyspedic mouse (RYR1-knock out) have been used as recipients for the expression of cDNA constructs (Avila et al. 2001). Again, cDNA construct and genetic background are well defined and standardised. The genetic
expression profile of myotubes may be closer to mature muscle. For this reason, results may not be directly comparable to the HEK system.

- Assays of RYR1 function in *ex vivo* tissues.
  Calcium measurements ligand binding studies have been performed on tissues from MHS patients with characterised RYR1 mutations:
  In myotubes (Brinkmeier et al. 1999, Wehner et al. 2002),
  in microsomal SR preparations from muscle biopsies (Richter et al. 1997), and
  in lymphoblasts (Girard et al. 2001, Tilgen et al. 2001).
  Read-out parameters were Ca\(^{2+}\) flux and resting [Ca\(^{2+}\)] or ryanodine binding to SR-RYR1 preparations. Myotubes and lymphoblasts were derived from individual patients and, therefore, the potential influence of other individual genetic factors cannot be excluded. For the SR preparations, muscle biopsies of several patients were pooled thus eliminating individual variation.
  In order to avoid the interference of genetic factors other than RYR1, it is recommended that all assays which are based on cells taken from patients should be performed on samples from at least two independent patients with the same mutation.

3. Publication of results.

For the time being it is requested that mutations are eligible for the list of “Mutations for genetic testing” only if results of their genetic and functional characterisation have been published in the scientific literature. It can be envisaged that it may become difficult to publish individual RYR1 mutations in the future. The EMHG will then design a protocol following the above criteria for the submission of mutations.
List of novel RYR1 mutations recommended for use of predictive genetic testing within pedigrees.

<table>
<thead>
<tr>
<th>Exon</th>
<th>Nucleotide</th>
<th>Protein</th>
<th>Phenotype</th>
<th>Assay system(s)</th>
<th>Reference</th>
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<tbody>
<tr>
<td>39</td>
<td>6502 G→A</td>
<td>V2168M</td>
<td>MH</td>
<td>Ca$^{2+}$ release in mutant lymphoblastoid cells and myotubes from several mutation carriers</td>
<td>Girard et al. 2001; Girard et al. 2001</td>
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<tr>
<td>40</td>
<td>6617 C→T</td>
<td>T2206M</td>
<td>MH</td>
<td>Ca$^{2+}$ release in mutant myotubes cultured from several mutation carriers</td>
<td>Rueffert et al. 2002; Wehner et al. 2002</td>
</tr>
<tr>
<td>44</td>
<td>7048 G→A</td>
<td>A2350T</td>
<td>MH</td>
<td>Mutant rabbit RYR1 expressed in HEK-293cells.</td>
<td>Sambuughin et al. 2001</td>
</tr>
<tr>
<td>100</td>
<td>14387 A→G</td>
<td>Y4796C</td>
<td>CCD &amp; nemaline rods</td>
<td>Mutant rabbit RYR1 expressed in HEK-293cells.</td>
<td>Monnier et al. 2000</td>
</tr>
<tr>
<td>101</td>
<td>14512 C→G</td>
<td>L4838V</td>
<td>MH</td>
<td>Mutant rabbit RYR1 expressed in CHO cells.</td>
<td>Oyamada et al. 2002</td>
</tr>
<tr>
<td>101</td>
<td>14582 G→A</td>
<td>R4861H</td>
<td>CCD</td>
<td>Measured changes in intracellular [Ca$^{2+}$] of mutant lymphoblastoid cells</td>
<td>Tilgen et al. 2001</td>
</tr>
<tr>
<td>102</td>
<td>14693 T→C</td>
<td>I4898T</td>
<td>MH / CCD</td>
<td>Mutant rabbit RYR1 expressed in HEK-293 cells and in dyspedic myotubes</td>
<td>Lynch et al. 1999; Avila et al. 2001</td>
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