Ryanodine receptor gene point mutation and malignant hyperthermia susceptibility

Abstract Malignant hyperthermia (MH) is a rare clinical syndrome, triggered in susceptible subjects by a variety of anaesthetic agents and muscle relaxants, and is the commonest cause of death due to general anaesthesia. Previous studies have reported that inherited mutations in the ryanodine receptor (RYR1) gene co-segregated, in some families, with MH susceptibility; lack of linkage between MH and the RYR1 gene in some other families indicates a heterogeneous genetic basis for the syndrome. The in vitro contracture test (IVCT) on muscle biopsy specimens is considered to be the most reliable test for establishing the diagnosis of MH. With the identification of RYR1 point mutations this might in turn result in non-invasive methods for the presymptomatic diagnosis of MH. In the present study we investigated four families suspected to be at risk of MH susceptibility; in all subjects histopathological examination and IVCT were performed on muscle biopsy specimens. We undertook a mutation analysis of RYR1 gene testing for the presence of five point mutations; in one pedigree a C1840→T point mutation was detected, strictly segregating with in vitro MH susceptibility.

Key words Malignant hyperthermia • In vitro contracture test • Ryanodine receptor • Central core disease

Introduction

Malignant hyperthermia (MH) is an autosomal dominant disorder of skeletal muscle, commonly triggered by inhalation anaesthetics (e.g. halothane) and depolarizing muscle relaxants (e.g. succinylcholine) [16].

The clinical syndrome is characterized by marked variability in the severity of symptoms, which include the classical fulminant episode, with early evidence of severe acidosis, muscle rigidity, hypermetabolism and elevation of temperature; a masseter muscle spasm form; a delayed presentation, with onset in the postoperative period, with myalgia, high creatine kinase (CK) and myoglobinuria blood levels [10, 20, 40]. Patients carrying the MH trait do not show any clinical sign of myopathy and are usually asymptomatic, except when exposed to a triggering agent [18, 41]. The in vitro contracture test (IVCT) performed on fresh muscle biopsy specimens is still the method for detecting MH susceptibility in probands at risk [23, 35]. A standardised protocol has been adopted by the European Malignant Hyperthermia Group (EMHG) since 1984; in this test MH susceptibility (MHS) is determined by the contracture response of fresh muscle samples to increasing concentrations of caffeine and halothane [11].

Although the biochemical defect responsible for MH is not known, current evidence indicates that an abnormality in the calcium release channel of skeletal muscle sarcoplasmic reticulum (SR), commonly known as the ryanodine receptor (RYR1), may account for the disorder [12, 32]. The RYR1 gene encoding the skeletal muscle ryanodine receptor has been localized to human chromosome...
19q13.1, and a positive linkage has been demonstrated in several families affected by MH [30, 31]. A single point mutation that causes a substitution of Cys for Arg 614 in the RYR1 gene was first identified in association with MH in families where it segregated with MHS [14, 22]. A second mutation, an Arg for Gly 248 substitution, was demonstrated in a single MHS pedigree [15]. Three additional single alterations have recently been identified in families with MH and central core disease (CCD): Arg for His 2434 (present in a single family) [44]; Arg for Cys 163, and Ile for Met 403 [39].

In the present study we have undertaken a mutation analysis of the RYR1 gene, testing for the presence of potential alterations in four families affected by MH susceptibility. We report a large human pedigree in which the Arg614Cys mutation was present in three generations co-segregating with MHS status.

Materials and methods

Case histories

We studied 27 subjects from four families (probands and close relatives) referred to our centre because of a personal or family history of suspected MH anaesthetic reactions. The pedigrees are shown in Figs. 1 and 2.

Family I

While undergoing diagnostic arthroscopy the proband (female, aged 13 years) was administered succinylcholine 50 mg i.v. and forane 1%; at the end of the surgical procedure she developed hyperpyrexia, generalized muscle rigidity, acidosis, tachycardia and arrhythmia. CK levels were measured at 3000 IU/l a few hours later, and at 50000 IU/l the day after the acute episode. Glutamic oxalacetic transaminase (GOT) levels were elevated to 960 IU/l. The patient was resuscitated with medical therapy (dantrolene was not used), and underwent muscle biopsy 2 years later. Both parents were studied with the IVCT.

Family II

The proband, a 16-year-old healthy boy, had experienced a postoperative acute rhabdomyolysis a few hours after general anaesthesia for tonsillectomy; CK levels were elevated to 33000 IU/l; GOT levels to 422 IU/l and lactate dehydrogenase (LDH) to 1870 IU/l; massive myoglobinuria was observed and severe diffuse myalgia and generalised weakness were reported for several days. The urine was discoloured 2 days later, and the CK level was normal 10 days later. Both halogenate gas (forane 1%) and succinylcholine (50 mg i.v.) were used for the anaesthetic procedure. The proband and five relatives were investigated with the IVCT.

Family III

The proband (male, aged 40 years) suffered from two episodes of postoperative rhabdomyolysis after halothane and succinylcholine anaesthesia for abdominal surgery. Data from the last intervention showed that the CK level was increased to 5200, GOT to 1106 and LDH to 2400; dark urine and generalised muscle pain were evident a few hours after anaesthesia. Basal CK levels were slightly elevated (250 IU/l) in the proband and his son; the IVCT was carried out in the two subjects.

Family IV

We studied a large three-generation family, in which two subjects died as a result of MH fulminant crises during general anaesthesia several years ago (III/35, III/36). Detailed data about the clinical episodes were not available. Up to now 16 relatives have undergone diagnostic muscle biopsy and an in vitro test. Most of them have experienced uneventful anaesthesias in the past.

Clinical examination did not show any sign or symptom of muscular involvement in any of the patients, probands or relatives examined. Family histories were negative for muscular disorders.