J Anesth (2008) 22:70–73
DOI 10.1007/s00540-007-0575-1

Journal of
Anesthesia

Malignant hyperthermia susceptibility diagnosed with a family-specific ryanodine receptor gene type 1 mutation

Takahiro Tanabe[1], Makoto Fukusaki[1], Yoshiaki Terao[1], Kazunori Yamashita[1], Koji Sumikawa[2], Keiko Mukaida[3], Carlos A. Ibarra[4], and Ichizo Nishino[4]

[1]Department of Anesthesia, Nagasaki Rosai Hospital, 2-12-5 Setogoshi, Sasebo 857-0134, Japan
[2]Department of Anesthesiology, Nagasaki University School of Medicine, Nagasaki, Japan
[3]Division of Anesthesia, Hiroshima Prefectural Rehabilitation Center, Hiroshima, Japan
[4]Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan

Abstract
Malignant hyperthermia (MH) is an autosomal dominant disorder of skeletal muscle calcium regulation, and the rate of calcium-induced calcium release (CICR), determined by using skinned fibers of skeletal muscle, has been employed as a diagnostic test for MH susceptibility in Japan. The ryanodine receptor (RYR1), encoding the major calcium-release channel in skeletal muscle sarcoplasmic reticulum, has been shown to be mutated in a number of MH pedigrees. We experienced the detection of accelerated CICR and/or an *RYR1* mutation in a patient with an MH episode and his family. Accelerated CICR and an *RYR1* mutation (c.14512C>G, p.L4838V) were found in the patient and his father. The MH-causative mutation (c.14512C>G, p.L4838V) was also found in his brother and his son (resulting in the diagnosis of MH without the CICR test), but the mutation was not found in his mother or two daughters. With the detection of the family-specific mutation in other family members, the diagnosis of MH was made without the invasive CICR test.

Key words Malignant hyperthermia · Ryanodine receptor gene type 1 · Calcium-induced calcium release · Genetic diagnosis of malignant hyperthermia

Introduction

Malignant hyperthermia (MH) is an autosomal dominantly inherited disorder that is considered to originate from the abnormal regulation of skeletal muscle calcium release [1,2].

The diagnosis of susceptibility to MH is achieved by the whole-muscle caffeine-halothane contracture test (CHCT) in North America [3] and by the in vitro contracture test (IVCT) in Europe [4], whereas in Japan, it is determined by detecting an acceleration of the rate of calcium-induced calcium release (CICR) from the sarcoplasmic reticulum (SR) in comparison to reference values previously measured in healthy controls [5,6].

The ryanodine receptor (*RYR1*) gene encodes the key channel which mediates calcium release in skeletal muscle during excitation-contraction coupling, and mutations in this gene are considered to account for susceptibility to MH [7].

We herein report a case of an MH family with detection of accelerated CICR and an *RYR1* mutation.

Case report

A 35-year-old man, weighing 55 kg, was admitted to our hospital for surgery for an upper limb bone fracture. He had a past history of MH, which had been diagnosed 15 years previously during inhalation anesthesia for surgery for a lower limb bone fracture, and he had been treated with dantrolene at a university hospital. He did not receive any specific test for MH thereafter. For the present surgery, we maintained anesthesia with total intravenous anesthesia (TIVA) including fentanyl, propofol, and vecuronium bromide, and he showed no MH symptoms perioperatively. After the operation, a skeletal muscle biopsy test was performed for the measurement of the CICR rate with chemically skinned muscle fibers from the patient; skeletal muscle fibers were also obtained from his parents in order to carry out these measurements. The measurements of the CICR rate were performed by Dr. Keiko Mukaida at the Division of Anesthesia, Hiroshima Prefectural Rehabilitation Center. The CICR rate test was performed according to the previously established protocols [8]. Details are given in the report of Oku et al. [9]. If the CICR rate at each Ca concentration exceeded or was equal to the mean value plus two SDs from the normal control data of 12 subjects diagnosed as MH-negative according to the CHCT in the report of Oku et al. [9], the specimen

Address correspondence to: T. Tanabe
Received: February 28, 2007 / Accepted: September 3, 2007

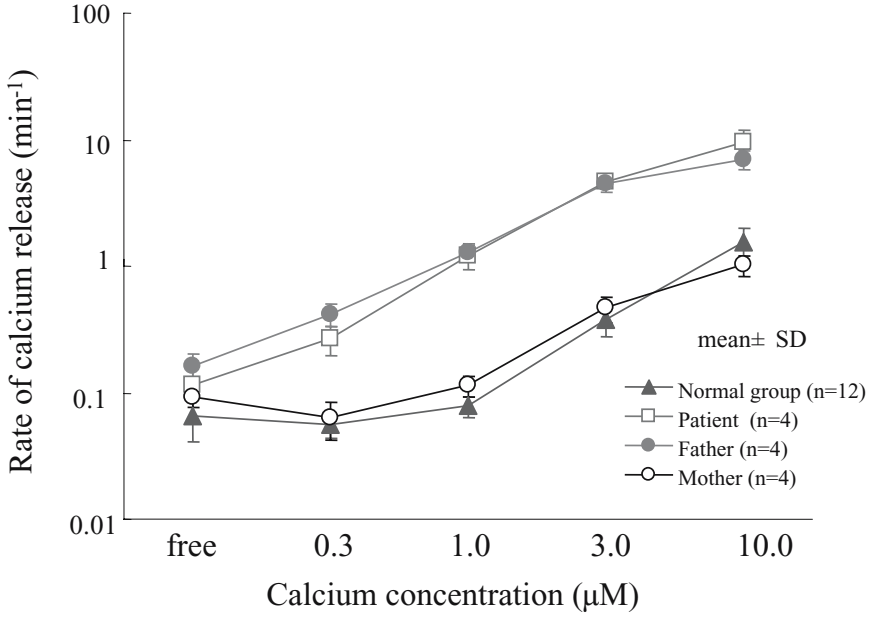

Fig. 1. Calcium-induced calcium release (CICR) rates in the patient and his parents at four calcium concentrations. Data values are expressed as means ± SD for four determinations at each concentration ($n = 4$) in each individual. For comparison, the average CICR rates of 12 patients who were diagnosed as being malignant hyperthermia (MH)-negative according to the whole-muscle caffeine-halothane contracture test (CHCT) in the report of Oku et al. [9] are also shown (*normal group*). If the CICR rate at each Ca concentration exceeded or was equal to the mean value plus two SDs from the data of the normal group, the specimen was classified as showing an accelerated rate. The CICR rates in the patient and his father were significantly accelerated, but that of the mother was not accelerated. CICR test data were presented by K.M. (Hiroshima University) and are reproduced with her permission

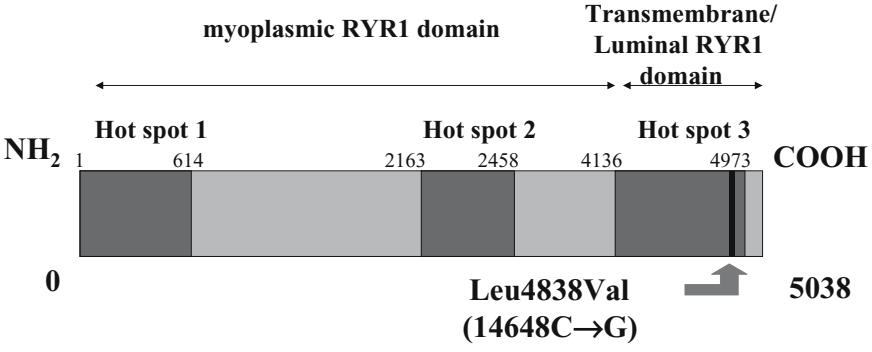

Fig. 2. The genetic mutation for malignant hyperthermia (MH) in the ryanodine receptor gene (*RYR1*) in this family. In exon 101 within the C-terminal channel region, an MH-causative mutation (c.14512C>G, p.L4838V) was identified. *RYR1* data for the MH family were provided by C.A.I. and I.N. (Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan) and are reproduced with their permission

was classified as showing an accelerated CICR rate. The patient and his father, but not his mother, showed significant acceleration in the CICR rate (Fig. 1). Mutation detection in the *RYR1* gene was performed in the patient and his parents, and an *RYR1* mutation (c.14512C>G, p.L4838V) in exon 101 within the C-terminal channel region was found in the patient and his father (Fig. 2). The same mutation study was performed in the patient's other family members, i.e., two daughters (aged 12 years and 8 years), a son (age 6 years), and a younger brother. The *RYR1* gene was studied by Drs. Carlos A. Ibarra and Ichizo Nishino at the Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan. Genomic DNA was extracted from peripheral blood lymphocytes and the entire *RYR1* coding region was sequenced according to a previously established protocol [10]. Intronic primers, approximately 25-bp-long, were designed to amplify all 106 *RYR1* exons with their flanking intron boundaries by polymerase chain reaction (PCR). Details are given in the report of Ibarra et al. [11]. The family-specific mutation (c.14512C>G, p.L4838V) was also detected in the patient's younger brother and his son (Fig. 2), but it was not detected in his mother or two daughters. With the detection of the MH causative mutation in his brother and his son, they were diagnosed as having MH without the CICR test (Fig. 3).

Discussion

In their daily lives, predisposition to MH does not pose a threat to MH-susceptible (MHS) individuals. Thus, the major goal of MH diagnosis is to identify susceptible patients before the administration of trigger agents. In Japan, the accepted standard test for MH susceptibility is the CICR test. However, the CICR test is performed on skinned fibers prepared from patients' skeletal muscle [5,6], so a less invasive diagnostic test for MH has been actively sought for many years. After the introduction of molecular genetics into MH

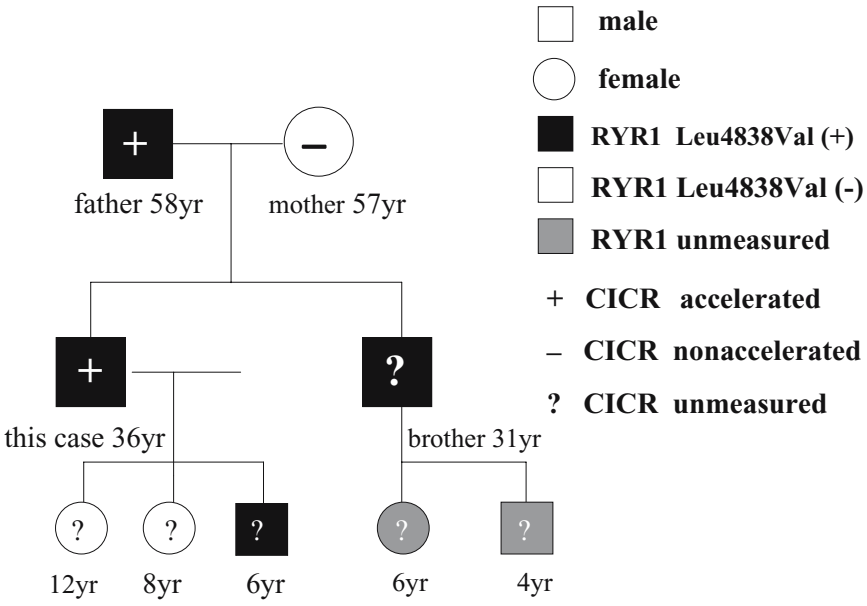

Fig. 3. The family pedigree, showing results of CICR tests and genetic test

research, guidelines for the molecular genetic diagnosis of MH susceptibility were published by two MH study groups [12,13]. According to these guidelines, once a mutation is identified in the affected member, family members will be offered testing for the presence of the family-specific mutation; those in whom the mutation is found are diagnosed as having MH without in vitro contracture testing. However, to avoid the danger of a false-negative diagnosis, it will remain necessary to perform the CHCT for the diagnosis of those family members who do not carry the familial *RYR1* mutation.

Here, an *RYR1* gene mutation (p.L4838V) was found in the patient and his father, who had clearly accelerated CICR rates, and in his son and his younger brother, for whom the CICR test was not performed. The patient's son and his brother were diagnosed as MHs individuals due to the identification of the causative MH mutation. Although the *RYR1* mutation was not detected in the patient's daughters, the guidelines call for performing the IVCT when mutation analysis results are negative.

RYR1 is a homotetrameric protein. Each subunit has a molecular weight of 560 kDa (5038 amino acids), making it one of the largest proteins known. Located on chromosome 19, the *RYR1* gene spans 160 000 nucleotide bases, and consists of 106 exons; as such, it is one of the most complex human genes [7]. To date, more than 80 mutations in the huge gene that encodes RYR type 1 (*RYR1*) have been associated with MH. Most of the MH-related mutations have been found mainly in three regions or "hot spots": domain 1, in the cytoplasmic aspect of the protein near the N-terminus between residues p.M1 and p.R614; domain 2, in the central region (p.R2163–p.R2458); and domain 3, in the C-terminal region (p.R4136–p.4973) that encodes the transmembrane pore-forming segment [14] (Fig. 2).

However, it is known that the location and distribution of mutations in the *RYR1* gene in MH patients is highly variable among populations. Particularly, in Japanese patients, the distribution of mutations is not limited to the widely accepted hot spots, even though they occur more frequently in some regions of the gene [11]. Although the vast size of the *RYR1* gene makes it painstaking to screen the entire gene, it is still important to screen the whole open reading frame of the *RYR1* gene for genetic investigations in MHs individuals. In the MH family in the present study, the entire *RYR1* coding region from genomic DNA was sequenced, and a family-specific mutation (p.L4838V) was identified. The p.L4838V mutation is the one that was identified in a Japanese MHs pedigree and found to be responsible for MH in the study done by Oyamada et al. [15]. If other members in the family in the present study receive general anesthesia, the screening test at the same specific mutation only would be acceptable. Although family members who are positive for the same mutation could be regarded as MH-positive, the CICR test would be needed in mutation-negative family members.

In summary, an MH-causative mutation was identified, by sequencing the entire *RYR1* coding region, in a patient with a clearly accelerated CICR rate. With the detection of the family-specific mutation in other family members, it was possible to make the diagnosis of MH without performing the invasive CICR test.

References

1. Nelson TE (2002) Malignant hyperthermia A pharmacogenetic disease of Ca++ regulating proteins. Curr Mol Med 2:347–369
2. Yuge O, Mukaida K (2002) Malignant hyperthermia. Nippon Rinsho (Jpn J Clin Med) 60 (Suppl 1):635–642
3. Larach MG (1989) Standardization of the caffeine halothane muscle contracture test. North American Malignant Hyperthermia Group. Anesth Analg 69:511–515
4. Ording H, for the European Malignant Hyperthermia Group (1997) In vitro contracture test for the diagnosis of malignant hyperthermia following the protocol of the European MH Group: results of testing patients surviving fulminant MH, and unrelated low-risk subjects. Acta Anaesthesiol Scand 41:955–966
5. Endo M, Yagi S, Ishizuka T, Horiuti K, Koga Y, Amaha K (1983) Changes in the Ca-induced Ca release mechanism in the sarcoplasmic reticulum of the muscle from a patient with malignant hyperthermia. Biomed Res 4:83–92
6. Kikuchi H, Matsui K, Morio M (1987) Diagnosis of malignant hyperthermia in Japan by the skinned fiber test. In: Britt B (ed) Malignant hyperthermia. Martinus Nijhoff, Boston, pp 279–294
7. Fill M, Copello JA (2002) Ryanodine receptor calcium release channels. Physiol Rev 82:893–922
8. Endo M, Yagi S, Ishizuka T, Horiuti K, Koga Y, Amaha K (1983) Changes in the Ca-induced Ca release mechanism in the sarcoplasmic reticulum of the muscle from a patient with malignant hyperthermia. Biomed Res 4:83–92
9. Oku S, Mukaida K, Nosaka S, Sai Y, Maehara Y, Yuge O (2000) Comparison of the in vitro caffeine-halothane contracture test with the Ca-induced Ca release rate test in patients suspected of having malignant hyperthermia susceptibility. J Anesth 14:6–13
10. Sambrook J, Russell DW (2001) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, New York, pp 6.4–6.12
11. Ibarra CA, Wu S, Murayama K, Minami N, Ichihara Y, Kikuchi H, Noguchi S, Hayashi YK, Ochiai R, Nishino I (2006) Malignant hyperthermia in Japan: mutation screening of the entire ryanodine receptor type 1 gene coding region by direct sequencing. Anesthesiology 104:1146–1154
12. Urwyler A, Deufel T, McCarthy T, West S (2001) Guidelines for molecular genetic detection of susceptibility to malignant hyperthermia. Br J Anaesth 86:283–287
13. Sei Y, Sambuughin N, Muldoon S (2003) Malignant Hyperthermia Genetic Testing in North America Working Group Meeting. Anesthesiology 100:464–465
14. Treves S, Anderson AA, Ducreux S, Divet A, Bleunven C, Grasso C, Paesante S, Zorzato F (2005) Ryanodine receptor 1 mutations, dysregulation of calcium homeostasis and neuromuscular disorders. Neuromuscul Disord 15:577–587
15. Oyamada H, Oguchi K, Saitoh N, Yamazawa T, Hirose K, Kawana Y, Wakatsuki K, Oguchi K, Tagami M, Hanaoka K, Endo M, Iino M (2002) Novel mutations in C-terminal channel region of the ryanodine receptor in malignant hyperthermia patients. Jpn J Pharmacol 88:159–166