Hiroshima J. Med. Sci.
Vol. 71, No. 1·2, 31~38, June, 2022
HIMJ 71–4

Genetic screening for malignant hyperthermia and comparison of clinical symptoms in Japan

Rieko KANZAKI[1)], Hiroyuki MORINO[2)], Keiko MUKAIDA[1)], Kodai KUME[3)], Ryosuke OHSAWA[3)], Yuko NODA[1)], Sachiko OTSUKI[1)], Hirotsugu MIYOSHI[1)], Takashi KONDO[1)], Ryuji NAKAMURA[1)], Masashi KAWAMOTO[4)], Hideshi KAWAKAMI[3)], and Yasuo TSUTSUMI[1,*)]

1) Department of Anesthesiology and Critical Care, Hiroshima University Hospital, Kasumi, Minami-ku, Hiroshima, 734-8551, Japan
2) Institute of Biomedical Sciences, Tokushima University, 770-8503, Japan
3) Department of Epidemiology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Kasumi, Minami-ku, Hiroshima 734-8553, Japan
4) Director, JR Hiroshima Hospital, 3-1-36 Futabanosato, Higashi-ku, Hiroshima 732-0057, Japan

ABSTRACT

Malignant hyperthermia (MH) is an anaesthetic complication that causes an abnormal hypermetabolic state. *RYR1* encoding ryanodine receptors of the sarcoplasmic reticulum and *CACNA1S* encoding α subunits of dihydropyridine receptors are known to be associated with MH pathogenicity. We performed genetic screening using next-generation sequencing to evaluate the prevalence of genes associated with MH pathogenicity and clinical symptoms. This was a retrospective cohort study wherein next-generation sequencing data of 77 families diagnosed with MH predisposition by calcium-induced calcium release (CICR) tests from 1995 to 2019 was used to search for *RYR1* and *CACNA1S* variants. Furthermore, the clinical symptoms and predisposition tests in participants with *RYR1* and *CACNA1S* variants were compared. In the 77 families, 44.2%, 7.8%, and 48.1% individuals had *RYR1, CACNA1S*, and neither *RYR1* nor *CACNA1S* variants, respectively. Clinically significant differences were found in the maximum body temperature, maximum elevated body temperature for 15 min, creatinine kinase level, and CICR rate between the *RYR1* and *CACNA1S* groups. The prevalence of pathogenic *CACNA1S* variants appears to be prominent in Japan. The severity of clinical symptoms and the CICR rate were greater in individuals with *RYR1* variants than in those with *CACNA1S* variants, likely due to more direct regulation of calcium levels by ryanodine receptors than by dihydropyridine receptors. Genetic analysis of MH in future studies may help identify other genes associated with MH, which will further clarify the relationship between genotypes and MH symptoms and contribute to safer anaesthesia practice.

Key words: *Malignant hyperthermia*, *Next Generation Sequencing*, *RYR1*, *CACNA1S*

INTRODUCTION

Malignant hyperthermia (MH) is a rare anaesthetic complication (1 in 10,000 to 250,000[25)]) caused by the administration of inducing agents such as volatile anaesthetics and depolarising muscle relaxants to MH-susceptible patients. Patients may have clinical symptoms such as abnormal temperature rise, tachycardia/arrhythmia, muscle rigidity, and skeletal muscle breakdown, which may be fatal without effective treatment[16)]. MH develops as a potentially fatal hypermetabolic crisis associated with a rapid and uncontrollable increase in calcium (Ca^{2+}) in skeletal muscle cells[19)].

The mechanism of MH is mainly an abnormality in Ca^{2+} metabolism caused by pathogenic variants in *RYR1* (gene encoding ryanodine receptor 1 in the sarcoplasmic reticulum) and *CACNA1S* (gene encoding the alpha subunit of the dihydropyridine receptor (DHPR) in the plasma membrane of skeletal muscle cells). It has also been reported that *STAC3* is involved in the development of MH[10)]. Although STAC3 is known to interact with DHPR, its precise functional role in MH is still unknown. Many individuals (34–86%) with pathogenic variants in *RYR1*[27)], and some (1%) with pathogenic variants in *CACNA1S* show predisposition to MH[29)]. *CACNA1S* is a rare gene associated with MH, and few studies have examined the clinical characteristics of MH caused by *CANCA1S* variants[8)]. *CACNA1S* is the second most common gene associated with MH, and it is important to examine the distinct clinical symptoms of MH caused by genes such as *RYR1* and *CANCA1S*. In this study,

* Corresponding author: Yasuo Tsutsumi
Department of Anesthesiology and Critical Care, Hiroshima University Hospital, Kasumi, Minami-ku, Hiroshima 734-8551, Japan
E-mail: yasuo223@hiroshima-u.ac.jp

we performed genetic screening using next-generation sequencing to evaluate the prevalence of genes associated with MH (*RYR1* and *CACNA1S*) and corresponding clinical symptoms of MH in Japan. We excluded *STAC3* from this study because there are no reports associating *STAC3* with MH in Asia.

METHODS

Patients and DNA extraction

This was a retrospective cohort study. This study was approved by the ethics committee of Hiroshima University, Japan (ethical approval #151). DNA was extracted from the blood, muscle tissues, and cells of 49 MH patients and 41 family members (Supplementary Table 1) with a predisposition to MH, as determined by the calcium-induced calcium release (CICR) test at Hiroshima University Hospital from 1995 to 2019. Seventy-seven independent families were included in this study. The automated DNA separation system Quick Gene-610L and Quick Gene DNA Whole Blood Kit L (KURABO, Kurashiki, Japan) were used for DNA extraction from the blood, and DNeasy Blood and Tissue Kits (QIAGEN, Hilden, Germany) were used for DNA extraction from muscles and cells.

Genetic screening using next-generation sequencing

DNA samples were screened for pathogenic variants in *RYR1* (NM_000540) and *CACNA1S* (NM_000069) using a next-generation sequencer (Ion Personal Genome Machine (PGM), Life Technologies, Carlsbad, CA, USA). In total, 231 primer pairs for the exonic regions of *RYR1* and *CACNA1S* were designed using the Ion AmpliSeq designer (https://www.ampliseq.com/) as two subgroups—primer pool 1 for 116 amplicons and primer pool 2 for 115 amplicons (GRCh37/hg19) (Supplementary Table 2). Eight exonic regions in *RYR1* and *CACNA1S* were not covered by the designed primers (Supplementary Table 3). The uncovered regions were amplified by polymerase chain reaction (PCR) and sequenced directly using Sanger sequencing using an Applied Biosystems 3130 DNA sequencer (Life Technologies).

Ion PGM sequencing was performed using the Ion AmpliSeq Library Kit 2.0 (Life Technologies) and Ion PGM Hi-Q sequencing kit (Life Technologies), according to the manufacturer's protocols. Variants were identified using the TorrentSuite Software 5.0.5 (Thermo Fisher Scientific, Waltham, MA, USA).

Variants were filtered based on the criteria of combined annotation-dependent depletion (CADD, https://cadd.gs.washington.edu/) score ≥ 16, rare exome variant ensemble learner (REVEL, https://sites.google.com/site/revelgenomics/downloads) score > 0.5, and minor allele frequency (MAF) < 0.1% in the Genome Aggregation Database (gnomAD v2.1.1, https://gnomad.broadinstitute.org/). These variants were confirmed by standard PCR-based amplification followed by Sanger sequencing.

We evaluated the confirmed variants based on the EMHG scoring matrix for identifying genetic variants conferring malignant hyperthermia susceptibility (https://www.emhg.org/genetic-scoring-matrix). To be evaluated as "Pathogenic" and "Likely pathogenic" according to EMHG scoring matrix, it is necessary to satisfy the conditions of "Pathogenic Strong" or "Pathogenic Moderate". Pathogenic Strong requires a diagnostic mutation in EMHG. To be pathogenic moderate, the prevalence of the variant was significantly increased ($P < 1 \times 10^{-7}$) compared with the prevalence of a relevant low-risk population. In previous studies, CICR-positive variants have also been found to be positive on in vitro contracture testing (IVCT)[26]. Therefore, we classified CICR-positive cases, that is, all cases in this study, as "Pathogenic supporting": association with a clinical reaction consistent with malignant hyperthermia under anaesthesia and confirmed by a positive IVCT in the EMHG scoring matrix. We chose "Pathogenic" and "Likely pathogenic" variants on the EMHG scoring matrix as pathogenic variants. CICR-negative samples from 17 independent MH patients and their families, not included in this study, were also genetically screened.

Evaluation of the association between variants and clinical symptoms

Clinical symptoms, namely, maximum body temperature (Max BT), maximum elevated body temperature for 15 min (°C/15 min), maximum creatine kinase value (Max CK), clinical grading scale (CGS)[17], maximum serum K concentration, maximum $PaCO_2$, and minimum pH data, were extracted from anaesthesia charts and medical records. The Mann–Whitney U test was performed to assess the differences in the clinical symptoms of the *RYR1* and *CACNA1S* groups. For comparison with normal controls, we used samples diagnosed as MH-negative (MHN) by IVCT and CHCT in a previous study as the normal group[26].

Calcium-induced calcium release (CICR) test

Ninety patients and their family members underwent skeletal muscle biopsies and were examined using the CICR test[11,26].The CICR test was performed according to the method described by Endo and colleagues[5] with chemically skinned fibres. The CICR rates were assessed by measuring the induced calcium release from the sarcoplasmic reticulum at five different calcium concentrations (0, 0.3, 1.0, 3.0, and 10 μM). The CICR rate was considered to be increased when the CICR values were two standard deviations (SD) above the average values of the normal group at two or more calcium concentrations. Normal CICR values were derived from 12 MHN individuals previously excluded by IVCT[14].

Data analysis

Data from the CICR test and clinical symptoms were analysed using the PRISM 7.0 package (USA, San Diego, CA) and EZR plug-in in the R software[13]. $P < 0.05$, as calculated by the Mann–Whitney U test, was considered significant.

Table 1 Variant List

Position	Sample	No. of families	rsID	AA change	report	REVEL	CADD	gnomAD MAF (east Asia)	gnomAD (East Asia) p-value	EMHG scoring matrix	SIFT	MTPred	PP2Pred	ToMMo MAF
RYR1 chr19:38934851C>T	M80	1	rs118192161	p.R163C	EMHG[11]	0.959	25.7		$< 1 \times 10^{-7}$	Pathogenic	N.S.	D	D	
RYR1 chr19:38939352G>A	M68	1	rs121918592	p.G341R	EMHG[15]	0.864	26.0		$< 1 \times 10^{-7}$	Pathogenic	N.S.	D	D	
RYR1 chr19:38939352G>C	M35	1	rs121918592	p.G341R	EMHG[11]	0.876	25.7		$< 1 \times 10^{-7}$	Pathogenic	N.S.	D	D	
RYR1 chr19:38946112G>A	M60	1	rs144336148	p.R533H	EMHG[11]	0.824	24.2	0	$< 1 \times 10^{-7}$	Pathogenic	N.S.	D	D	
RYR1 chr19:38948186G>T	M21, 25, 27, 69	4	rs193922772	p.R614L	EMHG[6]	0.931	28.4		$< 1 \times 10^{-7}$	Pathogenic	N.S.	D	D	
RYR1 chr19:38989863G>A	M43	1	rs112563513	p.R2336H	EMHG[27]	0.903	25.4		$< 1 \times 10^{-7}$	Pathogenic	N.S.	D	D	
RYR1 chr19:38991539G>A	M46	1	rs193922818	p.R2508H	EMHG[21]	0.898	25.7		$< 1 \times 10^{-7}$	Pathogenic	N.S.	D	D	
RYR1 chr19:39002961G>A	M32, 33	2	rs193922832	p.E3104K	EMHG[27]	0.868	26.0	0	$< 1 \times 10^{-7}$	Pathogenic	N.S.	D	D	
RYR1 chr19:39071010C>G	M11, 12, 13, 14, 15(Family8), 16, 17, 19(Family9)	2	rs193922878	p.L4838V	EMHG[11]	0.882	28.4		$< 1 \times 10^{-7}$	Pathogenic	N.S.	D	D	
CACNA1S chr1:201061121G>A	M6, 7(Family5), 24, 75	3	rs772226819	p.R174W	EMHG[4]	0.91	29.6	0.000109	$< 1 \times 10^{-7}$	Pathogenic	D	D	D	0.0012
RYR1 chr19:38934827C>A	M44, 71	2	rs193922750	p.Q155K	Reported[11]	0.94	25.7		$< 1 \times 10^{-7}$	Likely pathogenic	N.S.	D	D	
RYR1 chr19:38934892G>T	M23	1	rs1568436081	p.E176D	Reported[23]	0.504	20.9		$< 1 \times 10^{-7}$	Likely pathogenic	N.S.	D	D	
RYR1 chr19:38935311G>A	M78	1	rs771058055	p.E209K	Reported[15]	0.711	23.6	5.46E-05	$< 1 \times 10^{-7}$	Likely pathogenic	N.S.	B	D	
RYR1 chr19:38939431G>A	M38	1	rs113332073	p.R367Q	Reported[7]	0.651	24.6	0	$< 1 \times 10^{-7}$	Likely pathogenic	N.S.	D	D	
RYR1 chr19:38973997C>T	M60, 84	2	rs193922777	p.P1592L	Reported[11]	0.927	27.7	0	$< 1 \times 10^{-7}$	Likely pathogenic	N.S.	D	D	
RYR1 chr19:38987541G>A	M51	1	rs193922797	p.V2280I	Reported[18]	0.641	25.3	0	$< 1 \times 10^{-7}$	Likely pathogenic	N.S.	D	D	
RYR1 chr19:38990281G>C	M74	1		p.S2345T	Reported[28]	0.744	24.7		$< 1 \times 10^{-7}$	Likely pathogenic	N.S.	D	D	
RYR1 chr19:38990282C>A	M42	1	rs1448949048	p.S2345R	Reported[22]	0.747	22.9		$< 1 \times 10^{-7}$	Likely pathogenic	N.S.	D	D	
RYR1 chr19:38990285delGGA	M40	1	rs193922800	p.E2347del	Reported[24]		21.3		$< 1 \times 10^{-7}$	Likely pathogenic	D	D		
RYR1 chr19:38990344C>G	M82	1	rs193922806	p.P2366R	Reported[27]	0.88	23.7		$< 1 \times 10^{-7}$	Likely pathogenic	N.S.	D	D	
RYR1 chr19:38993557C>T	M51	1	rs377686237	p.R2625C	Reported[27]	0.785	29.1	0.000109	$< 1 \times 10^{-7}$	Likely pathogenic	N.S.	D	D	
RYR1 chr19:39009935A>G	M3,37	1	rs118192126	p.K3367R	Reported[27]	0.632	18.9		$< 1 \times 10^{-7}$	Likely pathogenic	N.S.	D	B	
RYR1 chr19:39034034A>G	M34	1		p.N3913D	Reported[31]	0.924	26.9		$< 1 \times 10^{-7}$	Likely pathogenic	N.S.	D	D	
RYR1 chr19:38934864A>G	M39	1		p.D167G	—	0.905	25.5		$< 1 \times 10^{-7}$	Likely pathogenic	N.S.	D	D	
RYR1 chr19:38948155T>C	M88	1	rs901536375	p.S604P	—	0.951	28.1		$< 1 \times 10^{-7}$	Likely pathogenic	N.S.	D	D	
RYR1 chr19:38990320T>C	M22	1		p.I2358T	—	0.912	25.1		$< 1 \times 10^{-7}$	Likely pathogenic	N.S.	D	D	
RYR1 chr19:38990626C>A	M90	1		p.D2431E	—	0.743	23.1		$< 1 \times 10^{-7}$	Likely pathogenic	N.S.	D	D	
RYR1 chr19:38991282C>G	M29	1		p.R2454G	—	0.893	26.1		$< 1 \times 10^{-7}$	Likely pathogenic	N.S.	D	D	
RYR1 chr19:39078017C>G	M70	1		p.A5025G	—	0.605	31.00		$< 1 \times 10^{-7}$	Likely pathogenic	N.S.	D	D	
CACNA1S chr1:201019613T>A	M66	1		p.D1382V	—	0.971	28.4		$< 1 \times 10^{-7}$	Likely pathogenic	D	D	D	0.0006
CACNA1S chr1:201028359G>C	M30	1		p.F1161L	—	0.923	26.7		$< 1 \times 10^{-7}$	Likely pathogenic	D	D	D	
CACNA1S chr1:201046197C>T	M89	1	rs763794604	p.A560T	—	0.876	27.7	0.0001	$< 1 \times 10^{-7}$	Likely pathogenic	D	D	D	0.0003

We selected variants with CADD ≥ 16, REVEL > 0.5, and MAF in gnomAD (East Asia) < 0.1% in 90 subjects from 77 families. These variants were evaluated using the EMHG scoring matrix and “Pathogenic ” and ”Likely Pathogenic” variants were extracted. Evaluation using three algorithms (SIFT; Mutation Taster, MTPred; PolyPhen-2, PP2Pred), and MAF with the Tohoku Medical Megabank Organization (ToMMo, https://jmorp.megabank.tohoku.ac.jp/), a genomic study using a large Japanese cohort evaluation is shown for reference. The underlines represent members of the same family.
Abbreviations: EMHG, listed as mutation in EMHG; Reported, reported in other papers but not listed as mutation in EMHG; N.S., not scored; B, benign; D, disease-causing.

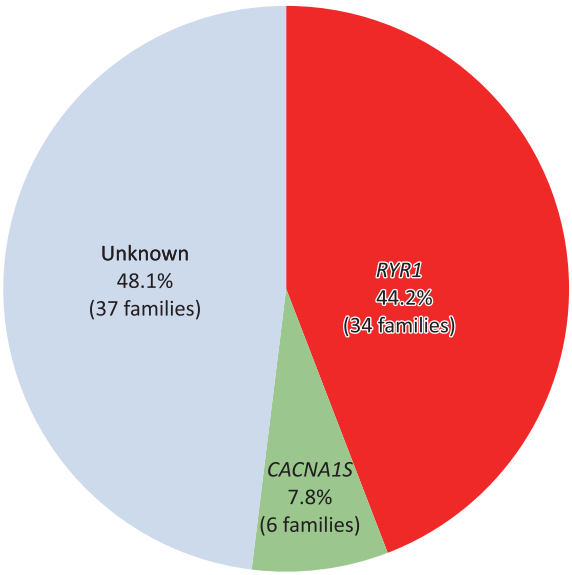

Figure 1 Percentage of genes associated with MH pathogenicity in MH patients and families. Screening results of genes associated with MH pathogenicity in 77 families showing that 34 families carried variants in *RYR1* and six families in *CACNA1S*. The remaining 37 families carried no variants in *RYR1* or *CACNA1*S (unknown group).

RESULTS

Screening of *RYR1* and *CACNA1S* variants

Thirty-two variants with a CADD score ≥ 16, REVEL score ≥ 0.5, and MAF ≤ 0.1% in gnomAD were selected, and all variants were confirmed by Sanger sequencing. Evaluation of these variants based on EMHG scoring, 28 *RYR1* variants and four *CACNA1S* variants classified as "Pathogenic" and "Likely pathogenic" were found in 90 subjects (49 MH patients, 41 MH family members) from 77 families (Table 1). Of these, nine *RYR1* and one *CACNA1S* variant have been reported by the European Malignant Hyperthermia Group (EMHG, https://www.emhg.org/diagnostic-mutations), thirteen *RYR1* variants have been reported in previous studies[9,11], and six *RYR1* and three *CACNA1S* variants were novel (Table 1, Supplementary Table 1). Thirty-four families had *RYR1* variants (41 individuals, 28 variants; *RYR1* group), six families had *CACNA1S* variants (seven individuals, four variants; *CACNA1S* group), and 37 families did not have *RYR1* or *CACNA1S* variants (42 individuals, Unknown group; Figure 1, Supplementary Table 1). Two individuals in the *RYR1* group had two *RYR1* variants (Supplementary Table 1, M51, 60). In the 77 families, 44.2%, 7.8%, and 48.1% individuals had *RYR1*, *CACNA1S*, and neither *RYR1* nor *CACNA1S* variants, respectively (Figure 1). Although variants associated with MH pathogenicity are abundant in the *RYR1* hotspot region, variants have also been frequently identified outside the hotspot (hotspot region I, p.M1-p.R614; region II, p.R2163-p.R2458; region III, p.R4136-P4973). We screened 17 CICR-negative MH patients and their families and found no likely pathogenic variants.

Evaluation of clinical symptoms

Max BT was recorded in 36 cases (18 cases from the *RYR1*, four cases from the *CACNA1S*, and 14 cases from the unknown group), and was significantly different between the *RYR1* and *CACNA1S* groups ($p = 0.0003$) (Figure 2A, Supplementary Table 1). Furthermore, °C/15 min was recorded in 32 cases (16 cases from the *RYR1* group, four cases from the *CACNA1S* group, and 12 from the unknown group), and was significantly different between the *RYR1* and *CACNA1S* groups ($p = 0.0239$) (Figure 2B, Supplementary Table 1). Maximum CK was recorded in 30 cases (15 cases from the *RYR1* group, three cases from the *CACNA1S* group, and 12 cases from the unknown group), and was significantly different between the *RYR1* and *CANCA1S* groups ($p = 0.0098$) (Figure 2C, Supplementary Table 1). There was no significant difference in CGS between the *RYR1* and *CACNA 1S* groups (18 cases from the *RYR1* group, five from the *CACNA1S* group, and 15 from the unknown group) ($p = 0.1140$) (Figure 2D, Supplementary Table 1).

Comparison of CICR rate between pathogenic variants

The CICR rates at five calcium concentrations were examined in all 90 participants. Under Ca-free conditions, samples generally did not react, even in the presence of MH predisposition, and no significant difference was found between the *RYR1* and *CACNA1S* groups using the Mann–Whitney U test (Figure 3A). However, significant differences were observed between the two groups at other four calcium concentrations (0.3 μM, $p = 0.0240$; 1.0 μM, $p = 0.0284$; 3.0 μM, $p = 0.0016$; 10 μM, $p = 0.0011$). Figure 3B shows a representative graph of CICR at calcium 3.0 μM.

DISCUSSION

We screened the most common genes, namely *RYR1* and *CACNA1S*, associated with MH pathogenicity in the Japanese population and analysed the frequency of these gene and their correlation with clinical symptoms in MH. The prevalence of pathogenic *CACNA1S* variants associated with MH pathogenicity was higher in Japan than in other countries. Patients with pathogenic *RYR1* variants had more severe MH than those with pathogenic *CACNA1S* variants, as determined by clinical symptoms and predisposition tests.

In the 77 families tested, 44.2%, 7.8%, and 48.1% individuals had *RYR1*, *CACNA1S*, and neither *RYR1* nor *CACNA1S* variants, respectively. We observed the prevalence of *RYR1* variants to be lower than that reported previously in the Japanese population[11]. Previous studies have reported pathogenic variants in *RYR1* as the most common in MH patients—it has been reported to be 76% in the UK (546 of 722 families)[20], 52% in the USA (62 out of 120 families)[1], 34% in Australia (33 out of 96 patients)[8,9], 57% in Japan (33 of 58 patients)[11], 72% in Italy (31 of 43 patients)[7], 86% in Canada (31 of 36 patients)[15], and 50% in Sweden (7 of 14 patients)[2]. We used the REVEL score, CADD score, and MAF in

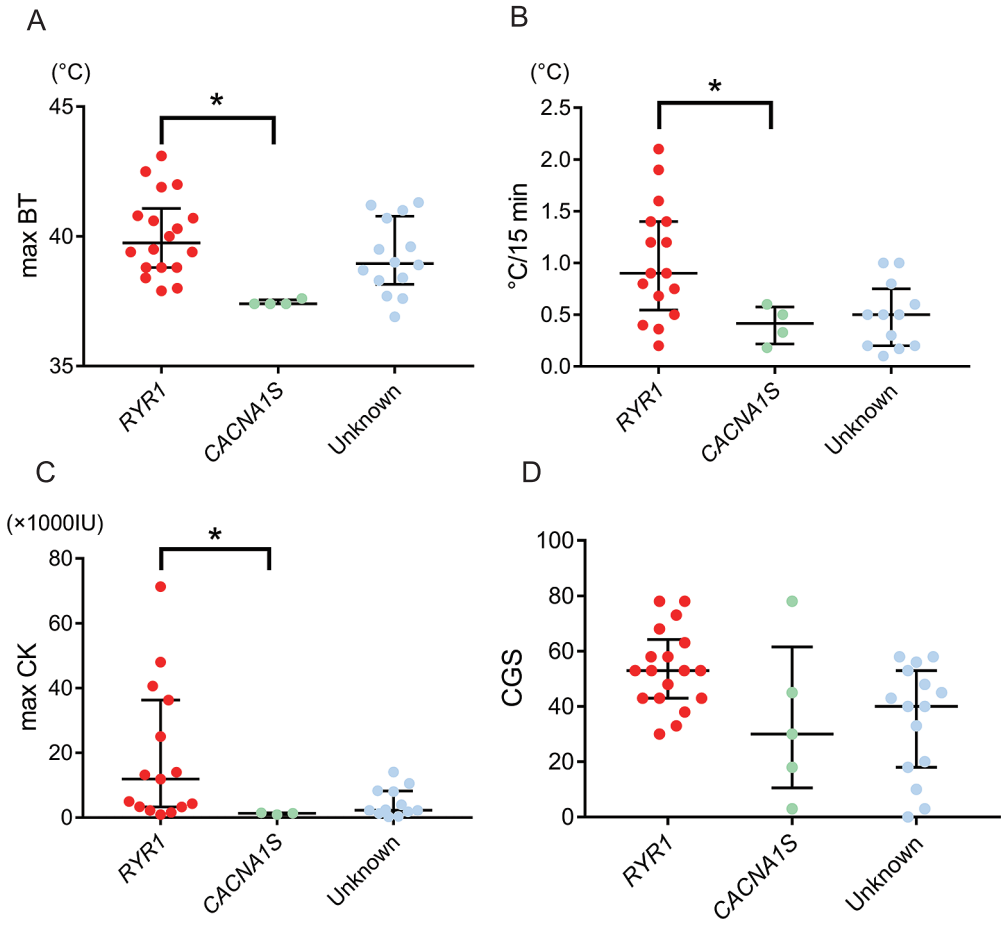

Figure 2 Comparison of the clinical symptoms corresponding to variants in each gene associated with MH pathogenicity. (A–D) Comparison of clinical symptoms corresponding to variants in each gene associated with MH pathogenicity (Max BT, °C/15 min, Max CK, CGS). The value of each item is represented by a dot and the interquartile range is represented by a line. The Mann–Whitney U test was conducted for comparing *RYR* and *CACNA1S* groups. *p < 0.05. Abbreviations: Max BT, maximum body temperature; °C/15 min, elevated body temperature for 15 min; Max CK, maximum creatine kinase; CGS, Clinical Grading Scale

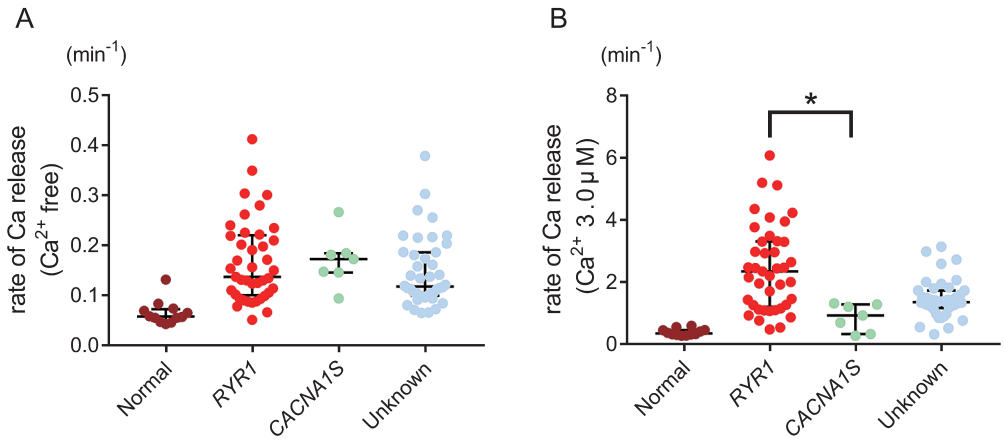

Figure 3 Comparison of CICR for each gene associated with MH pathogenicity. A comparison of the CICR values for each causative gene is shown. The CICR was examined at five Ca concentrations (0, 0.3, 1.0, 3.0, and 10 μM). (A) Rate of Ca release at 0 μM Ca (Ca-free) was not different the *RYR1* and *CACNA1S* groups in relation to MH status. (B) Rate of Ca release for each gene associated with MH pathogenicity at the other four Ca concentrations was significantly different between the *RYR1* and *CACNA1S* groups. Ca release at 3.0 μM Ca is shown as a representative.

gnomAD to assess the pathogenicity of the variants. The prevalence of *RYR1* variants is lower than in previous studies because our study not only excludes variants with high MAF but also excludes variants by bioinformatics. This may also be affected by the difference between CICR and IVCT/CHCT. However, few studies have examined the frequency of pathogenic *CACNA1S* variants, and a previous study reported that approximately only 1% of MH patients carried *CACNA1S* variants[29]. There have been no studies on the prevalence of genes associated with MH in Asia, except in Japan; thus, the prevalence of pathogenic *CACNA1S* variants in Asia is unknown.

In this study, the prevalence of *CACNA1S* variants was high, suggesting that *CACNA1S* variants may be more frequent in the Japanese population than in Western populations. The number of families without variants in either *RYR1* or *CACNA1S* was also higher than that reported in previous studies. It is difficult to make a simple comparison based solely on frequency because the criteria for determining the genes associated with MH pathogenicity and the method for selecting cases varied. The prevalence of pathogenic variants and their frequency change depending on the criteria for classifying a variant as pathogenic.

We compared the clinical symptoms and predisposition test results of each group and found that patients with *CACNA1S* variants had milder symptoms in that they had lower Max BT, °C/15 min, and Max CK. A previous study showed that patients with *RYR1* variants were likely to exhibit high CGS[8], however, we did not find any significant difference in CGS between *RYR1* group and *CACNA1S* group, probably because of the type of anaesthetic used and the timing of therapeutic interventions. Therefore, studies involving more patients are needed for gaining clarity in this regard. A previous study comparing the IVCT results of MH patients with *RYR1* and *CACNA1S* variants rather than clinical symptoms showed that patients with *CACNA1S* variants showed lower responses to both caffeine and halothane in IVCT[8]. We also found that the *CACNA1S* group showed milder abnormalities than the *RYR1* group in CICR, as well as in terms of the aforementioned clinical symptoms. DHPR encoded by *CACNA1S* does not directly participate in calcium dynamics but acts indirectly through RYR1. Consequently, unlike *RYR1* variants, *CACNA1S* variants may not directly alter RYR1 activity.

In a previous study, MH phenotype caused by *RYR1* variants varied depending on the location of the variants[3]. Our results also showed that the CICR data and clinical symptoms in the *RYR1* group were highly variable, which may be due to the different locations of *RYR1* variants (Figure 2, 3). In the unknown group, the CICR data and clinical symptoms were also widely distributed, probably because of the involvement of multiple novel genes associated with MH pathogenicity and different location of variants (Figure 2, 3).

The penetrance of genes most commonly associated with MH pathogenicity is low, and recent studies have shown that the penetrance of *RYR1* variants is 40.6%, and the probability of developing MH upon exposure to triggers is only 25% in predisposed individuals[12]. Patient age (young), sex (male), and use of succinyl choline also affected the onset of MH. Owing to the low penetrance, patients may be susceptible to MH but may not develop MH under general anaesthesia. In addition, not all individuals with pathogenic variants have experienced general anaesthesia. In such cases, the patient is not aware of the predisposition to MH. Genetic analysis is non-invasive compared with tests that require muscle biopsy (CICR, IVCT, and CHCT) and can identify the genes associated with MH pathogenicity in more than 50% of predisposed individuals. In addition to genetic diagnosis and bioinformatics analysis, it is desirable to perform a combination of predisposition tests (CICR, IVCT, and CHCT), MAF evaluation, and functional analysis depending on the situation[30]. As the investigation for new genes associated with MH pathogenicity progresses and genetic diagnosis becomes easier, predisposition to MH can be diagnosed before surgery, enabling safer anaesthesia.

In this study, we demonstrated that MH with *RYR1* variants (MH-RYR1) or *CACNA1S* variants (MH-CACNA1S) may have different clinical symptoms. We particularly focused on the different properties of MH-RYR and MH-CACNA1S symptoms to further clarify the differences in each pathological condition. Differences in pathogenesis due to the genes associated with MH pathogenicity can be useful in determining clinical treatment. In contrast, by determining features not related to the genes associated with MH pathogenicity, common characteristics of MH can be deduced and applied as a more useful diagnostic criteria. Further studies are required to examine MH-RYR1 and MH-CACNA1S in more detail. As the correlation between the genotype and phenotype of MH becomes more apparent, genetic diagnosis can be used to determine treatment strategies and predict MH severity and prognosis.

CONCLUSIONS

In this study, we investigated the genetic predispositions to MH using targeted sequencing. Pathogenic *CACNA1S* variants appear to be more prominent in Japanese than in Western populations. The clinical symptoms and predisposition test (CICR) results were more severe for MH-RYR1 than for MH-CACNA1S. This is probably because the ryanodine receptor encoded by *RYR1* has a more direct effect on the regulation of Ca^{2+} than DHPR encoded by *CACNA1S*. Further studies using larger samples can reveal more details regarding the relationship between genotypes and clinical symptoms of MH. Moreover, advances in genetic analysis, including the identification of novel genes responsible for MH, will improve the diagnostic rate of genetic screening and contribute to safer anaesthesia practice.

DETAILS OF AUTHOR'S CONTRIBUTIONS

Study design: R.K., H.M., K.M., H.K.
Data collection: R.K., K.M., S.O.
Data analysis: R.K., H.M., K.M.
Manuscript drafting: R.K., Y.N., T.K.
Manuscript revision: all authors

ACKNOWLEDGMENTS

We thank the families involved in this research and Ms. Eiko Nakajima for technical assistance.

Declaration of interest

The authors declare that they have no conflict of interest.

Funding

This study was supported by a Grant-in-Aid for Young Scientists (grant number: 17K16733 to Y.N. and 20K17783 to R.K.) from the Japan Society for the Promotion of Science and by the Takeda Science Foundation (H.K.).

Supplementary Tables

Supplementary tables are available on the website of the Hiroshima Journal of Medical Science. Please refer to the following URL: https://www.jstage.jst.go.jp/browse/hjms/list/-char/ja

(Received December 27, 2021)
(Accepted March 7, 2022)

REFERENCES

1. Brandom, B.W., Bina, S., Wong, C.A., Wallace, T., Visoiu, M., Jsackson, P.J., et al. 2013. Ryanodine receptor type 1 gene variants in the malignant hyperthermia-susceptible population of the United States. Anesth. Analg. 116: 1078–1086.
2. Broman, M., Gehrig, A., Islander, G., Bodelasson, M., R-Twetman, E., Ruffert, H., et al. 2009. Mutation screening of the RYR1-cDNA from peripheral B-lymphocytes in 15 Swedish malignant hyperthermia index cases. Br. J. Anaesth. 102: 642–649.
3. Carpenter, D., Robinson, R.L., Quinnell, R.J., Ringrose, C., Hogg, M., Casson, F., et al. 2009. Genetic variation in RYR1 and malignant hyperthermia phenotypes. Br. J. Anaesth. 103: 538–548.
4. Danielle, C., Christopher, R., Vincenzo, L., Andrew, M., Rachel, L., Robinson., P., Jane, H., et al. 2009. The role of CACNA1S in predisposition to malignant hyperthermia. BMC Med. Genet. 10: 104.
5. Endo, M. and Iino, M. 1988. Measurement of Ca2+ release in skinned fibers from skeletal muscle. Methods Enzymol. 157: 12–26.
6. Gillard, E.F., Otsu, K., Fujii, J., Khanna, V.K., de Leon, S., Derdemezi, J., et al. 1991. A substitution of cysteine for arginine 614 in the ryanodine receptor is potentially causative of human malignant hyperthermia. Genomics 11: 751–755.
7. Galli, L., Orrico, A., Lorenzini, S., Censini, S., Falciani, M., Covacci, A., et al. 2006. Frequency and localization of mutations in the 106 exons of the RYR1 gene in 50 individuals with malignant hyperthermia. Hum. Mutat. 27: 830.
8. Gillies, R.L., Bjorksten, A.R., Du, Sart. D. and Hockey, B.M. 2015. Analysis of the entire ryanodine receptor type 1 and alpha 1 subunit of the dihydropyridine receptor (CACNA1S) coding regions for variants associated with malignant hyperthermia in Australian families. Anaesth. Intensive Care 43: 157–166.
9. Gillies, R.L., Bjorksten, A.R., Davis, M. and Du, Sart. D. 2008. Identification of genetic mutations in Australian malignant hyperthermia families using sequencing of RYR1 hotspots. Anaesth. Intensive Care 36: 391–403.
10. Horstick, E.J., Linsley, J.W., Dowling, J.J., Hauser, M.A., Mcdonald, K.K., Ashley-Koch, A., et al. 2013. Stac3 is a component of the excitation-contraction coupling machinery and mutated in Native American myopathy. Nat. Commun. 4: 1952.
11. Ibarra M, C.A., Wu, S., Murayama, K., Minami, N., Ichihara, Y., Kikuchi, H., et al. 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. Ibarra Moreno, C.A., Hu, S., Kraeva, N., Schuster, F., Johannsen, S., Rüeffert, H., et al. 2019. An assessment of penetrance and clinical expression of malignant hyperthermia in individuals carrying diagnostic ryanodine receptor 1 gene mutations. Anesthesiology 131: 983–991.
13. Kanda, Y. 2013. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant. 48: 452–458.
14. Kobayashi, M., Mukaida, K., Migita, T., Hamada, H., Kawamoto, M. and Yuge, O. 2011. Analysis of human cultured myotubes responses mediated by ryanodine receptor 1. Anaesth. Intensive Care 39: 252–261.
15. Kraeva, N., Riazi, S., Loke, J., Frodis, W., Crossan, M.L., Nolan, K., et al. 2011. Ryanodine receptor type 1 gene mutations found in the Canadian malignant hyperthermia population. Can. J. Anaesth. 58: 504–513.
16. Larach, M.G., Brandom, B.W., Allen, G.C., Gronert, G.A. and Lehman, E.B. 2014. Malignant hyperthermia deaths related to inadequate temperature monitoring, 2007–2012: a report from the North American malignant hyperthermia registry of the malignant hyperthermia association of the United States. Anesth. Analg. 119: 1359–1366.
17. Larach, M.G., Localio, A.R., Allen, G.C., Denborough, M.A., Ellis, F.R., Gronert, G.A., et al. 1994. A clinical grading scale to predict malignant hyperthermia susceptibility. Anesthesiology 80: 771–779.
18. Galli, L., Orrico, A., Cozzolino, S., Pietrini, V., Tegazzin, V. and Sorrentino, V. 2002. Mutations in the RYR1 gene in Italian patients at risk for malignant hyperthermia: evidence for a cluster of novel mutations in the C-terminal region. Cell Calcium 32: 143–151.
19. Maclennan, D.H. and Zvaritch, E. 2011. Mechanistic models for muscle diseases and disorders originating in the sarcoplasmic reticulum. Biochim. Biophys. Acta 1813: 948–964.
20. Miller, D.M., Daly, C., Aboelsaod, E.M., Gardner, L., Hobson, S.J., Riasat, K., et al. 2018. Genetic epidemiology of malignant hyperthermia in the UK. Br. J. Anaesth. 121: 944–52.
21. Miyoshi, H., Yasuda, T., Otsuki, S., Kondo, T., Haraki, T., Mukaida, K., et al. 2015. Several Ryanodine Receptor Type 1 Gene Mutations of p. Arg 2508 Are Potential Sources of Malignant Hyperthermia. Aneth. Analg. 121: 994–1000.
22. Snoeck, M., van Engelen, B.G.M., Küsters, B., Lammens, M., Meijer, R., Molenaar, J.P.F., et al. 2015. RYR1-related myopathies: a wide spectrum of phenotypes throughout life. Eur. J. Neurol. 22: 1094–1112.
23. Witting, N., Laforêt, P., Voermans, N.C., Roux-Buisson, N., Bompaire, F., Rendu, J., et al. 2018. Phenotype and genotype of muscle ryanodine receptor rhabdomyolysis-myalgia syndrome. Acta Neurol. Scand. 137: 452–461.
24. Sambuughin, N., Mc.Williams, S., de Bantel, A.,

Kumaraswamy, S. and Thomas, N.E., et al. 2001. Single- amino- acid deletion in the RYR1 gene, associated with malignant hyperthermia susceptibility and unusual contraction phenotype. Am. J. Hum. Genet. 69: 204–208.
25. Ording, H. 1985. Incidence of malignant hyperthermia in Denmark. Anesth. Analg. 64: 700–704.
26. Oku, S., Mukaida, K., Nosaka, S., Sai, Y., Maehara, Y. and 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.
27. Robinson, R., Carpenter, D., Shaw, M.A., Halsall, J. and Hopkins, P. 2006. Mutations in RYR1 in malignant hyperthermia and central core disease. Hum. Mutat. 27: 977–989.
28. Sano, K., Miura, S., Fujiwara, R., Fujioka, R., Yorita, A., Noda, Kazuhito., et al. 2015. A novel missense mutation of RYR1 in familial idiopathic hyper CK-emia. J. Neurol. Sci. 356: 142–147.
29. Stewart, S.L., Hogan, K., Rosenberg, H. and Fletcher, J.E. 2001. Identification of the Arg1086His mutation in the alpha subunit of the voltage-dependent calcium channel (CACNA1S) in a North American family with malignant hyperthermia. Clin. Genet. 59: 178–184.
30. Schiemann, A.H. and Stowell, K.M. 2016. Comparison of pathogenicity prediction tools on missense variants in RYR1 and CACNA1S associated with malignant hyperthermia. Br. J. Anaesth. 117: 124–128.
31. Tian, X., Liang, W.-C., Feng, Y., Wang, J., Zhang, V.W., Chih-Hung, C., et al. 2015. Expanding genotype/phenotype of neuromuscular diseases by comprehensive target capture/NGS. Neurol. Genet. 1: e14.