Original Article

Whole Exome Sequencing Revealing *RYR1* Pathogenic Variant in an Exceptional Family with Malignant Hyperthermia Susceptibility

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Objective: To use whole exome sequencing (WES) in identifying pathogenic allele(s) in a challenging family of which specimen of the malignant hyperthermia (MH)-suspected case was not available. Points related to genetic counseling were also discussed.

Materials and Methods: A counselee walked in for genetic counseling and genetic test as there was a "deadly allergic reaction to anesthesia" in one of the family relatives. Multiple sessions of pre-test counseling were provided to the whole family. WES with targeted analysis of 23 genes associated with malignant hyperthermia susceptibility was carried out in nine individuals including one of the adult children, full sibs, and half-sib of the index case. Fourteen computational prediction programs, which included REVEL, DANN, DEOGEN2, FATHMM-MKL, LIST-S2, M-CAP, MVP, PolyPhen2, MutationTaster, BayesDel_addAF, EIGEN, MutationAssessor, PrimateAI, and SIFT, were used for pathogenicity prediction.

Results: The family consisted of 4-generation pedigree and comprised of 36 blood-related individuals. The authors identified two *RYR1* variants, c.550G>A (p.Ala184Thr) and c.1840C>T (p.Arg614Cys). The inferred genotype was heterozygous p.Arg614Cys for the index case and compound heterozygous p.Ala184Thr/p.Arg614Cys for the father of the individual. The p.Arg614Cys allele was previously proved to be pathogenic. Therefore, the p.Ala184Thr, which was not present in Thai reference genomes, was concluded to be non-pathogenic. Five additional family members were found to carry the *RYR1* pathogenic variant and were given comprehensive post-test counseling.

Conclusion: WES analysis is a powerful tool for determining the pathogenic allele in MH susceptibility family, even in the absence of specimen of the index case. However, comprehensive pre- and post-test genetic counseling and careful selection of the individuals to be tested is mandatory.

Keywords: Thai; malignant hyperthermia; *RYR1*; *CACNA1S*

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Malignant hyperthermia (MH) is a rare pharmacogenetic disorder characterized by acute hyper-metabolic state within skeletal muscle, which occurs during or immediately after the application of depolarizing muscle relaxant, succinylcholine, or potent inhalation anesthetics such as halothane, isoflurane, sevoflurane, or desflurane.

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Pathophysiology of MH includes defect in calcium homeostasis and hyper-metabolic state in the muscle as a result of continued contraction of the skeletal muscle, leading to massive carbon dioxide (CO_2) production, skeletal muscle rigidity, tachyarrhythmia, respiratory acidosis, lactic acidosis, fever, multi-organ failure, and eventually death[1-3]. The disorder is inherited in an autosomal dominant manner with incomplete penetrance. Mortality rate is high at 70% to 80%[2,4].

Clinical diagnosis of MH is based on established clinical grading scale[5]. These comprise objective parameters noted by anesthesiologists, including masseter rigidity, muscle breakdown as indicated by elevated serum creatine kinase and myoglobinuria, respiratory acidosis as shown by elevated blood $PaCO_2$, high fever, cardiac arrhythmia, arterial blood gas parameters, and response to intravenous dantrolene[5]. Clinical findings of the patient were then ranked into MH score, with the score of 5 or

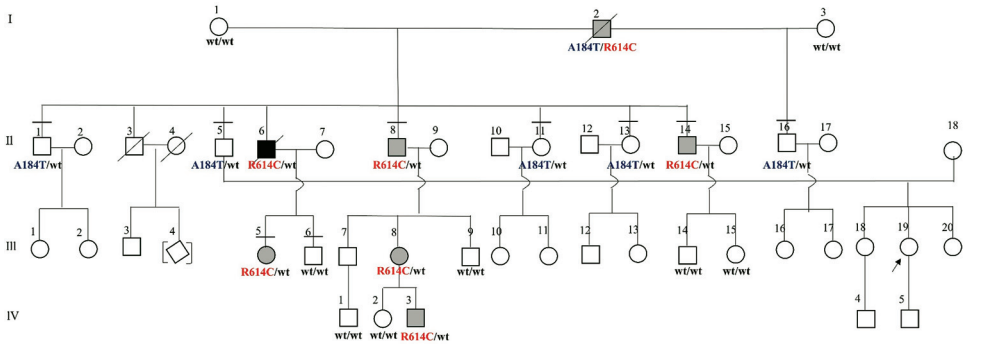

Figure 1. Pedigree of the study family. Arrow indicating the counselee (III-19). Genotype of each individual shown under their symbol. Black-filled square representing the index case (symptomatic); grey-filled square/circle for those with pathogenic allele c.Arg614Cys (R614C), but having no symptomatic experience; square indicating male and circle indicating female. Horizontal bars above the symbols indicating those receiving pre-test counseling followed by whole exome sequencing.

more indicating MH-suspected cases while those with score of 4 or less represents MH-like cases[5]. The gold standard confirmatory test for MH susceptibility (MHS) is an in vitro measurement of contracture response of biopsied muscle to varying concentrations of caffeine or halothane, known as caffeine/halothane contracture test (CHCT) or in vitro contracture test (IVCT)[1-3]. This test is invasive, costly, and available in only some countries, limiting the confirmation of MHS in most cases.

The most common responsible gene found to be associated with MH is *RYR1* (ryanodine receptor 1) accounting for 50% to 60% of those confirmed by the muscle contracture tests, followed by *CACNA1S* (calcium channel, voltage-dependent, L type, alpha-1s subunit) and *STAC3* (SH3 and cysteine-rich domains 3), each in less than 1% of the cases[1,2,6]. To date, over 181 *RYR1* variants have been reported as pathogenic or likely-pathogenic allele-associated with MHS, as listed in the Human Gene Mutation Database (HGMD) and ClinVar database[7,8]. Among these, only 48 variants have been functionally proven to be pathogenic, according to the European Malignant Hyperthermia Group (EMHG; https://www.emhg.org/diagnostic-mutations).

The exact incidence of MH is unknown. The incidence of MH is estimated 1 in 10,000 anesthetics in children and 1 in 50,000 anesthetics in adults[2,3]. A recent study from UK indicates an incidence of 1 in 250,000 anesthetics[4]. The incidence of MH in Thailand is estimated at 1 in 150,000 anesthetics, as reported by the Thai Anesthesia Incidents Study (THAI study)[9,10].

RYR1 gene (MIM#180901) located on chromosome 19q13.2, is a large gene, comprising of 106 exons and spanning 153.87 kb with mRNA size of 15.39 kb. Conventional Sanger screening of the entire *RYR1* and other MH-related genes is laborious, time-consuming, and costly. With the advance of the next generation sequencing (NGS), genetic test has been increasingly used to confirm diagnosis in MH.

Herein, the authors describe the application of whole exome sequencing (WES) in identification of pathogenic allele(s) in a challenging family of which specimen of the MH-suspected case was not available. Points related to genetic counseling were also discussed.

Materials and Methods

Ethical statement

The research protocol was approved by the Ramathibodi Hospital Human Research Ethics Committee (MURA2021/19). Medical records and genetic test reports of all family members that underwent the clinical genetic testing were retrospectively reviewed. Written informed consents for publication were obtained from the individuals with positive pathogenic *RYR1* allele, following the approval of the Institutional Research Ethics Committee.

Participating subjects and specimens

A counselee (individual III-19) (Figure 1) was a 35-year-old woman who walked in for genetic counseling and genetic test for her child, as there was a "deadly allergic reaction to anesthesia" in one of her paternal uncles (II-6). Since clinical data were greatly limited and that the counselee was not a child of the index case, the authors suggested gathering more clinical data and reaching out to the immediate family of the index case.

Subsequently, one of the adult children (III-5)

of the index case, accompanied by the counselee, came to the genetic clinic. The authors counseled them that genetic testing using WES approach was possible to identify genetic defect-related to MH, with limitations. The individuals went back to discuss with their family.

Later, eleven family members including all the living full-sibs, half-sib, and adult children of the index case came for a group pre-test counseling. The authors addressed the necessity of obtaining medical record of the index case from the hospital that the unfortunate event of MH took place and that the gold standard in vitro muscle contracture test was not available in the country. The authors explained the challenges and limitations including interpretation of the genetic test due to unavailable specimen of the index case, the absence of a known familial mutation, the possibility of extending WES to multiple family members before discovery of a pathogenic allele, and that negative data was possible as high as 40% to 50%.

The family appeared to understand the limitations and the stepwise approach of the test offered. They opted not to pursue further communication with the previous hospital and did not consent us to obtain medical data because the event occurred over ten years earlier and that they had a difficult time with the hospital due to the unexpected tragedy. Therefore, the authors did suggest doing WES in the two adult children of the index case first, if positive, then the authors could just perform Sanger sequencing for the targeted mutation in the rest of family members. In the case of negative result, the authors would extend WES to few additional family members at the time. However, the oldest brother of this family and other family members, which included 11 persons, who attended the pre-test counselling, of which nine individuals expressed their willingness to have WES study done, all at once. They said they did not want to wait longer and unintentionally put the few tested persons in the spot of attention and anxiety alone. The authors informed the family that negative WES result excluded neither MH in the index case nor MHS in the offspring.

At the end of counseling session, the family asked the authors to proceed on identifying the genetic defect running in their family by doing WES study for nine individuals at-risk for the mutation, including the living full-sibs, which were individuals II-1, II-5, II-8, II-11, II-13, and II-14, the half-sib, which was II-16, and the adult children of the index case, which were III-5 and III-6, all at once, on their own expense. The full pedigree was taken. After the results of genetic test were finalized, post-test genetic counseling by a clinical geneticist (DW) and an anesthesiologist (TV) was provided to the family.

Whole exome and Sanger sequencing

DNA was extracted from peripheral blood, using Gentra® Puregene® kit (QIAGEN®, Germany). WES was performed on Illumina HiSeq2000 by Macrogen® (Seoul, Republic of Korea), using Agilent's SureSelect (V5+UTR) for target enrichment (100bp Pair End mode and 125x coverage of target regions). The exome data were quality assessed by using the FastQC package and read alignment against a reference genome (hg19 from UCSC genome browser database) by using Burrows-Wheeler aligner (BWA, version 0.5.9); SAMTOOLS for variant identification; ANNOVAR for variant annotation, filtering and prioritizing the potential variants called for further analysis, following Broad Institute's best practice guidelines for GATK v3.4 (https://www.broadinstitute.org/) and the previous established protocols[11].

WES data were analyzed using vcf files and an online software, BaseSpace Variant Interpreter program (https://variantinterpreter.informatics.illumina.com). A human phenotype ontology term "malignant hyperthermia" HP:0002047 including 23 genes known to be linked with MHS was used for the analysis. They were *ABCA12*, *BIN1*, *CACNA1S*, *CHRNA1*, *CHRND*, *CHRNG*, *CLCF1*, *CRLF1*, *DNM2*, *EDAR*, *EDARADD*, *ELP1*, *HSPG2*, *KDF1*, *MTMR14*, *MYH3*, *NALCN*, *PGM1*, *RYR1*, *SCN4A*, *SCN5A*, *STAC3*, *TRAPPC9*. The authors spent the initial focus on the most common MH-related genes, *RYR1*, *CACNA1S*, and *STAC3*.

The variants detected were then classified according to the 2015 guidelines of the American College of Medical Genetics and Genomics and the Association of Molecular Pathology (ACMG/AMP)[12], using VarSome, a variants prediction software (https://varsome.com/). A set of 14 computational prediction programs, which included REVEL, DANN, DEOGEN2, FATHMM-MKL, LIST-S2, M-CAP, MVP, PolyPhen2, MutationTaster, BayesDel_addAF, EIGEN, MutationAssessor, PrimateAI, and SIFT were used in the pathogenicity prediction.

Once a genetic variant(s) was disclosed by WES, standard PCR-Sanger sequencing was performed to validate the identified variant(s) and to confirm the presence/absence of the variants in the other family members. Intronic flanking primers were designed

Table 1. Primer sequences used in the present study

Primer	Sequences (5' to 3')	Product size (bp)	AT (°C)
For *RYR1* variants detected			
7F	TGATGACTCTGTCTCCCATCT	311	60
7R	GAGGTTCCAAGGCTCCATTT		
17F	CCCTTTAACCTCTGACCTTGAC	342	60
17R	GACAGAACAAGAGGAGTGGATG		
For *RYR1* regions with incomplete coverage by WES			
33F	CTTGACCCATGTGTGTCTCTC	309	60
33R	CCAGAGGGCTTGCAACA		
53F	CCCTAAGACCCTTAGCTTGTTC	325	60
53R	AACCCACAGATCCACCTAGA		
64F	TGTACATCTGCTTGCTCTTCC	214	60
64R	ATGGCTCCCTCTCCTTACTT		
91F	TCATCTTCGACGTGGTGAAC	894	60
91R	TAGCCAGTTCTCTCCTCTGT		

AT=annealing temperature; bp=base pair

using PRIMER3 (http://frodo.wi.mit.edu). Primer sequences are shown in Table 1. GenBank reference sequences of the present study were NM_000540, and NP_000531. Moreover, to complete the study of the entire coding segments, PCR-Sequencing of the exons with partial coverage was performed in an individual who was positive for pathogenic allele. If the result indicated additional variant(s) identified, the test would be extended to the other family members.

Results

Pedigree and genetic counseling

The full pedigree comprised of four generations, 36 blood-related individuals, and one index case (II-6) (Figure 1). The index case was reported as having "abrupt fever, acidosis, and cardiac death" in the operating room following the initiation of general anesthesia for surgical removal of a postauricular cystic mass. In the present family, all the women who experienced giving childbirth had local or regional anesthesia for their delivery-related procedures without problems. Individuals II-11 and II-13 had had general anesthesia without having adverse effects. Individual II-8 had spinal anesthesia without complications. None of the family members was known to have history of bleeding disorders, myopathies, or exertional rhabdomyolysis.

The family described their feeling of fear in living with the uncertainty of diagnosis, not knowing their individual risk of developing the "deadly allergic reaction to anesthesia", not knowing where and how to get a definitive test without available specimen of the index case, and how to get safe anesthesia for themselves. The family expressed their experience of having difficulty in finding doctors/hospitals in the region who would accept to provide medical/surgical care for them and that they often had to go outside the region to seek medical care. The counselee was afraid that her child could someday need surgery requiring general anesthesia for acute conditions in children such as acute appendicitis and that they would not have proper preparation for the safe anesthesia for her child.

Mutation data from WES analysis

WES showed 96% coverage of the *RYR1* region with an average read depth at 50x in all individuals tested. The segments with incomplete coverage were exons 33, 53, 64, and 91 (Figure 2). As for *CACNA1S*, consisting of 44 exons and *STAC3* comprising 12 exons, WES coverage was 100% and 92%, respectively with *STAC3* Exon 1-partially covered (data not shown).

By filtering out synonymous and deep intron variants, it yielded two *RYR1* and three *CACNA1S* alleles as follow, c.550G>A and c.1840C>T of *RYR1*, and c.4113+7T>C, c.2748C>T, c.1828-5T>C of *CACNA1S* gene. After filtering out variants with minor allele frequency (MAF) higher than 0.03, the *CACNA1S* alleles were excluded (MAF 0.880391, 0.0539137, 0.759585 for c.4113+7T>C, c.2748C>T, c.1828-5T>C, in respective order).

The *RYR1* c.550G>A and c.1840C>T variants carried low MAF at 0.000036 and 0.000106, as noted by the Genome Aggregation Database (gnomAD; (https://gnomad.broadinstitute.org).

The c.550G>A variant in exon 7, yielding an amino acid substitution of threonine for alanine at codon 184 (p.Ala184Thr), was classified as likely-pathogenic based on the 2015 ACMG/AMP criteria and was present in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar) as variant of uncertain significance (Table 2, Figure 3a, b).

The variant c.1840C>T in exon 17, leading to an amino acid replacement of cysteine for arginine at codon 614 (p.Arg614Cys), was classified to be pathogenic and listed in ClinVar database as pathogenic allele (Table 2, Figure 3c, d). Eight of 14 prediction programs indicated deleterious effects of the p.Ala184Thr allele while 13 of 14 suggested harmful effect of the p.Arg614Cys variant.

The p.Ala184Thr variant was present in individuals II-1, II-5, II-11, II-13, and II-16, while the p.Arg614Cys was found in individuals II-8,

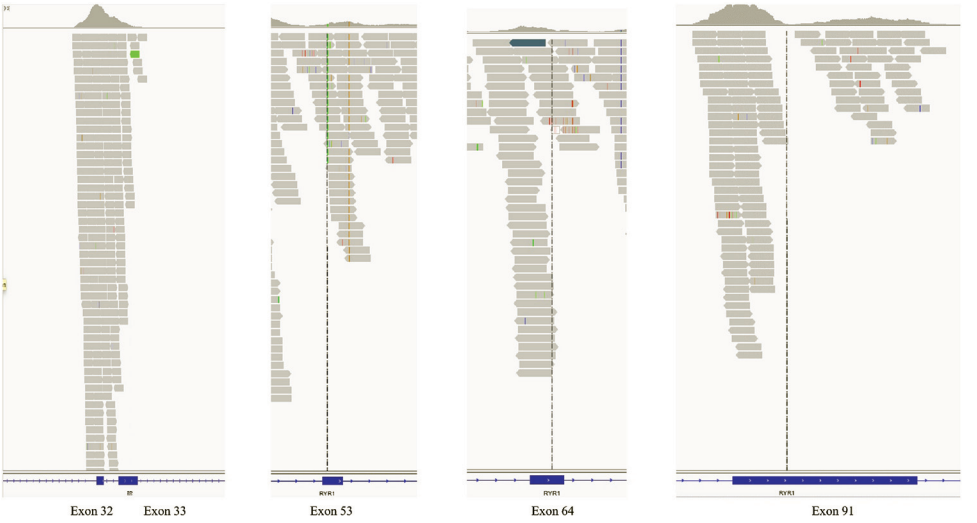

Figure 2. WES of *RYR1* exons 33, 53, 64, and 91. Noted part of the exons 33, 53, and 64 having low read depth <20x, and part of exon 91 having low read depth and/or zero read, indicating an incomplete coverage by WES. This pattern was seen in all individuals tested with WES.

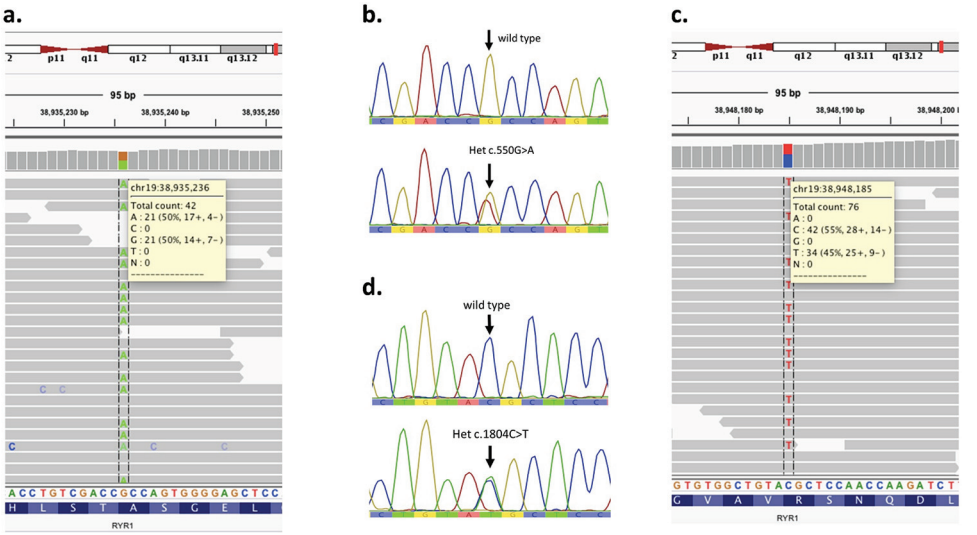

Figure 3. WES and Sanger sequences of *RYR1* variants identified. (a) WES showing c.550G>A, with read depth 42, and G/A allele= 21/21. (b) Chromatogram of c.550G>A. (c) WES indicating c.1840C>T, with read depth 76, and C/T allele=34/42. (d) Chromatogram of c.1840C>T.

II-14, and III-5. Based on identity by descent, it suggested that the index case (II-6) had genotype, heterozygous p.Arg614Cys. Subsequently, Sanger sequencing for p.Arg614Cys was extended to seven additional relatives at risk and identified two more positive individuals (III-8 and IV-3). To further identify which parent passed on the p.Ala184Thr and p.Arg614Cys alleles, genotype study of the living individuals in older generation, I-1 and I-3, was performed and revealed only wild type allele. This information was used to infer the genotype to the individual I-2 as having both variants in trans or compound heterozygous p.Ala184Thr/p.Arg614Cys. The PCR-Sanger sequencing of the exons 33, 53, 64, and 91 in an individual (III-5) with p.Arg614Cys revealed no additional *RYR1* variants. The authors did not do a PCR sequencing for *STAC3* exon 1-partially covered because a pathogenic variant in *RYR1* was already confirmed, and that *STAC3* was one of the least prevalent cause of MH at less than 1%.

Table 2. Characteristics of two *RYR1* variants identified in the present study

Comparison parameter	*RYR1* variant	
	c.550G>A	c.1840C>T
SNP#	rs766256366	rs118192172
gnomAD, MAF	0.000036	0.000106
T-REx, MAF[a]	0	0
Protein change	p.Ala184Thr	p.Arg614Cys
ClinVar	Uncertain significance RCV001127337	Pathogenic VCV000012964
2015 ACMG/AMP, general criteria	Likely-pathogenic (PM1, PM2, PP2, PP3)	Pathogenic (PM1, PM2, PM5, PP2, PP3, PP5)
2021 ACMG/AMP, *RYR1*-specific criteria	Uncertain significance (PM1)	Pathogenic (PS3_moderate, PS4, PM5, PP1_strong, PP3_moderate)
Prediction programs		
REVEL[b]	0.518	0.9269
DANN[b]	0.9933	0.9985
DEOGEN2	Damaging	Damaging
FATHMM-MKL	Damaging	Damaging
LIST-S2	Damaging	Damaging
M-CAP	Damaging	Damaging
MVP	Pathogenic	Pathogenic
PolyPhen2	Probably damaging	Probably damaging
MutationTaster	Disease causing	Disease causing
BayesDel_addAF	Tolerated	Damaging
EIGEN	Benign	Pathogenic
MutationAssessor	Low	Medium
PrimateAI	Tolerated	Damaging
SIFT	Tolerated	Damaging

[a] From 2,184 total alleles; [b] Value 0 to 1, with 1 given to the variants predicted to be the most damaging

Discussion

The present study demonstrated the success in using WES analysis to identify *RYR1* pathogenic allele in a challenging family of which DNA of the index case was not available. It led to an identification of five living family members with the *RYR1* pathogenic (p.Arg614Cys) variant, solving the enigma of the family. The authors showed a robust pedigree construction, pedigree analysis, careful selection of individuals for WES, and pre- and post-test counseling were key successful factors in such case. In case of available specimen of the index case, WES would be started from the index patient only, followed by Sanger sequencing of the mutation identified to the remaining family members, which would yield cost-saving at least 10x to 15x.

NGS-based targeted exome (TES) has been recently used as the first-tier diagnostic test for patients with MHS in several countries, as it provides cost-effectiveness and non-invasive procedure[1]. TES is generally considered as having higher sensitivity than WES because of its higher coverage. With the lowering cost of WES and its improving coverage and accuracy, WES with targeted gene analysis has become an alternative to TES[13]. In the authors context, we used more of WES in genetic diagnosis for known rare and undiagnosed disorder, because it can be accessed easier locally, with affordable price, and flexibility in the analysis beyond the initial list of genes of interest as needed. It is not known if TES or WES with targeted gene analysis being more cost-effective for diagnosis of MHS, due to restricted data.

As for why the family opted to have WES performed in nine at-risk individuals all at once instead of few family members first for cost-saving, the authors think that it could reflect the "family's values of integrity and unity", as they had shared suffering and fear for over a decade, and that they did not want to prolong that feelings or unintentionally put any family member in that situation alone while waiting for the genetic test result. The authors respected the family's decision and believed that this case represents an excellent example of "autonomy" in making decisions that are right for themselves. The lay description of "deadly allergic reaction to anesthesia" is not an uncommon situation happening

to families affected with MHS worldwide. This unexpected catastrophe could sometimes result in conflict between the family and the treating physicians/hospitals and uncooperative investigation for the cause of unfavorable outcome, leaving the rest of the family members and succeeding generations under a life-time fear/stress. In some country including Thailand, testing for MHS is not widely available and specimens of the deceased index cases were often not collected for further testing.

Although guidelines for MHS have been developed for diagnostic and management process including how to make referral of the patient for diagnosis, the real-word experiences could be different. The present study demonstrates the other side of the patient's family, which may have been overlooked and need to be addressed. Collaboration between clinical geneticists and anesthesiologists could advance the pragmatic approach for diagnosis and holistic management for MHS. As for the present family, they received post-test counseling from clinical geneticist and anesthesiologist, including an anesthesia alert card for confirmed heterozygous individuals. The authors support that data registry and systematic management for MHS family is much worthy and should be set up to fit the local context as it has been done in some countries[4,9,14].

The identification of positive *RYR1* pathogenic (p.Arg614Cys) variant in five family members has benefited them, thus considering change of anesthesia management. Each positive individual was given a one-page identification including their name and positive *RYR1* test, the risk of having MHS, the signs and symptoms that may occur, the anesthetic agents to be avoided, and the direct contact of the authors Department of Anesthesiology in case of emergency or if help is needed. A 13-paged clinical guideline (Thai version) by the Thai Royal College of Anesthesiology for anesthesia management for individual-at-risk of MHS and for management of MH symptoms, was given to the individuals so that they can share with the treating physicians prior to a plan for surgical procedure at-risk. In brief, the safe anesthesia management plans include 1) choosing local anesthesia instead of general anesthesia, if possible, 2) anesthetic agent should be removed for the machine and circuits following the guidelines and new equipment including corrugated tubes, reservoir bag, ventilator bellow, and soda lime should be employed, 3) prepare dantrolene and other medicinal drugs and equipment for emergency use, according to the detailed guideline, 4) avoid using depolarizing agent succinylcholine or trigger anesthetic agents such as potent volatile anesthetic agents as halothane, sevoflurane, desflurane, enflurane, and isoflurane, 5) close monitoring of MH symptoms for at least 24 hours after the initiation of general anesthesia and close monitoring of the temperature and end tidal carbon dioxide for individuals undergoing general anesthesia that exceeds 30 minutes duration, 6) prophylactic dantrolene is not recommended, and 7) if signs of MH occurred, dantrolene should be given immediately.

The p.Ala184Thr and p.Arg614Cys variants are located in hot-spot regions I, between codon 63 in exon 2 and codon 614 in exon 17 of *RYR1*[15]. The p.Ala184Thr has not been functionally characterized while the p.Arg614Cys allele has been proved for its pathogenicity, as described in the EMHG, North American MH Consensus, and earlier publications[16-18]. An in vitro expression study has shown that p.Arg614Cys led to an increased sensitivity of *RYR1* channels in activating $Ca2^{+}$ release from the sarcoplasmic reticulum, via gain of function mechanism[19].

Though, one would think that only one allele, either p.Ala184Thr or p.Arg614Cys is truly representing pathogenic variant in the present family, others could argue that both alleles could be pathogenic as it appeared in different individuals and that they were classified as likely pathogenic and pathogenic, in respective order. The authors attempted to find further supporting evidence for more definitive conclusion before genetic counseling given. By using MAF as supporting evidence of pathogenicity, it was not useful in differentiating the two alleles because both were not present in the Thai Exome Reference Database (T-REx; https://trex.nbt.or.th; 2184 reference alleles). The presence of both alleles in trans in individual I-2 suggests that only one of them is the pathogenic allele. Based on the existing information, the authors precluded p.Ala184Thr from being the pathogenic allele in the present family. Additionally, the individuals II-11 and II-13 carrying p.Ala184Thr variant did not exhibit MH-related symptoms under general anesthesia. Limitation of the present study includes the absence of specimens from the index case and individual I-2 to confirm our conclusion of their genotypes.

The ACMG/AMP recommendation for *RYR1* pathogenicity classifications in MH was released in early 2021, of which seven criteria were adopted from 2015 ACMG/AMP general guideline without change, 10 were adopted with *RYR1*-specific modifications,

and nine were removed[20]. When applying the *RYR1*-specific pathogenic classifications to assess the variants, the c.1840C>T is still classified as pathogenic with some detail changed, whereas the c.550G>A is shifted from the category of likely-pathogenic to variant of uncertain significance (Table 2). The result of the reanalysis further supports the exclusion of the c.550G>A (p.Ala184Thr) as being a pathogenic allele.

A recent review suggests expanding phenotypes of *RYR1*-pathogenic variants, these include bleeding disorder, varying types of rare myopathies, heat/exercise induced exertional rhabdomyolysis, and atypical periodic paralysis[1]. None of these manifestations was observed in the present family. Moreover, homozygous *RYR1*-pathogenic alleles have been associated with other rare disorders such as MHS plus congenital ptosis and scoliosis, lethal multiple pterygium syndrome, fetal akinesia, and arthrogryposis multiplex congenita[1,21]. Data on recessive mutations of *RYR1* are still limited but it appears to be linked with complete penetrance. Compound heterozygous between p.Ala184Thr and p.Arg614Cys disclosed in the individual I-2 in the present family is unlikely to cause a recessive disorder as there was no relevant medical problems reported in that person.

To the authors knowledge, this is the first comprehensive genetic study of a Thai family with MH. There have been few clinical reports of MH from Thai population and a report of c.946C>T (p.Arg316Cys) in a sporadic patient, detected by partial *RYR1* gene screening[10,22-26].

Conclusion

WES analysis could be used successfully in determining the pathogenic allele in affected MHS family despite absence of specimen of the index case. However, this must be done with robust pedigree construction, careful selection of blood relatives of the index case for testing, and effective collaborative counseling provided by clinical geneticists and anesthesiologists. Identification of an individual risk status is not only valuable for prevention of anesthesia-related complications for the persons harboring a pathogenic allele, but also adding substantial psychological relieve for the whole family.

What is already known on this topic?

Twenty-three genes are known to be associated with MHS. In real-world practice, specimens of the index cases are often unavailable, leading to difficulty in identifying the pathogenic allele of the affected families.

What this study adds?

A combination of WES, robust pedigree construction, careful selection of blood-relatives of the index case for testing, and comprehensive pre- and post-test counseling led to success in determining the pathogenic allele in an affected family despite absence of the specimen of the index case.

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Authors' contributions

AK performed molecular analysis and prepared the manuscript draft. NK collected pedigree and clinical data. TT assisted with molecular analysis. TV and DW provided genetic counseling. DW designed the study concept, supervised data analysis, and revised the manuscript. All authors reviewed and approved the final manuscript.

Conflicts of interest

All the authors declare no competing interest.

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