Can J Anesth/J Can Anesth (2010) 57:689–693
DOI 10.1007/s12630-010-9314-4

CASE REPORTS/CASE SERIES

A report of fulminant malignant hyperthermia in a patient with a novel mutation of the *CACNA1S* gene

Un cas d'hyperthermie maligne fulminante chez une patiente présentant une nouvelle mutation du gène *CACNA1S*

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Received: 5 March 2010 / Accepted: 12 April 2010 / Published online: 30 April 2010

Abstract

Purpose *To report the identification of a novel mutation in the CACNA1S gene that encodes the alpha-1-subunit (Cav1.1) of the voltage-gated skeletal muscle L-type calcium channel in a patient with malignant hyperthermia.*

Clinical findings *An otherwise healthy 34-yr-old female developed fulminant malignant hyperthermia (MH) under sevoflurane anesthesia during laparoscopic donor nephrectomy. The first sign was an increase in end-tidal CO_2. Malignant hyperthermia was suspected early, and resuscitative measures, including supportive and specific treatment, were successfully implemented. The patient rejected the open muscle biopsy for the Caffeine-Halothane Contracture Test (CHCT); therefore, only molecular genetic testing was performed. Sequencing of the entire ryanodine receptor type 1 transcript did not reveal any MH causative mutations. However, a novel homozygous mutation, p.Arg1086Ser, was identified in the CACNA1S gene that encoded for the alpha-1-subunit of the skeletal muscle L-type calcium channel (Cav1.1). A CACNA1S mutation, p.Arg1086His, involving the same Arg1086 residue that is mutated in our patient has previously been reported in association with MH in three independent families.*

Conclusion *The homozygous p.Arg1086Ser mutation of CACNA1S, the gene that encodes the alpha-1-subunit of the voltage-gated skeletal muscle L-type calcium channel, is a novel mutation associated with malignant hyperthermia.*

Résumé

Objectif *Signaler l'identification d'une nouvelle mutation du gène CACNA1S, qui code la sous-unité alpha 1 (Cav1.1) du canal calcique voltage-dépendant de type L des cellules musculaires squelettiques chez une patiente souffrant d'hyperthermie maligne.*

Éléments cliniques *Une femme de 34 ans généralement en santé a développé une hyperthermie maligne (HM) fulminante alors qu'elle se trouvait sous anesthésie au sévoflurane pendant une néphrectomie laparoscopique pour don d'organe. Le premier signe s'est manifesté sous la forme d'une augmentation du CO_2 télé-expiratoire. Un diagnostic présumé d'hyperthermie maligne a été effectué de manière précoce, et des méthodes de réanimation, y compris des traitements de soutien et des traitements spécifiques, ont été adoptées avec succès. La patiente a refusé la biopsie musculaire ouverte pour le test de contracture à la caféine-halothane (CHCT); par conséquent, seules les analyses moléculaires génétiques ont été effectuées. Le séquençage de l'ensemble du transcrit du récepteur de ryanodine de type 1 n'a révélé aucune mutation associée à la HM. Cependant, une nouvelle mutation homozygote, p.Arg1086Ser, a été identifiée dans le gène CACNA1S, qui code la sous-unité alpha 1 du canal calcique de type L des cellules musculaires squelettiques (Cav1.1). Une mutation du gène CACNA1S, p.Arg1086His, impliquant*

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le même résidu Arg1086 qui a muté chez notre patiente, a précédemment été signalée en association avec la HM chez trois familles indépendantes.

Conclusion *La mutation p.Arg1086Ser homozygote de CACNA1S, le gène qui code la sous-unité alpha 1 du canal calcique voltage-dépendant des cellules musculaires squelettiques, est une nouvelle mutation associée à l'hyperthermie maligne.*

Susceptibility to malignant hyperthermia (MHS) is potentially a fatal, pharmacogenetic disorder of skeletal muscles. Exposure of MH susceptible individuals to anesthetic agents (halogenated anesthetics and the depolarizing muscle relaxant, succinylcholine) triggers a hypermetabolic crisis[1,2] that reflects deregulation of excitation-contraction (EC) coupling and calcium homeostasis in skeletal muscles.[3]

Susceptibility to malignant hyperthermia is inherited as an autosomal dominant trait that is mapped to six genetic loci.[4] The ryanodine receptor (*RYR1)* gene encoding the calcium release channel of the sarcoplasmic reticulum represents a major MH locus with more than 200 MH-associated mutations identified to date.[5] The other MH gene with an identified MH-associated mutation is the *CACNA1S* gene encoding the α1-subunit of the skeletal muscle L-type calcium channel.[6-8] Here we describe the identification of a novel *CACNA1S* mutation in a patient with a clinical episode of MH.

The authors received written permission from the patient to report the case.

Case description

A 34-yr-old female patient of South Asian origin presented for a laparoscopic donor nephrectomy. Her preoperative evaluation was significant for chronic anemia, impaired glucose tolerance, and mild gastroesophageal reflux disease. She had previously undergone three general anesthetics for appendectomy, Cesarean delivery, and tubal ligation; all were uneventful. The patient weighed 78 kg, and her body mass index was 29.7 $kg \cdot m^{-2}$.

In the operating room, standard monitors were placed, including non-invasive blood pressure cuff, five-lead electrocardiogram, and pulse oximeter, and peripheral venous access was established. Anesthesia was induced with midazolam 1 mg, fentanyl 250 μg, propofol 200 mg, and rocuronium 50 mg. The patient's trachea was intubated uneventfully and positive pressure ventilation was established. Anesthesia was maintained using sevoflurane (2% maximum end-tidal concentration) in an air/oxygen mixture of 1 $L \cdot min^{-1}$ each, with minute ventilation at 4.5 $L \cdot min^{-1}$. After anesthesia induction, a radial arterial line and esophageal temperature probe were placed. The initial temperature was 35.8°C.

The patient was positioned in the right lateral position and pneumoperitoneum was established and maintained at 12 mmHg. The procedure proceeded uneventfully for approximately two hours after induction when the patient's end-tidal CO_2 increased to 50 mmHg. This was attributed initially to the CO_2 used to create the pneumoperitoneum, and minute ventilation was increased to 6 $L \cdot min^{-1}$. There was no sign of tachycardia or fever at that time. However, over the next thirty minutes, end-tidal CO_2 increased to 60 mmHg, with a rise in temperature to 38.5°C and a drop of systolic blood pressure to 60 mmHg associated with ST depression. The pneumoperitoneum was released, and the decision was made to terminate the procedure and to treat the patient for presumed malignant hyperthermia.

The treatment for MH included the following steps. Sevoflurane was discontinued immediately, and hyperventilation (minute ventilation was increased to 8.5 $L \cdot min^{-1}$) was started simultaneously with an F_iO_2 of 100%. The patient was returned to a supine position, and all laparoscopic access ports were removed and incisions closed. The surgical staff established a femoral vein access. An operating room emergency was called, and extra staff were called to the room with the MH cart. Blood gases were taken and analyzed using a Point of Care (Bayer Rapidpoint® 405, Siemens Medical Solutions, Deerfield, IL, USA). The patient was found to have profound acidosis (Table 1).

Dantrolene sodium 2.5 $mg \cdot kg^{-1}$ (JHP Pharmaceuticals, LLC, Rochester, MI, USA) was prepared and administered (total = 400 mg). Epinephrine boluses of 4-8 μg were given, and a dopamine infusion at 4-8 $\mu g \cdot kg^{-1} \cdot min^{-1}$ was started to maintain systolic blood pressure >90 mmHg. To treat hyperkalemia and acidosis, 8.4% sodium bicarbonate 100 mL was given. Regular insulin 20 units, 50% dextrose-water 50 mL, and calcium chloride 1 gram were given to further treat hyperkalemia. Active cooling was initiated by packing the patient in ice and administering

Table 1 Results of arterial blood gas analysis

	2 hr	2.5 hr	3 hr	12 hr
pH	7.19	6.90	7.30	7.51
$PaCO_2$ (mmHg)	60	74	35	34
PaO_2 (mmHg)	210	426	407	114
HCO_3 ($mmoL \cdot L^{-1}$)	22	17	17	26
Base excess ($mmoL \cdot L^{-1}$)	−5.4	−14	−9.2	
Potassium	4.32	6.83	5.28	3.9

Time in hours after induction of anesthesia. $PaCO_2$ = partial pressure of carbon dioxide; PaO_2 = partial pressure of oxygen; HCO_3 = bicarbonate

cold normal saline lavage via the nasogastric tube and urinary catheter. Two units of packed red blood cells were infused to expand intravascular volume and improve oxygen-carrying capacity.

With these measures, the patient's temperature returned to normal, acidosis was reversed, end-tidal CO_2 was decreased to 32 mmHg, blood pressure was stabilized, and ST depression resolved. Ninety minutes after the first CO_2 increase, the patient was transferred to the intensive care unit (ICU) in a stable cardiopulmonary condition, sedated with propofol infusion.

As a result of rhabdomyolysis, creatine kinase increased to a maximum of 583 (normal range < 195), but there was no rise in creatinine or blood urea nitrogen. Dantrolene infusion (2.5 $mg \cdot kg^{-1}$ every six hours) was continued for 24 hr, and the patient's trachea was extubated 24 hr after the crisis. Creatine kinase returned to a normal level on postoperative day 3. Investigations in the ICU ruled out sepsis, hyperthyroidism, and pheocromocytoma, and the patient was transferred to a regular surgical ward on postoperative day two. She developed diarrhea associated with *Clostridium difficile* that responded promptly to oral metronidazole, and she was discharged home on postoperative day 6 in good physical condition. The patient was diagnosed subsequently with a deep vein thrombosis (DVT) at the site of the femoral vein puncture, and she required three months of anticoagulation with warfarin.

Using the MH clinical grading scale,[9] our patient's clinical scenario gave us a calculated score of 63 that classifies the clinical episode as almost certain MH. Moreover, the hemodynamic instability in our patient was resolved, and her temperature normalized once she received the treatment with dantrolene sodium.

With the patient's consent, two ethylenediaminetetraacetic acid (EDTA) blood samples were drawn for isolation of total ribonucleic acid (RNA) and preparation of genomic deoxyribonucleic acid (DNA). The entire *RYR1* transcript was amplified by a reverse-transcriptase - polymerase chain reaction (RT-PCR) and sequenced as previously described.[10] The patient's genomic DNA was used as a template for amplification by polymerase chain reaction (PCR) of *RYR1* exons that were missing in the transcript due to incorrect splicing, as well as exon 26 of *CACNA1S* where the known MH mutation was located.

Sequence analysis of the entire coding region of the patient's *RYR1* gene did not reveal any missense sequence variants. One silent polymorphism (c.3459C > T p.Asp1153Asp) was identified, which was unlikely to be causative of MH. On the other hand, a novel variant in exon 26 of the *CACNA1S* gene (c.3332C > A in the homozygous state) was identified, which results in the Arg1086 to Ser amino acid substitution (Figure 1A). In order to eliminate any sequence error resulting from DNA polymerase activity, three independently amplified products of two different DNA fragments encompassing the putative mutation site were analyzed by sequencing. Additionally, the presence of the novel mutation was confirmed by restriction analysis (Figure 1B). Bioinformatic analysis[11] demonstrated that the mutated residue was invariant across available sequences of the $alpha_1$-subunits (GenBank, National Center for Biotechnology Information - NCBI) and was embedded in a short amino acid stretch featuring extreme evolutionary conservation (Figure 1C).

Discussion

According to the European Malignant Hyperthermia Group guidelines,[12] molecular genetic testing should be performed only if the patient has an MHS status or if his/her relative's MHS status has been confirmed by a positive Caffeine Halothane Contracture test (CHCT). So far, the molecular genetic basis of MH can be identified in 50% of MH families, and only 29 MH-associated mutations have been proven causative. Therefore, molecular genetic investigations have a high probability of being inconclusive in patients with suspected clinical MH episodes. However, the chance to identify an MH causative mutation increases with a higher probability of a true MH event.

Due to the clinical picture, the relative contraindication to muscle biopsy due to the patient's recent history of DVT, and her refusal for muscle biopsy, we followed the Malignant Hyperthermia Association of the United States recommendations[13] and proceeded directly to molecular genetic testing.

Malignant hyperthermia exhibits locus heterogeneity, and MH-associated mutations have been identified in two MH loci. A locus on chromosome19q was identified as the *RYR1* gene encoding the skeletal muscle ryanodine receptor (RyR1), and a locus on chromosome1q was identified as the *CACNA1S* gene encoding the alpha1subunit (Cav1.1) of the voltage-gated calcium channel receptor. More than 200 *RYR1* variants have been identified in different MH families around the world. However, to date only a single *CACNA1S* mutation, p.Arg1086His, has been identified in association with MH in three independent families.[6-8]

Although sequencing of the entire *RYR1* transcript in our patient did not reveal any mutations, we detected a novel p.Arg1086Ser mutation in our patient's $alpha_1$ subunit of voltage-sensitive L-type calcium channel gene. This mutation affects the same Arg1086 residue, which substitution for histidine, is associated with MH. Functional studies have shown that, similar to mutations in *RyR1*, the p.Arg1086His mutation increases the sensitivity of the SR calcium release to activation by both caffeine and voltage.[14]

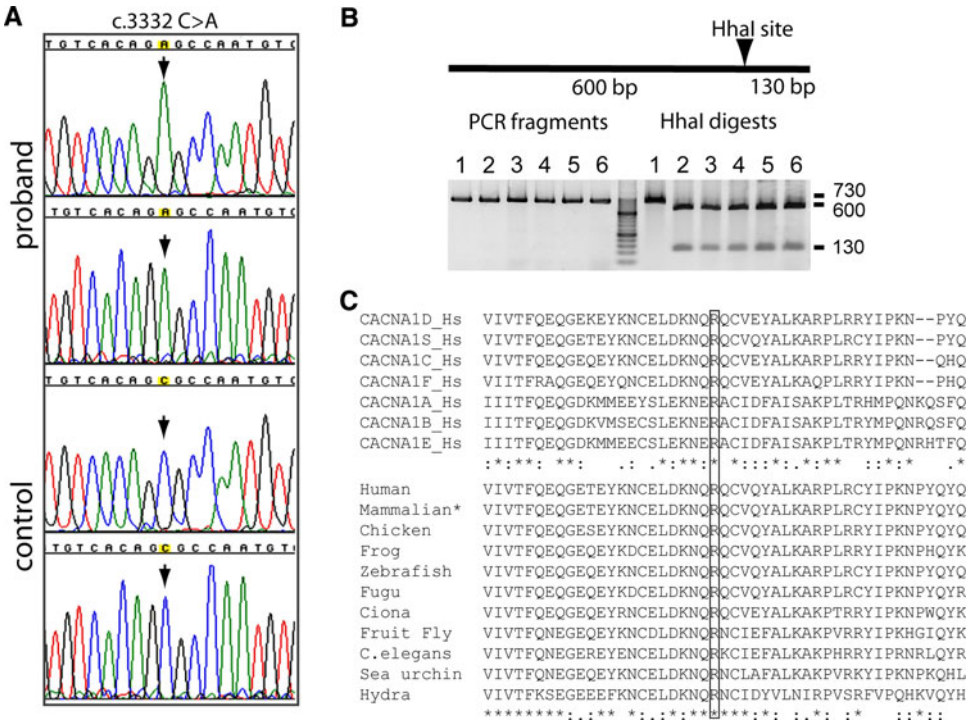

Fig. 1 **A**. Chromatograms of the sequencing analysis of DNA in the region of the new mutation. The position of the altered base is shown by arrows. **B**. Design and results of the restriction fragment length polymorphism analysis, showing predicted structure of the PCR fragment, the location of the HhaI cleavage site (top) and the analysis of DNA of the control and mutant subjects by gel electrophoresis. As seen from the diagram and on the gel, HhaI digestion of the 730-bp PCR fragments, amplified from genomic DNA of individuals carrying normal c.3332C allele, generates two (600-bp and 130-bp) fragments. Presence of the c.3332C > A in homozygous state in the proband's DNA results in loss of HhaI site and yields the uncut 730-bp fragment after HhaI digestion. 2% agarose gel; SybrGreen staining; 50-bp DNA marker XIII. **C**. Alignment of protein sequences in the mutated region of the alpha1-subunit of the voltage-gated calcium channel Cav1.1. The top portion of the alignment shows comparison of the sequences of the known alpha1-subunits of the voltage-gated calcium channels encoded in the human genome. The bottom portion displays comparison of the human protein sequence with the representative sequences of the orthologous proteins from various systematic groups of animals

Bioinformatic analysis[11] predicted that the novel p.Arg1086Ser mutation would be damaging for the protein function. Functional testing of the mutant protein in a suitable expression system will be required to confirm this prediction. To prove the causative nature of this mutation, it has yet to be established that the mutation segregates with the MHS phenotype (determined by CHCT) within the patient's family and that its frequency is below 1% in a group of presumably MH negative individuals of the same ethnicity.

In conclusion, our knowledge of the etiology of MH is still quite limited, and there are many unidentified mutations that potentially can cause MH. Therefore, awareness of MH and fast recognition of its clinical signs are essential in giving a safe anesthetic. The low incidence of MH, the vast number of associated genetic changes, and the limited ability of healthcare systems (in much of the world) to investigate patients will limit the pace at which our understanding of MH genetics grows. As a result, evaluation of patients such as ours is essential in continuing the slow and steady increase of our understanding.

Competing interests None declared.

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