Using DNA Techniques for Detecting Susceptibility of Malignant Hyperthermia

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List of Abbreviations

$[Ca^{+2}]i$	Intra-cellular Calcium
ASC	Ambulatory Surgical Center
bp	Base pair (unit of genome measurement)
Ca^{+2}	Calcium
CACNA1S	Calcium Channel, Voltage-dependent, L type, Alpha 1S subunit
cADPr	Cyclic ADP Ribose
CASQ1	calsequestrin (a calcium-binding protein of the SR)
Cd^{+2}	Cadmium
CHCT	Caffeine-Halothane Contracture Test
CHO	Chinese Hamster Ovary
CICR	Calcium-induce Calcium Release
Ck	Creatine Kinase
CO_2	Carbon Dioxide
DHPR	Dihydropyridine Receptor
EC	Excitation Contraction
ECCE	Excitation-Coupled Calcium Entry
EMHG	European Malignant Hyperthermia Group
$ETCO_2$	End Tidal Carbon Dioxide level
HMG-CoA	3-hydroxy-3methylglutaryl-Co-enzyme A
IP3	Inositol-3-Phosphate
IP3R	Inositol-3-Phosphate Receptor
IVCT	In-vitro Contracture Test
K^+	Potassium
MAC	Minimum Alveolar Concentration
MHAUS	Malignant Hyperthermia Association of the United States

List of Abbreviations (Cont.)

MDMA	3,4-methylenedioxymethamphetamine
Mg^{+2}	Magnesium
MH	Malignant Hyperthermia
MHCSA	Malignant Hyperthermia Centre of South Africa
MHE	Malignant Hyperthermia Equivocal
MHN	Malignant Hyperthermia Negative
MHS	Malignant Hyperthermia Susceptible
NAADP	Nicotinic Acid Adenine Dinucleotide Phosphate
NAMHG	North American Malignant Hyperthermia Group
NGS	Next Generation Sequencing
pCO^2	Partial Pressure of Carbon Dioxide
PCR	Polymerase Chain Reaction
ppm	parts-per-million
RHCF	Receiving Health Care Facility
RYR1	Ryanodine Receptor type 1 (skeletal muscle)
RYR2	Ryanodine Receptor type 2 (cardiac muscle)
SERCA	Sarco-Endoplasmic Reticulum Ca^{2+} -ATPase
SNP	Single Nucleotide Polymorphism
SOC	Store-Operated Channels
SOCE	Store-Operated Channels Entry
SR	Sarcoplasmic Reticulum
STIM1	Stromal interaction molecule 1
TT	Transverse Tubule

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Introduction

Malignant hyperthermia (MH) is one of the most devastating anesthesia-related complications. This fulminant syndrome is a pharmacogenetic clinical syndrome that, in its classic form, occurs during anesthesia with a volatile halogenated alkane such as halothane and/or the administration of the depolarizing muscle relaxant succinylcholine. The fulminant MH episode observed clinically produces rapidly increasing body temperature (by as much as 1° C in 5 minutes) and extreme acidosis as a result of an acute loss of control of intracellular calcium levels and compensatory uncontrolled increases in skeletal muscle metabolism that may proceed to severe rhabdomyolysis. Although MH was initially associated with a mortality rate of 60%, earlier diagnosis and the use of dantrolene have reduced the mortality to about 5% ***(Brady et al., 2009).***

Malignant hyperthermia is a disorder that can be considered a gene-environment interaction. In most people with malignant hyperthermia susceptibility, they have few or no symptoms unless they are exposed to a triggering agent. So, the detecting of susceptibility of MH before undergoing surgery under general anesthesia is very essential and important to avoid fatal complication from an episode of MH. The gold standard for diagnosis of MH is the in-vitro contracure test (IVCT) using skeletal muscle biopsy tissue after the European protocol or the caffeine halothane contracture test (CHCT) after the North American protocol. Both tests have limitations in sensitivity and specificity. They are, however, extremely invasive and are subject to between-center variation. The ability to replace these physiological tests with a simple DNA test has been a major goal since

1990, when the first gene associated with MH susceptibility was identified **(*Litman et al., 2005).***

The advent of the polymerase chain reaction and the availability of data from various global human genome projects should make it possible, using a DNA sample isolated from white blood cells, to diagnose rapidly and accurately almost any monogenic condition resulting from single nucleotide changes. DNA-based diagnosis for malignant hyperthermia (MH) is an attractive proposition, because it could replace the invasive and morbid caffeine-halothane in vitro contracture tests of skeletal muscle biopsy tissue. Moreover, MH is preventable if an accurate diagnosis of susceptibility can be made before general anesthesia, the most common trigger of an MH episode ***(Stowell, 2014).***

Aim of the Essay

This essay is directed toward highlighting the recent diagnostic techniques for detecting the susceptibility of malignant hyperthermia using DNA techniques and detect if it is capable to replace the standard in vitro contractures.

Chapter one
Pathophysiology of Malignant Hyperthermia

Malignant hyperthermia (MH) is a rare life-threatening condition that is usually triggered by exposure to certain drugs used for general anesthesia, specifically the volatile anesthetic agents and succinylcholine, a neuromuscular blocking agent. In susceptible individuals, these drugs can induce a drastic and uncontrolled increase in oxidative metabolism in skeletal muscle, which overwhelms the body's capacity to supply oxygen, remove carbon dioxide, and regulate body temperature, eventually leading to circulatory collapse and death if not immediately treated. ***(Diptiman et al., 2013).***

Current cases of MH are restricted in severity because of diagnostic awareness, early detection through end-expired carbon dioxide (CO_2), the use of less potent anesthetic triggers, and the administration of drugs that attenuate the progression of the fulminant episode. The incidence of fulminant MH was reported to be 1 case per 62,000 anesthetics administered when triggering agents were not used, but the number of suspected cases was 1 case per 4500 anesthetics administered when triggering agents were administered ***(Diptiman et al., 2013).***

MH has virtually no characteristic phenotype before exposure to the triggering agent and is truly an example of the interaction of genes and the environment. It is caused by dysregulation of excitation contraction (EC) coupling in skeletal muscle as a result of altered control of sarcoplasmic reticulum (SR) Ca^{2+} release. Mutations have been identified in MH-susceptible (MHS) individuals in two key proteins of

EC coupling, the Ca^{2+} release channel of the SR, ryanodine receptor type 1 (RyR1) and the α1 subunit of the dihydropyridine receptor (DHPR, L-type Ca^{2+} channel) ***(Stowell, 2014).***

❖ ***Calcium homeostasis & excitation-contraction coupling***

Variations in intracellular calcium ion concentration are directly or indirectly related to many cellular functions, including muscle contraction and energy metabolism. In the resting state, the cytosolic calcium concentration is maintained at around the 100 nM level against approximately 10,000-fold concentration gradients between both the external fluid and the intracellular stores. Calcium release from these stores is triggered by a number of second messengers, including nicotinic acid adenine dinucleotide phosphat (NAADP), inositol-3-phosphate (IP3), cADP-ribose (cADPr) and Ca^{2+}. IP3 acts on the IP3 receptor (IP3R) rather than RyR1, while NAADP and cADPr appear to act via RyR1, although their exact mechanisms and functional roles in skeletal muscle are unknown ***(Pan and Ma, 2003).***

The major calcium store in skeletal muscle is the SR. Calcium release from the SR is predominantly regulated by physical communication between two channels: the L‑type voltage-dependent Ca^{2+} channel, DHPR in the T-tubule membrane and the Ca^{2+} release channel, RyR1 located at the terminal cisternae of the SR. Thus, a functional interaction known as excitation-contraction (EC) coupling occurs between these two multi subunit channels in response to electrical stimulation. This is in contrast to the situation in cardiac muscle where the two channels communicate solely by calcium-induced calcium release (CICR) with no actual physical contact. The result is a release of Ca^{2+} into the

cytoplasm from stores in the SR (orthograde conformational coupling), which triggers muscle contraction and has a range of other functional consequences including increased flux through the glycolytic pathway and apoptosis. Ca^{2+} is returned to the SR by a sarco/endoplasmic reticulum Ca^{2+} - ATPase (SERCA) ***(Pan and Ma, 2003).***

A consequence of calcium release from intracellular stores is the concomitant decrease in stored calcium. This results in the activation of store-operated channels (SOC) channels on the plasma membrane, resulting in an influx of extracellular calcium. Studies of the polypeptide components comprising these channels, as well as the signalling mechanisms responsible for their activation, suggest both heterogeneity and complexity ***(Pan and Ma, 2003).***

While SOC entry has long been associated with non-excitable cells, SOC influx associated with electrical stimulation was first demonstrated in skeletal muscle in 2001, and later shown to be coupled to the IP3R. More recently, a calcium sensor for the ER/SR has been identified as stromal interaction molecule 1 (STIM1) ***(Pan and Ma, 2003).***

A number of the polypeptides constituting the skeletal muscle calcium-release channel, including RyR1, have also been shown to have a role in regulating the entry of calcium into the cell via SOC channels. RyR1 activation has been shown to activate SOC release in primary human muscle cells, and the foot region of RyR1 appears to be required for this functional coupling ***(Antaramian et al., 2005),***

More recently, the calcium sensor STIM1 has been shown to localize on the terminal cisternae of the SR with RyR1. Therefore, it is clear that SOC channels play an important role in overall calcium homeostasis and need to be

considered as possible effectors or modulators of an MH episode ***(Hawkins et al., 2008).***

As well as SOC entry, an alternative mechanism of Ca^{2+} entry into skeletal muscle myotubes has recently been recognized. This alternative pathway occurs in the absence of store depletion and is triggered by membrane depolarization, a process that normally blocks standard SOC entry. Thus, it has been suggested that Ca^{2+} entry occurs through an alternative plasma membrane channel that interacts with the DHPR and RyR1. This discovery has implications for the pathophysiology of MH, since the RyR1 exhibits calcium-induced as well as excitation-coupled calcium release ***(Murayama and Ogawa, 2004).***

❖ ***Different mechanisms of EC coupling***

The two types of striated muscle in vertebrates, skeletal and cardiac muscle, differ in their mechanism of EC coupling. In both tissues, Ca^{2+} release is controlled by the α1-subunits (cardiac: α1C, skeletal muscle: α1S) of voltage-sensitive L-type Ca^{2+} channels [called dihydropyridine receptors (DHPR) because of their binding affinity for this class of drugs] located in the transverse tubules (TT), i.e. narrow invaginations of the plasma membrane, make contact with the terminal cisternae of the SR ***(Beam and Dirksen, 1999).***

The cardiac L-type channel opens rapidly on membrane depolarization. The Ca^{2+} inward current triggers Ca^{2+} release through the cardiac-type ryanodine receptor (RyR2) in a process called Ca^{2+} -induced Ca^{2+} release (CICR). The L-type channel of skeletal muscle, on the other hand, opens much slower on depolarization .Here, Ca^{2+} release is activated before Ca^{2+} inflow from the extracellular