Anesthesia in Patients with Mitochondrial Disease

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The University of Texas McGovern Medical School
Neurometabolic & Mitochondrial Center

Clinical Research Protocol

Title:	**Anesthesia in Patients with Mitochondrial Disease**
Investigational Drug(s):	**Sevoflurane, Propofol, Dexmedetomidine**
IND/EudraCT Number:	**Not Applicable**
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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ATP	Adenosine Triphosphate
DNA	Deoxyribonucleic Acid
FADH2	Flavine Adenine Dinucleotide
mtDNA	Mitochondrial DNA
NADH	Nicotinomide Adenine Dinucleotide
nDNA	Nuclear DNA
RNA	Ribonucleic Acid
rRNA	Ribosomal RNA
tRNA	Transfer RNA
VA	Volatile agents
EC50	Half Maximal Effective Concentration
PRIS	Propofol infusion syndrome
MAC	Monitored Anesthesia Care
CNS	Central Nervous System

I. SUMMARY

This pilot study is a prospective, randomized clinical trial to evaluate the effect of anesthesia in the mitochondrial dysfunction patient. Subjects undergoing a routine medical care non-emergent procedure will be randomized into three different groups to receive one of the following anesthetic agents; Sevoflurane, Propofol or Dexmedetomidine.

The primary outcome of this pilot study is to evaluate and compare the incidence of adverse events in mitochondrial patients undergoing a diagnostic or therapeutic procedure up to 48 hours post anesthesia. The secondary outcome is to compare the metabolic derangements between three study groups by comparing changes in blood sugar, serum pH, serum bicarbonate, serum lactate and serum pyruvate levels before, during and after anesthesia in the groups.

II. BACKGROUND INFORMATION

INTRODUCTION

A. MITOCHONDRIA

Mitochondria are membrane bound organelles found inside most eukaryotic cells. Mitochondria provide cells with an advanced system for energy production, i.e., oxidative phosphorylation. Oxidative phosphorylation is the process in which ATP is formed as a result of the transfer of electrons from NADH or $FADH_2$ to oxygen by a series of electron carriers, i.e., the electron transport chain. The process of oxidative phosphorylation is the major source of ATP in aerobic organisms. Other intra-mitochondrial processes include the Krebs cycle, beta-oxidation and urea cycle. In addition to producing cellular energy, mitochondria are involved in cell signaling, cell differentiation, cell death, and control of the cell cycle and growth. In the absence of oxygen, pyruvate formed from glycolysis is converted to lactate or alanine producing only a small source of ATP

Mitochondria contain their own DNA with 16,569 base pairs. The mtDNA codes for 37 proteins, including specific subunits for several complexes of the electron transport chain (ETC), ribosomal RNA (rRNA), and transfer RNA (tRNA) (Figure 1). A defect in any of these genes can produce clinical disease. About 1100 other gene products within the organelle are encoded by genes within the nuclear genome [1, 2] and they follow the classical Mendelian inheritance patterns [3]. Nuclear DNA (nDNA) contains genes involved in mitochondrial function that code for the remainder of the specific complex subunits. Mutations in either mtDNA or nDNA can result in a deficit of a specific electron transport chain complex or can give rise to global mitochondrial dysfunction via signaling, transcription, or translation.

Cells and tissues can have both normal and mutant mtDNA. The state of having more than one type of mtDNA within a given cell is termed "heteroplasmy" [3] (Figure 2). If only some of the inherited mitochondria contain mutant mtDNA, there could be an uneven distribution into tissues, with varied thresholds for displaying a physiologic defect. This could result in different offspring from a single mother demonstrating strong variation in phenotype despite being genetically similar [3].

B. MITOCHONDRIAL DISORDERS

Mitochondrial disorders are a heterogeneous group of clinical diseases resulting from dysfunction of the mitochondria. They occur as a result of pathogenic defects in the mtDNA and nuclear DNA that code for specific complex subunits involved in mitochondrial function. Initially described as a rare disease, Elliot et al. reported the prevalence of pathogenic mitochondrial DNA mutations to be at least 1 in 200 people(4), and the incidence of respiratory chain disorders have been estimated to be within range of other well-known neurologic diseases [5, 6]. The study of mitochondrial disease however remains in infancy [5, 6].

The first disorder of mitochondrial function was described in 1959 by Luft [7]. It was in a young woman with generalized weakness, inability to gain weight, and

extensive perspiration. Her muscle pathology demonstrated large accumulations of variable sized mitochondria containing paracrystalline inclusions [7]. Over time, many cases of mitochondrial dysfunction were described and the term "mitochondrial disorder" arose to describe defects in the mitochondrial electron transport chain [8]. The sequence and organization of the human mitochondrial genome was first defined by Anderson in 1981[9]. By 1989 Holt had described the first deletion in the mitochondrial genome associated with clinical pathology [10].

Patients with mitochondrial dysfunction, most of which are children, typically have multi-systemic, multi-organ involvement, with the organs requiring more energy being mostly affected. Mitochondrial disease is now well recognized as an important cause of neurologic, muscular, cardiac, endocrine, hepatic, and renal disorders [11]. Diagnosis of mitochondrial disorders has remained difficult due to the wide range of clinical presentation these patients may have. Traditionally patients have been diagnosed using the modified Walker criteria that classify patients into either a "Definite" or a "Probable" category based on major and minor clinical and histopathological findings. More recently, advanced technologies in molecular studies have enabled definite genetic diagnosis of pathogenic mtDNA and nDNA mutations.

C. ANESTHETIC AGENTS

1. SEVOFLURANE

Sevoflurane is a volatile halogenated general inhalation anesthetic drug used for induction and maintenance of general anesthesia. It is a nonflammable liquid administered by vaporization.

Volatile agents (VA) promote extensive neuro-apoptosis by promoting the release of intracellular calcium from the endoplasmic reticulum. This increase in calcium load results in loss of mitochondrial integrity, release of pro-apoptotic factors, impairment of ATP synthesis, and enhanced accumulation of reactive oxygen species [11]. Sevoflurane like other VAs

in use currently is capable of interacting negatively with a mitochondrial myopathy [3].

Studies have consistently shown Complex I to be most sensitive to inhibition by VAs [12-15]. Complex I inhibition have been demonstrated with VA concentrations that range from 1 to 2 times the EC50 of an organism. Other components of the ETC are affected with increasing concentrations of VAs [3].

Niezgoda et al noted that "as VAs exert much of their effects in high-energy tissues; it is possible that patients with mitochondrial disease may have an abnormal sensitivity to VAs" [3]. All of the volatile anesthetics depress respiration to varying degrees [16]. More direct muscle relaxation, although with small differences, is seen in Sevoflurane than other VAs [17, 18]. Currently used volatile anesthetics are exhaled and do not require metabolism for excretion, an advantage over intravenous anesthetics, which are dependent on energy-requiring metabolism [3]. This to an extent lowers the risk from exposure to Sevoflurane in comparison to intravenous agents for mitochondrial disease patients [19, 20].

2. PROPOFOL

Propofol is a short-acting, intravenous sedative-hypnotic agent used for induction and maintenance of anesthesia or sedation. It has its primary effects on ligand-gated ion channels or G-protein-coupled receptors similar to other parenteral sedatives [3]. Its excretion is metabolism dependent. Propofol has many of the same side effects as volatile anesthetics, with the exception of muscle relaxation [3]. It is however capable of decreasing ventilatory drive, cardiac output and contractility [3].

Propofol inhibits complex I and IV functions, as well as carnitine transport of fatty acids into the mitochondria blocking beta-oxidation. It also uncouples the electron transport chain, preventing ATP synthesis and generating free radicals [21-25].

Propofol infusion syndrome (PRIS) is a serious reaction in patients receiving long-term infusions. The syndrome comprises of severe lactic acidosis, liver and renal failure, rhabdomyolysis, hyperlipidemia and refractory bradycardia which can lead to, cardiovascular collapse and death [3, 26]. PRIS is assumed to occur from long-chain acylcarnitine esters transport inhibition coupled with an indirect effect on complexes I and II [26, 27]. Risk factors for developing PRIS include; young age, high dose of propofol, prolonged duration of propofol administration, underlying, neurologic disorder and concomitant steroid infusion [3].

As of 2007, 61 cases had been reported in the literature, with 62% mortality (20 children & 18 adults. Although generally thought to only occur during prolonged propofol administration, 7 (11%) of these patients developed PRIS during routine anesthesia [19]. Two independent clinical reports have documented mitochondrial dysfunction during PRIS [28, 29]**.** Several authors have proposed that patients who develop PRIS actually have undiagnosed mitochondrial disease and recommend that all patients who develop PRIS should be screened for mitochondrial dysfunction [28].

3. DEXMEDETOMIDINE (PRECEDEX®)

Dexmedetomidine is a centrally acting α-2 receptor agonist with an unusual ability to provide sedation without causing respiratory depression [30-32]. It has an analgesic-sparing effect, thereby reducing opioid requirements during and after surgery [33-38] and a sympatholytic effect that can reduce the stress response to surgery such as tachycardia and hypertension [34, 37]. It is increasingly being used as a sedative for Monitored Anesthesia Care (MAC) because of the advantage it has over

other drugs such as midazolam, propofol, and fentanyl, all of which cause respiratory depression [39, 40]. There have been several reports on its successful use as the primary sedative drug for diagnostic and therapeutic procedures [37, 38, 41-44]. Reported adverse effects with Dexmedetomidine infusion include bradycardia, sinus arrest and hypotension [45].

A randomized, multicenter, double-blind, Phase III study evaluating the safety and efficacy of Dexmedetomidine in patients requiring MAC showed it had an effective baseline sedation with less opioid requirements, and less respiratory depression than placebo rescued with midazolam and fentanyl [45]. However, there is no data in the literature currently on its safety in myopathic and mitochondrial patients.

D. ANESTHESIA AND MITOCHONDRIAL DYSFUNCTION

Patients with mitochondrial disorders often present to the radiology suite and operating room for diagnostic and therapeutic - elective and emergent - procedures that require general anesthesia. The vast majority of them have an uneventful surgery and anesthesia course [46]. There are reports however of profound and unexpected complications occurring during and following anesthetic exposure, some of which have been fatal [47-49].

As with many disorders of metabolism, children with mitochondrial disease are at risk of metabolic decompensation. This risk increases during times of stress, such as with an inter-current illnesses, reduced oral intake or fasting, and surgery. A mitochondrial crisis occurs when multiple organs and physiologic systems begin failing. It can be precipitated by a minor illness, metabolic stressor or exposure that tips the patient over their baseline threshold, and develops hours or weeks following the insult.

Prolonged respiratory depression and Central Nervous System (CNS) white matter degeneration have been reported in patients seemingly only mildly affected preoperatively with relatively uneventful anesthetic courses during surgery [47-

49]. Respiratory depression can occur as a result of muscle weakness seen in any myopathic state, as well as from the combination of anesthetics [3]. It is well documented that mitochondrial patients may face an increased risk in the operating room from a combination of both surgical stress and anesthetic exposure [50-52]. General anesthetic agents could tip a patient past their threshold resulting in decompensation hours or days post-operatively.

Volatile anesthetics and propofol have been shown to depress mitochondrial function in vitro. Several reports have shown that exposure to general anesthetics at critical stages of brain development can cause neurotoxicity to immature neurons [53]. Other authors have reported the safe use of different anesthetic drugs and approach in mitochondrial patients [19, 54]. Based on these reports, an author has suggested that "patients with myopathies and mitochondrial disease usually do well regardless of the specific anesthetic approach that is chosen" [46]. As Niezgoda et al. stated; *it would be unwise to infer that an anesthetic agent or approach used safely with one mitochondrial dysfunction patient would be equally safe in all other patients with mitochondrial disease, or even in siblings with similar genetic mutations because of heteroplasmic variations that typically occur in cells and tissues [3].*

With the limited data available on the effects of anesthesia in mitochondrial disease, current recommendations are based on the biochemical understanding of mitochondrial function and *in vitro* knowledge regarding anesthetic agents [3, 55-57].

Mitochondrial patients may be more susceptible to Propofol Infusion Syndrome and avoidance of propofol use has been recommended when possible.

Mitochondrial patients may also have underlying myopathy, sub-clinical or undiagnosed cardiac disease (cardiomyopathy or arrhythmia) making them more sensitive to neuromuscular blockage. These patients may also not be able to tolerate the lactate load from lactated fluids. Fasting periods should be minimized, with dextrose-containing fluids administered pre-operatively except in cases where ketogenic diet is indicated.

III. PRELIMINARY DATA

There are no prospective studies assessing the use and effects of anesthetic agents in mitochondrial patients. There are several case reports, case series, and review articles describing the use of various anesthetic agents (presented in Table 1). These reports have shown contradictory and inconclusive findings on the effect of general anesthesia in patients with mitochondrial dysfunction. While some have reported profound clinical complications including death, others have reported their safe use. It is important to note that the absence of published reports of adverse effects with a given agent does not confirm that the agent is safe; it may instead simply reflect a publication or sampling bias Other limitations of these reports include their small sample size and study design. Most of the reports do not extend beyond the immediate operative period and therefore would miss a mitochondrial crisis that occurs hours to days following anesthesia administration. They have also not been able to adequately measure the incidence, nor characterize the peri-operative complications in patients with mitochondrial dysfunction. All of which has created an information gap in the peri-operative management of mitochondrial disease patient with regards to anesthesia.

There is a general consensus among anesthesiologists that this patient population is at an increased risk of peri-operative complications from both anesthetic and surgical stress [3, 47-50, 55, 56, 58]; including but not limited to respiratory failure, cardiac depression, conduction defects and dysphagia. Results from a national survey of pediatric anesthesiologists in the United States done by Rafique MB et al. to investigate their current practice in regards to anesthesia in mitochondrial patients is presented in Table 2 [59]. Table 3 shows the results of a survey of the clinical directors of the recognized mitochondrial centers in the United States and Canada done by the mitochondrial medicine society of mitochondrial disease specialists to determine practices for clinical care of patients with mitochondrial disease pertaining to anesthesia [60].

IV. STUDY OBJECTIVES

1. The primary outcome of this pilot study is to evaluate and compare the incidence of adverse events in mitochondrial patients undergoing a diagnostic or therapeutic procedure up to 48 hours post anesthesia.
2. The secondary outcome is to compare the metabolic derangements between three study groups by comparing changes in blood sugar, serum pH, serum bicarbonate, serum lactate and serum pyruvate levels before, during and after anesthesia in the groups.

V. STUDY DESIGN

A. SELECTION AND WITHDRAWAL OF SUBJECTS

1. STUDY SUBJECT RECRUITMENT

Institutional approval and written informed consent from parents/ legal guardians of children 0- 17 years of age with definitive, probable or suspected diagnosis of mitochondrial disease (Walker's criteria) will be obtained. Subjects 0-17 years, both genders, scheduled for diagnostic or therapeutic procedure requiring general anesthesia that is estimated to last for at least one hour will be included in the study. Minor assent will be obtained from children 7 – 17 years. Pregnant females, nursing females and subjects who have participated in the same study within 48 hours will be excluded. This pilot study is a prospective, randomized clinical trial and we need permission from CPHS to enroll 60 patients. We need permission to approach 100 families considering screen failures, study withdrawals and drop outs. Copies of a flyer with explanation of the study will be provided at the office of pediatric neurologist at UT Mitochondrial clinic, surgeons at UT professional building, pediatric floors at MHH and anesthesia clinic. We will communicate with the nurses' of the above mentioned offices and ask them to give the flyer to the parents, and adolescents. The flyer will be sent to the IRB for review and approval.

The PI, Co PI and the research coordinator will invite parents of children, and adolescents with mitochondrial disease in the pediatric neurology clinic, in the DSU, anesthesia clinic and floor to participate in the study. Ample time will be given to read the consent forms and ask questions about the study. HIPPA consent will also be obtained.

2. INCLUSION OF WOMEN & MINORITIES

Female subjects and minorities will be enrolled in the study based on the inclusion/exclusion criteria described below.

3. INCLUSION OF CHILDREN

Children up to 17 years of age will be included in the study based on the inclusion/exclusion criteria described below.

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4. INCLUSION CRITERIA

Subjects must fulfill the following inclusion criteria:

a. Subject is informed and given ample opportunity to consider his/her participation and has given his/her written consent.
b. Subject is willing and able to comply with all study requirements.
c. Subject is between 0 - 17 years of age.
d. Subject has been diagnosed with mitochondrial dysfunction based on modified Walker criteria.
e. Subject is scheduled to have a non-emergent diagnostic or therapeutic procedure for routine medical care requiring general anesthesia. ~~estimated to last at least one hour.~~
f. Subject is classified ASA I - IV

5. EXCLUSION CRITERIA

Subjects are not permitted to enroll in the study if any of the following criteria are met:

a. Subject is older than 17 years
b. Subject is pregnant
c. Subject is a nursing female and
d. Subject has participated in the same study within 48 hours
e. Subject is allergic or has had any adverse effect to any of the study agents in the past
f. ~~Anesthesia time is less than one hour~~
~~g.~~f. Subject is classified ASA V

6. CRITERIA FOR WITHDRAWAL

Participation in this study may be discontinued for any of the reasons listed below.

- The subject is unwilling or unable to continue and withdraws his/her consent.

B. STUDY GROUPS

Consented subjects will be randomized in to three study groups using a computer generated randomization list.

1. STUDY GROUP I -SEVOFLURANE

These subjects will receive Sevoflurane gas.
In the Operating Room (OR) after applying routine monitors, anesthesia will be induced with a mask using Sevoflurane up to 8%. Sevoflurane will be used for maintenance for the rest of the procedure with dosage between 1.5% and 4%. Vitals will be monitored. Fentanyl will be used as needed.

Rocuranium will be used as needed for muscle relaxation. At the end of the procedure twitches will be evaluated and muscle relaxation will be reversed. Sevoflurane will be turned off at the end of procedure.

2. STUDY GROUP 2 – DEXMEDETOMIDINE (PRECEDEX®)

These subjects will receive Dexmedetomidine intravenously.
In the OR routine monitors will be placed. Vitals will be monitored. A loading dose of Precedex 1mcg/ kg will be given over 10 minutes. Infusion will be started using Precedex 1mcg/ kg/ hr and can be increased or decreased as needed. Fentanyl will be used as needed. Rocuranium will be used as needed for muscle relaxation. At the end of the procedure twitches will be evaluated and muscle relaxation will be reversed. Precedex infusion will be stopped at the end of the procedure.

3. STUDY GROUP 3 – PROPOFOL

These subjects will receive propofol for anesthesia maintenance.
In the OR routine monitors will be placed. Vitals will be monitored. A loading dose of Propofol 2mg/ kg will be given over 2 minutes. Infusion will be started using Propofol 50 mcg/ kg/min and can be increased or decreased as needed (range 50-300mcg/kg/min). Fentanyl will be used as needed. Rocuranium will be used as needed for muscle relaxation. At the end of the procedure twitches will be evaluated and muscle relaxation will be reversed. Propofol infusion will be stopped at the end of the procedure.

4. ALL STUDY GROUPS

As a standard for all patients with mitochondrial disease every study subject will have IV access placed prior to induction. All subjects without IV line will have 1-2 lignocaine patches before premedication. All subjects will receive po midazolam 15 minutes before IV placement. If

midazolam is contraindicated, then premedication will be administered at the discretion of the anesthesiologist. All subjects will receive regional anesthesia as indicated per standard of care. All groups will receive normal saline (NS) infusion at the rate of 10 ml/kg/hr to maintain normovolemia. If needed 5% dextrose with NS not to exceed 10ml/kg/hr will be given to maintain normoglycemia. In open abdominal procedures fluid may be increased or decreased as required.

C. EVALUATIONS AND ASSESSMENTS

1. PREOPERATIVE:

Before the induction of anesthesia, after midazolam is given, and IV access has been established, baseline blood glucose, serum lactate and serum pyruvate, serum bicarbonate and pH will be obtained

2. INTRAOPERATIVE:

Blood glucose, serum lactate and serum pyruvate, serum bicarbonate and pH will be obtained ~~1 hour~~15 minutes after start of anesthesia. These parameters will be repeated every hour till the end of anesthesia.
Blood glucose, serum lactate and serum pyruvate, serum bicarbonate and pH will be obtained 30 minutes after the end of anesthesia.

3. POST OPERATIVE:

a) 12 hours follow up post anesthesia

- Blood glucose, serum lactate and serum pyruvate, serum bicarbonate and pH will be obtained 6 hours after end of anesthesia if patient is in house or next morning at the UT Mitochondrial Clinic if discharged.

- **Admitted patient**: The patient will be monitored up to 12h after end of anesthesia by an independent blinded observer for feeding difficulties, temperature >100 F, tachycardia, vomiting, nausea, lethargy, change in admission plan and any other change in baseline as per parent. The parents will be blinded to the anesthetic procedure. If any adverse events occur the treating physicians will follow the standard of care and the investigators will be notified.

- **Discharged patient**: The patient will be monitored by the parent every 4 h for feeding difficulties, temperature >100 F, tachycardia, vomiting, nausea, lethargy, any other change in baseline. The patient will be seen the next morning at the UT Mitochondrial clinic by the blinded pediatric neurologist and reevaluated for feeding difficulties, temperature >100 F, tachycardia, vomiting, nausea, lethargy, hospital readmission/ ER visit and any other change in baseline as per parent. The parents will be blinded to the anesthetic procedure.

b) 48 hours follow up post anesthesia

- For in-patients the child will be followed from 48 hours post anesthesia by the independent blinded observer and the blinded pediatric neurologist for feeding difficulties, temperature >100 F, vomiting, nausea, lethargy, change in admission plan and any other change in baseline as per parent. Blood glucose, serum lactate and serum pyruvate, serum bicarbonate and pH will be also obtained
- If patient is discharged then the independent observer will follow up for the above mentioned parameters. Blood glucose, serum lactate and serum pyruvate, serum bicarbonate and pH

will be obtained 48 hours after end of anesthesia if patient is in house or 2nd post-operative day at the UT Mitochondrial Clinic if discharged.

D. ADVERSE EVENTS

An adverse event (AE) is any untoward medical occurrence in a subject, compared with pre-existing condition that occurs during any phase of the research study. An AE is defined as being independent of assumption of any causality. The following laboratory values and physical findings are to be considered AEs:

a. Laboratory value(s) that change from a subject's baseline by greater than 10% and is outside the normal range.
b. Any of the following parameters; feeding difficulties, temperature >100 F, vomiting, nausea, lethargy, change in admission plan and any other change in baseline that are clinically relevant.

E. PROCEDURES FOR RECORDING AND REPORTING ADVERSE EVENTS

All problems having to do with subject safety must be documented and reported to the investigators (Dr. Maria Matuszczak, [redacted]; Dr. Mary Kay Koenig, [redacted]; Dr. Jael Carbajal, 713-500-6200, Jael.G.Carbajal@uth.tmc.edu). Specifically the following must be reported:

a. All serious adverse events associated with the study procedures.
b. All deaths, whether or not they are directly related to study procedures.
c. Any incidents or problems involving the conduct of the study or subject.

If a subject is develops an adverse event, the subject will be followed as frequently as necessary until resolution of the event or for as long as the investigators deem necessary.

F. POWER AND SAMPLE SIZE CALCULATION

Since the current study is a pilot study, 60 patients will be enrolled and randomized to Sevoflurane, Propofol and Dexmedetomidine in a 1:1:1 ratio using a block randomization with block size of 6 (20 patients per group).

G. DATA ANALYSIS

All statistical analyses will be performed in SAS 9.3 (Cary, NC). Descriptive statistics; mean (± standard deviation) for continuous variables and frequency (percent) for discrete variables, will be summarized for all study variables. Before performing any analysis, the distribution of all variables will be examined for appropriateness of distribution assumptions. Extreme values and influential data points will be carefully examined for accuracy. The primary endpoint is to evaluate and compare the incidence of adverse events up to 48 hours post anesthesia. Intention to treat principle will be applied for the primary analysis. A binomial confidence interval approach will be used to estimate the incidence rate of adverse events as well as 95% confidence interval for each group. To compare the incidence rate with a pre-specified safety cutoff, 0.10, a binomial test will be applied. Secondary analysis of the primary outcome will include an unadjusted analysis using an extension of Fisher's exact test and covariate adjusted analysis through logistic regression, evaluating potential confounding variables. The exploratory analysis for the secondary outcome include comparison the metabolic derangements between three groups. Analysis of variance method will be performed to compare the changes in blood sugar, serum PH, serum bicarbonate, serum lactate and serum pyruvate levels before and after anesthesia. Data will be sub-analyzed in three categories based on the duration of exposure to anesthesia. The categories will consist of: (a) Total anesthesia time is 30 minutes or less; (b) total anesthesia time is more than 30 minutes but less than one hour; and (c) total anesthesia time is up to an hour or more.

VI. RISKS

Study participants are not subjected to any additional risks other than those that are associated with receiving general anesthesia for routine medical care, including but not

limited to an increased risk of metabolic decompensation in children with mitochondrial disease.

VII. BENEFITS

A. STUDY SUBJECTS

Study subjects are not able to directly benefit from participating in the study.

B. MITOCHONDRIAL DISORDER COMMUNITY

Study findings may provide knowledge on the comparative safety amongst different anesthetic agents and approach for patients with mitochondrial dysfunction.

It will also add to the body of knowledge on the effects of anesthetic agents in mitochondrial disease.

VIII. ETHICS

A. INFORMED CONSENT

Institutional approval and written informed consent from parents/legal guardians of children 0 - 17 years of age with definitive, probable or suspected diagnosis of mitochondrial disease (Walker's criteria) will be obtained. Subjects 0-17 years, both genders, scheduled for diagnostic or therapeutic procedure requiring general anesthesia will be included in the study. Minor assent will be obtained from children 7-17 years.

Subject's informed consent will be obtained and documented in accordance with local regulations and the ethical principles that have their origin in the Declaration of Helsinki. Prior to obtaining informed consent, information will be given in a language and at a level of complexity understandable to the subject in both oral and written form by the investigator. Each subject will have the opportunity to discuss the study and its alternatives with the investigator.

Prior to participation in the study, the written informed consent form will be signed and dated by the subject, and/or his/her legal guardian, and by the person who conducted the informed consent discussion (investigator or designee). The subject and/or his/her legal guardian will receive a copy of the signed and dated informed consent form.

For minors and cognitively disabled subjects, assent will be obtained from the subject in addition to consent from the legal guardian.

B. INTERNAL REVIEW BOARD

The study will be conducted under the auspices of the University of Texas Health Science Center Houston CPHS and in accordance with the ethical principles that have their origin in the Declaration of Helsinki. Before initiating the study, the investigator will have written and dated full approval from the CPHS for the protocol.

The investigator will promptly report to the CPHS all changes in the study, all unanticipated problems involving risks to human subjects or others, and any protocol deviations to eliminate immediate hazards to subjects.

C. SUBJECT PRIVACY

Study investigators will affirm and uphold each subject's confidentiality. Every effort to maintain confidentiality will be taken. Upon enrollment subjects will be assigned a subject identification (ID) number. Files matching subject name with the subject ID number will be kept in the office of the PI (limited access) in a locked file, as well as in a spread sheet on the computer of the PI. Subjects will not be personally identified in any reports or publications that may result from this study. Information identifying subjects will not appear on records viewed by anyone other than study personnel. Names will not appear on any of the summaries or statistical analyses.

APPENDIX

FIGURES AND TABLES

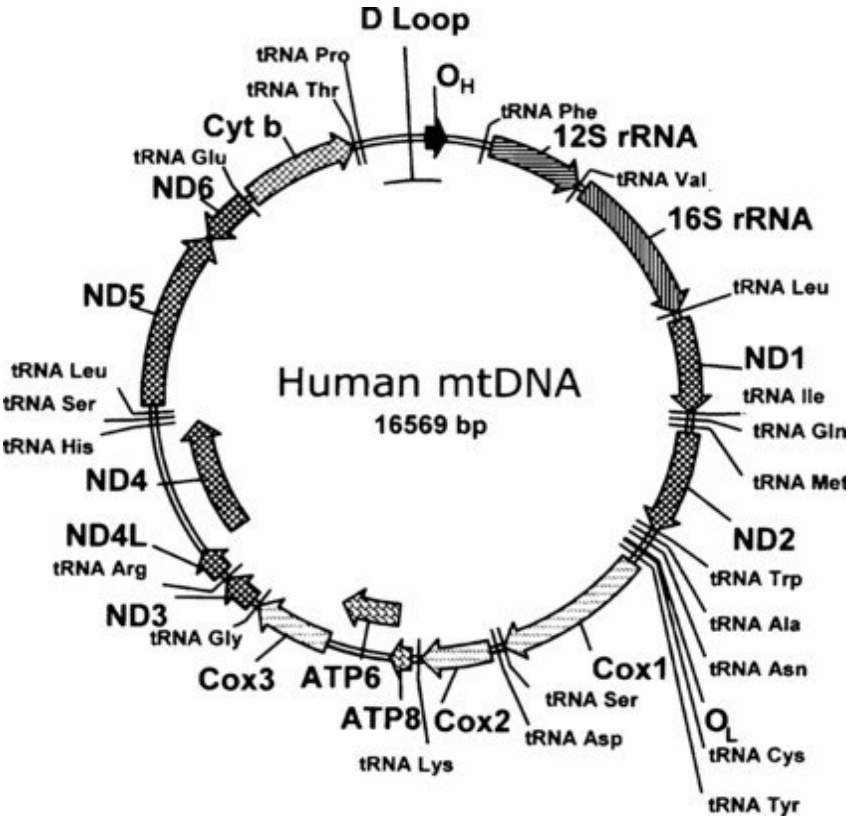

Figure 1. Human Mitochondrial DNA.

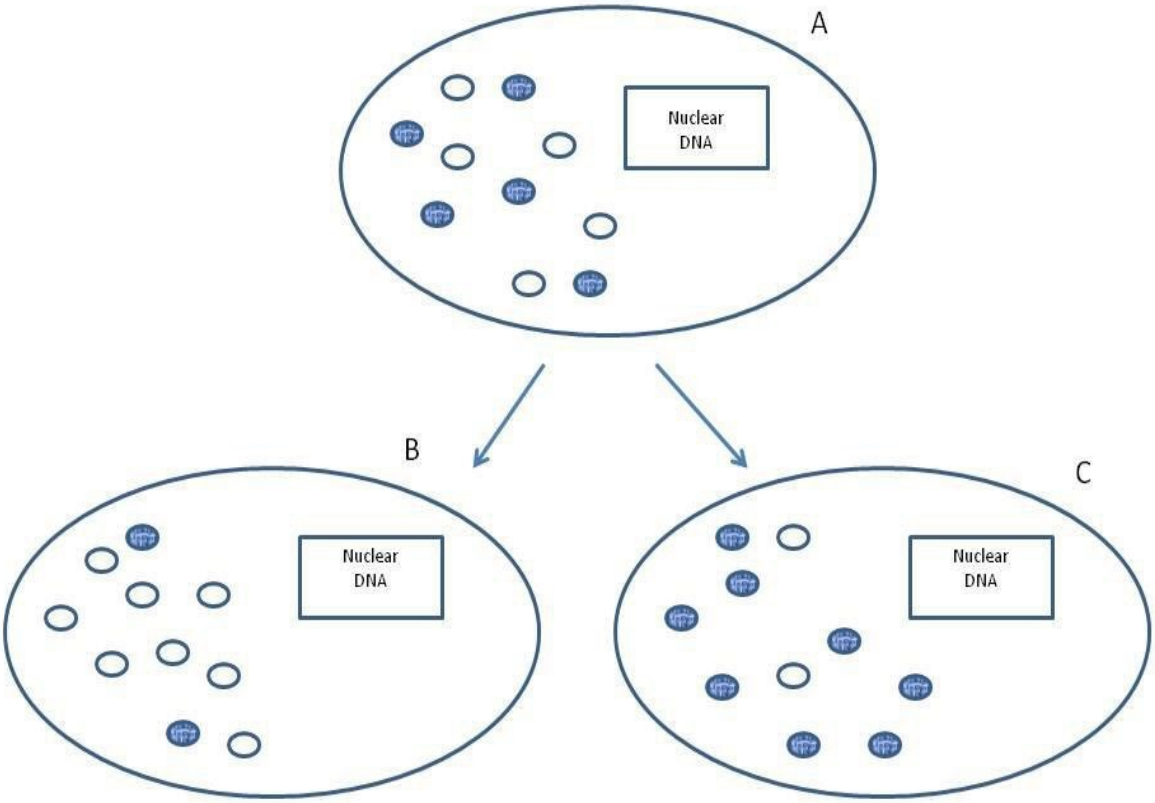

Figure 2. Mitochondrial Heteroplasmy. A. Original cell with 50% mixture of wild type (○) and mutant (●) mtDNA. B. Daughter cell with 80% wild type (○) and 20% mutant (●) mtDNA. C. Daughter cell with 20% wild type (○) and 80% mutant (●) mtDNA.

Table 1: Classification of case reports and series on inhaled and intravenous anesthetic agents for patients with mitochondrial dysfunction into safe and unsafe categories

Volatile agents - Sevoflurane		Intravenous agents - propofol		Iv - ketamine	Muscle relaxants - rocuronium	Case series (multiple agents)	
Safe	Unsafe	Safe	Unsafe	Safe	Unsafe	Safe	Unsafe
Burns AM and MP . Anaesthesia for patients with mitochondrial myopathy. Anaesthesia 1989;44:975-977. Single case; Thiopentone induction; Nitrous oxide & Isoflurane maintenance; No complications Pvalizza EG, KJ Ando, and MS . Kearns-Sayre syndrome and cardiac anesthesia. Journal of cardiothoracic and vascular anesthesia 1995;9:189-191. Single case report; Induction and maintenance with sevoflurane; No complications Vilela H, Garcia-Fernandez J, Parodi E, Reinoso-Barbero F, P Duran, and F . Anesthetic management of a patient with MERRF syndrome. Pediatric Anesthesia 2005;15:77-79. Single case report; Sevoflurane induction and maintenance; No complications.	Casta A, EJ Quackenbush, CS Houch, and MS . Perioperative white matter degeneration and death in a patient with a defect in mitochondrial oxidative phosphorylation. Anesthesiology 1997;87:420-425. Single case; Thiopental induction; Isoflurane maintenance; Neurologic deterioration post-op with death at 5 weeks	Thompson VA and JA . Anesthetic considerations in patients presenting with MELAS syndrome. Anesthesia and Analgesia 1997;85:1404-1406. Single case report; Propofol induction and maintenance; No complications Cheam EWS and LAH . Anesthesia for a child with complex I respiratory chain enzyme deficiency. Journal of clinical anesthesia 1998;10:524-527. Single case. Propofol for induction and maintenance; No complications Sasano N, Y Fujita, MH So, K Sobue, H Sasano, and H . Anesthestic management of a patient with MELAS during laparotomy. Journal of Anesthesia 2007;21:72-75. Single report; Propofol induction and maintenance; Lactated Ringers; No complications	Vanlander AV, PG Jorens, J Smet, B De Paepe, W Verbrugghe, GG Van Den Eynden, F Meire, P Pauwels, N Van der Aa, S Seneca, W Lissens, JG Okun, and R Van . Inborn ox phos defect as risk factor for propofol infusion syndrome. Acta Anaesthesiologica Scandinavica 2012;56:520-525. Single case of patient with LHON developing PRIS; Long-term propofol use following TBI (>88 hours) Mehta N, DeMunter C, Habibi P, Nadel S, and J . Short-term propofol infusion in children. The Lancet 1999;354:866-867. Single case; Propofol induction and maintenance Developed severe metabolic decompensation; Found to have Complex IV deficient mitochondrial disease	Z Shenkman, I Krichevski, ON Elpeleg, A Joseph, and A . Anaesthetic management of a patient with Leigh's syndrome. Canadian Journal of Anesthesiology 1997;44:1091-1095. Single report; Ketamine induction and propofol/nitrous oxide maintenance; No complications Farag E, Argalious M, Narouze S, DeBoer GE, and J . The anesthetic management of ventricular septal defect repair in a child with mitochondrial cytopathy.Obstetrical and Pediatric Anesthesia 2002;49:958-962. Single report; Ketamine induction and maintenance; No complications	Finsterer J, U Stratil, R Bittner, and P . Increased sensitivity to rocuronium and atracurium in mitochondrial myopathy. Canadian Journal of Anesthesiology 1998;45:781-784. Single case report; Propofol induction and maintanence Increased sensitivity to rocuronium and atracurium	Driessen J, S Willems, S Dercksen, J Giele, F Van der Staak, and J . Anesthesia-related morbidity and mortability after surgery for muscle biopsy in children with mitochondrial defects. Pediatric Anesthesia 2007;17:16-21. Retrospective review of 122 muscle biopsies in patients with mitochondrial disease Induction: propofol, thiopental, sevoflurane, halothane Maintenance – propofol, sevoflurane, isoflurane, halothane; No complications; Mean duration of procedure was 36 minutes	Footitt EJ, MD Sinha, JAJ Raiman, A Dhawan, S Moganasundram, and MP . Mitochondrial disorders and general anesthesia: a case series and review. British journal of anesthesia 2008;100:436-441. Retrospective review of 58 cases of anesthesia in 38 patients with mitochondrial disease Did not specify agents used; There were no adverse events during the anesthesia but post-operatively there was one metabolic decompensation and one patient who developed acute renal failure (3%); Both cases used propofol for induction; one used sevoflurane and one used isoflurane for maintenance Gurrieri C, JE Kivela, K Bojanic, RH Gavrilova, RP Flick, J Sprung, and TN (70). Anesthetic considerations in MELAS: a case series. Canadian Journal of Anesthesia 2011;58:751-763. Retrospective review of 20 cases of anesthesia in 9 patients with MELAS; Did not specify agents used; There were no adverse events during the anesthesia but post-operatively there were two cases of acute renal failure (10%)

Table 2: Results from a national survey of pediatric anesthesiologists in the United States to investigate their current practice in regards to anesthesia in mitochondrial

Total number of respondents	503
Reports having institutional guidelines for anesthesia management in mitochondrial patients	11%
Reports the use of standard NPO guidelines with liberal oral fluids until 2 hours pre-operatively	80%
Reports taking precautions for malignant hyperthermia	18%
Considers Sevoflurane as the safest inhaled agent for induction	90%
Considers Sevoflurane as the safest inhaled agent for maintenance	79%
Considers Ringers Lactate fluid safe	49%
Use dextrose containing fluids	48%

Table 3: Results from a survey of the clinical directors of the recognized mitochondrial centers in the United States and Canada pertaining to anesthesia in mitochondrial

Total number of respondents	32
Recommends anesthesia "precautions" in patients with possible or diagnosed mitochondrial disease Specific recommendations varied but generally involved - Restriction of select anesthetic agents - Limiting pre and post-operative fasting - Maintaining IV hydration with dextrose containing fluids	100%
Avoidance of Propofol for long term sedation	100%
Restricts Propofol use for short term sedation (less than 2 hours)	27%
Avoids Succinylcholine	16%
Avoids Sevoflurane	9%
Provide patients with written anesthesia protocol including information about preferred anesthetic agents and risks of fasting	72%
Routinely admit patients prior to surgery for IV hydration - Remaining 49% only admits patients pre-operatively if the patient reports a problem with fasting in the past	59%

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