Boston University Theses & Dissertations

2023

Review of mitochondrial DNA and mitochondrial-associated disorders

BOSTON UNIVERSITY

ARAM V. CHOBANIAN & EDWARD AVEDISIAN SCHOOL OF MEDICINE

Thesis

REVIEW OF MITOCHONDRIAL DNA AND MITOCHONDRIAL-ASSOCIATED DISORDERS

by

OPEOLUWA OLUKOREDE

B.S., Johns Hopkins University, 2017

Submitted in partial fulfillment of the

requirements for the degree of

Master of Science

2023

Approved by

First Reader ______________________________

Gwynneth Offner, Ph.D.
Associate Professor of Medicine

Second Reader ______________________________

David Flynn, M.S.
Assistant Director of Library and Information Management Education

DEDICATION

I would like to dedicate this work to my family, both near and far, for their unwavering support.

ACKNOWLEDGMENTS

I am eternally grateful to my readers, Dr. Gywnneth Offner and David Flynn as well as Mildred Agosto for their enduring patience and dedication to helping me finish this work.

REVIEW OF MITOCHONDRIAL DNA AND MITOCHONDRIAL-ASSOCIATED DISORDERS

OPEOLUWA OLUKOREDE

ABSTRACT

Mitochondrial diseases are caused by gene mutations in either mitochondrial DNA (mtDNA) or nuclear DNA (nDNA) and they are among one of the most common forms of inherited disorders. It is estimated that 1 out of every 5000 individuals will develop a mitochondrial disease in their lifetime. Due to the crucial and widespread functionality of mitochondria in human cells, prolonged diseases of the mitochondria affect cells of the brain, heart, liver, muscles and kidneys and can lead to multi-organ failure in some patients. Inherited or acquired mitochondrial diseases can present at any stage of life, affecting both children and adults. Since its discovery, the mitochondrial genome has been analyzed and sequenced with increasing ease and this process has helped recognize various mitochondrial disorders as the root of genetic diseases.

This paper will explore the unique properties of the mitochondrion and its genome, examine the relationship between mtDNA and some common myopathies such as Leigh syndrome (LS) or maternally inherited Leigh syndrome (MILS), mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) in order to explore commonalities and differences in their inheritance patterns and their effect on mitochondrial function. Although studies have shown that these conditions generally affect the process of oxidative phosphorylation in mitochondria, because of the wide

variety of presentations of this disease, further research is needed to understand the different etiologies, as well as to explore novel therapies to treat them.

PREFACE

Sometimes one thinks it will be easier to give up. Just throw in the towel. Pack up your things and go home. You tell yourself, "at least you tried your best." Sometimes you try to look to people- mentors and guardians for words of wisdom or to the internet for a video or meme to keep you going. But what you really need is already inside of you. Once you look within and ask yourself "why" and "for whom," you will start to realize that what you are doing is bigger than you and bigger than this moment. It is for those who will come after you. Those who may never know what you did and how you did it, but nevertheless, will feel the impact. What you are doing will not be missed.

I am blessed to be a part of the Graduate Medical Sciences Department that gave me the academic and professional tools that I will use throughout my lifetime. I am also grateful to be a part of this institution, Boston University. Go Terriers!

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AD .... Alzheimer's Disease

ALS .... Amyotrophic lateral sclerosis

ATP .... Adenosine Triphosphate

DM2 .... Type 2 Diabetes Mellitus

DNA .... Deoxyribonucleic acid

ETC .... Electron Transport Chain

LS .... Leigh Syndrome

MELAS .... Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes

MERRF .... Myoclonic epilepsy with ragged-red fibers

MIDD .... Maternally Inherited Diabetes and Deafness

MILS .... Maternally Inherited Leigh Syndrome

MODY .... Maturity Onset Diabetes of the Young

MT-ATPase 6 .... Mitochondrially encoded ATPase6 subunit

mtDNA .... Mitochondrial DNA

MTOR .... Mammalian Target of Rapamycin

mtRNA .... Mitochondrial transfer RNA

NADH .... Nicotinamide adenine dinucleotide

nDNA .... Nuclear DNA

PCR .... Polymerase Chain Reactions

PD .... Parkinson's Disease

RNA .... Ribonucleic acid

ROS .... Reactive Oxygen Species

TCA .... Tricarboxylic Acid Cycle

tRNA .... Transfer RNA

CHAPTER ONE

Introduction

Mitochondria have been identified as a dominant driver for producing the energy required to fuel biological functions through oxidative phosphorylation, in the form of adenosine triphosphate (ATP). This process is primarily carried out in the inner mitochondrial membrane using the electron transport chain (ETC). The ETC contains a respiratory chain of four complexes, generating a proton gradient that drives a fifth complex, an ATP synthase, to produce ATP. Research on mitochondrial DNA (mtDNA) and its associated diseases has been on the forefront of scientific study for many decades. In the 1960s, these double-membrane bound organelles were discovered to contain their own DNA, and in the 1980s, the first human mtDNA was sequenced and published (Smith, 2016).

In addition, mitochondria have been found to be under dual genetic control: some core mitochondrial proteins are encoded by mtDNA while most functional proteins are encoded by nuclear DNA (nDNA) (Ng & Turnbull, 2016). Although the mitochondrial genome contains over 16,000 DNA molecules, they only make up thirty-seven genes. These genes encode thirteen proteins- seven subunits of Complex I, one subunit of Complex III, three subunits of Complex IV, and two subunits of the F1F0- ATPase (Complex V), along with the 22 tRNAs and 2 rRNAs as shown in Figure 1 (Schubert Baldo and Vilarinho, 2020). The other proteins involved in the regulatory function of the mitochondria such as assisting in assembly, maintenance, DNA transcription or translation and other functions are encoded in the cell's nDNA, synthesized in the cytosol, and transported into the mitochondria (Mokranjac, 2005).

Beyond this important role of oxidative phosphorylation, mitochondria are also responsible for other metabolic functions such as the regulation of fatty acid oxidation, regulation of pyruvate, calcium, amino acids, lipid, cholesterol metabolism, initiation and modulation of cell death, initiation of cell signaling, formation of reactive oxygen species (ROS), among others (Sun et al., 2018).

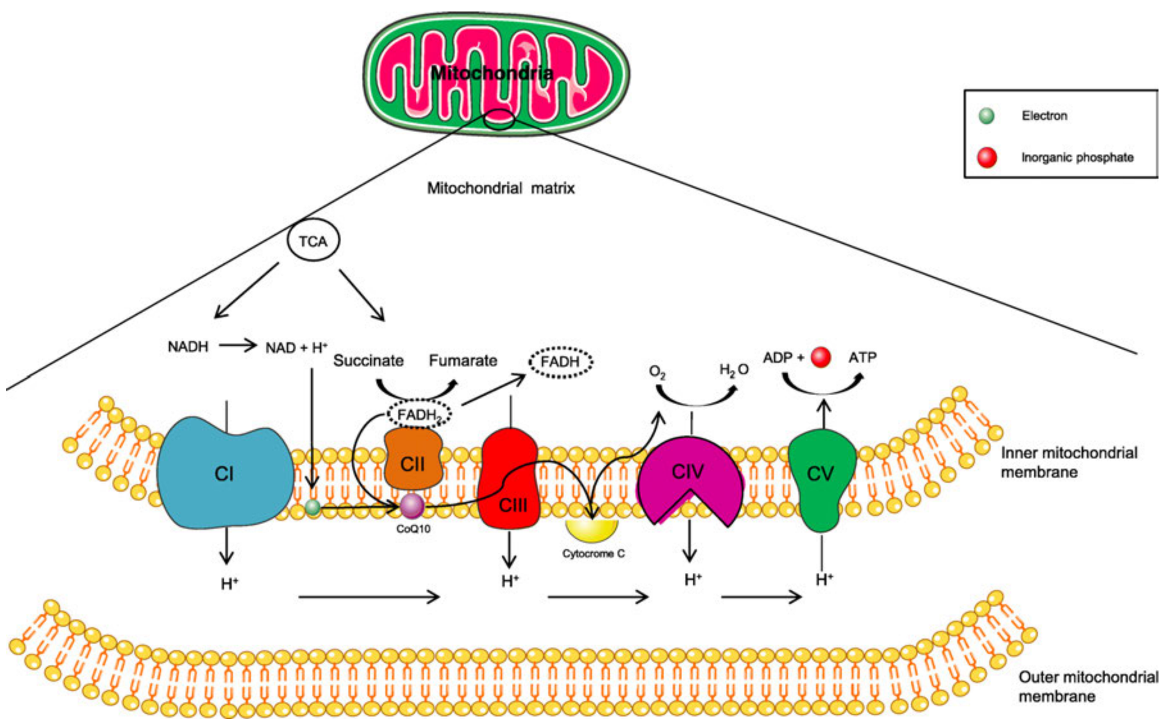


Figure 1: Representation of oxidative phosphorylation in mitochondria. CI: Complex I, CII: Complex II, CIII: Complex III, CIV: Complex IV, CV: Complex V. TCA: The Citric Acid (Cycle). Taken from (Schubert Baldo and Vilarinho, 2020).

Mitochondrial Genetics

The mitochondrial genome is unique, complex and crucial to the proper function of our cells. mtDNA is double stranded, replicated independent of the cell cycle, and packaged into nucleoids in the mitochondrial matrix. One of the unique properties of mtDNA includes having multiple copies of DNA in one cell (Ng & Turnbull, 2016) and each cell having a heterogenous mixture of DNA as shown in Figure 2.

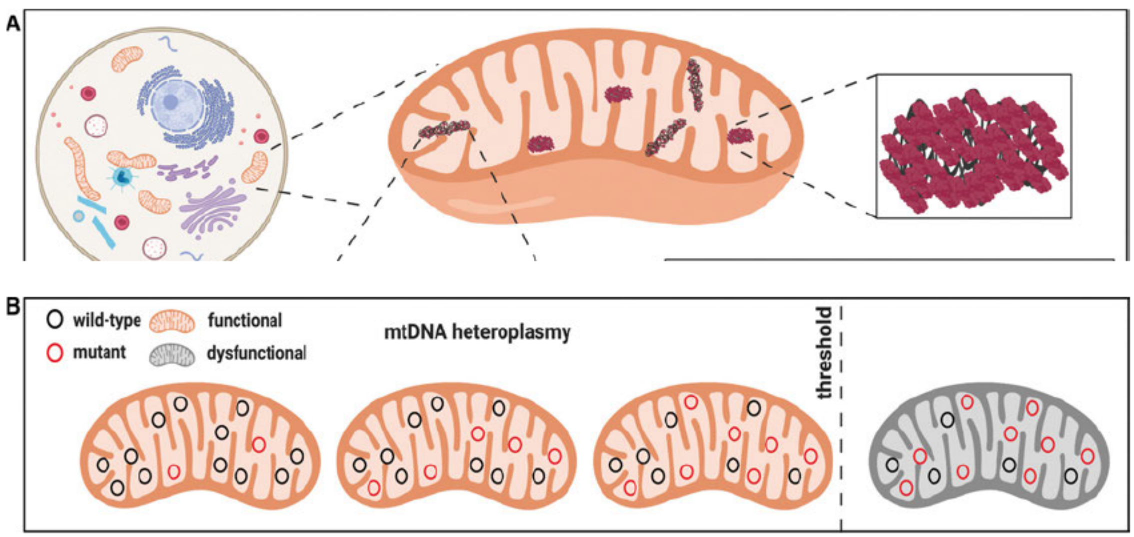


Figure 2: (A) A human cell with mitochondria showing multiple copies of mtDNA, in the relaxed or compacted state (useful for replication accessibility). (B) Heteroplasmy showing a mixture of mtDNA with wild type genes and mutant genes. There can be different combinations of heteroplasmy until the mitochondria reaches a "threshold" where the mutations outnumber the wildtype mtDNA and can render the mitochondria dysfunctional (shown in grey) due to defects in the oxidative phosphorylation system, depending on what part of the system is affected. Adapted from (Filograna et al., 2021).

Filograna et al. (2021) and Lightowlers et al. (2015) explained how a high number of copies during replication can result in increased DNA mutation and an increased risk of myopathic diseases such as Leber hereditary optic neuropathy which can lead to painless blurred vision or in Pearson's syndrome in which mutations affect hematopoietic stem cells and lead to anemia and weakness in children. Conversely, Bonnen et al. (2013) and Emperador et al. (2020) also discuss how damage to mtDNA can cause widespread deletions in the genome leading to a decreased copy of mtDNA. These depletion syndromes can then go on to cause loss of function in mutations such as in the case of Encephalomyopathic mitochondrial DNA (mtDNA) depletion syndrome 13 (MTDPS13) (Emperador et al., 2020).

Mitochondria also have a small but compact genome, with their own unique genetic code. Unlike the human nuclear genome which contains thousands of genes, the mitochondrial genome only contains thirteen important genes and lacks any introns. Although this seemingly simply system with no untranslated sequences makes the mtDNA genome easy to sequence and study, it can also lead to increased frequency of mutations. Figure 3 illustrates a human mitochondrial DNA map with some of the pathogenic mtDNA mutations and polymorphisms, populations affected, along with the nucleotide position of the mutations. To further demonstrate this complexity, Figure 4 also shows the different genes that have been researched to be associated with mitochondrial dysfunction according to the location of the defect in the mitochondria.

These mutations can arise from defects in the mitochondrial genome due to adaptive base substitution mutations and it is evident that depending on where the mutations arise from, there can be multiple different presentations of mitochondrial associated diseases (Wallace and Chalkia, 2013). Some of the most reported mutations in mtDNA include m.1555A>G which is associated with aminoglycoside induced deafness; m.3243A>G which is associated with Myoclonic epilepsy with ragged-red fibers syndrome and Maternally inherited diabetes and deafness; m.3460G>A and m.11778A>G which are associated with Leber hereditary optic neuropathy (Ng & Turnbull, 2016).

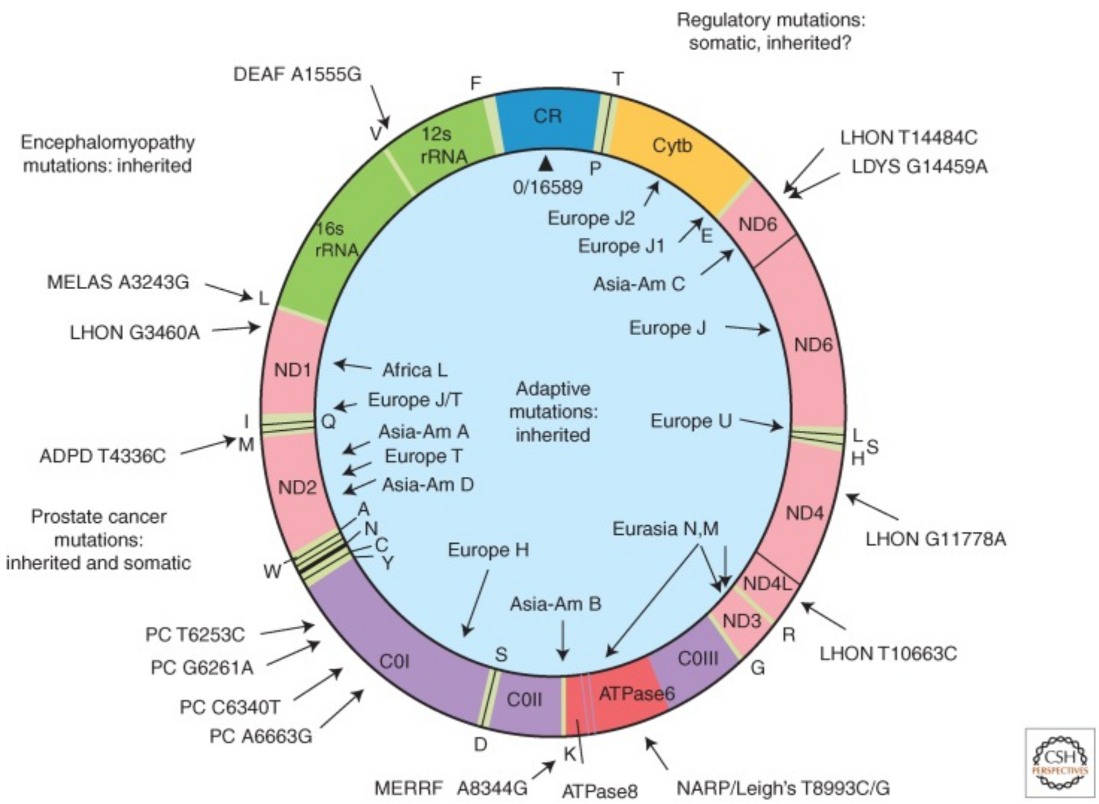


Figure 3: Map of human mitochondrial DNA showing common pathogenic mutations and nucleotide positions. CR- control region/ D-loop. DEAF- deafness; MELAS- mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; LHON- Leber hereditary optic neuropathy; ADPD- Alzheimer's disease and Parkinson's disease; MERRF- myoclonic epilepsy and ragged red fiber disease; NARP- neurogenic muscle weakness, ataxia, retinitis pigmentosum; LDYS- LHON + dystonia; PC- prostate cancer. Taken from (Wallace and Chalkia, 2013).

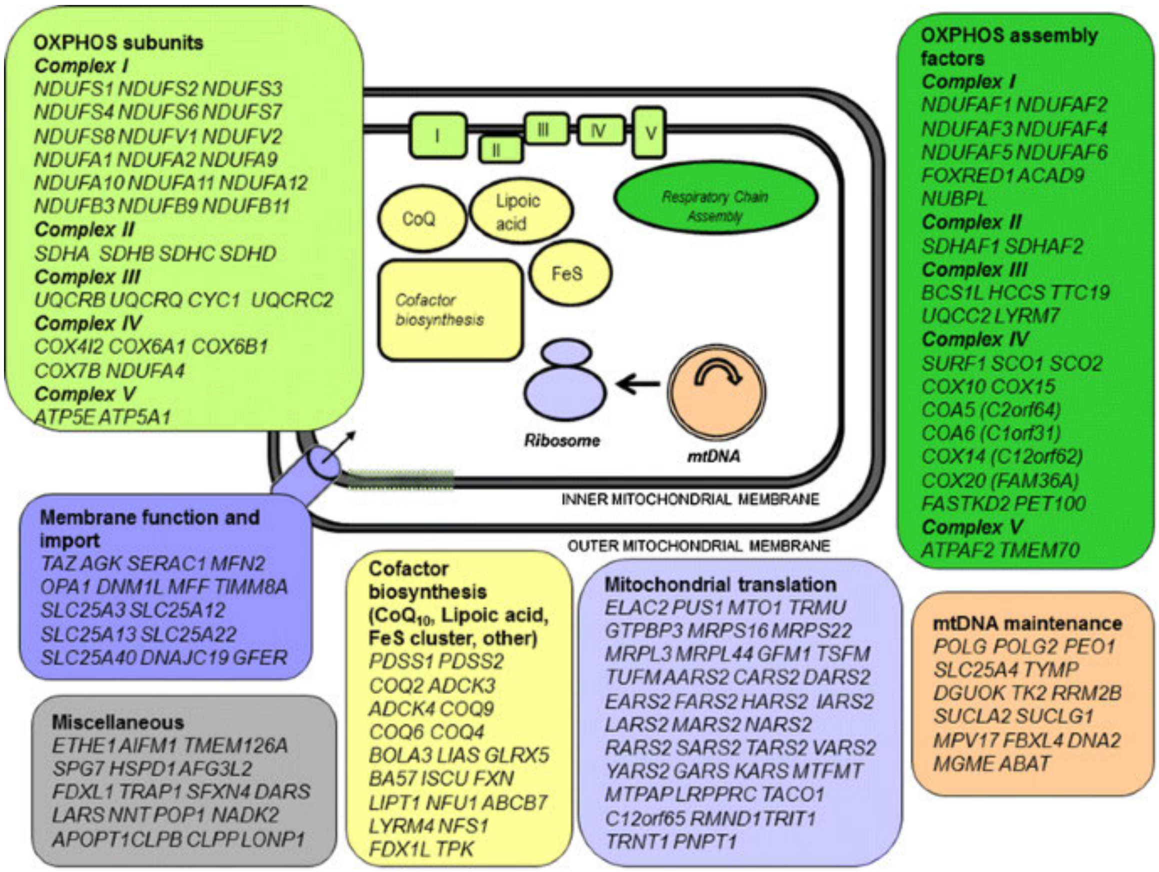


Figure 4: Nuclear genes that lead to mitochondrial defects according to their pathomechanism. OXPHOS-oxidative phosphorylation. Taken from (Rahman, 2015).

Heteroplasmy

Heteroplasmy occurs when a cell contains a mixed genome of mutated mtDNA and non-mutated or wild-type mtDNA (Ng & Turnbull, 2016). Homoplasmy, on the other hand, describes when the mtDNA in a cell is genetically identical. Heteroplasmy has been shown to be mostly predominant in post mitotic tissue such as muscle or brain tissue (Thyagaraiani & Byrne, 2002). Scientific research in the area of mitochondrial heteroplasmy has been on the rise due to the increased risk and severity of mitochondrial associated diseases depending on the magnitude of heteroplasmy. Luo et al. (2018) have shown that in the neurometabolic disorder of Leigh syndrome, when heteroplasmy for the m.8993T > G mutation is less than 30%, the patient can be asymptomatic; but if the heteroplasmy for the same mutation is greater than 60%, the symptoms of mental and motor skills become more severe.

The m.3243A>G mutation in mtDNA is the most common heteroplasmic mtDNA disease mutation; however, the cause of the different disease burdens and prognosis is poorly understood (Grady et al., 2018). From posttranscriptional modification analysis of the mtDNA tRNA$^{Leu(UUR)}$ m.3243A > G mutation, it has been found that this mutation can present with either insulin insufficiency, a neurodegenerative disorder, or a lethal disease depending on the degree of heteroplasmy which stabilizes or destabilizes histone acetylation and leads to gene modifications and altered disease presentations (Kopinski et al., 2019).

In addition, it is possible that even low levels of heteroplasmy may be enough to cause significant mitochondrial disease. In 2016, molecular genetic testing on the whole blood of a 54-year-old female revealed that only 31% of heteroplasmy of the m.3243A > G variant resulted in multiple epileptic episodes suggesting that this heteroplasmy likely caused the patient’s Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) (Sunde, et al., 2016). Due to the discovery of the m.3243A > G mutation, it has been hypothesized that measuring heteroplasmic levels can be a useful tool in helping elucidate questions about the mutant DNA, although the correlation between heteroplasmy and clinical presentation is still not completely well-defined.

Fortunately, there are different techniques that can be implored to reduce the heteroplasmic levels such as using mitochondrial-targeted restriction endonucleases, zinc-finger endonucleases, transcription activator-like effectors nucleases and clustered regularly interspace palindromic repeats (CRISPR)/Cas 9 (Hirano et al., 2018). However, research on how these heteroplasmic cells lead to loss or gain of mutations and increased disease severity as well as therapies to shift heteroplasmic load is still underway.

Mitochondrial Inheritance Patterns

Although mitochondrial diseases can arise from defects in mitochondrial encoded proteins, there are also mutations that arise from nuclear-encoded proteins that can cause disease (DiMauro et al., 2003). nDNA encode genes for structural subunits, assembly factors and translation factors for proper mitochondrial function (Chinnery et al., 2014). Gorman et al. (2015) showed that adults in North East England had an increased prevalence of 2.9:100,000 for mutations in nuclear genes associated with mitochondrial dysfunction. Due to the different origins of mutation, mitochondrial-associated diseases can be inherited in a maternal, autosomal or acquired pattern (Rusecka et al., 2018). Specifically, it has been found that mutations in mtDNA are more likely to be inherited in a maternal pattern while mutations in nDNA can be inherited in an autosomal dominant, autosomal recessive, or in a sex-linked inheritance pattern (Chinnery et al., 2014).

Although researchers had previously determined that mtDNA inheritance was strictly through maternal transmission (Birky, 1995), recent studies have started to challenge this theory, showing that there might be some exceptions. The understanding of uniparental inheritance of mtDNA was derived from a study that showed that mitochondria from sperm are selectively degraded in early embryogenesis and the oocyte mitochondria was the dominant driver for mitochondrial inheritance (Birky, 1995).

However, using polymerase chain reaction (PCR) in mouse models, it was discovered that paternal mtDNA might also play a role in inheritance because low levels of paternal transmission of mtDNA have been observed to be passed down after specific backcrosses of several generations (Gyllensten, et al., 1991). In Drosophila models, Kondo et al. (1990) showed an incomplete maternal DNA inheritance and paternal DNA leakage. In a unique case report, Schwartz et al. (2002) presented a 28-year-old man with a mutation in the gene associated with a subunit of Complex I that led to significant mitochondrial myopathy, and using genomic sequencing, found that 90% of the patient's mutated DNA was of paternal origin.

In the parasitic *Schistosoma mansoni* species, Jannotti-Passos et al. (2001) showed a biparental inheritance of mtDNA in offspring of the F2 and F3 generations. There has also been biparental inheritance shown in yeast (Breton et al., 2015). One notable study found multiple cases of biparental inheritance of mtDNA passed down in three unrelated, multiple generational families in an autosomal dominant pattern, though these cases depended on a higher percentage of heteroplasmic mtDNA (Luo et al., 2018). Although the underlying mechanism for selective elimination of sperm mtDNA in human embryos has not been discovered, further investigation into paternal mtDNA inheritance would be beneficial in understanding the etiology of paternal sporadic mitochondrial mutations that cause disease.

Clinical Features of Mitochondrial Disease

Mitochondrial diseases are multifaceted. Most of the disorders are caused by genetic mutations and due to the large quantity of mitochondria for high oxygen demand in tissues such as the heart, muscle and brain, these organs are most commonly affected (Tarnopolsky & Raha, 2005). Most mitochondrial diseases lead to either myopathic dysfunction, central nervous system dysfunction or they affect other tissues such as the eyes causing ophthalmoplegia or the endocrine organs causing diabetes. It is important to be aware of certain principles when considering the presentation of mitochondrial disease. Firstly, the overall presentation for different populations and age of onset, the variety and severity of the clinical syndromes, as well as the overlapping presentations in multiple disease syndromes. For instance, in MELAS, Myoclonic epilepsy with ragged-red fibers (MERRF) and Alperts Syndrome, all the patients might present with seizures, but deafness and muscle weakness are only associated with MELAS and MERRF (Thyagaraiani & Byrne, 2002).

Although there are specific syndromes that manifest with mitochondrial diseases, it is also important to be aware of presentations that involve certain systems (Table 1). These systems include the central nervous system which presents with strokes or developmental delay or the peripheral nervous system which presents as peripheral neuropathy; the visual and auditory system which can present with blindness and hearing loss; the neuromuscular system which can present with exercise intolerance, muscle weakness and wasting; the gastrointestinal system which can present with delayed gastric emptying, constipation, diarrhea and malabsorption (Thyagaraiani & Byrne, 2002).

Table 1. Some Clinical Manifestations of Mitochondrial Diseases. Adapted from (Thyagaraiani & Byrne, 2002).

CLINICAL MANIFESTATIONS

System/organ	Manifestations
CNS	Seizures, stroke-like episodes, dementia, sensorineural deafness, movement disorders including ataxia, myoclonus, dystonia, chorea, migraine, psychomotor regression/retardation, Parkinsonism
Skeletal muscle	Hypotonia, myopathy, ptosis, CPEO, recurrent myoglobinuria
Peripheral nerves	Neuropathy
Bone marrow	Pancytopaenia, sideroblastic anaemia
Kidney	De-Toni-Fanconi renal tubular acidosis
Endocrine	Type II diabetes mellitus, hypoparathyroidism, growth hormone deficiency
Heart	Cardiomyopathy, conduction defect
Gastrointestinal system	Pancreatic failure, pseudo-obstruction, hepatopathy
Eye	Retinal pigmentary degeneration, optic atrophy, cataract
Systemic	Systemic lactic acidosis

CHAPTER TWO

Acquired mtDNA Associated Diseases

mtDNA associated diseases can be acquired as seen in premature aging and age-related diseases such as Type 2 Diabetes Mellitus (DM2), neurodegenerative diseases and cancer pathologies (Taylor et al., 2007). When mitochondria become defective or dysfunctional in a cell, there are increased toxic accumulations and activated aging-related features that ultimately result in cell death. Trifunovic et al. (2004) found that mice bred with a knock-in gene that was defective for mitochondrial DNA polymerase (Polymerase gamma) proofreading abilities, accumulated point mutations and deletions in mtDNA in a variety of tissues that resulted in reduced lifespan and premature onset of diseases such as alopecia, kyphosis, anemia and cardiomyopathy.

Rao et al. (2018) found that in both experimental models and humans with advanced chronic kidney disease, there was disrupted mitochondrial metabolism and uncoupling of oxidative phosphorylation due to mitochondrial structure, function and composition abnormalities. In these patients, their advanced disease was also associated with acquired mitochondrial myopathy leading to skeletal muscle atrophy, skeletal muscle weakness and decreased exercise capacity (Rao et al., 2018). In one of the first studies examining mitochondrial volume in skeletal muscle of patients undergoing dialysis, Gamboa et al., (2016) also found that there was decreased mitochondrial DNA density and copy number, structural abnormalities, increased oxidative stress, and an increased marker of mitophagy abnormalities in skeletal muscles of the patients compares to patients with normal kidney function.

In addition, mitochondrial abnormalities have been indicated in endocrine disorders such as Diabetes Mellitus. In order for pancreatic beta cells to release insulin, they need a high ATP/ADP ratio and defects in the mitochondria's ability to form ATP can lead to decreased insulin release and therefore decreased clearance of glucose in the blood (Watada & Fujitani, 2015). The mitochondria of these patients with DM2 have also been found to have a downregulation of tightly-regulated and highly-expressed genes involved in mitochondrial oxidative phosphorylation (Mootha et al., 2003). Maternally Inherited Diabetes and Deafness (MIDD) is an inherited single gene disorder associated mutations such as m.1555A>G and m.3243A>G leading to insulin secretory defect and early onset diabetes due to a disruption in oxidative phosphorylation (Schaefer et al., 2013). Disruption of mitophagy, the process of degrading damaged mitochondria, has also been found to accelerate diabetes and diabetic cardiomyopathy (Yu et al., 2017).

Defects in mtDNA have also been known to play significant roles in aging associated with neurodegenerative diseases such as Parkinson's disease (PD), Alzheimer's disease (AD), Amyotrophic lateral sclerosis (ALS), Friedreich's ataxia and Charcot-Marie-Tooth disease (Chaturvedi, et al., 2013). In patients with PD, there were large areas of deletions in mtDNA in substantia nigra neurons in the basal ganglia in the brain compared to the brain of control patients (Bender et al., 2006). In patients with AD, there were mutations in mtDNA leading to deficiencies in cytochrome oxidase, a key protein in the ETC leading to defective oxidative phosphorylation in neuronal cells (Parker et al., 1990, Parker et al., 1994). In ALS patients, dysregulated mitochondrial function has been linked to increased motor neuron death, accumulation of dysfunctional mitochondria coupled with impaired ATP production and release contributing to increased cell damage and disease progression of ALS (Muyderman, 2014).

Further research into the accumulation of mutations in mtDNA in cancer cell lines is still underway but it has been found that these mutations occur at high frequency and without selection (Coller et al., 2001). In some cancers such as gastric cancer, a study found a positive correlation between dysfunction of mismatch repair genes that produces nuclear genome and mitochondrial genome instability, an important marker in human cancer (Bianchi el al., 2001). In addition, a collection of somatic mutations in mtDNA was associated with ten colorectal cancer cell lines (Polyak et al., 1998) and seven papillary thyroid carcinoma cell lines (Abu-Amero et al., 2005).

Somatic mutations of mitochondrial DNA have been found in different cancers such as in hepatocellular cancer, breast cancer due to decreased mtDNA copy numbers, defects in mitochondrial enzymes, increase in oxidative stress, altered calcium metabolism and a dysregulation of tumor suppressor/oncogenic genes (Hsu et al., 2016). More research still needs to be conducted to determine the main role of mtDNA mutations in aging, neurodegenerative diseases and human cancers and to answer questions about how some diseases have a positive correlation with mtDNA dysfunction compared to others.

mtDNA Damage and Repair

mtDNA and nDNA encode genomic information for proteins necessary for the proper function of the mitochondria. The mtDNA component is more susceptible to damage because of the increased rate of DNA mutation (up to 20 times more) compared to nDNA (Annesley and Fisher, 2019). This high rate of mutagenesis and lack of adequate DNA repair mechanisms ultimately leads to increased levels of pathological dysfunction (Annesley and Fisher, 2019). Unlike the rest of the nuclear genome, mtDNA replicates without introns or histones and because of the genome's close proximity to the ETC, the mutations impact the complexes leading to an increased production of reactive oxygen species (DeBalsi et al., 2017).

Most of the oxygen consumed by the human body is taken up by the mitochondria (Annesley and Fisher, 2019). This oxygen is first converted to superoxide ($O_2^{\bullet -}$) and this free radical is then converted to hydrogen peroxide (H_2O_2) by superoxide dismutase and later detoxified by catalase or glutathione peroxidase to water and oxygen; if the hydrogen peroxide is allowed to accumulate due to a dysfunctional detoxification process, it will be converted to the hydroxyl radical ($HO^{\bullet}$) through the Fenton reaction which will be detrimental to the cell (DeBalsi et al., 2017).

Accumulation of ROS increases the mutation rate in mtDNA leading to more production of ROS in a detrimental feedback loop (Kwong and Sohal, 2000). Ultimately, the accumulation of damaged mtDNA whether due to ROS or increased mutations have been shown to be associated with acquired mtDNA diseases (DeBalsi et al., 2017).

Mitophagy

Mitophagy, coined by Lemasters, is a process by which mitochondria are targeted for degradation via autophagy in a cell (Lemasters, 2005). Mitochondrial degradation could occur as a result of normal cell turnover or recycling, cell or mitochondrial damage, or cell death due to starvation (Dombi et al., 2018). The process of autophagy is conserved and is as a result of signaling pathways that ultimately lead to programmed cell death. There are various mitophagy proteins used to form an autophagosome, and research into understanding the machinery of the pathways are still underway. Dombi et al. (2018) discuss the two classifications of mitophagy, namely Type 1 which occurs due to the lack of nutrients and depends on the phosphoinositide 3-kinase

(PI3K) complex, compared to Type 2 which occurs due to photodamage and uses PTEN-induced putative kinase 1 (PINK 1) and Parkin in order to clear damaged mitochondria. In Parkin dependent pathways, substrates become ubiquitinated and Parkin acts to recruit other autophagic machinery to the mitochondria to begin the process of mitophagy; however, in Parkin-independent pathways, there are receptor mediated and ligase mediated mechanisms that lead to the process of mitophagy (Liu et al., 2019).

Due to the widespread effect of dysfunctional mitochondria, research into the modulation of mitophagy has become more important. Kerr et al. (2017) show that impaired mitophagy has been linked to the synaptic dysfunction and cognitive decline observed in Alzheimer's patients. In addition, when the dysfunctional mitochondria that should be targeted for degradation through mitophagy accumulate, the increase in reactive oxygen species as well as decreased cellular ATP levels and accumulation of toxic metabolites can further exacerbate mitochondrial damage, leading to increased accumulation of AD defining proteins (Kerr et al., 2017).

Furthermore, Fivenson et al. (2017) discuss how aging and age-related diseases might be associated with impaired mitochondrial autophagy and how decreased autophagy plays a role in neurodegenerative diseases such as AD, PD and Huntington’s disease. Gene mutations in PD proteins play a role in mitochondrial dysfunction and disordered mitophagy through a disruption of the ubiquitination process, a dysregulation of mitophagy associated protein recruitment and the accumulation of toxic compounds in cell due to lack of degradation (Liu et al., 2019). In *Caenorhabditis elegans* models, it was found that mitochondria accumulate with age, possible due to decreased mitochondrial clearance mechanisms; however an induction of mitophagy was observed in of *C. elegans* mutants who lived longer than controls (Fivenson et al., 2017).

In studying therapeutic options for patients with acquired or inherited mitochondrial disease, it is important to explore the modulation of mitophagy and mitochondrial recycle to improve mitochondrial homeostasis which leads to improved cellular stability. Some therapies have included Coenzyme Q and the quinine, idebenone, that could be used to upregulate mitophagy by targeting damaged mitochondria in mitochondrial disease, thereby increasing functional mitochondria for the benefit of overall mitochondrial function in the cell (Dombi et al., 2018). However, the process by which mitophagy plays a role in improving mitochondrial-associated diseases continues to be investigated.

CHAPTER THREE

Objective

Due to the extensive functionality of mitochondria, there are multiple genetic and acquired diseases that arise from mitochondrial dysfunction as shown in Table 2 (Chinnery et al., 2014). This paper will examine two mitochondrial associated diseases known as Leigh Syndrome (LS) and Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), discussing the similarities and differences between the two diseases. Although they have been found to affect the same subunit (Complex I) of the electron transport chain in mitochondria, they present with different clinical symptoms as well as at different stages of life (Chinnery et al., 2014). Furthermore, this paper will examine mitochondrial inheritance patters in relation to the myopathies and briefly review current therapies to treat LS and MELAS. Treatment of mitochondrial-associated diseases have proved to be a challenge because current research is predominately cellular based research with few transgenic mouse models, though these models do not always mimic human disease presentations (Taylor et al., 2007). Nonetheless, these therapies continue to enlighten our current understanding on the various mitochondrial associated diseases.

Table 2. Clinical Syndromes of Mitochondrial Diseases showing similarities and differences in primary features. Table adapted from (Chinnery et al., 2014).

Disorders	Primary Features	Additional Features
Alpers-Huttenlocher syndrome	• Hypotonia • Seizures • Liver failure	Renal tubulopathy
Chronic Progressive External Ophthalmoplegia	• External ophthalmoplegia • Bilateral ptosis	Mild proximal myopathy
Infantile myopathy and lactic acidosis (fatal & non-fatal forms)	• Hypotonia in 1st yr. of life • Feeding & respiratory difficulties	Fatal form may be associated w/cardiomyopathy &/or Toni-Fanconi-Debre syndrome.

(Table 2 Continued)

Pearson syndrome	• Sideroblastic anemia of childhood • Pancytopenia • Exocrine pancreatic failure	• Renal tubular defects
Leigh syndrome	• Subacute relapsing encephalopathy • Cerebellar and brain stem signs • Infantile onset	• Basal ganglia lucencies • Maternal history of neurologic disease or Leigh syndrome
Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes	• Stroke-like episodes at age <40 years • Seizures &/or dementia • Ragged-red fibers &/or lactic acidosis	• Diabetes mellitus • Cardiomyopathy (initially hypertrophic; later dilated) • Bilateral deafness • Pigmentary retinopathy • Cerebellar ataxia
Myoclonic Epilepsy Myopathy Sensory Ataxia	• Myopathy • Seizures • Cerebellar ataxia	• Dementia • Peripheral neuropathy • Spasticity

(Table 2 Continued)

Myoclonic Epilepsy with Ragged-red Fibers	• Myoclonus • Seizures • Cerebellar ataxia • Myopathy	• Dementia • Optic atrophy • Bilateral deafness • Peripheral neuropathy • Spasticity • Multiple lipomata
Leber hereditary optic neuropathy	• Subacute painless bilateral visual failure • Males:females ~4:1 • Median age of onset 24 yrs	• Dystonia • Cardiac pre-excitation syndromes

Mitochondrial Myopathies

Myopathies are clinical disorders of skeletal muscle tissue. Skeletal muscle fibers contain a large number of mitochondria because of their high demand for ATP synthesis during oxidative phosphorylation to fuel voluntary movement. As a result of this high energy demand, mitochondrial myopathies are one of the most common manifestations of adult-onset mitochondrial diseases (Ahmed, 2018). Research in recent decades have revealed that proper mitochondrial structure and function is also required for skeletal muscle homeostasis in areas such as the regulation of calcium homeostasis, redox processes, release of pro-apoptotic factors during cell death, and others (Vincent, 2016).

Some symptoms associated with mitochondrial myopathies include exercise intolerance, fatigue and cramps with chronic progressive external ophthalmoplegia, proximal myopathy, seizures and dementia. These symptoms are as a result of progressive weakness of skeletal muscle due to a depletion in mitochondrial function from mutations or deletions in mtDNA or nDNA (Ahmed, 2018). Diagnostic approaches include biochemical studies to assess for enzyme and complex activity, whole genome sequencing, real time PCR to determine mtDNA copy number, lactate measurement or an exercise test (Ahmed, 2018).

Leigh Syndrome

History and Presentation

Leigh syndrome (LS), also known as subacute necrotizing encephalomyelopathy, gets its name from Denis Leigh's first postmortem investigation of a 7-month old male infant in London who presented with the symptoms in 1947 (Leigh, 1951). Leigh took interest in this patient because after his presentation, his condition rapidly declined from being comatose to later dying from terminal hyperpyrexia within three days (Leigh, 1951). LS is most commonly found in previously healthy pediatric patients between three and twelve months of age; it has an estimated prevalence of 1 in 40,000 live births and is typically fatal (Lake et al., 2016).

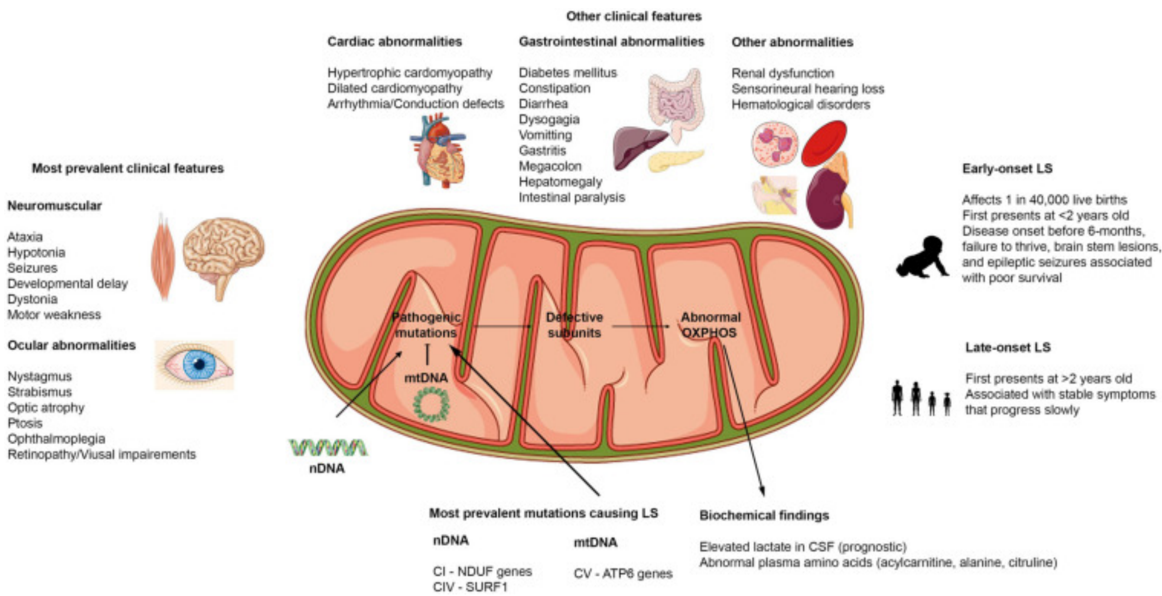


Figure 5: Clinical Features of Leigh Syndrome as well as the presentation, biochemical findings and most common mutations. Taken from (Bakare et al., 2021).

After a normal delivery and development, most LS patients present with developmental delay and regression before the age of two, in addition to neurological symptoms that quickly progress to multi-organ disorders such cardiac, hepatic, gastrointestinal and renal dysfunction as shown in Figure 5.

Since Leigh's research and publication of LS in the 1950s, there have been more investigations into the syndrome as well as a classification of the symptoms based on the variability of the disease as shown in the Table 3 (Lim and McFarland, 2019). It was found that patients with "Leigh-like syndromes" could present with some of the clinical features but may not completely fit into the three diagnostic criteria listed in Table 3 for Leigh Syndrome (Lim and McFarland, 2019).

Table 3. Diagnostic criteria proposed for Leigh Syndrome. Table taken from (Lim & McFarland, 2019).

1. Stepwise clinical deterioration	Progressive neurological disease characterised by *stepwise* decompensation during intercurrent illness. Motor and intellectual developmental delay or regression is often followed by a period of stabilisation or recovery before another decline. Seizures and peripheral neuropathy are often associated
2. Bilateral neuroradiological features	Signs and symptoms of brain stem and/or basal ganglia disease (dystonia spasticity, movement disorder, ataxia and nystagmus). These correspond with bilateral symmetric hyper-intense signal changes in the brain stem and/or basal ganglia on T2-weighted magnetic resonance imaging (MRI) of the brain. Proton magnetic resonance spectroscopy could pick up areas of raised lactate levels
3. Abnormal mitochondrial energy metabolism	Elevation of blood and/or CSF lactate levels. Increased lactate is more consistent in CSF than blood. Plasma amino acid might show raised alanine level. These result from defective oxidative phosphorylation (OXPHOS) or pyruvate dehydrogenase complex (PDHc) activities
"Leigh-like syndrome" terminology is frequently used if patients have features strongly suggestive of Leigh syndrome but do not satisfy all these criteria. These may be due to atypical/normal neuroimaging, normal blood/CSF lactate levels or unusual neuropathology	

Patients with LS also have problems with feeding and vomiting leading to failure to thrive, as well as a combination of disorders of the nervous and neuromuscular system that manifest as nystagmus, strabismus, ptosis, dystonia, ataxia, spasticity and seizures (Ruhoy and Saneto, 2014). While there are a lot of complications with this disease, including increased co-morbidity factors, often the main cause of death is respiratory failure (Ruhoy and Saneto, 2014).

Molecular Basis of LS

LS is a heterogeneous progressive neurodegenerative disorder caused by a defect in the energy production role of mitochondria and research on its genetic etiologies are still underway. LS can present with variable inheritance patterns such as autosomal recessive, x-linked, and maternal inheritance (Darin et al., 2001). Over 75 genes have been identified including nuclear genes involved in oxidative phosphorylation, as well as genes for other mitochondrial functions that cause Leigh or Leigh-like syndrome (Table 2, Lake et al., 2016). As shown in Table 4, there are many different genes responsible for subunits of the electron transport that can be affected in Leigh Syndrome after a mutation in one or more mitochondrial or nuclear genes.

Table 4. Genes associated with Leigh Syndrome. Taken from (Ruhoy and Saneto, 2014).

Gene	**Defect**	**Location**
MT-ATP6	Complex V	Mitochondrial
MT-ND1	Complex I	Mitochondrial
MT-ND2	Complex I	Mitochondrial
MT-ND3	Complex I	Mitochondrial
MT-ND4	Complex I	Mitochondrial
MT-ND5	Complex I	Mitochondrial
MT-ND6	Complex I	Mitochondrial
MT-ATP6	Complex V	Mitochondrial
MT-ND1	Complex I	Mitochondrial

(Table 4 Continued)

Gene	**Defect**	**Location**
MT-ND2	Complex I	Mitochondrial
MT-ND3	Complex I	Mitochondrial
MT-ND4	Complex I	Mitochondrial
MT-ND5	Complex I	Mitochondrial
MT-ND6	Complex I	Mitochondrial
NDUFA9	Complex I	Nuclear
NDUFA10	Complex I	Nuclear
NDUFS1	Complex I	Nuclear
NDUFS2	Complex I	Nuclear
NDUFS3	Complex I	Nuclear
NDUFS4	Complex I	Nuclear
NDUFS7	Complex I	Nuclear
NDUFS8	Complex I	Nuclear
NDUFV1	Complex I	Nuclear
PDHA1	Pyruvate dehydrogenase deficiency	X-linked

(Table 4 Continued)

PDHB	Pyruvate dehydrogenase deficiency	X-linked
PDHX	Pyruvate dehydrogenase deficiency	X-linked
PDSS2	Ubiquinone deficiency	Nuclear
PET100	Complex IV	Nuclear

Most commonly, however, a defect in Complex I has been shown to be the most frequent clinical presentation of LS (Lake et al., 2016). Complex I has forty-five subunits encoded by different genes and one of the genes, encoded by nuclear DNA, translates into a subunit called Nicotinamide adenine dinucleotide dehydrogenase (NADH) (ubiquinone) Iron-sulfur protein 4 (Ndufs4) that when mutated can be inherited in an autosomal recessive manner leading to inherited LS (Quintana et al., 2010). In homozygous children, the mutation affects the phosphorylation site of Complex I and therefore impairs the normal assembly of the complex leading to a fatal neurological syndrome (Papa, 2002). The deficiency in Complex I presents with bilateral basal ganglia lesions (Figure 6), stroke-like lesions if involved with mtDNA and leukoencephalopathy if involved with nDNA (Schubert Baldo and Vilarinho, 2020), including brain stem lesions of white and grey matter (Arii and Tanabe, 2000).

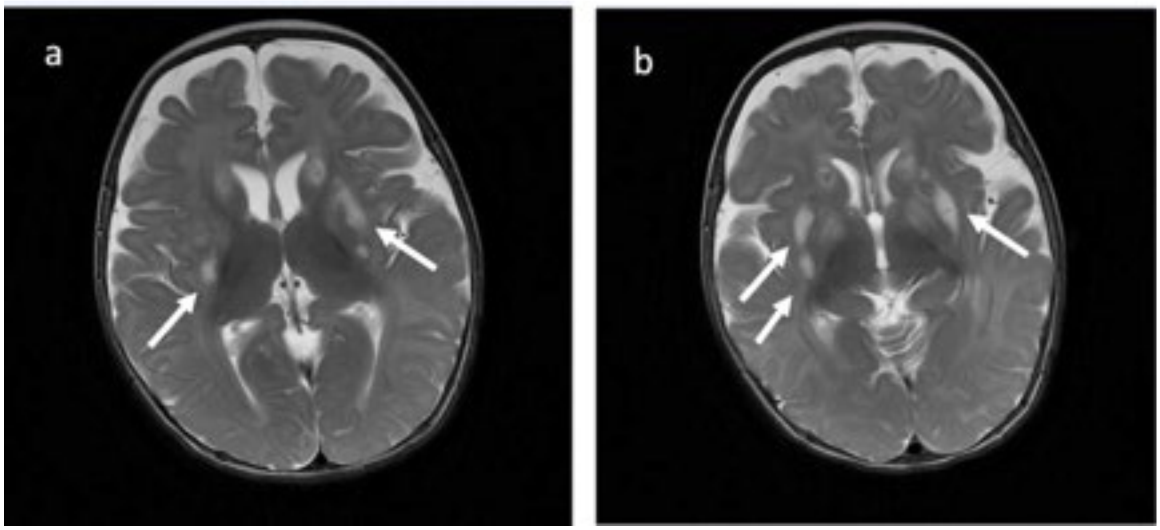


Figure 6: Brain MRI images showing bilateral basal ganglia lesions (white arrows, a and b). Case courtesy of Dr. M. Venkatesh, Radiopaedia.org, rID: 27512. Taken from (Schubert Baldo and Vilarinho, 2020).

In addition to Ndufs1, mutations in another nuclear gene encoded subunit in Complex I known as NADH dehydrogenase (ubiquinone) flavoprotein 1 (Ndufv1) has also been found to cause LS (Lake et al., 2016). Both cases present with an early onset of LS- with the Ndufv1 mutation, the disease presents at the average age of 8.5 months and ends with fatality at 4 years old whereas with the Ndufs1 mutation, the patient lives a little longer into late childhood (Fassone and Rahman, 2012).

LS can also be caused by deficiencies in the PDH complex leading to a reduction in TCA activity and early onset LS or by mutations in Complex II, a complex regulated by nuclear encoded subunits leading to mitochondrial dysfunction or in a thiamine transporter gene SLC19A3 (c.20 c>A) that leads to a premature stop codon, decreased thiamine transport needed for the respiratory complex of the mitochondria (Ruhoy and Saneto, 2014).

Table 5. Characteristic LS Disease Features, Affected Ethnicities, and Founder Genotypes Associated with the Most Common Nuclear Disease Genes. Taken from (Lake et al., 2016).

Gene	Biochemical Defect	Characteristic Disease Features	Ethnicities Notably Affected	Founder/Frequent Genotypes
NDUFS4	CI	"Typical" LS disease course with death by 3 years	Ashkenazi Jews	c.462delA with an estimated carrier frequency of 1:1,000[25]
SURF1	CIV	Hypertrichosis, cognitive sparing	Pan-ethnic	c.311_312insATdel10 in Caucasian Europeans[35]
LRPPRC	CIV	Mild facial dysmorphism, liver pathology, and a course punctuated by episodes of acute metabolic decompensation	Saguenay-Lac-Saint-Jean region of Quebec, Canada	c.1119C>T, p.A354V with an estimated carrier frequency of 1:23[3,37]
SCO2	CIV	Hypertrophic cardiomyopathy, spinal muscular atrophy pattern of histopathology in skeletal muscle	Pan-ethnic	c.1541G>A, p.E140K[39]
PET100	CIV	Seizures	Lebanese	c.3G>C, p.Met1?[114]
ETHE1	CIV	Ethylmalonic aciduria, acrocyanosis, petechiae, gastrointestinal involvement	Mediterranean basin and Arabian peninsula regions	c.487C>T, p.R163W[38]
SUCLA2	CI, CIII, CIV, CV	Methylmalonic aciduria, dystonia, deafness, often Leigh-like presentation, cerebral atrophy, onset in early infancy with long disease duration	Faroe Islanders	c.534 + 1G>A with an estimated carrier frequency of ~1:33 people[4,115]
MTFMT	CI, CIII, CIV, CV	Microcephaly frequently observed	Europeans	c.626C>T with an estimated carrier frequency of 1:100 in Europeans[51]
SERAC1	Multiple	Hypoglycemia, liver involvement, behavioral disturbance	Pan-ethnic	—
PDHA1	PDHc	Lactate:pyruvate ratio < 20, agenesis of corpus callosum frequently observed	Pan-ethnic	—
PDHX	PDHc	Lactic acidosis crisis in newborn or in early infancy, cerebral palsy–like presentation	Roma children from Bulgaria	c.1336C>T, p.R446*[116]

CI–CV = oxidative phosphorylation complexes I–V; LS = Leigh syndrome; PDHc = pyruvate dehydrogenase complex.

Maternally Inherited Leigh Syndrome

Molecular Basis of Maternally Inherited Leigh Syndrome (MILS)

Like LS, (MILS) is also a fatal neurodegenerative disease that presents during infancy and leads to bilateral lesions in the basal ganglia and brainstem (Uittenbogaard et al., 2018). MILS is maternally inherited and closely associated with mutations in the mitochondrially encoded ATPase6 subunit (MT-ATPase 6) (Carrozzo et al., 2004). There are many gene mutations that affect the mitochondrial ATPase and some common ones include L217R (Vazquez-Memije et al. 1998; Carrozzo et al., 2001), or T8993G which was the first report of proven MILS in Hong Kong in 2009 (Shuk-kuen Chau et al., 2010), and has been documented in multiple case reports on families with MILS. In most cases reported, up to 80% of the family members were found to have the T8993G after presenting with symptoms of MILS (Mkaouar-Rebai et al., 2009; Santorrelli et al., 1993).

The most common genetic mutation is the T8993G variant that has been found to be a common cause of MILS and shown to have a profound defect in ATP synthesis (Santorrelli et al., 1993). In the final part of oxidative phosphorylation carried out by mitochondria, there is an ATP synthase composed of two functional domains, called F_0 and F_1 (Sgarbi et al., 2006). The F_0 domain is the proton channel while the F_1 domain is the catalytic site for ATP synthase. The MT-ATPase 6 subunit is part of the F_0 domain; it contains four transmembrane helices that couple proton translocation with rotation of the F_0 subunit c-ring which converts mechanical energy into chemical energy for ATP synthesis (Sgarbi et al., 2006). The L156R substitution, caused by the m.8993T>G variant, prevents MT-ATPase 6 from inducing rotation of the c-ring which leads to a defective ATP synthase machinery and decreased ATP synthesis (Santorrelli et al., 1993).

In comparing MILS and LS, MILS patients have been found to have greater than 90% heteroplasmy of mtDNA in their cells (Lake et al., 2016). Like LS, they present with a rapidly progressive, degenerative disease that can be fatal. Although the mutations in nuclear genome are more closely associated with LS, five to ten percent of patients who have MILS have disease processes that are closely associated with mutations in mtDNA which is maternally inherited (Ruhoy and Saneto, 2014). There are variable results in the MRI findings of patients with LS, Leigh-like syndromes as well as MILS and the etiology of the differences are the still being researched (Ruhoy and Saneto, 2014).

Mutations in the nucleotide 8993 that usually lead to MILS is also closes associated with a neuropathy, ataxia and retinitis pigmentosa (NARP) syndrome. Like MILS, this syndrome is caused by a mutation in the MT-ATP6 gene but it is characterized by proximal neurogenic muscle weakness with sensory neuropathy, ataxia, and pigmentary retinopathy, seizures (Thorburn et al., 2003).

Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS)

History and Presentation

MELAS was first presented and characterized in the literature in 1984 by Steven Pavlakis (Pavlakis et al, 1984). In his team's novel finding, there were 11 cases of patients with MELAS who presented with normal early development, short stature, seizures, hemiparesis, lactic acidosis hemianopia or cortical blindness (Pavlakis et al, 1984). His team also found ragged red fibers on muscle biopsy, a possible evidence of a defect in the ETC and now a hallmark of MELAS disease processes (Pavlakis et al, 1984). This process is characterized by hypertrophic mitochondria attempting to compensate for poor ATP production and they appear bright red under the Gomori trichome stain and are termed "ragged red fibers" (Pia and Lui, 2020).

Like most mitochondrial diseases, MELAS can also present as a multiorgan disease with varying severity in different patients. MELAS can present both in pediatric and adult patients. In the juvenile form, which is more likely to be fatal, most patients characteristically present with short stature, while the adult form is frequented with hearing loss, cortical blindness and diabetes mellites (Yatsuga et al., 2012). Some patients of varying ages can also present with other co-morbidities such as fever, vasculitis, encephalitis and migraine (Goodfellow et al, 2012), exercise intolerance, cardiomyopathy, ocular motility abnormalities and gastrointestinal changes (Lorenzoni et al., 2015).

Molecular Basis of MELAS

MELAS has been discovered to be one of the most common mitochondrial diseases with an incidence of 1in 4000 (Pia and Lui, 2020). The disease affects all genders equally and although MELAS is maternally inherited, it can also arise from a sporadic mutation (Pia and Lui, 2020). This condition is associated with various point mutations in mitochondrial transfer RNA (mtRNA). Eighty percent of mutations in MELAS patients are associated with a m.3243A>G variant which causes disruption of the dihydrouridine loops of the mttRNA gene as shown in Table 6 (Lorenzoni et al., 2015). This structural mutation is associated with disruption of the protein assembly of the ETC, decreased glycolysis, increased production of lactic acid and accumulation of metabolic/assembly intermediates (Frey et al., 2017).

Table 6. Common genetic mutations associated with MELAS. Taken from (Lorenzoni et al., 2015).

Gene	Mutation	Proportion of affected individual
MT-TL1 [$tRNA^{Leu(UUR)}$]	3243 A > G	~80%
	3271 T > C	~7.5%
	3252 A > G	< 5%
	3256 C > T 3260 A > G 3291 T > C 3302 A > G	< 10%
MT-ND5	13513 G > A	< 10%
	12770 A > G 13042 A > T 13045 A > C 13046 T > C 13084 A > T 13514 G > A 13528 A > G	< 5%

Most of the mutations present in the MELAS syndrome affect protein assembly and function and therefore affect the process of oxidative phosphorylation, with about half of patients affected with decreased Complex I activity, and less than one-third with decreased Complex III or Complex IV activity (Santa, 2010). These deficiencies ultimately lead to a variety of symptoms including but not limited to cardiomyopathy, Wolff-Parkinson-White syndrome, heart failure, lactic acidosis, constipation, vomiting, exercise intolerance, optic atrophy, pulmonary hypertension or sensorineural hearing loss (Santa, 2010).

There are strict criteria that have been developed to diagnose MELAS in order to differentiate this diagnosis from an ischemic attack or other brain pathologies. The criteria taken from (Pia and Lui, 2020) and (Lorenzoni et al., 2015) includes a combination of molecular and clinical findings such as:

1. Normal birth and development
2. Stroke-like episode before 40 years of age
3. Encephalopathy (dementia and/or seizures)
4. Evidence of mitochondrial dysfunction such as blood lactic acidosis, ragged red fibers on muscle biopsy
    - Lactate might also be elevated in patient serum or cerebrospinal fluid (Lorenzoni et al., 2015).

In addition, for evaluation purposes, an MRI can be performed to evaluate the presence of areas of cortical necrosis, diffuse cortical atrophy in both the cerebral hemispheres and the cerebellum but sparing the brainstem in contrast to LS and MILS as shown in Figure 7 (Lorenzoni et al., 2015). In all of these investigations, MELAS has no single defining feature and continues to be a compilation of symptoms such as seizures and stroke-like episodes and it is ultimately a diagnosis of exclusion (Santa, 2010).

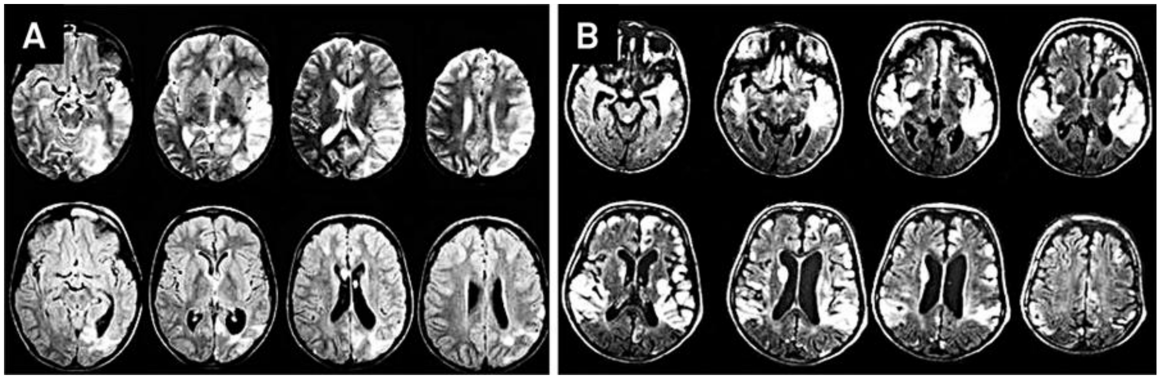
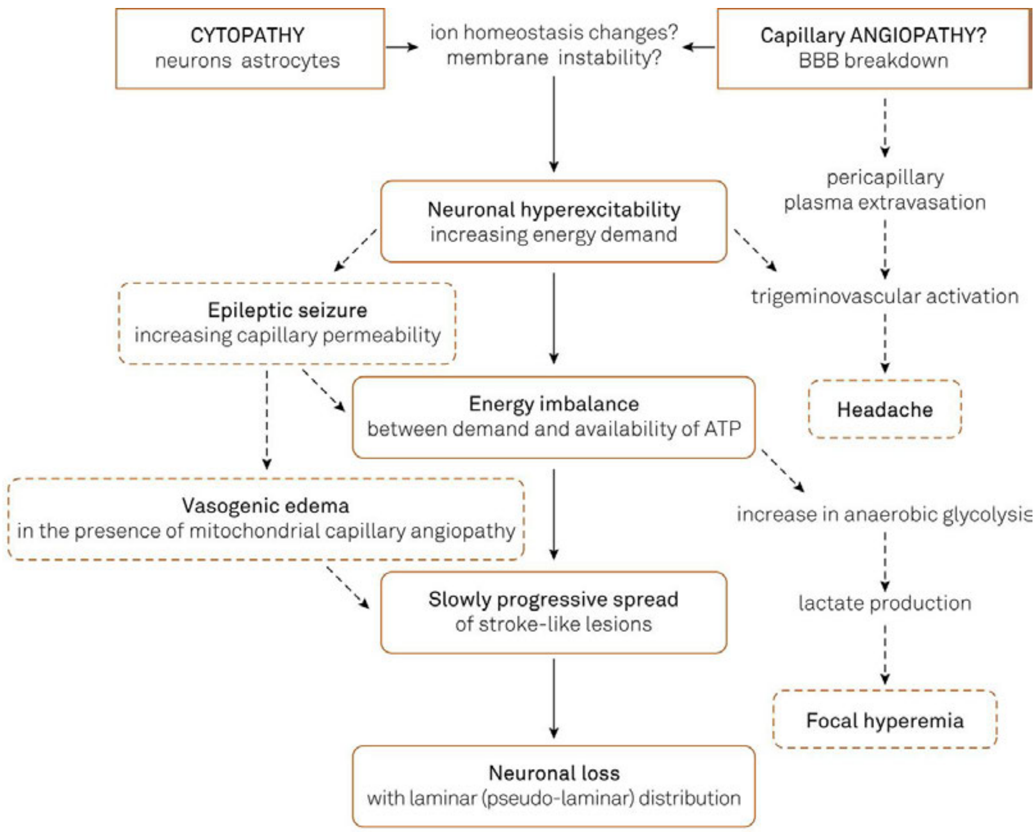


Figure 7: Imaging features in brain MRI showing unilateral (A) or bilateral (B) lesions in MELAS patients. Taken from (Lorenzoni et al., 2015).

Although the genetic basis for MELAS has been identified and continues to be explored, the pathogenesis of the disease is still not understood. Lorenzoni et al. (2015) describe the brain lesions as a possible mechanism for the stroke-like episodes due to energy imbalance and later neuronal loss, as shown in Figure 8, while Lizuka and Sakai (2005) described an increased electrical activity in neuronal tissue manifested in epilepsy, ultimately leading to cortical atrophy and neuronal vulnerability and neuronal loss. MELAS continues to be considered in differential diagnosis of acute stroke cases in young people and it is expected that more research on the disease's molecular basis will grow in the coming years.



BBB: blood-brain barrier.

Figure 8: Proposed mechanism for pathogenesis of stroke-like episodes in MELAS patients. Taken from (Lorenzoni et al., 2015).

Mitochondrial Disease Workup

Due to the severity, multi-organ involvement and fatality of mitochondrial associated diseases, there are important questions and laboratory investigations that should be considered when one of these diseases is suspected (Thyagaraiani & Byrne, 2002). These investigations can include but are not limited to:

1. A review of patient's clinical history and family history
2. Serum lactate after light exercise (it would rise if there is skeletal muscle pathology)

3. Associated symptoms such as hearing loss, endocrine dysfunction, vision problems and cardiac dysfunction
4. MRI might be indicated for neurologic pathology, for instance in LS, there would be lesions in deep grey matter while in MELAS, there would be signs of cerebellar atrophy
5. Mitochondrial biopsy might be indicated for mitochondrial DNA studies such as histochemical analysis to find ragged-red fibers, prominent in MERRF or MELAS
6. Electron microscopy can be used if histochemical studies have been negative although this is not uniformly done
7. Southern Blot can be used to find large mutation deletions in skeletal muscle DNA
8. Counseling the patient and patient's family about the disease

Treatment

Pharmacological and Non-Pharmacological Therapies

Like most of the mitochondrial diseases, there is no definitive treatment for LS, MILS or MELAS. Most of the current treatments are related to alleviating the variety of symptoms caused by the disease syndromes rather than treating the actual disease (Slone et al., 2018). It is possible that exercising can be beneficial for patients with less severe disease phenotypes since it was shown to delay or prevent premature mortality in mouse models with mutated mitochondria that would normally lead to a fatal outcome. (Safdar et al., 2011). However, patients with severe diseases especially at a young age are still in need of both non-pharmacological and pharmacological interventions in order to improve their quality of life and possibly prevent death.

In addition to exercise, Rodriguez et al. (2007) proposed a combination therapy of vitamins and supplements such as creatinine monohydrate, coenzyme Q_{10} and lipoic acid in order to increase ATP production, increase reliance on other anaerobic energy sources and decrease ROS leading to a counteractivity of impaired mitochondrial function. Other formulations include using L-carnitine, coenzyme Q_{10}, riboflavin, thiamine, as well as antioxidants such vitamin C, and vitamin E (Slone et al., 2018).

Research on other novel therapies to treat mitochondrial diseases have been developing in the past decade. Johnson et al. (2013) studied how Ndufs4 knock-out mice interact with rapamycin, an MTOR (mammalian target of rapamycin), a cell growth regulator, to improve the lifespan of mouse models with LS. In these models, there was a delay in the onset of neurological symptoms with fewer brain lesions, a decrease in inflammation, and less buildup of toxic glycolytic intermediates due to a metabolic shift towards amino acid catabolism instead of glycolysis (Johnson et al., 2013). In repeat experiments, it also shown that MTOR inhibition is beneficial in mitochondrial disease because it improves the clearance of defective mitochondria through mitophagy and also increases lysosomal biogenesis, improving the respiratory chain function by circumventing oxidative phosphorylation (Sage-Schwaede et al., 2019). Although, it is possible that the capacity of oxidative phosphorylation in these patients' post-treatment with rapamycin was not affected and in fact that the effect of rapamycin acts on immunosuppression rather than actually improving mitochondrial function (Rahman, 2015).

Ferrari et al. (2017) showed that the use of normobaric 11% oxygen at an early age can prevent neurological disease and improve survival in mice with LS after a study using mice that were knocked out for the Complex I subunit and Ndufs4, a condition that usually causes an encephalopathy that resembles LS, lived longer than mice in the normoxia (21%) or hyperoxia (55%) states with statistical significance. In addition, knockout mice in the hyperoxia state showed no signs of neurological lesions while mice in the normoxia states developed lesions in the brain stem, specifically in the vestibular nuclei; therefore, it would be important to explore the state of hypoxia and how this treatment could be effective in preventing the neurological impacts of LS (Ferrari et al., 2017).

Nicotinamide adenine dinucleotide (NAD^+) is an important electron carrier in the electron transport chain; it plays a significant role in the metabolic pathway of the mitochondria and it is also used as an enzyme in post-translational modification, DNA repair, and the process of glucose metabolism. Supplementation of NAD^+ has been studied in multiple pathologies including fixing redox balance in heart failure, protecting the blood brain barrier after cerebral ischemia (Wei et al., 2019), reversing vascular dysfunction (de Picciotto et al., 2016) and oxidative stress in aging mice (Lee et al., 2019). Furthermore, the supplementation of NAD^+ was shown to extend the lifespan of Ndufs4 knockout mice by bypassing the loss of a deficient Complex I, normalizing the redox balance in the inner mitochondrial membrane and assisting in the continuation of oxidative phosphorylation (Lee et al., 2019).

Patients with LS who specifically have PDH deficiency were found to have high lactate, pyruvate and alanine levels increasing the risk of seizures and failure to thrive (Schubert Baldo and Vilarinho, 2020). For these patients, there were basal ganglia and corpus collusum abnormalities and treatment usually includes dietary modification by increasing lipid, thiamine intake and monitoring lactate and pyruvate levels (Schubert Baldo and Vilarinho, 2020).

For patients with MELAS, aerobic exercise was also found to be beneficial. Patients who participated in traditional cognitive training, computerized cognitive training and computerized cognitive training plus a low intensity aerobic motor exercise showed greater improvement of the cognitive deficits of the disease (De Luca et al., 2016). Due of the accumulation of oxidative phosphorylation intermediates in MELAS, Frey et al. (2017) found that the addition of ketone bodies and reducing the glucose load in cells alleviates the dysfunction by reducing the NADH/NAD+ ratio, increasing ATP synthesis and restoring Complex I activity in neurons (Frey et al., 2017).

In addition to the options for LS also used for MELAS syndrome, several treatment options continue to be explored to reduce endothelial dysfunction, reduce ROS and improve stroke-like symptoms (Lorenzoni et al., 2015). Some of these options including anti-epileptics, L-arginine, CoenzymeQ10, L-citrulline and Vitamin B are listed in Table 7, with their dosages, mechanisms and adverse effects.

Table 7. Treatment options for MELAS Syndrome. Taken from (Santa, 2015).

Treatment	Adult Dosage	Pediatric Dosage	Mechanism	Adverse Effects
L-Arginine	Acute stroke: 500 mg/kg i.v. q6h for 1–3 days Maintenance: 150–300 mg/kg/day p.o. or i.v. in 2–3 divided doses	Same as adult dosage	Vasodilation, increases nitric oxide synthesis	i.v. formulation: hypotension, headaches, metabolic acidosis p.o. formulation: nausea, vomiting
Ascorbic acid (vitamin C)	250–4000 mg/day p.o. in 1–3 divided doses	5 mg/kg/day p.o. in 1–3 divided doses	Antioxidant; improves mitochondrial energy transport	Doses > 3 g/day may cause diarrhea; prolonged high doses may cause kidney stones
Cytochrome c	6.25 mg i.v. q.d.	No data available	Augments mitochondrial respiratory chain function	None reported
Coenzyme Q_{10}	200–600 mg p.o. q.d.	2–15 mg/kg/day p.o. in 2 divided doses	Antioxidant; electron carrier in the mitochondrial respiratory chain	None reported
Corticosteroids	Dexamethasone 12–16 mg/day p.o. in 2–4 divided doses Prednisone 2–4 mg/kg p.o. q.d. Methylprednisolone 1 g p.o. q.d.	No data available	Exact mechanism unknown; may stabilize mitochondrial membranes by modulating peroxidation of phospholipids and inhibiting phospholipases	Headache, edema, immunosuppression, glucose intolerance, nausea, vomiting
Creatine	20 g/day p.o. for 2 wks followed by 2–10 g/day p.o. in 2 divided doses	100 mg/kg/day p.o. in 2 divided doses	Phosphate donor, regenerates ATP	Use with caution in patients with renal dysfunction; gastrointestinal distress
Idebenone	90–270 mg p.o. q.d.	Same as adult dosage	Antioxidant; electron carrier in the mitochondrial respiratory chain	Gastrointestinal complaints, headache, drowsiness, anxiety, tachycardia
Levocarnitine	100–1000 mg p.o. or i.v. b.i.d.–t.i.d. (maximum 3 g/day); i.v. infusion: 100–300 mg/kg/day in 4–6 divided doses	50–100 mg/kg/day p.o. or i.v. in 2–3 divided doses (maximum 3 g/day)	Facilitates fatty acid transport into the mitochondria; replaces carnitine stores	Diarrhea with high oral doses, fishy odor; long-term use of high doses in patients with renal dysfunction can result in accumulation of toxic metabolites
Menadione (vitamin K_3)	40–80 mg p.o. q.d.	No data available	Stimulates oxygen utilization in the mitochondria	May produce hemolytic anemia, kernicterus, and hyperbilirubinemia; contraindicated in newborns, pregnant women, and patients taking warfarin
Nicotinamide (vitamin B_3)	50–500 mg p.o. q.d.	50 mg p.o. q.d.	Cofactor for electron transport in mitochondrial respiratory chain	Elevated liver function tests, nausea, vomiting, diarrhea, dizziness, headache, flushing
Riboflavin (vitamin B_2)	50–400 mg p.o. q.d.	Same as adult dosage	Cofactor for electron transport in mitochondrial respiratory chain	Abdominal pain, nausea and vomiting with high doses (> 200 mg/day)
Sodium dichloro-acetate	25–50 mg/kg/day p.o. in 2 divided doses	Same as adult dosage	Reduces conversion of pyruvate to lactate	Peripheral neuropathy, liver dysfunction
Succinate	6 g p.o. q.d.	No data available	Augments mitochondrial respiratory chain function	None reported
Thiamine (vitamin B_1)	50–300 mg p.o. q.d.	Same as adult dosage	Cofactor for electron transport in mitochondrial respiratory chain	None reported

Gene Therapy for Mitochondrial Diseases

In looking to the future, some researchers and clinicians are considering gene therapy as a possible treatment for mitochondrial-inherited diseases, especially the syndromes with severe symptoms or that are difficult to treat. High and Roncarolo (2019) describe the two strategies used in gene editing: an integrating gene-delivering vehicle (a vector) inserted into a precursor or stem cell where every progeny of that cell will have the vector, or a non-integrating vector delivered and stabilized outside of the chromosome into a slowly-dividing or post mitotic cell which keeps the vector alive for a longer period of time.

In other areas of disease, there are currently ongoing gene therapies for Sickle Cell Disease and Beta thalassemia targeting different genes in order to "correct" the blood cells and reset the disease; in addition, these corrected cells have also been used to treat adrenoleukodystrophy and metachromic leukodystrophy, although requiring a high viral copy number and full myeloablation (High and Roncarolo, 2019). Although gene therapy has previously proved to be promising, it faces a lot of challenges in mitochondrial associated diseases due to the wide-spread effect of the syndromes, especially while requiring a need for a vector that can be expressed in multiple tissues in the body rather that in one specific location (Slone and Huang, 2020).

Other challenges that must be considered in this case include, the circular nature of the mitochondrial genome, the heterogeneity of the different diseases, and the various inheritance patterns of mitochondrial diseases. In regards to the bi-genomic control of mitochondrial function, most of the proteins required for mitochondrial function are encoded in the cell's nucleus and later imported through the mitochondria's outer membrane complex. Once inside the matrix, the vector would need to travel to its respective complex in the inner mitochondrial membrane into the mitochondria so finding a vector that can mimic this process seems to be key in the process of gene editing.

Recently, the mitochondrial disease known as Leber's hereditary optic neuropathy has been involved in a clinical trial using vectors called Adeno-associated virus (AAV) vectors (Slone and Huang, 2020). These vectors are paired with mitochondrial-encoded NADH-ubiquinone oxidoreductase chain 4 protein gene coding sequences, which is usually deficient in LHON, and some patients have shown improvement in the patient's visual function, especially if the disease presented less than 2 years before treatment (Zhang et al., 2017). With the recent developments in CRISPR technology in areas of gene replacement and gene editing, there might be some potential at impacting syndromes such as LS and MELAS in the future; however, the research today, although ongoing, continues to be ambiguous.

DISCUSSION

Proper mitochondrial function is necessary for proper cellular function. Mitochondria provide the energy fuel for cellular processes through oxidative phosphorylation by making ATP. Any defect in the process towards making this ATP results in loss of efficient cellular function and can lead to multi-organ failure. In addition, the loss of oxidative phosphorylation processes results in increased levels of

ROS and other toxic metabolic intermediates that can further intoxicate low functioning cells, accelerating cell death.

Mitochondrial defects can occur as a result of inherited or sporadic mutations in nDNA or mtDNA. For maternal inherited diseases, most of the mutations arise in mtDNA, while for autosomal inheritance or sex-linked inheritance, most of the mutations arise in nDNA. As stated previously, patients with mitochondrial associated diseases might have normal birth and development for a few months and then as the disease progresses, the symptoms become more apparent and depending on the severity, can be fatal in childhood.

mtDNA associated diseases have been linked not only to mitochondrial myopathies, but also neurodegenerative diseases such as AD, PD, and ALS, including cancers such as gastric and thyroid cancer, and age-related diseases such as Type 2 Diabetes Mellitus. Therefore, studying mitochondrial-associated diseases and their pathogenesis continues to be important in order to understand the disease mechanisms and discover novel therapeutic options to combat the syndromes.

This paper explored two common types of mitochondrial inherited diseases: Leigh Syndrome and Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-Like Episodes, as well as a variant of Leigh Syndrome known as Maternal Inherited Leigh Syndrome. In exploring these diseases, it was found that LS, MILS and MELAS contained genetic mutations that disrupted the functional subunits of the ETC which when functioning appropriately, directs oxidative phosphorylation and fuels bodily cells.

For LS, the most common defect was a gene mutation in a subunit for Complex I, also known as NADH dehydrogenase. Without this first subunit to accept electron donors, the efficiency of the ETC is decreased and this leads to decreased ATP synthesis. Complex I has multiple core subunits; some of the proteins are encoded by mtDNA while the others are encoded by nDNA. Because of this bi-genomic origin, defects in either mtDNA or nDNA will affect the integrity of Complex I and decrease the process of oxidative phosphorylation.

For MILS, the most common defect was in the gene necessary for proper function of the ATPase segment, the final component of the ETC. Without the coupling energy of the pump, the mitochondria cannot generate enough ATP to fuel the cell. On the contrary for MELAS, the most common defect was a mutation in the gene for the tRNA protein that helps assemble the subunits for the ETC. Without the tRNA and assembled subunits, the ETC cannot function properly and oxidative phosphorylation is decreased immensely.

Both LS and MELAS can result in lactic acidosis but they had no other outstanding commonalities. Imaging studies of patients with LS or MILS present with bilateral lesions in the basal ganglia and brainstem region of the brain while patients with MELAS present with cortical atrophy in the cerebellar hemispheres, for the most part, sparing the brainstem region. Through the examination of recently published research, while LS and MELAS both affect the ETC, they might affect different complexes and present with different symptoms that ultimately lead to a decrease in oxidative phosphorylation. It is clear that the pathogenesis in LS localizes the atrophy to neurons in the basal ganglia while the process in MELAS is more diffuse, although sparing the brainstem. In addition, the stroke like episodes in the adult onset MELAS patients are not seen in LS patients. It is also possible that the loss of a Complex I subunit alone as seen in the LS disease process is not as significant as in the MELAS process, where the dysfunction is due to a tRNA responsible for assembling all the subunits of the ETC. This could explain why MELAS is a more progressive disease rather than a localized neuronal loss. However, the pathogenesis is still not clear and further research is warranted based on the potential of these studies.

In summary, there are many limitations to studies on mitochondria for many reasons. Firstly, every human cell needs mitochondria to function so when there is a defect in any part of the system, it affects multiple organs which can be difficult to control, predict and treat. In addition, there are multiple ways mitochondria can acquire a defect. It can be either through a mutation in different parts of the genome and either mutation can present as a different disease type. Also, these mutations can occur sporadically, be inherited maternally, paternally, in sex-linked or autosomal fashion, genetic counseling, though difficult, is truly important.

BIBLIOGRAPHY

Abu-Amero KK, Alzahrani AS, Zou M, Shi Y. High frequency of somatic mitochondrial DNA mutations in human thyroid carcinomas and complex I respiratory defect in thyroid cancer cell lines. Oncogene. 2005 Feb 17;24(8):1455-60. doi: 10.1038/sj.onc.1208292. PMID: 15608681.

Ahmed, S. T., Craven, L., Russell, O. M., Turnbull, D. M., & Vincent, A. E. (2018). Diagnosis and Treatment of Mitochondrial Myopathies. *Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics*, *15*(4), 943–953. https://doi.org/10.1007/s13311-018-00674-4

Annesley, S. J., & Fisher, P. R. (2019). Mitochondria in Health and Disease. Cells, 8(7), 680. https://doi-org.rossuniversity.idm.oclc.org/10.3390/cells8070680

Arii J, Tanabe Y. Leigh syndrome: serial MR imaging and clinical follow-up. *American Journal of Neuroradiology*. 2000;21(8):1502-1509.

Bakare AB, Lesnefsky EJ, Iyer S. Leigh Syndrome: A Tale of Two Genomes. Frontiers in Physiology. 2021 Aug 11;12:693734. doi: 10.3389/fphys.2021.693734. PMID: 34456746; PMCID: PMC8385445.

Bender A, Krishnan KJ, Morris CM, Taylor GA, Reeve AK, Perry RH, Jaros E, Hersheson JS, Betts J, Klopstock T, Taylor RW, Turnbull DM. High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. Nature Genetics. 2006 May;38(5):515-7. doi: 10.1038/ng1769. Epub 2006 Apr 9. PMID: 16604074.

Bianchi NO, Bianchi MS, Richard SM. Mitochondrial genome instability in human cancers. Mutation Research. 2001 Mar;488(1):9-23. doi: 10.1016/s1383-5742(00)00063-6. PMID: 11223402.

Birky C. W., Jr (1995). Uniparental inheritance of mitochondrial and chloroplast genes: mechanisms and evolution. *Proceedings of the National Academy of Sciences of the United States of America*, *92*(25), 11331–11338. https://doi.org/10.1073/pnas.92.25.11331

Bonnen PE, Yarham JW, Besse A, Wu P, Faqeih EA, Al-Asmari AM, Saleh MA, Eyaid W, Hadeel A, He L, Smith F, Yau S, Simcox EM, Miwa S, Donti T, Abu-Amero KK, Wong LJ, Craigen WJ, Graham BH, Scott KL, McFarland R, Taylor RW. Mutations in FBXL4 cause mitochondrial encephalopathy and a disorder of mitochondrial DNA maintenance. Am J Hum Genet. 2013 Sep 5;93(3):471-81. doi: 10.1016/j.ajhg.2013.07.017. Epub 2013 Aug 29. Erratum in: American Journal of Human Genetics. 2013 Oct 3;93(4):773. PMID: 23993193; PMCID: PMC3769921.

Breton, S., & Stewart, D. T. (2015). Atypical mitochondrial inheritance patterns in eukaryotes. Genome, 58(10), 423–431. https://doi-org.rossuniversity.idm.oclc.org/10.1139/gen-2015-0090

Caplan, L. & Biller, J. & Leary, M. & Lo, E. & Thomas, A. & Yenari, M. & Zhang, J. (2017). Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-Like Episodes (MELAS). Primer on Cerebrovascular Diseases (2nd ed.). Elsevier Science and Technology. https://www-r2library-com.rossuniversity.idm.oclc.org/Resource/Title/0128030585/ch0107s1234

Carrozzo R, Rizza T, Stringaro A, et al. Maternally-inherited Leigh syndrome-related mutations bolster mitochondrial-mediated apoptosis. *Journal of Neurochemistry*. 2004;90(2):490-501. doi:10.1111/j.1471-4159.2004.02505.x

Chaturvedi RK, Flint Beal M. Mitochondrial diseases of the brain. Free Radical Biology & Medicine. 2013 Oct;63:1-29. doi: 10.1016/j.freeradbiomed.2013.03.018. Epub 2013 Apr 6. PMID: 23567191.

Chinnery PF. Mitochondrial Disorders Overview. 2000 Jun 8 [Updated 2014 Aug 14]. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2020. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1224/

Coller HA, Khrapko K, Bodyak ND, Nekhaeva E, Herrero-Jimenez P, Thilly WG. High frequency of homoplasmic mitochondrial DNA mutations in human tumors can be explained without selection. Nature Genetics. 2001 Jun;28(2):147-50. doi: 10.1038/88859. PMID: 11381261.

Dakubo, G.D. (2010). Basic Mitochondrial Genetics, Bioenergetics, and Biogenesis. In: Mitochondrial Genetics and Cancer. Springer, Berlin, Heidelberg. https://doi-org.ezproxy.bu.edu/10.1007/978-3-642-11416-8_1

Darin N, Oldfors A, Moslemi AR, Holme E, Tulinius M. The incidence of mitochondrial encephalomyopathies in childhood: clinical features and morphological, biochemical, and DNA abnormalities. *Annals of Neurology*. 2001;49(3):377-383.

DeBalsi, K. L., Hoff, K. E., & Copeland, W. C. (2017). Role of the mitochondrial DNA replication machinery in mitochondrial DNA mutagenesis, aging and age-related diseases. *Ageing research reviews*, *33*, 89–104. https://doi-org.rossuniversity.idm.oclc.org/10.1016/j.arr.2016.04.006

De Luca R, Russo M, Leonardi S, Spadaro L, Cicero C, Naro A, Bramanti P, Calabrò RS. Advances in the Treatment of MELAS Syndrome: Could Cognitive Rehabilitation Have a Role? Applied Neuropsychology Adult. 2016;23(1):61-4. doi: 10.1080/23279095.2014.960522. Epub 2015 Sep 21. PMID: 26391741.

de Picciotto NE, Gano LB, Johnson LC, Martens CR, Sindler AL, Mills KF, Imai S, Seals DR. Nicotinamide mononucleotide supplementation reverses vascular dysfunction and oxidative stress with aging in mice. Aging Cell. 2016 Jun;15(3):522-30. doi: 10.1111/acel.12461. Epub 2016 Mar 11. PMID: 26970090; PMCID: PMC4854911.

Dombi E, Mortiboys H, Poulton J. Modulating Mitophagy in Mitochondrial Disease. Current Medicinal Chemistry. 2018;25(40):5597-5612. doi: 10.2174/0929867324666170616101741. PMID: 28618992.

Emperador, S., Garrido-Pérez, N., Amezcua-Gil, J., Gaudó, P., Andrés-Sanz, J. A., Yubero, D., Fernández-Marmiesse, A., O'Callaghan, M. M., Ortigoza-Escobar, J. D., Iriondo, M., Ruiz-Pesini, E., García-Cazorla, A., Gil-Campos, M., Artuch, R., Montoya, J., & Bayona-Bafaluy, M. P. (2020). Molecular characterization of new FBXL4 mutations in patients with mtdna depletion syndrome. Frontiers in Genetics, 10. https://doi.org/10.3389/fgene.2019.01300

Fassone E, Rahman S. Complex I deficiency: clinical features, biochemistry and molecular genetics. Journal of Medical Genetics. 2012 Sep;49(9):578-90. doi: 10.1136/jmedgenet-2012-101159. Erratum in: J Med Genet. 2012 Oct;49(10):668. PMID: 22972949.

Ferrari M, Jain IH, Goldberger O, Rezoagli E, Thoonen R, Cheng KH, Sosnovik DE, Scherrer-Crosbie M, Mootha VK, Zapol WM. Hypoxia treatment reverses neurodegenerative disease in a mouse model of Leigh syndrome. Proceedings of the National Academy of Sciences of the United States of America. 2017 May 23;114(21):E4241-E4250. doi: 10.1073/pnas.1621511114. Epub 2017 May 8. Erratum in: Proc Natl Acad Sci U S A. 2017 Jun 13;114(24):E4894-E4896. Erratum in: Proceedings of the National Academy of Sciences of the United States of America. 2018 Jan 30;: PMID: 28483998; PMCID: PMC5448167.

Filograna, R., Mennuni, M., Alsina, D., & Larsson, N. G. (2021). Mitochondrial DNA copy number in human disease: the more the better?. Federation of European Biochemical Societies letters, 595(8), 976–1002. https://doi.org/10.1002/1873-3468.14021

Frey S, Geffroy G, Desquiret-Dumas V, Gueguen N, Bris C, Belal S, Amati-Bonneau P, Chevrollier A, Barth M, Henrion D, Lenaers G, Bonneau D, Reynier P, Procaccio V. The addition of ketone bodies alleviates mitochondrial dysfunction by restoring complex I assembly in a MELAS cellular model. Biochimica et Biophysica Acta Molecular Basis of Disease. 2017 Jan;1863(1):284-291. doi: 10.1016/j.bbadis.2016.10.028. Epub 2016 Nov 1. PMID: 27815040.

Gamboa, J. L., Billings, F. T., 4th, Bojanowski, M. T., Gilliam, L. A., Yu, C., Roshanravan, B., Roberts, L. J., 2nd, Himmelfarb, J., Ikizler, T. A., & Brown, N. J.

(2016). Mitochondrial dysfunction and oxidative stress in patients with chronic kidney disease. *Physiological reports*, *4*(9), e12780. https://doi.org/10.14814/phy2.12780

Goodfellow, John, Dani, Krishna, Stewart, Willie, Santosh, Celestine, McLean, John, Mulhern, Sharon, et al. (2012). Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes: an important cause of stroke in young people. Postgraduate Medical Journal, 88, 326-334. https://doi.org/10.1136/postgradmedj-2011-130326

Gorman, G. S., Schaefer, A. M., Ng, Y., Gomez, N., Blakely, E. L., Alston, C. L., Feeney, C., Horvath, R., Yu-Wai-Man, P., Chinnery, P. F., Taylor, R. W., Turnbull, D. M., & McFarland, R. (2015). Prevalence of nuclear and mitochondrial DNA mutations related to adult mitochondrial disease. *Annals of neurology*, *77*(5), 753–759. https://doi.org/10.1002/ana.24362

Grady, J. P., Pickett, S. J., Ng, Y. S., Alston, C. L., Blakely, E. L., Hardy, S. A., Feeney, C. L., Bright, A. A., Schaefer, A. M., Gorman, G. S., McNally, R. J., Taylor, R. W., Turnbull, D. M., & McFarland, R. (2018). mtDNA heteroplasmy level and copy number indicate disease burden in m.3243A>G mitochondrial disease. European Molecular Biology Organization molecular medicine, 10(6), e8262. https://doi-org.rossuniversity.idm.oclc.org/10.15252/emmm.201708262

Gyllensten U, Wharton D, Josefsson A, Wilson AC. Paternal inheritance of mitochondrial DNA in mice. Nature. 1991 Jul 18;352(6332):255-7. doi: 10.1038/352255a0. PMID: 1857422.

Haas, R. H., Parikh, S., Falk, M. J., Saneto, R. P., Wolf, N. I., Darin, N., & Cohen, B. H. (2007). Mitochondrial disease: a practical approach for primary care physicians. *Pediatrics*, *120*(6), 1326–1333. https://doi.org/10.1542/peds.2007-0391

High KA, Roncarolo MG. Gene Therapy. New England Journal of Medicine. 2019 Aug 1;381(5):455-464. doi: 10.1056/NEJMra1706910. PMID: 31365802.

Hirano, M., Emmanuele, V., & Quinzii, C. M. (2018). Emerging therapies for mitochondrial diseases. *Essays in biochemistry*, *62*(3), 467–481. https://doi-org.rossuniversity.idm.oclc.org/10.1042/EBC20170114

Hsu, C. C., Tseng, L. M., & Lee, H. C. (2016). Role of mitochondrial dysfunction in cancer progression. *Experimental biology and medicine (Maywood, N.J.)*, *241*(12), 1281–1295. https://doi.org/10.1177/1535370216641787

Iizuka T, Sakai F. Pathogenesis of stroke-like episodes in MELAS: analysis of neurovascular cellular mechanisms. Current Neurovascular Research. 2005 Jan;2(1):29-45. doi: 10.2174/1567202052773544. PMID: 16181098.

Jannotti-Passos LK, Souza CP, Parra JC, Simpson AJ. Biparental mitochondrial DNA inheritance in the parasitic trematode Schistosoma mansoni. J Parasitol. 2001 Feb;87(1):79-82. doi: 10.1645/0022-3395(2001)087[0079:BMDIIT]2.0.CO;2. PMID: 11227906.

Johnson SC, Yanos ME, Kayser EB, et al. mTOR inhibition alleviates mitochondrial disease in a mouse model of Leigh syndrome. *Science*. 2013;342(6165):1524-1528. doi:10.1126/science.1244360

Kerr, J. S., Adriaanse, B. A., Greig, N. H., Mattson, M. P., Cader, M. Z., Bohr, V. A., & Fang, E. F. (2017). Mitophagy and Alzheimer's Disease: Cellular and Molecular Mechanisms. Trends in neurosciences, 40(3), 151–166. https://doi.org/10.1016/j.tins.2017.01.002

Kopinski, P. K., Janssen, K. A., Schaefer, P. M., Trefely, S., Perry, C. E., Potluri, P., Tintos-Hernandez, J. A., Singh, L. N., Karch, K. R., Campbell, S. L., Doan, M. T., Jiang, H., Nissim, I., Nakamaru-Ogiso, E., Wellen, K. E., Snyder, N. W., Garcia, B. A., & Wallace, D. C. (2019). Regulation of nuclear epigenome by mitochondrial DNA heteroplasmy. Proceedings of the National Academy of Sciences of the United States of America, 116(32), 16028–16035. https://doi-org.rossuniversity.idm.oclc.org/10.1073/pnas.1906896116

Kwong, L. K., & Sohal, R. S. (2000). Age-related changes in activities of mitochondrial electron transport complexes in various tissues of the mouse. *Archives of biochemistry and biophysics*, *373*(1), 16–22. https://doi-org.rossuniversity.idm.oclc.org/10.1006/abbi.1999.1495

Lake NJ, Compton AG, Rahman S, Thorburn DR. Leigh syndrome: One disorder, more than 75 monogenic causes. *Annals of Neurology*. 2016;79(2):190-203. doi:10.1002/ana.24551

Lee CF, Caudal A, Abell L, Nagana Gowda GA, Tian R. Targeting NAD+ Metabolism as Interventions for Mitochondrial Disease. Sci Rep. 2019 Feb 28;9(1):3073. doi: 10.1038/s41598-019-39419-4. PMID: 30816177; PMCID: PMC6395802.

Leigh D. (1951). Subacute necrotizing encephalomyelopathy in an infant. *Journal of neurology, neurosurgery, and psychiatry*, *14*(3), 216–221. https://doi.org/10.1136/jnnp.14.3.216

Lemasters JJ. Selective mitochondrial autophagy, or mitophagy, as a targeted defense against oxidative stress, mitochondrial dysfunction, and aging. Rejuvenation Res. 2005 Spring;8(1):3-5. doi: 10.1089/rej.2005.8.3. PMID: 15798367.

Lightowlers RN, Taylor RW, Turnbull DM. Mutations causing mitochondrial disease: What is new and what challenges remain? Science. 2015 Sep 25;349(6255):1494-9. doi: 10.1126/science.aac7516. Epub 2015 Sep 24. PMID: 26404827.

Lim, A.Z., McFarland, R. (2019). Leigh Syndrome. In: Mancuso, M., Klopstock, T. (eds) Diagnosis and Management of Mitochondrial Disorders. Springer, Cham. https://doi-org.ezproxy.bu.edu/10.1007/978-3-030-05517-2_10

Lorenzoni PJ, Werneck LC, Kay CS, Silvado CE, Scola RH. When should MELAS (Mitochondrial myopathy, Encephalopathy, Lactic Acidosis, and Stroke-like episodes) be the diagnosis? Arquivos de Neuro-psiquiatria. 2015 Nov;73(11):959-67. doi: 10.1590/0004-282X20150154. PMID: 26517220.

Luo, S., Valencia, C. A., Zhang, J., Lee, N. C., Slone, J., Gui, B., Wang, X., Li, Z., Dell, S., Brown, J., Chen, S. M., Chien, Y. H., Hwu, W. L., Fan, P. C., Wong, L. J., Atwal, P. S., & Huang, T. (2018). Biparental Inheritance of Mitochondrial DNA in Humans. Proceedings of the National Academy of Sciences of the United States of America, 115(51), 13039–13044. https://doi-org.rossuniversity.idm.oclc.org/10.1073/pnas.1810946115

Mkaouar-Rebai E, Chaari W, Younes S, Bousoffara R, Sfar MT, Fakhfakh F. Maternally inherited Leigh syndrome: T8993G mutation in a Tunisian family. *Pediatric Neurology*. 2009;40(6):437-442. doi:10.1016/j.pediatrneurol.2009.01.004

Mokranjac D, Neupert W. Protein import into mitochondria. Biochem Soc Trans. 2005 Nov;33(Pt 5):1019-23. doi: 10.1042/BST20051019. PMID: 16246036.

Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, Puigserver P, Carlsson E, Ridderstråle M, Laurila E, Houstis N, Daly MJ, Patterson N, Mesirov JP, Golub TR, Tamayo P, Spiegelman B, Lander ES, Hirschhorn JN, Altshuler D, Groop LC. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet. 2003 Jul;34(3):267-73. doi: 10.1038/ng1180. PMID: 12808457.

Muyderman, H., & Chen, T. (2014). Mitochondrial dysfunction in amyotrophic lateral sclerosis - a valid pharmacological target?. *British journal of pharmacology*, *171*(8), 2191–2205. https://doi.org/10.1111/bph.12476

Ng, Y. S., & Turnbull, D. M. (2016). Mitochondrial disease: genetics and management. *Journal of neurology*, *263*(1), 179–191. https://doi.org/10.1007/s00415-015-7884-3

Pavlakis SG, Phillips PC, DiMauro S, De Vivo DC, Rowland LP. Mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes: a distinctive clinical syndrome. Ann Neurol. 1984 Oct;16(4):481-8. doi: 10.1002/ana.410160409. PMID: 6093682.

Pia S, Lui F. Melas Syndrome. 2020 Jul 6. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan–. PMID: 30422554.

Polyak K, Li Y, Zhu H, Lengauer C, Willson JK, Markowitz SD, Trush MA, Kinzler KW, Vogelstein B. Somatic mutations of the mitochondrial genome in human colorectal tumours. Nat Genet. 1998 Nov;20(3):291-3. doi: 10.1038/3108. PMID: 9806551.

Quintana A, Kruse SE, Kapur RP, Sanz E, Palmiter RD. Complex I deficiency due to loss of Ndufs4 in the brain results in progressive encephalopathy resembling Leigh syndrome. Proceedings of the National Academy of Science U S A. 2010 Jun 15;107(24):10996-1001. doi: 10.1073/pnas.1006214107. Epub 2010 Jun 1. PMID: 20534480; PMCID: PMC2890717.

Rahman S. Emerging aspects of treatment in mitochondrial disorders. *Journal of Inherited Metabolic Disease*. 2015;38(4):641-653. doi:10.1007/s10545-015-9855-3

Rao M, Jaber BL, Balakrishnan VS. Chronic kidney disease and acquired mitochondrial myopathy. Current Opinion in Nephrology and Hypertension. 2018 Mar;27(2):113-120. doi: 10.1097/MNH.0000000000000393. PMID: 29266014.

Ruhoy, Ilene S, and Russell P Saneto. “The genetics of Leigh syndrome and its implications for clinical practice and risk management.” *The application of clinical genetics* vol. 7 221-34. 13 Nov. 2014, doi:10.2147/TACG.S46176

Rusecka J, Kaliszewska M, Bartnik E, Tońska K. Nuclear genes involved in mitochondrial diseases caused by instability of mitochondrial DNA. Journal of Applied Genetics. 2018 Feb;59(1):43-57. doi: 10.1007/s13353-017-0424-3. Epub 2018 Jan 17. PMID: 29344903; PMCID: PMC5799321.

Safdar A, Bourgeois JM, Ogborn DI, Little JP, Hettinga BP, Akhtar M, Thompson JE, Melov S, Mocellin NJ, Kujoth GC, Prolla TA, Tarnopolsky MA. Endurance exercise rescues progeroid aging and induces systemic mitochondrial rejuvenation in mtDNA mutator mice. Proceedings of the National Academy of Science U S A. 2011 Mar 8;108(10):4135-40. doi: 10.1073/pnas.1019581108. Epub 2011 Feb 22. PMID: 21368114; PMCID: PMC3053975.

Sage-Schwaede A, Engelstad K, Salazar R, Curcio A, Khandji A, Garvin JH Jr, De Vivo DC. Exploring mTOR inhibition as treatment for mitochondrial disease. Annals of Clinical and Translational Neurology. 2019 Sep;6(9):1877-1881. doi: 10.1002/acn3.50846. Epub 2019 Aug 6. PMID: 31386302; PMCID: PMC6764630.

Santa KM. Treatment options for mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome. Pharmacotherapy. 2010 Nov;30(11):1179-96. doi: 10.1592/phco.30.11.1179. PMID: 20973690.

Santorelli FM, Shanske S, Macaya A, DeVivo DC, DiMauro S. The mutation at nt 8993 of mitochondrial DNA is a common cause of Leigh's syndrome. Annals of Neurology. 1993 Dec;34(6):827-34. doi: 10.1002/ana.410340612. PMID: 8250532.

Schaefer, A. M., Walker, M., Turnbull, D. M., & Taylor, R. W. (2013). Endocrine disorders in mitochondrial disease. *Molecular and cellular endocrinology*, *379*(1-2), 2–11. https://doi.org/10.1016/j.mce.2013.06.004

Schubert Baldo, M., & Vilarinho, L. (2020). Correction to: Molecular basis of Leigh syndrome: a current look. *Orphanet journal of rare diseases*, *15*(1), 77. https://doi.org/10.1186/s13023-020-1351-7

Schwartz M, Vissing J. Paternal inheritance of mitochondrial DNA. New England Journal of Medicine. 2002 Aug 22;347(8):576-80. doi: 10.1056/NEJMoa020350. PMID: 12192017.

Sgarbi G, Baracca A, Lenaz G, Valentino LM, Carelli V, Solaini G. Inefficient coupling between proton transport and ATP synthesis may be the pathogenic mechanism for NARP and Leigh syndrome resulting from the T8993G mutation in mtDNA. Biochem Journal. 2006 May 1;395(3):493-500. doi: 10.1042/BJ20051748. PMID: 16402916; PMCID: PMC1462703.

Shokolenko IN, Alexeyev MF. Mitochondrial DNA: A disposable genome? Biochimica et Biophysica Acta. 2015 Sep;1852(9):1805-9. doi: 10.1016/j.bbadis.2015.05.016. Epub 2015 Jun 10. PMID: 26071375; PMCID: PMC4523420.

Shuk-kuen Chau, C., Kwok, K., Ng, D.K. *et al.* Maternally inherited Leigh syndrome: an unusual cause of infantile apnea. *Sleep Breath* **14,** 161–165 (2010). https://doi-org.ezproxy.bu.edu/10.1007/s11325-009-0288-9

Slone J, Gui B, Huang T. The current landscape for the treatment of mitochondrial disorders. Journal of Genetics and Genomics. 2018 Feb 20;45(2):71-77. doi: 10.1016/j.jgg.2017.11.008. Epub 2018 Feb 14. PMID: 29502956.

Slone J, Huang T. The special considerations of gene therapy for mitochondrial diseases. NPJ Genomic Medicine. 2020 Mar 2;5:7. doi: 10.1038/s41525-020-0116-5. PMID: 32140258; PMCID: PMC7051955.

Smith, David Roy. “The past, present and future of mitochondrial genomics: have we sequenced enough mtDNAs?.” Briefings in functional genomics vol. 15,1 (2016): 47-54. doi:10.1093/bfgp/elv027

Sun, Hongzhi & Wang, Xiangdong. (2017). Mitochondrial DNA and Diseases. 10.1007/978-981-10-6674-0

Tarnopolsky, Mark & Raha, Sandeep. (2005). Mitochondrial Myopathies: Diagnosis, Exercise Intolerance, and Treatment Options. Medicine & Science in Sports & Exercise, 37(12), 2086-2093. Retrieved from http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=ovfth&NEWS=N&AN=00005768-200512000-00011.

Thorburn DR, Rahman J, Rahman S. Mitochondrial DNA-Associated Leigh Syndrome and NARP. 2003 Oct 30 [Updated 2017 Sep 28]. In: Adam MP, Mirzaa GM, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1173/

Thyagarajan D, Byrne E. Mitochondrial disorders of the nervous system: clinical, biochemical, and molecular genetic features. International Review of Neurobiology. 2002;53:93-144. doi: 10.1016/s0074-7742(02)53005-1. PMID: 12512338.

Trifunovic A, Wredenberg A, Falkenberg M, Spelbrink JN, Rovio AT, Bruder CE, Bohlooly-Y M, Gidlöf S, Oldfors A, Wibom R, Törnell J, Jacobs HT, Larsson NG. Premature ageing in mice expressing defective mitochondrial DNA polymerase. Nature. 2004 May 27;429(6990):417-23. doi: 10.1038/nature02517. PMID: 15164064.

Uittenbogaard, M., Brantner, C. A., Fang, Z., Wong, L. C., Gropman, A., & Chiaramello, A. (2018). Novel insights into the functional metabolic impact of an apparent de novo m.8993T>G variant in the MT-ATP6 gene associated with maternally inherited form of Leigh Syndrome. *Molecular genetics and metabolism*, *124*(1), 71–81. https://doi.org/10.1016/j.ymgme.2018.03.011

Vincent, A. E., Ng, Y. S., White, K., Davey, T., Mannella, C., Falkous, G., Feeney, C., Schaefer, A. M., McFarland, R., Gorman, G. S., Taylor, R. W., Turnbull, D. M., & Picard, M. (2016). The Spectrum of Mitochondrial Ultrastructural Defects in Mitochondrial Myopathy. *Scientific reports*, *6*, 30610. https://doi.org/10.1038/srep30610

Wallace, D. C., & Chalkia, D. (2013). Mitochondrial DNA genetics and the heteroplasmy conundrum in evolution and disease. *Cold Spring Harbor perspectives in biology*, *5*(11), a021220. https://doi-org.rossuniversity.idm.oclc.org/10.1101/cshperspect.a021220

Wei, C. C., Kong, Y. Y., Hua, X., Li, G. Q., Zheng, S. L., Cheng, M. H., Wang, P., & Miao, C. Y. (2017). NAD replenishment with nicotinamide mononucleotide protects blood-brain barrier integrity and attenuates delayed tissue plasminogen activator-induced haemorrhagic transformation after cerebral ischaemia. British journal of pharmacology, 174(21), 3823–3836. https://doi.org/10.1111/bph.13979

Yatsuga S, Povalko N, Nishioka J, Katayama K, Kakimoto N, Matsuishi T, Kakuma T, Koga Y; Taro Matsuoka for MELAS Study Group in Japan. MELAS: a nationwide prospective cohort study of 96 patients in Japan. Biochimica et Biophysica Acta. 2012 May;1820(5):619-24. doi: 10.1016/j.bbagen.2011.03.015. Epub 2011 Apr 2. PMID: 21443929.

Yu W, Gao B, Li N, Wang J, Qiu C, Zhang G, Liu M, Zhang R, Li C, Ji G, Zhang Y. Sirt3 deficiency exacerbates diabetic cardiac dysfunction: Role of Foxo3A-Parkin-mediated mitophagy. Biochim Biophys Acta Molecular Basis of Disease. 2017 Aug;1863(8):1973-1983. doi: 10.1016/j.bbadis.2016.10.021. Epub 2016 Oct 26. PMID: 27794418.

Zhang Y, Tian Z, Yuan J, Liu C, Liu HL, Ma SQ, Li B. The Progress of Gene Therapy for Leber's Optic Hereditary Neuropathy. Current Gene Therapy. 2017;17(4):320-326. doi: 10.2174/1566523218666171129204926. PMID: 29189152; PMCID: PMC5902861

CURRICULUM VITAE

[redacted]

[redacted]

[redacted]

[redacted]