

# Mitochondrial Diseases

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**Master's thesis / Diplomski rad**

**2024**

*Degree Grantor / Ustanova koja je dodijelila akademski / stručni stupanj:* **University of Rijeka, Faculty of Medicine / Sveučilište u Rijeci, Medicinski fakultet**

*Permanent link / Trajna poveznica:* <https://um.nsk.hr/um:nbn:hr:184:105439>

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*Download date / Datum preuzimanja:* **2025-03-23**



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**UNIVERSITY OF RIJEKA  
FACULTY OF MEDICINE**

**INTEGRATED UNDERGRADUATE UNIVERSITY STUDY OF  
MEDICINE IN ENGLISH**

**Vivien Martins**

**Mitochondrial Diseases**

**GRADUATION THESIS**

**Rijeka, 2024**

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SVEUČILIŠTE U RIJEKI - MEDICINSKI FAKULTET | UNIVERSITY OF RIJEKA - FACULTY OF MEDICINE

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The graduation thesis contains 27 pages, 1 figures, 0 tables, 50 references.



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### **Dedication**

I dedicate this thesis to my beloved mother, whose belief in me never wavered. Her departure came too soon, and she cannot witness the fruit of her unwavering support and hard work. This achievement is a tribute to her enduring love and faith in me.

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## **List of Abbreviations and acronyms**

mtDNA – mitochondrial DNA

OXPPOS – oxidative phosphorylation

LHON – Leber Hereditary Optic Neuropathy

Leigh Syndrome - LS

OMM – outer mitochondrial membrane

IMM – inner mitochondrial membrane

IMS – intermembrane space

ATP – Adenosine Triphosphate

ADP – adenosine diphosphate

rRNAs – ribosomal RNAs

tRNAs – transfer tRNAs

Mitochondrial disorders – MIDs

Electron transport chain – ETC

Mitochondrial Neurogastrointestinal Encephalomyopathy – MNGIE

Mitochondrial encephalomyopathy - MELAS

## **1. Introduction**

Mitochondrial diseases are characterized by a broad spectrum of clinical signs and complex genetic underpinnings, presenting a significant challenge in the field of genetic and metabolic disorders.

The vulnerability of mitochondrial DNA to mutations, heightened by its adjacent location to the electron transport chain and absence of histone protection, results in an elevated rate of mutations (1). This plays a key role in the development of mitochondrial diseases. Such mutations can drastically reduce the cell's ability to produce energy, impacting high-energy requiring tissues like the brain, heart, and muscles. Additionally, the presence of both mutant and normal mitochondrial DNA within cells, known as heteroplasmy, introduces further complexity by affecting how mitochondrial diseases manifest at the phenotypic level (2–4).

Mitochondrial diseases, that belong to the group of hereditary disorders, stand as some of the most frequently encountered genetic conditions, their occurrence rates on par with other diseases related to neurogenetics (5). The wide spectrum of clinical features, from isolated symptoms like Leber Hereditary Optic Neuropathy (LHON) to severe multisystem involvement as seen in Leigh Syndrome (LS), reflects the critical role of mitochondria in various cellular functions (6). The diagnostic challenge posed by mitochondrial diseases necessitates a comprehensive approach, integrating genetic, biochemical, and neuroimaging data to elucidate the underlying pathogenic mechanisms (5,7).

Therapeutic strategies for mitochondrial diseases have evolved from symptomatic treatments to targeted interventions, such as idebenone for LHON and potential gene therapy approaches (8). However, the heterogeneity and complexity of these disorders demand further research to develop effective treatments. Mitochondrial donation techniques offer a new horizon in preventing the transmission of mtDNA diseases, providing hope for affected individuals and families (5,9).



## **2. Aims and objectives**

In this thesis, the aim is present a review on the intricate structure and encoding capabilities of mitochondrial DNA (mtDNA) and its significant implications for mitochondrial diseases. The primary aim is to explain the unique structural and functional attributes that characterize the mtDNA, including its closed-circular, double-stranded nature, absence of introns, and its highly efficient and tightly packed gene organization. This exploration is pivotal to understanding the critical role of mtDNA in cellular energy production and the regulatory mechanisms that ensure mitochondrial functionality.

Furthermore, this thesis seeks to investigate the genetic underpinnings and clinical consequences of mtDNA mutations. By examining the spectrum of these mutations, we aim to uncover how they contribute to the diverse array of mitochondrial diseases, thereby establishing a clear link between specific genetic alterations and their phenotypic expressions. This analysis is crucial for shedding light on the complex relationship between mtDNA mutations and the resultant mitochondrial dysfunction, which is a hallmark of various mitochondrial diseases.

An additional focal point of this research is to assess the intricate interplay between the mitochondrial and nuclear genomes. This includes exploring how mutations in nuclear genes that impact mitochondrial function can lead to mitochondrial diseases and understanding the regulatory mechanisms governing the intergenomic communication essential for mitochondrial biogenesis and energy production.

To achieve these aims, the thesis is structured around several key chapters. Initially, we will characterize the structure and encoding functions of mtDNA, identifying and cataloging the range of mtDNA mutations, elucidating their mechanisms, and understanding their association with specific mitochondrial diseases.

Finally, an important component of this review involves evaluating current and emerging therapeutic strategies for mitochondrial diseases. This encompasses a review of existing treatments, such as dietary supplements and antioxidants, and a critical assessment of novel approaches like gene therapy and mitochondrial replacement therapy. The potential of these

therapies to address the underlying mitochondrial dysfunction and their future prospects will be discussed.

### 3. Literature Review: The Mitochondria

#### 3.1.1 Structure

Mitochondria, often described as the powerhouses of eukaryotic cells, play a pivotal role in energy conversion and metabolic regulation. These organelles are characterized by a unique structure that facilitates their function in oxidative phosphorylation and energy production (2,10). Central to this architecture are two unique membranes: the outer mitochondrial membrane (OMM) and the inner mitochondrial membrane (IMM), both of which enclose the mitochondrial matrix and form a space between the membranes known as the intermembrane space (IMS) (2).

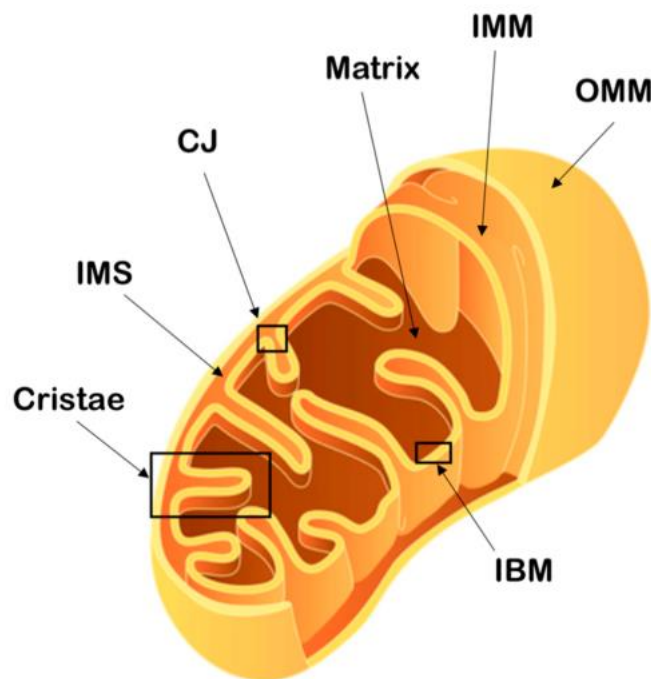


Figure 1. Diagrammatic illustration of mitochondrial structure (2)

The OMM is integral to the mitochondrion's interaction with the cytosol, containing proteins that allow for the exchange of ions and small molecules (11). This membrane is akin in lipid composition to those of eukaryotic cell membranes, serving as a semi-permeable barrier that regulates the entry and exit of substances. Notably, the OMM hosts enzymes vital for the elongation of fatty acids, highlighting its role in lipid metabolism (2,11).

Diverging in composition and function, the IMM is densely packed with proteins, exhibiting a higher protein-to-lipid ratio compared to the OMM (11). This membrane is reminiscent of bacterial membranes, containing cardiolipin, and is responsible for the critical functions of ATP synthesis and respiratory chain activities. The IMM is ingeniously folded into structures known as cristae, which significantly amplify the surface area available for oxidative phosphorylation, thereby enhancing the mitochondrion's capacity to produce ATP (2).

Within the convoluted folds of the IMM resides the machinery for oxidative phosphorylation, including complexes involved in the electron transport chain and ATP synthase. The arrangement of these complexes within the cristae is strategic, optimizing the efficiency of electron transfer and proton gradient formation essential for ATP generation (2).

The mitochondrial matrix, enclosed by the IMM, is a site of intense metabolic activity. It contains a plethora of enzymes necessary for the Krebs cycle, a central metabolic pathway that contributes to the production of electron carriers used in oxidative phosphorylation (12). Additionally, the matrix houses mitochondrial DNA (mtDNA), which encodes essential components of the respiratory chain and ATP synthase, underscoring the mitochondrion's semi-autonomous nature (2).

### 3.1.2 Function

Mitochondria have emerged as key players in cellular signaling and regulation. Beyond their well-known function in ATP synthesis, mitochondria are integral to calcium homeostasis, apoptosis, and the biosynthesis of critical compounds such as heme and iron-sulfur clusters, reflecting their multifaceted roles in cellular physiology (2).

The fundamental process of energy production in cells involves the conversion of adenosine triphosphate to adenosine diphosphate (ADP), a reaction that releases energy usable by the cell. To sustain cellular functions, ATP must be continually regenerated from ADP, a task predominantly accomplished by mitochondria through oxidative phosphorylation (2,13). The journey of energy production begins in the cytosol with glycolysis, an anaerobic process that breaks down glucose into pyruvate, yielding a modest gain of two ATP molecules per glucose molecule. Pyruvate then enters the mitochondria, where it is further processed in the

citric acid cycle, an aerobic pathway that produces high-energy electron carriers, nicotinamide adenine dinucleotide, and flavin adenine dinucleotide (2,13).

The electron transport chain is powered by carriers that initiate a series of oxidation-reduction reactions, ultimately leading to electrons being passed to oxygen, resulting in water formation. This movement of electrons creates a gradient of protons across the inner membrane of the mitochondria, which in turn propels the production of ATP through the action of ATP synthase. Through this sophisticated mechanism, a single molecule of glucose can yield around 30 molecules of ATP, underscoring the mitochondrion's essential function in the energy management within cells (2).

Mitochondria are also central to apoptosis, the programmed cell death is important for tissue development and homeostasis. They participate in both intrinsic and extrinsic apoptotic pathways by releasing factors that trigger caspase activation, leading to controlled cellular dismantling (14). This function underscores the mitochondrion's role in cellular quality control and the elimination of damaged or unnecessary cells (2).

In the realm of calcium signaling, mitochondria act as both buffers and regulators of intracellular calcium levels. Calcium ions play pivotal roles in numerous cellular processes, and their concentrations are tightly regulated by organelles like mitochondria and the endoplasmic reticulum. Mitochondrial calcium uptake influences various metabolic pathways by modulating the activity of enzymes within the citric acid cycle, thus linking calcium signaling directly to cellular energy status (2,15).

Furthermore, mitochondria are involved in the synthesis of heme, a vital component of hemoglobin, cytochromes, and other heme proteins. The process of heme synthesis is bifurcated between the mitochondria and the cytosol, with mitochondria hosting key steps, including the incorporation of iron into protoporphyrin IX by ferrochelatase (16). This function is critical not only for oxygen transport and storage but also for the electron transport chain's operation, thereby linking heme synthesis to mitochondrial energy production and broader cellular functions (2).

### 3.1.3 Mitochondrial DNA -Structure and Encoding

Mitochondrial DNA is a closed-circular, double-stranded molecule comprising approximately 16,569 base pairs (17). This compact genome is remarkably efficient in its organization, lacking introns and containing tightly packed genes with minimal or no intergenic sequences. The mtDNA is predominantly polycistronic, where each gene is directly contiguous to the next, and in some instances, genes overlap, maximizing the use of the available genetic material (2,4).

The mtDNA molecule is characterized by a heavy (H) strand and a light (L) strand, differentiated by their guanine-plus-thymine (G+T) base composition. This difference results in different levels of buoyancy when conditions cause denaturation, with the majority of genetic data being carried on the denser strand (18). The denser strand carries the code for two ribosomal RNAs (rRNAs), 14 transfer RNAs (tRNAs), and 12 out of the 13 essential polypeptides needed for the process of oxidative phosphorylation. Conversely, the L strand is responsible for encoding a single polypeptide and eight tRNAs (3,10).

The human mitochondrial genome is responsible for coding 37 genes: 13 polypeptides, 22 tRNAs, and 2 rRNAs. These genes are integral components of the OXPHOS system, which is central to mitochondrial energy production. The 13 polypeptide genes are core subunits of the OXPHOS complexes I, III, IV, and V. It is noteworthy that the mtDNA genetic code exhibits slight deviations from the universal genetic code, with unique codons for amino acids such as tryptophan and methionine, and a reduced number of stop codons (3,18).

A significant regulatory element within the mtDNA is the displacement loop, a non-coding region that serves as the main control site for mtDNA replication and transcription (4). This region underscores the autonomous regulatory capacity of the mitochondrial genome, albeit within the context of a tightly integrated nuclear-mitochondrial genetic system (2).

Mitochondrial DNA is exclusively maternally inherited, a phenomenon attributed to the significant discrepancy in mtDNA copy numbers between sperm cells (50-75 copies) and oocytes (approximately 100,000 copies) (19). This vast difference likely results in the dilution of paternal mtDNA to levels below detectable limits, thus establishing the maternal lineage as the sole contributor to offspring mtDNA (10).

Despite its autonomous genetic machinery, the mitochondrion is not an isolated entity. Most proteins found in mitochondria, especially the ones that form part of the respiratory chain, are produced from genes located in the cell nucleus. These proteins are initially created in the cytosol and then transported into the mitochondria. This interdependence highlights the sophisticated level of coordination between the mitochondrial and nuclear genomes, ensuring the proper functioning and regulation of mitochondrial activities (4).

### **3.2. Mutations in Mitochondrial DNA**

The understanding of mtDNA mutations and their implications has expanded significantly, revealing a complex relationship between these genetic alterations and a spectrum of clinical outcomes, including various diseases. Mitochondrial dysfunction, influenced by mtDNA mutations, is a contributing factor to conditions ranging from neurodegenerative diseases and metabolic disorders to aging and cancer (3).

Mitochondrial DNA is especially susceptible to changes due to a variety of inherent and external influences. It does not have protective histone proteins, is situated near the electron transport chain which produces reactive oxygen species, and faces ongoing exposure to oxygen free radicals (1). These conditions predispose mtDNA to a higher mutation rate compared to nuclear DNA. The mitochondrial genome encodes core components of the oxidative phosphorylation system, and mutations within mtDNA can severely impair cellular energy production, leading to the dysfunction of high-energy demand tissues like the heart, the brain and muscles (2,3).

Since the identification of the inaugural mutation in human mitochondrial DNA (mtDNA) in 1988, numerous mutations have been associated with distinct medical conditions. These mutations range from extensive deletions, exemplified by the alterations observed in Kearns–Sayre syndrome that impact muscle and ocular health, to specific point mutations that lead to conditions such as Leber's hereditary optic neuropathy and Leigh syndrome. Beyond these specific examples, alterations in mtDNA have also been connected to prevalent chronic illnesses, including diabetes, and to neurodegenerative disorders like Parkinson's and Alzheimer's disease, as well as various forms of cancer (3).

A unique aspect of mtDNA in pathology is the concept of heteroplasmy—the coexistence of wild-type and mutant mtDNA within the same cell. The ratio of mutant to wild-type mtDNA can greatly influence the severity and type of clinical manifestations, leading to a wide spectrum of phenotypes even within the same family (20). The threshold effect, where a certain proportion of mutant mtDNA must be exceeded to manifest symptoms, adds another layer of complexity to mitochondrial diseases. The bottleneck effect during oocyte maturation results in a random distribution of mtDNA mutations from mother to offspring, contributing to the variability in disease expression (7).

Mitochondrial diseases exhibit unique inheritance patterns due to the maternal transmission of mtDNA. This matrilineal inheritance means that mutations are passed from mothers to all their offspring, but only daughters can transmit the mutations to subsequent generations (21). Additionally, secondary mtDNA changes, such as multiple deletions and mtDNA depletion, can arise because of the mutations in nuclear genes which are involved in mtDNA replication and maintenance. These secondary changes often lead to multisystem diseases with variable onset and severity (2,7).

The heterogeneity of mitochondrial diseases, coupled with the complex interplay between nuclear and mitochondrial genomes, complicates the diagnosis and treatment of these conditions. The diversity of mtDNA mutations, their variable expression in different tissues, and the influence of nuclear genes on mitochondrial function make it challenging to identify common therapeutic targets or develop universal treatments for mitochondrial diseases (2,7).

### 3.2.1 Mitochondrial diseases

Primary mitochondrial disorders (MIDs) represent a diverse spectrum of diseases that arise from genetic dysfunctions within the mitochondria, the cellular organelles crucial for energy production. These disorders are part of a larger category of heritable diseases, notable for their heterogeneity in clinical presentation, genetic basis, and epidemiology. As a group, MIDs are among the most common inheritable diseases, underscoring the critical role of mitochondrial function in cellular health and organismal vitality (5).

The prevalence of mitochondrial diseases is on par with other neurogenetic disorders such as Charcot-Marie-Tooth disease, myotonic dystrophy, and various forms of muscular dystrophy. Recent studies, such as those conducted in North East England, have indicated that about 1 in 4,300 adults is affected by or at risk for mitochondrial disease, highlighting its significance in the adult population (22). Interestingly, primary mutations in mtDNA are more commonly observed in adults, whereas nuclear gene mutations predominate in pediatric cases, especially within consanguineous families (23). Despite the vast array of mutations identified since the first discovery in 1988, a few mutations occur with higher frequency and are associated with specific syndromes, though their clinical manifestation can vary widely, often influenced by factors such as environmental toxins and mutation load (7).

Mitochondrial diseases can affect virtually any organ system due to the ubiquitous presence of mitochondria the clinical features of the disorders are remarkably diverse, often mimicking other neurological or systemic diseases, which makes the clinical diagnosis challenging (5). The disease can manifest across a spectrum, from isolated symptoms such as Leber hereditary optic neuropathy (LHON) to severe multisystem involvement, particularly in pediatric cases where the disease course tends to be more aggressive and prognosis poorer (7).

Clinicians often face a diagnostic puzzle when presented with the non-specific constellation of symptoms that can accompany mitochondrial diseases. Common symptoms like deafness, diabetes, and myopathy, when occurring in combination, should prompt consideration of a mitochondrial disorder (24). A thorough system-based examination and extensive investigations are crucial for uncovering subtle or asymptomatic systemic involvements, such as endocrine dysfunctions, cardiac issues, or renal tubulopathy, which may not be apparent in the early stages of the disease (25). Utilization of disease rating scales can aid in documenting the extent of system involvement and monitoring disease progression, providing valuable insights for both pediatric and adult patients (7).

#### 3.2.1.1 Leber Hereditary Optic Neuropathy

Leber's Hereditary Optic Neuropathy (LHON) serves as a quintessential instance of primary mitochondrial diseases, marked by the sudden or gradual deterioration of central vision resulting from the atrophy of retinal ganglion cells and their axons. This mitochondrial genetic disorder predominantly affects young adult males, with a notable prevalence of



approximately 1 in 30,000, showcasing a distinct pattern of maternal inheritance due to its linkage to mtDNA mutations (5).

LHON is mainly linked to changes in mitochondrial DNA (mtDNA), especially in genes responsible for making parts of complex I in the mitochondrial respiratory chain. More than 90% of LHON instances can be traced back to one of three harmful point mutations in mtDNA: m.3460G>A within the MT-ND1 gene, m.11778G>A in the MT-ND4 gene, and m.14484T>C in the MT-ND6 gene. These mutations lead to impaired mitochondrial function, particularly affecting the optic nerve and retina—tissues with high metabolic demands (3,5,7).

Interestingly, LHON-associated mtDNA mutations are typically homoplasmic, meaning that nearly all mtDNA copies within a cell harbor the mutation. Despite this, the penetrance of LHON is incomplete, with approximately 50% of male carriers and 10% of female carriers manifesting the disease (26). Incomplete expression of the trait and the increased vulnerability in males, potentially influenced by elements such as estrogen, highlight the intricate relationship between genetic predispositions and environmental influences in the development of LHON (5).

LHON presents a unique clinical challenge due to its variable onset and progression. Patients typically experience bilateral, painless visual loss, with one eye often affected before the other. Initial symptoms can mimic optic neuritis, lacking overt optic nerve atrophy but possibly showing subtle signs like optic nerve swelling and peripapillary telangiectasia detectable through optical coherence tomography (5,6).

The diagnosis of LHON hinges on recognizing these clinical features and confirming the presence of one of the primary mtDNA mutations through genetic testing. Given the maternal transmission of mtDNA, family history plays a crucial role in the risk assessment and management of LHON (27).

Management strategies for LHON have evolved from symptomatic treatments, such as visual aids, to targeted interventions like idebenone—a synthetic analog of coenzyme Q10 (8). Idebenone facilitates electron transfer within mitochondria, potentially bypassing the complex I defect induced by LHON mutations. Its efficacy, backed by randomized controlled trials,

highlights the shift towards therapies that address the underlying mitochondrial dysfunction in LHON (6,7).

Moreover, the advent of gene therapy offers a promising horizon for LHON management. Techniques like intravitreal injection of gene therapy vectors aim to restore normal mitochondrial function in retinal cells. Early clinical trials have shown encouraging results, particularly for the m.11778G>A mutation in the MT-ND4 gene, with treated patients experiencing significant visual improvement (5,7).

### 3.2.1.2 Leigh Syndrome

Leigh Syndrome (LS), also known as Subacute Necrotizing Encephalomyelopathy, represents a paradigmatic model of mitochondrial dysfunction manifesting as a severe and early-onset neurodegenerative disorder. Characterized by bilateral central nervous system lesions and a variable symptomatology reflective of mitochondrial energy production impairment, LS underscores the intricate relationship between mitochondrial bioenergetics and neural integrity (29). This review delves into the genetic underpinnings of LS, highlighting the role of mutations in both mitochondrial DNA and nuclear DNA in the disease's pathogenesis, and explores the current understanding of its genetic heterogeneity and the resultant clinical implications (30).

Leigh Syndrome's genetic landscape, predominantly inherited in an autosomal recessive manner, is notably diverse (31). Pathogenic mutations span a wide range of genes, including those encoding components of the electron transport chain (ETC), as well as those involved in mitochondrial DNA maintenance, assembly of OXPHOS complexes, and additional mitochondrial functions. This genetic heterogeneity translates into a wide array of clinical manifestations, often dictated by the nature and location of the mutations (5,30).

Mutations in mtDNA are a significant contributor to LS, often involving genes that encode subunits of the ETC complexes. For instance, mutations at position m.8993 in mtDNA, affecting ATP synthase, are notably linked to LS, illustrating the direct impact of compromised ATP production on neural health (32). Moreover, mtDNA mutations can result in combined OXPHOS deficiencies due to their role in encoding multiple components of the ETC, further complicating the clinical picture (30).

Nuclear DNA mutations play a critical role in LS, affecting not only the structural components of the ETC complexes but also proteins essential for the assembly, maintenance,

and expression of mitochondrial machinery. Notably, mutations in SURF1, involved in complex IV assembly, and PDHA1, part of the pyruvate dehydrogenase complex, exemplify the diverse nDNA mutations leading to LS (33). These mutations underscore the intricate interplay between nuclear and mitochondrial genomes in maintaining mitochondrial function (9,30).

The genetic heterogeneity of LS is reflected in its clinical presentation, ranging from the classic early-onset form to atypical manifestations appearing later in life. This variability underscores the challenge in diagnosing and managing LS, necessitating a comprehensive approach that integrates genetic, biochemical, and neuroimaging data (30).

The diverse genetic landscape of LS poses significant diagnostic challenges, often requiring advanced molecular techniques such as Next Generation Sequencing to identify the underlying genetic cause. This complexity also impacts therapeutic strategies, which currently are mostly supportive and symptomatic, given the lack of curative treatments. Emerging therapies, including mitochondrial replacement and gene therapy, offer potential avenues for intervention, albeit with their own set of ethical, technical, and efficacy considerations (5,30).

### 3.2.1.3 Mitochondrial neurogastrointestinal encephalopathy

Mitochondrial Neurogastrointestinal Encephalomyopathy (MNGIE) epitomizes the complexity of mitochondrial disorders, where nuclear DNA mutations disrupt the delicate balance of mitochondrial function. MNGIE arises from mutations in the TYMP gene, leading to thymidine phosphorylase deficiency, which in turn causes systemic accumulation of thymidine and deoxyuridine (34). This accumulation disrupts mitochondrial DNA maintenance, leading to instability and a spectrum of clinical manifestations primarily affecting the gastrointestinal and nervous systems. The disease's autosomal recessive inheritance pattern underscores the importance of understanding the genetic basis for accurate diagnosis and potential therapeutic interventions (35).

The gene TYMP, found on the 22q13.33 location of chromosome 22, is responsible for producing thymidine phosphorylase, a crucial enzyme in the breakdown of nucleosides. Mutations in TYMP, either homozygous or compound heterozygous, result in a marked reduction or complete absence of thymidine phosphorylase activity. This enzymatic deficit

leads to the systemic build-up of thymidine and deoxyuridine, which are toxic to mitochondrial function. Elevated levels of these nucleosides in plasma and tissues induce nucleotide pool imbalances, contributing to mtDNA replication errors, deletions, and depletion, ultimately impairing mitochondrial bioenergetics and leading to cellular dysfunction (34,36).

MNGIE typically manifests in early adulthood, presenting a constellation of gastrointestinal and neurological symptoms, including gastrointestinal dysmotility, cachexia, peripheral neuropathy, and leukoencephalopathy (37). The gastrointestinal symptoms often mimic common disorders, leading to frequent misdiagnoses. Neurologically, patients may exhibit features such as ptosis, ophthalmoplegia, and demyelinating neuropathy, further complicating the clinical picture (35).

Diagnosis of MNGIE hinges on the detection of TYMP mutations and the biochemical hallmark of elevated plasma levels of thymidine and deoxyuridine. Reduced thymidine phosphorylase activity in leukocytes serves as a critical diagnostic criterion (34). Advanced molecular techniques, including sequencing of the TYMP gene and quantification of nucleoside levels in plasma, are essential for confirming the diagnosis (36).

The management of MNGIE remains challenging, primarily due to the systemic nature of the disorder and the lack of curative treatments. Current therapeutic strategies aim to mitigate the biochemical imbalance caused by nucleoside accumulation. Approaches such as allogeneic hematopoietic stem cell transplantation and liver transplantation have shown promise in restoring thymidine phosphorylase activity and normalizing nucleoside levels, albeit with significant risks and variable outcomes (38). Erythrocyte-encapsulated thymidine phosphorylase therapy offers a temporary solution, potentially serving as a bridge to more definitive treatments (36).

Emerging therapies, including gene therapy, hold promise for addressing the underlying genetic defect in MNGIE. By correcting the TYMP mutation or providing a functional copy of the gene, gene therapy aims to restore thymidine phosphorylase activity and achieve long-term disease stabilization. However, these approaches are still in the experimental stages, and further research is needed to ascertain their efficacy and safety (35,36).

#### 3.2.1.4 Mitochondrial encephalomyopathy (MELAS)

Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like Episodes (MELAS) is a multifaceted mitochondrial condition marked by a variety of symptoms that mainly impact the nervous system and muscular tissues. This disorder typically manifests in the first two decades of life, presenting a challenging prognosis with significant variability in clinical outcomes (5).

The primary cause of MELAS syndrome is often associated with changes in mitochondrial DNA, especially the m.3243A>G mutation found in the MT-TL1 gene (39). This gene is responsible for producing mitochondrial tRNA for leucine. The mutation causes a change in the structure of the tRNA, reducing its effectiveness in the mitochondria's protein production process. Mitochondria are crucial for generating energy within cells, so any disruption in their protein synthesis significantly impacts cell operations and energy generation (40).

The m.3243A>G mutation is responsible for over 80% of MELAS cases, highlighting its significance in the pathogenesis of the disorder. However, it is essential to recognize the existence of other mtDNA mutations contributing to MELAS, underscoring the genetic heterogeneity of the syndrome (39). The diagnosis of MELAS, therefore, relies heavily on genetic testing, with the detection of specific mutations in blood leukocytes or more sensitively in urine sediment, providing a definitive diagnosis (5).

As the Mitochondria are critical for energy production through oxidative phosphorylation in MELAS, mutations in mtDNA lead to dysfunctional mitochondria, manifesting in a range of symptoms due to energy deficits in the most metabolically active tissues, including the brain and muscles. The impaired energy production is further evidenced by elevated lactate levels, a hallmark of disrupted oxidative phosphorylation and reliance on anaerobic metabolism (5).

The heteroplasmic nature of mtDNA mutations in MELAS introduces variability in disease expression. The ratio of mutated to normal mtDNA can affect how severe the clinical features of a condition appear. This genetic complexity contributes to the broad spectrum of symptoms observed in MELAS patients and within affected families (40).

MELAS is characterized by a spectrum of symptoms ranging from muscle weakness, exercise intolerance, and gastrointestinal issues to more severe neurological manifestations such as stroke-like episodes, seizures, and cognitive decline. The episodes resembling strokes, which are characteristic of MELAS, do not adhere to the usual patterns associated with vascular regions, complicating the process of diagnosing and managing the condition clinically (5,11).

Management of MELAS remains largely symptomatic, with antiepileptic therapies being central to the treatment of seizures and prevention of stroke-like episodes (39). The use of newer antiepileptic drugs is preferred due to concerns over mitochondrial toxicity associated with older medications. Despite various treatment strategies explored, including supplements like coenzyme Q10 and L-carnitine, no therapy has yet shown efficacy in altering the disease course (41). The use of L-arginine, although controversial, has been explored given its role in nitric oxide metabolism and potential effects on vascular function in MELAS patients (9).

### **3.3 Therapies**

#### **3.3.1 Existing Therapeutic Approaches**

Traditionally, management strategies for mitochondrial diseases have been largely supportive and symptomatic. Pharmacological interventions often include a range of dietary supplements such as coenzyme Q10, riboflavin, and L-carnitine, aimed at augmenting mitochondrial function or mitigating oxidative stress (21). Despite their widespread use, the clinical efficacy of these supplements remains a topic of debate, with a Cochrane review indicating a lack of conclusive evidence supporting their effectiveness (9).

Notably, antioxidants have been a focal point in treating mitochondrial diseases due to the role of reactive oxygen species in mitochondrial pathology. Coenzyme Q10, idebenone, and other antioxidants aim to reduce oxidative damage, although their impact on disease progression is still under investigation (8). The Mitochondrial Medicine Society has issued consensus recommendations to standardize the use of such supplements, emphasizing the need for careful monitoring and gradual introduction to assess patient response (9,30).

In specific cases, targeted interventions have shown promise. For instance, high-dose coenzyme Q10 supplementation has been reported to yield variable improvements in primary coenzyme Q10 biosynthetic defects. Additionally, L-arginine administration during acute stroke-like episodes in patients with the m.3243A>G mutation has been suggested to offer benefits, although further validation is needed (30,40).

### 3.3.2 Emerging Therapeutic Strategies

In the dynamic field of mitochondrial medicine, the development of novel therapeutic strategies marks a significant advance towards addressing the complex nature of mitochondrial diseases. These strategies are broadly categorized into "non-tailored" and "disease-tailored" approaches, each targeting different aspects of mitochondrial pathology.

#### 3.3.2.1 Non-tailored Therapeutic Strategies

Non-tailored strategies are not specific to any single type of mitochondrial disease but rather aim to ameliorate mitochondrial dysfunction in a more general sense. Among these, the activation of mitochondrial biogenesis stands out as a promising avenue. This approach seeks to increase the overall mass and number of mitochondria within cells, thereby potentially enhancing cellular energy production. Agents such as Sirt1 activators and AMPK agonists, for instance, AICAR, are currently under investigation for their capacity to stimulate this process (42). By boosting mitochondrial biogenesis, it is hoped that the energy output of cells suffering from mitochondrial dysfunction can be significantly improved (9).

Another key area of focus is the regulation of mitophagy and mitochondrial dynamics. Mitophagy, the selective degradation of dysfunctional mitochondria, is crucial for maintaining mitochondrial quality and function (43). Enhancing this process through agents like rapamycin could lead to the removal of defective mitochondria and the preservation of mitochondrial health (9).

Bypassing defects in the oxidative phosphorylation system represents another innovative strategy. This involves utilizing alternative enzymes to circumvent dysfunctional components of the OXPHOS system. Such approaches have shown potential in preclinical models and offer a novel means of restoring energy production in affected cells (5,9).

Mitochondrial Replacement Therapy is a groundbreaking technique that involves replacing mutant mtDNA in oocytes or embryos. This approach aims to prevent the transmission of mitochondrial diseases from mother to offspring, offering hope for families affected by these conditions (9,30).

Lastly, the activation of hypoxic response pathways has emerged as a novel therapeutic strategy. By mimicking a hypoxic environment, it may be possible to induce adaptive responses that mitigate the effects of mitochondrial dysfunction (44). Preclinical models have shown promising results, suggesting that this approach could lead to new treatments for mitochondrial diseases (9).

### 3.3.2.2 Disease-tailored Therapeutic Strategies

Disease-tailored strategies, on the other hand, are designed to target the specific genetic or biochemical defects associated with particular mitochondrial disorders. In diseases characterized by mtDNA depletion, such as MNGIE and TK2 deficiency, supplementing nucleosides or nucleotides has been shown to correct mtDNA imbalances and stabilize mtDNA copy numbers (45). This approach directly addresses the underlying genetic defects and offers a targeted means of treatment (9).

Gene therapy represents one of the most precise disease-tailored strategies. Techniques employing adeno-associated virus vectors and the CRISPR-Cas9 system are being explored to correct nuclear DNA defects and specifically target mitochondrial DNA (46). This highly specific approach holds great promise for treating mitochondrial diseases at their genetic roots, offering the potential for long-lasting therapeutic effects (9).

The exploration of both non-tailored and disease-tailored therapeutic strategies represents a significant leap forward in the quest to manage and potentially cure mitochondrial diseases. By addressing the diverse mechanisms underlying mitochondrial dysfunction, these approaches offer hope for more effective treatments. As research in this area continues to evolve, the future of mitochondrial medicine looks increasingly promising, with the potential to transform the lives of those affected by these challenging disorders (9).



### 3.3.2.3 Reproductive Options and Genetic Counseling

Mitochondrial diseases, characterized by their complex inheritance patterns and diverse clinical manifestations, pose unique challenges in the realm of reproductive options and genetic counseling. The advent of mitochondrial donation techniques has revolutionized the field, offering new avenues for individuals carrying (mtDNA) mutations to have genetically related children while minimizing the risk of transmitting these diseases (9,30,47).

Mitochondrial donation, encompassing techniques such as pronuclear transfer and metaphase II spindle transfer (MST), represents a groundbreaking approach in the prevention of mtDNA disease transmission. These techniques involve the transfer of nuclear genetic material from a fertilized or unfertilized egg with mutated mtDNA into a donor egg with healthy mitochondria, which is then fertilized (if not already) and implanted into the mother's womb. This process ensures that the child inherits the nuclear DNA from its parents while the mtDNA comes from a healthy donor, effectively breaking the cycle of mtDNA disease transmission (30,48).

PNT is performed shortly after fertilization, where the pronuclei containing the nuclear DNA from both parents are transferred from a fertilized egg with defective mitochondria to an enucleated donor egg with healthy mitochondria. MST, on the other hand, involves transferring the spindle-chromosomal complex from an unfertilized egg of the mother with defective mitochondria to an enucleated donor egg, followed by fertilization with sperm (9,30,48).

In addition to mitochondrial donation, traditional reproductive options remain pivotal for individuals at risk of transmitting mitochondrial diseases. These include Prenatal testing and preimplantation genetic Diagnosis (PGD).

Prenatal testing techniques such as chorionic villus sampling and amniocentesis allow for the detection of mitochondrial mutations in the fetus. These tests provide valuable information but also pose ethical and emotional challenges, particularly in the context of variable disease expression and the impact of heteroplasmy levels on disease severity (47,49).

Preimplantation Genetic Diagnosis involves screening embryos created through in vitro fertilization for mitochondrial mutations before implantation. This technique can identify embryos with lower levels of mutated mtDNA, reducing the risk of disease transmission. However, the effectiveness of PGD is limited by the phenomenon of heteroplasmy and the genetic bottleneck, which can result in unpredictable shifts in mutation load between generations (47,50).

Genetic counseling plays a crucial role in guiding individuals and families through the complex landscape of mitochondrial diseases. Counseling provides education about the inheritance patterns, risks of transmission, and the implications of various reproductive options. It also offers emotional support to families grappling with the potential of passing on a mitochondrial disease (5).

For mitochondrial diseases resulting from nuclear DNA mutations, the counseling and reproductive options align with those for other genetic disorders, including the consideration of PGD and prenatal testing. However, for diseases caused by mtDNA mutations, the counseling process is more nuanced due to maternal inheritance patterns, the potential for heteroplasmy, and the variable expression of mtDNA diseases (5,9).

#### **4. Discussion**

The advancements in therapeutic strategies for mitochondrial diseases, as delineated in this thesis, underscore the progress made from supportive treatments to more targeted and innovative approaches. The dichotomy between existing pharmacological interventions, like antioxidants and dietary supplements, and emerging strategies, including mitochondrial biogenesis activation and mitochondrial replacement therapy, highlights a pivotal shift towards addressing the root causes of mitochondrial dysfunction.

Existing therapies, while widely utilized, have shown limitations in clinical efficacy, as evidenced by the lack of conclusive support from Cochrane reviews for supplements like coenzyme Q10 and riboflavin. This underscores the complexity of mitochondrial diseases and the challenge in finding universally effective treatments due to the heterogeneous nature of these disorders.

Conversely, emerging strategies offer a more nuanced approach. Non-tailored strategies, such as the activation of mitochondrial biogenesis and the regulation of mitophagy, target fundamental aspects of mitochondrial function, potentially offering broader applicability across various forms of mitochondrial disease. Disease-tailored strategies, including gene therapy and nucleoside supplementation, represent a more personalized approach, targeting specific genetic and biochemical defects.

Moreover, the advent of mitochondrial replacement therapy and the regulation of mitochondrial dynamics present new possibilities for not only managing but potentially preventing the transmission of mitochondrial diseases. These advances could transform the treatment landscape, offering hope to patients and families affected by these challenging disorders.

Despite these promising developments, there are inherent limitations to the current therapeutic strategies. The efficacy of many emerging treatments is still under investigation, with much of the evidence derived from preclinical models or small-scale clinical trials. The complexity of mitochondrial diseases, coupled with their diverse genetic causes and clinical manifestations, poses a significant challenge to developing universally effective treatments.

Furthermore, the ethical and technical complexities associated with mitochondrial replacement therapy and gene editing techniques, such as CRISPR-Cas9, warrant careful consideration. These approaches raise important ethical questions, particularly regarding the manipulation of germline DNA, which have yet to be fully addressed.

## **5. Conclusion**

In conclusion, the investigation into mitochondrial DNA has elucidated a critical nexus between the nuanced structures and functions of mtDNA and the spectrum of mitochondrial diseases. The mtDNA, with its unique closed-circular, double-stranded configuration, encodes essential components of the oxidative phosphorylation system, pivotal in cellular energy production. This efficient genetic organization, devoid of introns and characterized by tightly packed genes, underscores the mitochondria's integral role as the cell's powerhouse. The phenomenon of maternal inheritance and the concept of heteroplasmy introduce a layer of

complexity to the transmission and manifestation of mitochondrial diseases, highlighting the intricate interplay between genetic predispositions and clinical outcomes.

The diversity of mitochondrial disorders, from Leber Hereditary Optic Neuropathy to Leigh Syndrome and Mitochondrial Neurogastrointestinal Encephalomyopathy, exemplifies the broad impact of mitochondrial dysfunction across various organ systems. These disorders underscore the critical role of mitochondria in maintaining cellular and systemic homeostasis, with mutations in mtDNA leading to a wide range of pathophysiological states.

Current therapeutic approaches for mitochondrial diseases remain predominantly supportive and symptomatic, focusing on managing the clinical manifestations of the diseases. However, advances in mitochondrial medicine, including the exploration of gene therapy, mitochondrial replacement therapy, and targeted molecular interventions, offer promising avenues for more effective treatments. These emerging therapies aim to address the underlying genetic and biochemical defects, providing hope for individuals affected by these challenging conditions.

Despite the significant strides made in understanding mitochondrial genetics and pathology, considerable challenges persist in the accurate diagnosis, management, and treatment of mitochondrial diseases. The heterogeneity of clinical presentations, coupled with the complex interplay between mtDNA and nuclear DNA, complicates the development of universal therapeutic strategies. As research continues to unravel the multifaceted nature of mitochondrial dysfunction, it is imperative to foster multidisciplinary collaborations that will drive the discovery of innovative treatments and improve the prognosis for patients with mitochondrial disorders.

## **6. Summary**

This thesis provides a comprehensive review of mitochondrial DNA (mtDNA), elucidating its unique structure, encoding capabilities, and the implications of mtDNA mutations for mitochondrial diseases. Mitochondria, critical for cellular energy production, house a compact and efficient genome that is remarkable for its polycistronic nature and lack of introns, emphasizing the organelle's role as the cell's powerhouse.

A significant focus is placed on the susceptibility of mtDNA to mutations due to its proximity to the electron transport chain and lack of histone protection, which predisposes it to a higher mutation rate compared to nuclear DNA. These mutations are intricately linked to a spectrum of mitochondrial disorders, ranging from neurodegenerative diseases to metabolic dysfunctions and aging. The thesis delves into the concept of heteroplasmy and its impact on the clinical manifestation of mitochondrial diseases, alongside the unique maternal inheritance pattern that adds complexity to the diagnosis and management of these conditions.

The review extends to primary mitochondrial disorders (MIDs), emphasizing their prevalence and the diversity of clinical manifestations that complicate diagnosis and treatment. Notable conditions such as Leber Hereditary Optic Neuropathy (LHON), Leigh Syndrome, and Mitochondrial Neurogastrointestinal Encephalomyopathy (MNGIE) are examined to illustrate the varied impact of mitochondrial dysfunction.

Therapeutic strategies for mitochondrial diseases are critically assessed, from traditional symptomatic treatments to emerging approaches like gene therapy and mitochondrial replacement therapy, highlighting the advances and challenges in developing effective treatments. The thesis also explores the role of genetic counseling and reproductive options in managing mitochondrial diseases, considering the complex inheritance patterns and the potential of mitochondrial donation techniques to prevent disease transmission.

**Key-words:** mtDNA, mitochondrial diseases, mtDNA mutations

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## **8. Curriculum Vitae**

Vivien Martis, born and raised in Cologne Germany, commenced her academic journey at the primary school in Cologne, where they laid the foundational stones of their education. Her academic path took her through various institutions, including the Max Ernst school in Köln and the Escola Basica Integrada de Azambuja in Portugal. She continued her education at Ernst Simons school in Cologne, followed by an international experience at Dover Grammar School of Girls in England during 2013-2014. Miss Martins then attended the LVR- Anna Freud Gymnasium in Köln from 2014 to 2017, which culminated in her current pursuit of a degree in Human Medicine at the University of Rijeka, Croatia, since 2018.

In addition to her academic endeavors, she gained practical experience through various internships. She completed a nursing internship at St. Elisabeth hospital between November 2017 and February 2018. Further expanding her practical skills, she undertook an internship in General Surgery at university clinic Göttingen in August 2021 and in different clinics in the west of Germany in Internal Medicine throughout the years. These experiences have contributed to her practical understanding of the medical field, complementing her academic studies in Human Medicine.