

**Exploring the Reproductive Choices and Family Planning Experiences of Women who
have MELAS: A Qualitative Study**

by

Maria Elizabeth Rhine

BS Biological Sciences, University of Pittsburgh, 2019

Submitted to the Graduate Faculty of the
Department of Human Genetics – Genetic Counseling
Graduate School of Public Health in partial fulfillment
of the requirements for the degree of
Master of Science

University of Pittsburgh

2022

UNIVERSITY OF PITTSBURGH

GRADUATE SCHOOL OF PUBLIC HEALTH

This thesis was presented

by

Maria Elizabeth Rhine

It was defended on

April 12, 2022

and approved by

Robin E. Grubs, PhD, LCGC; Associate Professor, Department of Human Genetics, Graduate
School of Public Health, University of Pittsburgh

Elizabeth M. Felter, DrPH; Assistant Professor, Behavioral and Community Health Sciences,
Graduate School of Public Health, University of Pittsburgh

Thesis Advisor: Catherine M. Walsh Vockley, MS, LCGC; Adjunct Instructor, Department of
Human Genetics, Graduate School of Public Health, University of Pittsburgh

Copyright © by Maria Elizabeth Rhine

2022

Exploring the Reproductive Choices and Family Planning Experiences of Women who have MELAS: A Qualitative Study

Maria Elizabeth Rhine, MS

University of Pittsburgh, 2022

Mitochondria are cellular structures that are the powerhouses of the cell due to the large amounts of cellular energy that they produce. A harmful mutation in a mtDNA gene can cause mitochondrial conditions which have genetic complexities related to unique challenges around diagnosis, inheritance, and family planning. While much research has been done on the genetic complexities (heteroplasmy, threshold effect, genetic bottleneck) and reproductive options (oocyte donation and mitochondrial replacement therapy) related to mitochondrial conditions, there have been few studies on the psychosocial implications of receiving a diagnosis, how the diagnosis impacts family planning, or the parental attitudes about available reproductive options. One of the most common mitochondrial conditions is Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes (MELAS). For this study, women who have MELAS were interviewed to gain a more in-depth understanding of the psychosocial impact of receiving a diagnosis of a mitochondrial condition and of their experiences with genetic counseling, family planning, and reproductive decision-making. Interviews were conducted with two women who have MELAS, and interview transcripts were analyzed using thematic analysis. This study identified four themes related to the impact of a MELAS diagnosis on women and their families: impact of the range of severity of MELAS, familial implications and concern for family, intellectual curiosity and desire for knowledge, and implications for family planning and reproductive decision making. The experiences and opinions of these women were analyzed with a focus on ways in which genetic counselors and other healthcare providers can provide care and support to families with MELAS.

This study has Public Health significance because the results can be used to provide more personalized care to individuals with MELAS and may contribute to the development of updated educational materials or resources that are provided to individuals diagnosed with MELAS.

Table of Contents

Preface.....	x
1.0 Introduction.....	1
1.1 Specific Aims	3
2.0 Literature Review	5
2.1 Mitochondrial Disease.....	5
2.1.1 Mitochondrial Genetics	6
2.1.1.1 Maternal Inheritance.....	6
2.1.1.2 Heteroplasmy, Threshold Effect, and Genetic Bottleneck.....	7
2.2 Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes.....	9
2.2.1 Diagnosis	11
2.2.1.1 Clinical Diagnostic Criteria	11
2.2.1.2 Molecular Genetics	12
2.2.2 Treatment and Prognosis	13
2.3 Reproductive Options and Mitochondrial Disease.....	14
2.3.1 Oocyte Donation and Mitochondrial Replacement Therapy	15
2.4 Genetic Counseling for Mitochondrial Disease	18
3.0 Manuscript.....	20
3.1 Background	20
3.1.1 Thematic Analysis	23
3.2 Methods	24
3.2.1 Research Study Approval.....	24

3.2.2 Participant Recruitment	24
3.2.3 Participant Interviews	26
3.2.4 Thematic Analysis	27
3.3 Results.....	28
3.3.1 Demographics	28
3.3.2 Themes Identified in Analysis.....	29
3.3.2.1 Impact of the range of severity of MELAS	30
3.3.2.2 Familial implications and concern for family	33
3.3.2.3 Intellectual curiosity and desire for knowledge	35
3.3.2.4 Implications for family planning and reproductive decision making.....	37
3.4 Discussion	41
3.4.1 Impact of the range of severity of MELAS	42
3.4.2 Familial implications and concern for family.....	43
3.4.3 Intellectual curiosity and desire for knowledge.....	45
3.4.4 Implications for family planning and reproductive decision making	46
3.4.5 Limitations	48
3.4.5.1 Sample Size.....	48
3.4.5.2 Recruitment Methods.....	48
3.4.6 Future Directions	49
3.5 Conclusions	50
4.0 Research Significance to Genetic Counseling and Public Health.....	51
Appendix A IRB Approval Letter	54
Appendix B Department Discretionary Funding Approval	55

Appendix C Recruitment Letter for the United Mitochondrial Disease Foundation.....	56
Appendix D United Mitochondrial Disease Foundation Letter of Support	57
Appendix E Recruitment Letter	58
Appendix F Recruitment Flyer.....	59
Appendix G Participant Interview Introduction	60
Appendix H Participant Verbal Consent Script	61
Appendix I Participant Interview Guide	62
Bibliography	64

List of Tables

Table 1. Participant Demographics..... 29

Table 2. Summary of Identified Themes 30

Preface

I would like to take this opportunity to thank all of the individuals who participated in this project and also those who provided me with endless support and guidance throughout this project. Thank you to the interviewees who were willing to share their stories with me; your thoughts and experiences were the motivation for this project, and I am extremely grateful for your willingness to share such personal parts of your life with me. I would also like to thank my thesis advisor, Catherine Walsh Vockley, as well as my other committee members, Dr. Robin Grubs and Dr. Elizabeth Felter, who kept me focused and provided the guidance and feedback needed to finalize this project. Another special thank you to my program director, Jodie Vento, who initially helped conceptualize this project and connect me to the United Mitochondrial Disease Foundation (UMDF); this organization was essential for the recruitment of participants for my project, and I would like to specifically thank Margaret Moore at the UMDF for her commitment to assisting me with this project. Finally, to my family, friends, and classmates – I would not have had the strength to complete this project without your love, friendship, and encouraging words.

1.0 Introduction

Mitochondria are small organelles in most human cells. There can be hundreds to thousands of these structures which specialize in creating usable energy in the form of adenosine triphosphate (ATP) in a single cell. Each organelle has mitochondrial DNA (mtDNA) that encodes 37 genes important for normal functioning and energy production (Zou, Slone, Cao, & Huang, 2020). In most cases, the many mtDNA molecules within a single cell will be the same, a concept known as homoplasmy. However, due to a high mutation rate of the mitochondrial genome, heteroplasmy can occur, which is the presence of two or more mtDNA genotypes within a cell, tissue, or organ (Zou et al., 2020). Mitochondrial disease happens when a significant number of cells within a tissue or organ are impacted by disease-associated mtDNA mutations. The threshold effect describes the level of mutant mitochondrial DNA at which a particular cell becomes vulnerable to a deleterious mutation and can no longer produce sufficient amounts of energy to maintain cellular function. Heteroplasmy and threshold levels can vary among different areas of the body leading to variable effects on organs and thus in symptoms of mitochondrial disease (Craven, Alston, Taylor, & Turnbull, 2017). Higher heteroplasmy levels are usually associated with more severe clinical manifestations, and clinical symptoms often occur in the tissues and organs with the highest energy demands (e.g., brain, skeletal, muscles, heart) (Craven et al., 2017; Gorman et al., 2016).

Inheritance of the mitochondrial genome is unique in that it is almost solely maternally-inherited due to elimination of the paternal mtDNA during fertilization (Zou et al., 2020). The mitochondrial genetic bottleneck describes the random contribution of the mother's mitochondria

into each egg cell (Chiaratti et al., 2020). A random sample of the mother's mitochondria is contributed to each egg cell, and thus, if a mitochondrial mutation is present in the germline, there can be variability in the mutant load of individual egg cells. It is therefore possible for a woman to have a low level of heteroplasmy but pass on a random sample that includes a higher proportion of mutated mtDNA to offspring (Vento & Pappa, 2013).

One of the most common conditions caused by mutations in mtDNA is Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes (MELAS). MELAS can affect multiple organs of the body with symptoms that include stroke-like episodes, dementia, epilepsy, lactic acidemia, hearing impairment, diabetes, and other complications due to energy deficiency. There is significant phenotypic variability with this condition due to variable heteroplasmy with different percentages of mutation loads in tissues and organs, as well as varying threshold effects i.e., the mutant load needed for clinical symptoms to present (El-Hattab, Adesina, Jones, & Scaglia, 2015). The clinical and genetic complexity of this condition poses challenges for discussing recurrence risk, inheritance, reproductive options, and prenatal diagnosis with families in which this condition is being transmitted.

Much research has been done on mitochondrial disorders and the available reproductive options, such as mitochondrial replacement therapy; however, there have been few studies on the psychosocial implications of receiving a diagnosis of a mitochondrial condition. Genetic counselors are positioned to help families understand the implications of a mitochondrial condition and counsel them as they are making personal and medical decisions (Poulton, Finsterer, & Yu-Wai-Man, 2017). This project was designed to further explore the psychosocial implications following the diagnosis of MELAS, specifically focusing on women with a mitochondrial alteration and their experiences around family planning and the reproductive options that are

available for mitochondrial conditions. To recruit participants for this project, a letter and introductory flier was sent to MELAS families associated with the United Mitochondrial Disease Foundation (UMDF). A list of primarily open-ended questions was used in interviews with participants who have MELAS and was designed to elicit their experiences. The goal of this study was to explore the participants perspectives on reproductive choices, family planning, and genetic counseling. The transcripts of interviews were analyzed using thematic analysis to identify common themes. The results of this study may be useful to genetic counselors and other healthcare providers as they interact with and provide support to families who have MELAS.

1.1 Specific Aims

Specific Aim 1: Recruit women over the age of 18 who have a mitochondrial alteration resulting in MELAS

Specific Aim 2: Conduct interviews and analyze interview transcripts using thematic analysis to assess:

- Psychosocial implications and emotions following a diagnosis of MELAS and how this influenced further reproductive options and family planning.
- Experiences with genetic counseling and understanding of the genetic complexities associated with mitochondrial disease.
- The reproductive choices, family planning, and genetic counseling experiences of women with MELAS.

Specific Aim 3: Use the results to suggest additional beneficial resources for MELAS families, identify gaps in support, and assess ways genetic counselors can help improve outcomes for these families

2.0 Literature Review

2.1 Mitochondrial Disease

Mitochondrial disease encompasses a group of disorders that affect the function of mitochondria, which are organelles present in all cells of the body except red blood cells. Mitochondria are responsible for the production of cellular energy by oxidative phosphorylation (Alston, Rocha, Lax, Turnbull, & Taylor, 2017; Craven et al., 2017). Mitochondrial disease represents a group of clinically heterogeneous genetic disorders that can present with multisystem involvement, primarily affecting organs with high energy demands (e.g., brain, skeletal muscles, heart); there can be a wide range of symptoms that can present at any age (Craven et al., 2017; Gorman et al., 2016). Mitochondrial diseases can be caused by genetic mutations in either mitochondrial DNA (mtDNA) or nuclear DNA (nDNA), meaning that mitochondria are under dual genetic control (Ng & Turnbull, 2016). A major difference between the mitochondrial and nuclear genomes is that the mitochondrial genome is almost exclusively maternally inherited (Craven et al., 2017). The focus of this project is the specific mitochondrial disease Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes (MELAS). This condition is a mitochondrial disease caused by mutations in the mtDNA.

2.1.1 Mitochondrial Genetics

The mitochondrial genome is a double-stranded circular DNA molecule that is approximately 16.6kb in length (van den Aamele, Li, Ma, & Chinnery, 2020). There are 37 total genes that encode for 13 messenger RNAs (mRNA), 2 ribosomal RNAs (rRNA), and 22 transfer RNAs (trRNA) (Chiaratti et al., 2020). These genes are important for mitochondrial replication and transcription as well as for encoding proteins for the complexes of the electron transport chain, which is essential for the generation of cellular energy (Chiaratti et al., 2020; Zou et al., 2020).

A number of factors about the mitochondrial genome are unique, and recognizing these factors is essential to understanding maternally-inherited mitochondrial disease: (1) there are 100s to 1000s of copies of mtDNA present within each cell, (2) there can be a mixture of mutated and wild type mtDNA within a cell, which refers to a phenomenon called heteroplasmy, and (3) there are threshold effects in the presence of heteroplasmy that lead to variable clinical expression among different tissues (Ng & Turnbull, 2016).

2.1.1.1 Maternal Inheritance

Humans have two distinct genomes, nuclear and mitochondrial, that compose the total genetic instructions for growth, development, and functioning. While the nuclear genome is composed of genetic material from the mother and father, the mtDNA is almost solely maternally-inherited (van den Aamele et al., 2020; Zou et al., 2020). Oocytes can have up to thousands of mitochondria while sperm have a much smaller number of mitochondria (only dozens) (Chiaratti et al., 2020). Initially, researchers believed that this uneven distribution of mitochondria accounted for the maternal inheritance pattern, but it was later discovered that different species have varying

mechanisms to eliminate paternal mitochondria during fertilization; however, reasons for this process remain unclear. (Zou et al., 2020). Regardless, evidence shows that in most cases, only the oocyte contributes mtDNA to offspring during fertilization, and thus there is typically maternal inheritance of mtDNA related mitochondrial disease. A qualitative study of adults who are living with mitochondrial disease discussed that once the initial diagnosis was made in the family, individuals have been able to identify maternal families members who have also been affected by symptoms of the condition (Dimond, 2013).

2.1.1.2 Heteroplasmy, Threshold Effect, and Genetic Bottleneck

The phenomena of homoplasmy and heteroplasmy are key to mitochondrial genetics given that each cell can contain hundreds to thousands of mitochondria (Ng & Turnbull, 2016). Homoplasmy refers to when all of the mtDNA molecules are the same, while heteroplasmy describes the presence of two or more mtDNA genotypes within a cell, tissue, or organ (Zou et al., 2020). Mitochondrial DNA has a high mutation rate, approximately 10-17 times higher than the nuclear genome, which is a contributing factor, in addition to other factors like the genetic bottleneck effect, to increasing heteroplasmy levels over generations that may eventually become high enough to cause mitochondrial disease (Craven et al., 2017; Zou et al., 2020).

The threshold effect describes the proportion of mutant mitochondrial DNA at which a particular cell becomes vulnerable to a deleterious phenotype and can no longer produce sufficient amounts of energy to maintain cellular function (Zou et al., 2020). The threshold level that leads to clinical symptoms can be dependent on an individual's particular mutation and can vary significantly among different areas of the body with energy-intensive tissues (brain, skeletal, muscles, heart) being most vulnerable to clinical effects of disease (Craven et al., 2017; Gorman

et al., 2016). Additionally, higher levels of heteroplasmy are usually associated with more severe clinical manifestations (Craven et al., 2017). Prior to this critical threshold being reached, the wild-type mtDNA allows for enough energy production and prevents deleterious effects of the mutant mtDNA (Chiaratti et al., 2020).

In addition to threshold effects, there can be shifts in heteroplasmy levels both within and between generations. This can be explained by a concept known as the mitochondrial genetic bottleneck, which describes a reduction of a population of mtDNA in offspring/gamete (Craven et al., 2017). Research done on Holstein cows originally led to this mitochondrial genetic bottleneck hypothesis. Further studies on heteroplasmic pathogenic mtDNA mutation in humans provided support for this theory, although the exact mechanism of the mitochondrial genetic bottleneck has not been defined (Stewart & Chinnery, 2015). In the context of maternal transmission, the genetic bottleneck states that females pass down a limited number of mtDNA molecules; with random transmission of wild-type and mutant mtDNA molecules to offspring, mtDNA mutation frequencies can be quite different between a female and her offspring (Zaidi et al., 2019). Considering these mitochondrial genetic complexities, it becomes difficult to determine the risk to the offspring of a heteroplasmic female who carries a mixed population of wild-type mtDNA and mtDNA with a pathogenic mtDNA mutation. The genetic bottleneck can result in offspring who carry varying levels of heteroplasmy as compared to each other and that may be different from the heteroplasmy level of the mother. For example, a child could inherit a high enough frequency of mutated mtDNA to develop disease from a completely asymptomatic mother who has low heteroplasmy (Craven et al., 2017; Zaidi et al., 2019).

2.2 Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes

Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes (MELAS) is one of the most common maternally-inherited, multisystem mitochondrial disorders. This disorder is primarily characterized by neurological manifestations, but there can be a broad spectrum of features such as stroke-like episodes, dementia, epilepsy, lactic acidemia, myopathy, hearing impairment, and others (El-Hattab et al., 2015). A listing of the overall clinical symptoms and their frequencies in MELAS is as follows (El-Hattab et al., 2015):

- $\geq 90\%$: Stroke-like episodes, dementia, epilepsy, lactic acidemia, ragged red fibers, and exercise intolerance
- 75-89%: Hemiparesis, cortical vision loss, recurrent headaches, and hearing impairment
- 50-74%: Peripheral neuropathy, learning disability, memory impairment, recurrent vomiting, and short stature
- 25-49%: Basal ganglia calcification, myoclonus, ataxia, episodic altered consciousness, gait disturbance, depression, anxiety, psychotic disorders, and diabetes
- $< 25\%$: Optic atrophy, pigmentary retinopathy, progressive external ophthalmoplegia, motor developmental delay, cardiomyopathy, cardiac conduction abnormalities, nephropathy, and vitiligo

Affected individuals may present with a number of the following common initial symptoms: seizures, recurrent headaches, stroke-like episodes, cortical vision loss, muscles weakness, or recurrent vomiting (El-Hattab et al., 2015). A majority of individuals affected by MELAS present with symptoms between the ages of two and forty years of age, with 65-76% of affected individuals becoming symptomatic at or before age twenty (El-Hattab et al., 2015; Fan, Lee, Yue, & Chi,

2021). The prevalence of MELAS is reported to be approximately 0.2:100,000 in Japan, 1.41:100,000 in the north east of England, 2:100,000 in Sweden, 18.4:100,000 in Finland, and 236:100,000 in Australia (Fan et al., 2021). The epidemiological studies in Finland and England were based on case referrals and population census data. These prevalence estimates show a wide range likely because the data are based on an accurate count of case referrals among the total referrals at the collection center. However, complete ascertainment can still be difficult due to the phenotypic variability of MELAS. The true population prevalence is estimated be higher than these initial studies. The Australian study was conducted by collecting medical information and a sample for genetic testing for a large cohort of individuals in a defined urban area and thus may represent a more accurate assessment of prevalence (Manwaring et al., 2007).

2.2.1 Diagnosis

The phenotype of individuals with MELAS can be quite variable and may show some features that overlap with other mitochondrial or non-mitochondrial neurological conditions. Brain imaging, laboratory studies, and molecular genetic testing may be useful in establishing the diagnosis of MELAS. Stroke-like episodes are the most typical clinical manifestation of MELAS; other neurological manifestations include, dementia, recurrent headaches, altered mental status, cortical vision loss, hearing loss and motor weakness (Fan et al., 2021). Another cardinal feature of MELAS is lactic acidosis, and many patients with MELAS have elevated levels of lactic acid in both blood and cerebrospinal fluid (Fan et al., 2021). In the presence of suggestive findings, a diagnosis of MELAS can be established based on meeting clinical diagnostic criteria and identifying a pathogenic variant in one of the genes associated with MELAS.

2.2.1.1 Clinical Diagnostic Criteria

The most recent clinical diagnostic criteria for MELAS were published in 2012 by Yatsuga et al. The diagnostic criteria for MELAS is based on clinical findings of stroke-like episodes and evidence of mitochondrial dysfunction (Yatsuga et al., 2012). A definitive diagnosis of MELAS can be made with the presence of two findings from among clinical findings of stroke-like episodes and two items from evidence of mitochondrial dysfunction. MELAS should be suspected with the presence of at least three items with one item from clinical findings of stroke-like episodes and two items from evidence of mitochondrial dysfunction (Yatsuga et al., 2012).

- Clinical findings:
 - Headache

- Seizure
- Hemiplegia
- Cortical blindness
- Acute focal lesion
- Mitochondrial dysfunction:
 - High lactate levels or deficiency of mitochondrial-related enzymes
 - Abnormalities of mitochondria in muscle biopsy
 - mtDNA pathogenic variant related to MELAS

2.2.1.2 Molecular Genetics

MELAS is caused by mutations in the mitochondrial DNA (mtDNA); there have been at least 30 different mutations identified with about 80% of affected individuals having the m.3243A>G mutation in the *MT-TL1* gene (Fan et al., 2021). There have been other mutations within the *MT-TL1* gene reported to cause MELAS as well as other mitochondrial genes that have been associated with MELAS, but these causes are rare (El-Hattab et al., 2015).

It is important to consider sample type for genetic testing of MELAS given that mutant load can vary among tissue types. Mitochondria are present in almost all human cells, but the sample should preferably come from an accessible tissue, such as blood, urine, or buccal mucosa. Some studies have reported that urinary epithelial cells are the preferred sample type because this sample gives a more accurate and consistent level of heteroplasmy compared to other easily accessible tissues (de Laat et al., 2012). In addition, studies have shown that blood heteroplasmy levels decline by about 2% per year making blood a less accurate sample type for detection of mitochondrial disease, particularly as a person gets older (Grady et al., 2018).

2.2.2 Treatment and Prognosis

Treatment for MELAS is primarily supportive and requires a multidisciplinary approach which may include clinical genetics, neurology, cardiology, endocrinology, audiology, ophthalmology, physical and occupational therapy, psychology, and social work (El-Hattab et al., 2015; Fan et al., 2021). Following a diagnosis of MELAS, a comprehensive evaluation should be completed for potential multi-system involvement, as well as to develop a plan for regular follow-up visits (El-Hattab et al., 2015). Several medications and supplements are being used by MELAS patients, particularly L-arginine therapy to improve the frequency and severity of stroke-like episodes. Other studies have shown that Coenzyme Q₁₀ may be useful as symptomatic treatment for muscle weakness, fatigue, and lactate level, and L-Carnitine may be useful for patients with secondary carnitine deficiency (El-Hattab et al., 2015).

MELAS is a progressive condition with deterioration primarily related to the stroke-like episodes. Kaufmann et al (2011) reported an estimated overall median survival time of 16.9 years for fully symptomatic individuals from onset of focal neurological disease (Kaufmann et al., 2011). In contrast, a Japanese prospective cohort study of 96 individuals with MELAS showed a rapidly progressive course of disease with 20.8% of individuals passing away within a median time of 7.3 year from the time of diagnosis (Yatsuga et al., 2012). A more recent retrospective study of MELAS survival in a Chinese cohort found a high morbidity and mortality rate, particularly related to an acute stroke-like episode and/or status epilepticus. Of 28 deceased MELAS patients, the mean age of passing was 23.3 with decline occurring within 12 years of onset of the disease (Zhang et al., 2018).

A clinical trial of MELAS patients found a significant improvement in the 9-year-survival of individuals who were treated with oral or intravenous L-arginine as compared to individuals who were not treated with L-arginine. Additional studies found therapeutic efficacy of L-arginine for stroke-like episodes as well as improvements in the severity of disease progression in MELAS patients (Ikawa, Povalko, & Koga, 2020).

2.3 Reproductive Options and Mitochondrial Disease

There are reproductive options available for preventing transmission of genetic diseases. In the case of many genetic conditions that follow Mendelian inheritance, when a familial disease-causing variant has been identified, prenatal diagnosis or preimplantation genetic diagnosis are options for determining if a fetus or embryo will be affected by the genetic condition in the family; however, the utility and reliability of these options is more complex for maternally-inherited mitochondrial diseases (Zou et al., 2020). Prenatal testing for maternally-inherited mtDNA variants is not as straightforward because testing on a chorionic villus or amniocentesis sample may not accurately reflect the mutation load of all tissues at birth (Vento & Pappa, 2013). The same challenges arise when considering pre-implantation genetic testing; genetic testing cannot accurately predict the mutation load of all tissues in the resulting embryo. This process has also raised ethical concerns surrounding which embryo would be implanted to maximize the chance of having an unaffected child given a process that cannot accurately detect mutation load of the resulting embryo (Poulton et al., 2017). For example, Treff et. al. (2012) reported a case of pre-implantation genetic diagnosis for a 30-year-old woman with a 35% mutation rate for MELAS in

blood. A 12% mutation load embryo resulted in the birth of a baby boy who had several health complications and at 18-months-old showed heteroplasmic mutation loads of 46% in blood and 42% in urine samples (Treff et al., 2012). Due to multiple ethical and genetic concerns, the use of prenatal or preconception genetic testing remains a controversial technology that may not be reliable for the detection of a maternally-inherited mitochondrial conditions (Vento & Pappa, 2013; Zou et al., 2020).

2.3.1 Oocyte Donation and Mitochondrial Replacement Therapy

In the case of maternally-inherited mitochondrial conditions, the use of oocyte donation or mitochondrial replacement therapy may be more effective reproductive options for preventing passage of a mtDNA mutation from a mother to her offspring. Oocyte donation is a method of assisted reproductive technology in which a donated oocyte is fertilized with the intended father's sperm (Poulton et al., 2017). The resulting embryo may be carried by the intended mother, allowing for a woman with a mitochondrial condition to carry her child without transmitting her nuclear or mitochondrial genetic information. Oocyte donation represents a low-risk strategy for avoiding the transmission of a pathogenic mtDNA mutation; however, this technique does not allow for a woman who carries a mtDNA mutation to have a biological child (Poulton et al., 2017). The possibility for obstetric complications for women who carry a mitochondrial mutation is an important consideration when contemplating oocyte donation as a reproductive option. For example, one study reported complications such as preeclampsia, gestational diabetes, prematurity, and dysmaturity at a higher rate in women who carry the most common mutation causing MELAS as compared to healthy women (de Laat, Fleuren, Bekker, Smeitink, & Janssen,

2015). These results indicate that women with mitochondrial disease may be more likely to have high risk pregnancies and should be monitored more closely during pregnancy.

Mitochondrial replacement therapy is a technique that is a potential reproductive option to help women who carry a pathogenic mtDNA mutation have a biologically-related child (Zou et al., 2020). As of January 2021, there have been five different mitochondrial replacement techniques proposed with the similar goal of preventing vertical transmission of mitochondrial disease by transferring the nuclear DNA of the intended mother into a donor egg from which the nucleus/nuclear DNA has been removed (Sendra, García-Mares, Herrero, & Aliño, 2021; Vento & Pappa, 2013). The resulting embryo contains the nuclear genetic information of the biological/intended mother as well as cytoplasmic material that contains healthy mitochondria from the donor egg (Sendra et al., 2021). While this technique has shown promise in clinical trials for reducing the load of mutant mtDNA, there have also been clinical and ethical concerns raised around the use of mitochondrial replacement therapy. Some concerns from the scientific community include risks associated with assisted reproductive technologies and handling of an embryo in the laboratory, carryover of mutated maternal mtDNA molecules (approximately 1-5% of the total mtDNA), and undefined yet possible deleterious effects of mixing maternal nuclear DNA with the donor's mitochondrial DNA. The potential for carryover of mutant mtDNA molecules raises the possibility that even a small amount of such mtDNA could expand in future generations due to the bottleneck effect; therefore, additional research is focusing on determining an acceptable amount of carryover for mitochondrial replacement therapy with the lowest risk of deleterious effects and transmission to future generations. (Farnezi, Goulart, Santos, Ramos, & Penna, 2020; Sendra et al., 2021; Zou et al., 2020). There are also a number of ethical and legal aspects of mitochondrial replacement therapies that need to be considered, such as whether or not

mitochondrial replacement should be considered a form of germline gene therapy or if the term “three parent baby” is an appropriate representation of this technique. There continue to be public and political debates around the use of mitochondrial replacement therapy; however, it has been argued that the use of this technique is ethically justifiable given that for many mitochondrial conditions the only available treatment is supportive, with no curative treatments available (Sendra et al., 2021). In a study of 92 female carriers of a mtDNA mutation, 95% reported that the development of mitochondrial replacement therapy is “important and worthwhile,” and a majority of participants indicated support for the development of this assisted reproductive technology (Engelstad et al., 2016). In a survey of about 500 participants, one participant responded that they see an urgency for the FDA to approve the use of mitochondrial replacement therapy so that it can be taken advantage of before it is too late (Zilber & Yeske, 2020).

The United Kingdom became the first country to legalize the use of mitochondrial replacement therapies in 2015 with certain guidelines put in place to ensure the technique is utilized in appropriate situations. To meet the United Kingdom’s laws, a clinic must seek a license, be able to document that a prospective child has a significant risk to inherit a severe mitochondrial disease, and determine a plan for long-term follow-up care of children born by the use of this technology (Sendra et al., 2021). There was a child born in Mexico in 2016 as a result of mitochondrial donation that was reported to be a healthy 3-year-old as of the latest reported update in 2020. These results, although limited in number, do show the potential of this technique in clinical practice, although not much is known about parental attitudes towards this procedure (Vento & Pappa, 2013; Zou et al., 2020). A recent qualitative study in Canada explored attitudes around mitochondrial replacement therapy by interviewing experts, patients, egg donors, and the public. Results indicated that while there was support from participants for mitochondrial

replacement therapy, the responses varied about the possibility of implementing this into clinical practice considering ongoing debates and the current ban on mitochondrial replacement therapy in Canada (Noohi, Li, & Joly, 2021).

2.4 Genetic Counseling for Mitochondrial Disease

Genetic counselors are medical professionals who are specially trained to provide individuals and families with information on the implications of a genetic disorder to help them make informed decisions about their care (Poulton et al., 2017). The counseling process involves the genetic counselor explaining the clinical variability, inheritance, and reproductive testing options associated with mitochondrial disease that have been previously discussed, including heteroplasmy, the mitochondrial genetic bottleneck, and threshold effects. (Vento & Pappa, 2013). These factors can make genetic counseling challenging for mitochondrial disease, particularly due to uncertainty about prognosis and recurrence risk in the presence of a confirmed mitochondrial DNA (mtDNA) pathogenic variant (Poulton et al., 2017; Vento & Pappa, 2013).

In addition to the genetic complexities of mitochondrial disease that must be explained to families, there are often psychosocial issues, such as guilt, grief, and frustration, that may arise during a session. One study showed that adults with mitochondrial disease describe dealing with a significant amount of loss and changes to the expectations they had for their life due to the diagnosis of a mitochondrial condition (Noorda et al., 2012). Genetic counselors are trained to address these psychosocial issues and concerns with clients (Vento & Pappa, 2013). While research has been done on the genetic complexities and the available reproductive options, there

have been few studies that explore the psychosocial implications of receiving a diagnosis of a mitochondrial condition (Poulton et al., 2017). When discussing the role of the genetic counselor for families who have a mitochondrial condition, Vento (2013) states:

More immediate work is needed to achieve a better understanding of how patients and families deal with the uncertainty brought by this diagnosis and how well providers are able to facilitate coping strategies during the difficult diagnostic journey. Additional work needs to be conducted in order to better understand how this information is communicated to and understood by the families, and how it may affect their lives, health behaviors, and reproductive planning (p.249).

3.0 Manuscript

3.1 Background

Mitochondria are cellular organelles that play a key role in the generation of energy for the cell in the form of ATP (adenosine triphosphate); dysfunction of the mitochondria can lead to loss of essential energy and subsequently to a number of human diseases (Chiaratti et al., 2020; Ng & Turnbull, 2016). Mitochondrial diseases are caused by alterations in the mitochondrial and/or nuclear genomes (Vento & Pappa, 2013). Mutations in the mitochondrial DNA (mtDNA) follow a maternal pattern of inheritance and affect approximately 1 in 4,300 people around the world (Chiaratti et al., 2020). Maternally-inherited mitochondrial conditions are both clinically and genetically complex, causing unique challenges for families and healthcare professionals. There are up to 1000s of copies of mtDNA in a single cell, and pathogenic variants can be present in all copies of the mitochondrial genome in that cell (homoplasmy) or only in some copies of the mtDNA (heteroplasmy) (Ng & Turnbull, 2016; Poulton et al., 2017). When a pathogenic variant is present in the heteroplasmic state, the level of mutation must exceed a certain threshold for clinical symptoms to occur, which can vary in severity and systemic involvement (Poulton et al., 2017).

One of the most common maternally inherited mitochondrial conditions is Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes (MELAS). MELAS was first described in 1984 followed by the identification of its molecular basis and publishing of clinical diagnostic criteria (El-Hattab et al., 2015). This condition is a multisystem disorder with variable

age of onset and manifestations including stroke-like episodes, dementia, lactic acidosis, myopathy, recurrent headaches, hearing impairment, diabetes, short stature, and cortical vision loss (El-Hattab et al., 2015). The most common mutation causing MELAS is in a gene encoding a tRNA, *MT-TL1* m.3243A>G; this mutation is found in about 80% of individuals affected by MELAS. The prevalence of this condition is estimated to be 0.2:100,00 in Japan with the prevalence of the most common mutation estimated at 16-18:100,000 in Finland. Management of MELAS is largely symptomatic with involvement from a multidisciplinary team including clinical genetics, neurology, cardiology, endocrinology, audiology, ophthalmology, physical and occupational therapy, psychology, and social work. Management of the symptoms of MELAS may also include use of several medications and supplements (El-Hattab et al., 2015; Fan et al., 2021).

Genetic counseling for mitochondrial disorders typically includes discussions about recurrence risk and reproductive planning; however, the genetic and phenotypic complexity of mitochondrial disease make these conversations challenging (Poulton et al., 2017; Vento & Pappa, 2013). Options for preconception or prenatal diagnosis are limited for families who have a known mtDNA pathogenic variant causing a mitochondrial condition. For instance, there is limited utility of testing a prenatal sample, such as chorionic villi or amniocytes, for a mtDNA variant because those results may not accurately reflect the mutation load of all fetal tissues. Similar concerns arise when considering pre-implantation genetic testing (Vento & Pappa, 2013). For mitochondrial conditions such as MELAS, oocyte donation or mitochondrial replacement therapy may be more effective techniques for preventing the transmission of the condition from mother to offspring. Mitochondrial replacement therapy allows for a woman with a mtDNA mutation to have a biological child by transferring the nuclear DNA of the mother who has the mitochondrial disorder into a donor egg from which the nucleus/nuclear DNA has been removed (Sendra et al., 2021).

This technique is promising for families with a mitochondrial condition; however, it is not currently legal in the United States, and limited information is available about the parental attitudes regarding use of this technique as a reproductive option (Vento & Pappa, 2013). In a study of 92 female carriers of a mtDNA pathogenic variant, 95% reported that the development of mitochondrial replacement therapy is “important and worthwhile,” and a majority of participants indicated support for the development of this assisted reproductive technology (Engelstad et al., 2016). In a survey of about 500 participants, one participant responded that they see an urgency for the FDA to approve the use of mitochondrial replacement therapy so that it can be taken advantage of before it is too late (Zilber & Yeske, 2020).

The goal of this study was to further explore the psychosocial implications following the diagnosis of MELAS with specific focus on female experiences around family planning and reproductive options. Few studies have examined the implications of receiving a diagnosis of a mitochondrial condition and if/how the diagnosis influenced family planning (Vento & Pappa, 2013). Further, there is limited information on parental attitudes toward reproductive options for mitochondrial conditions, particularly mitochondrial replacement therapy. A recent qualitative study in Canada explored attitudes about mitochondrial replacement therapy by interviewing experts, patients, egg donors, and the public. Results indicated that while there was support from participants for mitochondrial replacement therapy, the responses varied about the possibility of implementing this into clinical practice considering ongoing debates and the current ban on mitochondrial replacement therapy in Canada (Noohi et al., 2021). Further evaluation of the perspectives and needs of families are critical for genetic counselors and other healthcare providers to recognize as they interact with and support families who have maternally-inherited mitochondrial conditions.

3.1.1 Thematic Analysis

Thematic analysis is a common qualitative method that allows for a flexible approach to analyzing and reporting themes within data (Braun & Clarke, 2006). Braun and Clarke state that thematic analysis can be applied within different theoretical frameworks; however, they also convey the importance of making the theoretical approach clear when utilizing thematic analysis. The analysis will either be (1) inductive or theoretical and (2) semantic or latent (Braun & Clarke, 2006). Identifying themes in thematic analysis can follow inductive analysis, which allows for data-driven coding that is not based on the researcher's theoretical interest in the topic. In contrast, theoretical coding is a more analyst-driven approach that uses the researcher's theoretical interest in the data to guide the analysis. Another decision involved in thematic analysis involves the level of theme identification. A semantic approach revolves around examining the surface meanings of the data while a latent approach focuses on identifying underlying ideas within the data (Braun & Clarke, 2006). This study was designed to gain a better understanding of the experiences of women who have MELAS, particularly related to their decisions around family planning and reproductive decision making. Therefore, the approach for this project was inductive analysis with semantic theme identification to assess psychosocial implications as well as reproductive choices, family planning, and genetic counseling experiences of women with MELAS.

3.2 Methods

3.2.1 Research Study Approval

The Institutional Review Board of the University of Pittsburgh approved this study (STUDY21100227- Appendix A). University of Pittsburgh Medical Center fiscal ancillary review requirements were met and approved the use of a third-party service for transcription of recorded audio from interviews (Appendix B). Verbal informed consent was obtained from all participants.

3.2.2 Participant Recruitment

The target population for this study included women over the age of 18 who have MELAS caused by a mitochondrial pathogenic variant. Women were recruited for this study because MELAS is a mitochondrial condition that is maternally inherited. The study focuses on the perspectives and challenges of women who may transmit this mitochondrial disorder, including psychosocial implications, particularly those associated with making decisions about reproductive options and family planning.

The primary recruiting was completed with the help of the United Mitochondrial Disease Foundation (UMDF). The Support and Education Associate at the UMDF was contacted by email to discuss the project and provided with a letter describing the study that could be reviewed for approval (Appendix C). The proposed project was then reviewed by the Science and Alliance Officer of the UMDF, and a signed letter of support for the project was provided in which the organization agreed to help facilitate distribution of recruitment materials to the members of the

organization through e-communications and/or member email lists (Appendix D). Recruitment materials that were circulated to UMDF members included an introductory letter providing additional details about the research study as well as a flier, both of which included contact information for the principal investigator (Appendices E, F). Potential eligible individuals were encouraged to contact the principal investigator if they were interested in participating in the study or for more information to help make a decision about participation. Three participants reached out via email to express interest in being interviewed for the study. The third individual who expressed interest in participating in the interview process was not eligible to participate because her diagnosis of MELAS was not confirmed despite extensive testing including molecular analysis and muscle biopsy. The participants were informed that following this initial contact to schedule an interview, their name or any identifying information would not be connected to any part of the research project. Participants were not compensated, and there were no direct benefits for their participation in this study.

Due to low recruitment with initial outreach, additional efforts were made to increase the number of participants. The UMDF was asked to re-circulate the recruitment letter and flyer to potentially reach any individuals who may have missed the original message. Finally, a modification was submitted to the Institutional Review Board to include snowball sampling as an alternate recruitment approach; any participant who agreed to be re-contacted during their initial interview would receive a follow-up email from the principal investigator asking for assistance in identifying other potential participants. The research participant would be provided with the recruitment letter and flyer and encouraged to pass it along to others who may be interested or eligible to participate in the research study. Additional attempts at recruitment through another support organization was not approved by the time the recruitment period had completed.

Snowball recruitment was approved by the Institutional Review Board; however, this approval was received outside of the allotted recruitment period and participants were not recontacted.

3.2.3 Participant Interviews

Participants were interviewed by telephone between January 2022 and March 2022 by the principal investigator, a genetic counseling student at the University of Pittsburgh. An introductory script was utilized to introduce the interviewer and the goals of the study (Appendix G). The interviewer proceeded to review a verbal consent script with the participant (Appendix H); consent to participate included permission to record and transcribe interviews for review of the conversations. Any identifying information from the transcripts was removed and a participant ID was assigned to each individual to preserve the confidentiality of the participant. All responses to interview questions were stored in a secure manner. An interview guide was developed primarily with open-ended questions, to elicit participants experiences related to a diagnosis of MELAS with particular focus on experiences with genetic counseling, family planning, and reproductive options. Initial drafts of the interview guide were reviewed by the supervisory team, including one supervisory team member who has extensive experience in conducting qualitative research. A final interview guide was developed and utilized in each of the two interviews (Appendix I); each interview lasted about one hour. Following each interview, the audio was sent to a third-party service, TranscribeMe, for transcription. The transcripts were reviewed and coded as the transcripts were received. As part of the consent process, each participant was asked if they were comfortable being re-contacted during the study period should the research team believe that

additional clarification or questioning was indicated during the analysis period; all participants agreed to be recontacted, if needed.

3.2.4 Thematic Analysis

The following six steps as described by Braun and Clarke were performed to analyze interview transcripts using thematic analysis (Braun & Clarke, 2006).

Becoming familiar with data: Following each interview, the audio files were uploaded to TranscribeMe for transcription. The files were then manually reviewed by the principal investigator while listening to the original recording to ensure accuracy and increase familiarity with the data. Prior to beginning the coding process, the transcripts were read in their entirety to begin to generate initial thoughts and impressions on the experiences of the participant.

Creating codes: Transcripts were coded by the principal investigator in Microsoft Word by using the comments feature. This method allowed the code to be linked to the specific quote from which it was derived. The initial codes remained as close as possible to the exact wording of the participant to preserve the meaning. The codes were then collected into a spreadsheet in Microsoft Excel to create a codebook of the data. The codebook contained a total of 207 codes that were identified from the two interviews conducted as part of this study. The principal investigator met regularly with the supervisory team to review the coded transcripts and codebook.

Identifying themes: Once the codes were generated in the codebook, they were sorted into broad categories to determine how codes combined into overarching themes. A thematic map was utilized to create a visual representation of the codes and to illustrate relationships among the codes. This process helped identify candidate themes from the data.

Examining themes: Quotes for each code were reviewed as themes were developed to ensure the data supported the nascent themes. Adjustments were made during this step to ensure codes and themes were grouped in a way that created an accurate representation of the experiences of the participants from the perspective of the principal investigator.

Describing and naming themes: The data set was reviewed and refined to create a finalized set of themes that best represented the data generated from interviews with participants.

Reporting the results: The following results and discussion sections comprise the produced report for this thematic analysis.

3.3 Results

3.3.1 Demographics

Two female participants who have MELAS were interviewed as part of this study. Both were married and in their 30s. Their relationship to the first person in the family diagnosed with MELAS differed. These details are summarized in Table 2.

Participant 1 is a 33-year-old married female who has some unexplained fatigue but is overall healthy. Her son passed away at 23 months from MELAS. Before he passed, her son had feeding intolerance, vomiting, hypotonia, and high lactic acid levels identified at 9 months that led to a genetic evaluation. There was no known prior family history of MELAS at that point and many healthy individuals in the family.

Participant 2 is a 39-year-old married female who is a physician and overall healthy. Her mother was diagnosed with MELAS later in life as compared to most individuals with MELAS. Her mother was 63 when she had her first stroke-like episode and 65 when she had her second stroke-like episode that led to the diagnosis of MELAS.

Table 1. Participant Demographics

Participant ID	Age	Relationship Status	Relationship to family proband
1001	33	Married	Mother
1002	39	Married	Daughter

3.3.2 Themes Identified in Analysis

Four themes emerged from the interviews with these participants: impact of the range of severity of MELAS, familial implications and concern for family, intellectual curiosity and desire for knowledge, and implications for family planning and reproductive decision making. These themes are summarized in Table 3.

Table 2. Summary of Identified Themes

Theme	Description
Impact of the range of severity of MELAS	Participants describe a range of symptoms that their family members experienced leading to the diagnosis of MELAS. They also discuss connecting other health concerns in the family to MELAS once they learned about the diagnosis.
Familial implications and concern for family	Participants described concern for their family members when they learned of the genetic diagnosis and the responses they received when attempting to communicate information about the diagnosis to family members.
Intellectual curiosity and desire for knowledge	Participants talked about their experiences with genetics providers and some of the resources they were given around the time of the diagnosis. They also discussed wanting to learn as much as possible about the condition.
Implications for family planning and reproductive decision making	Participants described a desire for children and the mental/emotional struggles that they experienced after receiving the diagnosis. They described a significant loss of reproductive hope with the diagnosis.

3.3.2.1 Impact of the range of severity of MELAS

The range of severity of the condition appears to have a significant impact on the participants as they experienced a family member exhibiting classic or more mild symptoms of the condition. The participants described a variety of symptoms that their family members experienced prior to the diagnosis and how health concerns were connected to MELAS once the diagnosis was confirmed. This theme refers to the types of MELAS symptoms the participants' family members experienced and the ways in which these health concerns impacted the family. One participant discussed how she learned about the condition and the diagnostic journey that led her family to a diagnosis of MELAS:

“And we actually found out about the genetic disease because of [son with MELAS]. Obviously, you probably know that MELAS is a generational thing passed on by the mother, but no one until [son with MELAS] has ever had any symptoms... So throughout

[son with MELAS's] life, we went through all the testing of everything and eventually led us to genetic testing, which is where we learned about the MELAS. He was nine months old when we started the whole genetic process and he passed at 23 months.” -1001

This participant described some of the features of the condition that her son experienced:

“He always had feeding intolerance, so lots of vomiting, discomfort. And then he ended up not taking anything by mouth, so he was tube-fed. He was delayed quite a bit and then like muscle tone issues, so hypotonia. I'm trying to think what else. They did kind of the big trigger for them when he was nine months old with his lactic acid levels in his blood.” -1001

The other participant shared a different experience with a family member being diagnosed with MELAS:

“And the way that I got diagnosed is my mom was diagnosed pretty late in life, actually. Relative to other people with MELAS. She was 63 when she had her first stroke-like event. And then the second one when she was 65 and that's when she got diagnosed... And sure enough, her lactate came back high. And when the stroke neurologist conferred with my brother, who's still in neurology training, they pretty much had a diagnosis for her because on imaging, it looked kind of classical.” -1002

The participants also described what it was like to have a family member diagnosed with MELAS and begin to connect other health concerns in the family to this diagnosis:

“My mom, who obviously was also affected, has had severe health issues her whole life, but they've never connected it back. And I was doing some research on her before we knew this about [son with MELAS] and MELAS did come up. And when I learned that it was

kind of shocking... And then she also was recently diagnosed with a type of dementia, and that kind of triggered more research, which led to the MELAS.” -1001

Participants were also able to recognize more mild features of the condition in their family members once they were aware of the diagnosis:

“We just didn't know because they were kind of common things that just run in the family. So, hearing loss is there, diabetes is there... So as far as I know, that's the only kind of thing that's pretty serious diabetes and its pretty common to have.” -1002

One participant highlighted the range of severity of the condition by discussing the heteroplasmy levels of herself and her unaffected child versus the heteroplasmy level of her son who passed away from MELAS:

“I mean, I do experience a little bit of fatigue that's not really explained. So, I kind of attributed back to that, but nothing major... So, when they took the heteroplasmy levels of this, his was very similar to mine. So, we were in the 20s. So, we're quite low. And then [speaker's son with MELAS] was at 90%.” -1001

A participant shared that with the initial diagnosis, the severity of the condition was not well communicated to her and her family by healthcare providers:

“Because I remember when we were first diagnosed, the severity of it didn't come across. I mean, [son with MELAS] was doing well-ish. I was fine and my family was fine. So, it wasn't communicated well that way.” -1001

One participant explained how this range of severity impacted her family members' perception of the diagnosis:

"I honestly think, for the most part, people were like, "Whatever, we're fine," because they haven't been affected. And I mean, [speaker's son with MELAS] was severely affected. So, there's that range where they're not quite ready to accept that." -1001

3.3.2.2 Familial implications and concern for family

This theme refers to the participants describing concern for their family members when they learned of the genetic diagnosis and the responses they received when attempting to communicate information about the diagnosis to family members. Recognizing that the participants were expressing concern for family members related to the MELAS diagnosis, they were asked about communication within their families about the diagnosis. Both participants shared that they had conversations with their family members and informed them about the diagnosis, but the participants were met with a range of reactions from family members, including denial and lack of acceptance. One participant talked about having a well-educated family with many physicians, but still having difficulty speaking with her family members about the condition:

"And the conversation is kind of weird. There are a lot of doctors in my family. My uncle is a doctor, so my mom's sister's husband is a retired pediatric emergency doctor. So, he's got medical training, and he kind of acts for my aunt. So, there's no frank discussion about MELAS. Like my dad basically said, "Hey, my mom has this you might want to check out [speaker's aunt]. And it was something that was met with denial at first when we talked to him, and he's a doctor. He said, "No way she has this. No way." And then she actually had it." -1002

The other participant shared that she informed family members about the genetic diagnosis, but many of them did not show concern, particularly because there was no prior family history of MELAS in this family and many individuals are healthy:

“I mean, my mom was my primary concern because she has had health issues. Everyone else in our family is quite healthy. So, I definitely informed them of this, but they didn't show concern... I think there are still many of my family members who kind of just think they don't have it, even though they have to have it, my grandmother being one of those.”

-1001

One participant described family members eventually coming to an understanding of the diagnosis but choosing not to seek care:

“And they've come around to kind of understanding that, but they're not so on board with giving her [maternal aunt] treatment. And I'm not her doctor. And my parents are definitely not. So, we've taken the approach of we love them very much, but we can't force you to take arginine or any of the cocktails that we take...” -1002

While some family members have met the diagnosis with denial or lack of acceptance, others refuse to talk about symptoms or listen to information about the condition, as highlighted by this participant:

My mother's sister's kids, she has two sons, and one of them is a neurosurgeon. He has a tremor, but we don't really talk about it if he has symptoms or not... And then in terms of my uncles, they just don't want to hear it. Like they're not medical at all. They don't want to hear it.” -1002

When asked if her family members who are hesitant to discuss the diagnosis or exhibit some denial about the diagnosis would be willing to meet with a genetics specialist to learn more about the condition, one participant stated:

“And if they are not open to it, I can't really force them to look.” -1002

3.3.2.3 Intellectual curiosity and desire for knowledge

This theme highlights the participants experiences with genetics providers and some of the resources they were given around the time of the diagnosis. It also defines the participants' desire to learn as much as possible about the condition. Both participants expressed a strong desire to learn about MELAS once the condition was identified in their family, and the need for healthcare providers to supply them with better resources to start their research. These participants discussed an interest in research and educational information. In addition, they both received information about the United Mitochondrial Disease Foundation (UMDF) support organization. The participants described doing self-driven research and having difficulty researching this complex genetic condition:

“Looking back at it, the only real resource that I was given was the UMDF website and some of the things that they offer. But I think looking back, I really would have liked just some places to start my research because, I mean, as a mom and it's affecting my other son, myself, my mom, that kind of stuff. I really wanted to learn as much as I could, and I had to dig for that myself. And it was kind of difficult.” -1001

With the medical background of one participant and her family, she discussed doing much research on her own to achieve a satisfactory level of understanding of the condition:

“At the level that we are in terms of the UMDF, it was a little too basic. It felt like they are supportive in terms of support like a support group does. But in terms of the level of understanding that we wanted for our family, a lot of it we had to do on our own. With a lot of medical training, right? There is a lot of medical training that we have.” -1002

One participant even described this search for knowledge when asked about how she coped with learning about the diagnosis of MELAS:

“I mean, in the beginning, I definitely overloaded myself with research, learning as much as I possibly could...” -1001

Around the time of the diagnosis in their family members, these participants saw different genetics providers. One participant and her family met primarily with a geneticist and she discussed the experience:

“She's the one who sort of recommended looking at UMDF initially, but she gives us scholarly articles, too... And in terms of her relationship with my family, she provides what she can.” -1002

Even though the geneticist was able to provide some resources for this participant and her family, she reported doing a lot of self-driven research:

“And then each time we see her, if we ask for more, we'll get more because an issue comes up. But we did all the research ourselves. My brother is a clinician researcher. So, we used our education to be able to inform ourselves, and we use our intellectual curiosity to do that.” -1002

Another participant described the interactions that she and her family had with a genetic counselor as part of the diagnostic process:

“We were given some resources, which were pretty helpful, but definitely, obviously, I felt comfortable contacting her if I had questions, but just in the general conversations, I didn't gain a whole lot of knowledge from her.” -1001

On the other hand, one participant explained how she could see the value in being referred to a genetic counselor as part of the diagnostic process:

“...and I was not offered a genetic counselor. I just went to see the genetic doctor. I was not offered a counselor. I was not offered somebody to kind of talk it out. And during my medical training, I never shadowed anybody like you. I don't know what it's like to be in your shoes or to see what you go through on a day-to-day basis. So, I think the patients need to trust in information that you can provide or that your team can provide. And not everybody's lucky enough to have a counselor because I certainly didn't get one.” -1002

One participant's advice to a family who has just received a new diagnosis revolved around identifying informative resources. She stated:

“I would probably stress doing your own research, but also reaching out for helpful resources right away... So, I think just doing your own research and realizing the severity and asking questions around that would probably be my biggest thing.” -1001

3.3.2.4 Implications for family planning and reproductive decision making

This theme depicts the participants' desire for children and the mental/emotional struggles that they experienced after receiving the diagnosis. It also reviews the significant loss of reproductive hope that participants described following the diagnosis. The participants in this study shared that they always wanted children and the diagnosis of MELAS had significant impacts on family planning and reproductive decision making. Participants described the mental and

emotional struggles they faced after receiving the diagnosis of MELAS, and the significant loss of reproductive hope they experienced following the diagnosis. One participant shared:

“Yeah, I always wanted to have kids. I didn't really have a huge preference, but two or three, something like that. Not too big, but not small.” -1001

“I always thought that I'd have two kids. My husband is one of three, so we always thought we would have more. It's a little hard for me.” -1002

With the diagnosis of MELAS, the participants described a strong feeling of not wanting to pass the condition on to more children:

“And then when we got the diagnosis, we definitely were like, “Nope, we can't pass this along to any other children.” -1001

“And it really affected my ability, once she got diagnosed, to have other kids... I knew as soon as she got diagnosed, I knew I couldn't have any more kids because I'd be passing it along. It was hard for me to understand that...” -1002

When asked about conversations with their reproductive partner, one participant explained:

“So, my husband and I are pretty good at communicating. And my husband is not a doctor. He's an engineer and very much understands what's going through this. He has stepped in wholeheartedly to be mom's caregiver if I can't or if dad can't. My husband is a great guy. So, he understood. It wasn't a decision that we had to make right? It was more of a he understood that it just can't happen.” -1002

Another participant shared that her husband was even more against having more children after the diagnosis of MELAS because of concerns for impacting a potential daughter's future reproductive decision making:

"I think he's even more against having another child because the thought of explaining to a daughter, like, "Hey, you probably shouldn't have kids because you're going to give them this, but we chose to have you even though we knew we were going to give it to you." So, he definitely is very against having any more kids." -1001

One participant shared that since her son's death, she has had some uncertainty about her decision to not have other children; however, because of her position in the family structure, she has the potential to stop this disease in the family and feels a responsibility to do so:

"Since his passing, I've had some wavering that I want to have another child. But again, you go back to not wanting to pass this along anymore because he would-- basically because the male can't pass on this gene, it stopped with me. I'm the only girl on my entire grandmother's side of the family. So, if I would have a girl, we continue this terrible disease." -1001

Participants described the challenges they faced with this overwhelming loss of reproductive hope. As one participant stated:

"I think it's so great that you're going through this because there was an element of, for me, it was very challenging mentally to understand that I couldn't have another kid... I understood that it was more the loss of reproductive hope or reproductive longevity. That was really, really hard for me mentally, and for me to admit that-- I was almost suicidal when I found out... and I was like, "Irrationally, I'm having thoughts that are not good." But it was an adjustment reaction. And I look back, and I'm like, "Wow. That is not me as a person. I'm not one to have-- I don't have depression or anxiety... But these were thoughts that I had never had ever in my life before when I got diagnosed because of my inability to have another kid." -1002

One participant talked with her reproductive partner about some alternate ways to complete their family, but nothing felt like a perfect option for them and ultimately, they came to the realization that their family was complete with their son:

“And then we talked about adopting, and it just wasn't the time... And also on a personal level, I'm not sure that we would want to adopt a kid because I had never thought about it before. I never thought about adoption. And then we talked about a surrogate. That wasn't an option. We just didn't want to because we were so happy with our son... The act of wanting a kid so, so bad for so long, and then when you actually have them, they're a lot of work, and I love it... I think that we understood that it doesn't have to be two kids exactly or three kids exactly.” -1002

The participants reported that they did not have any conversations with healthcare providers about reproductive decision making given the diagnosis of a mitochondrial condition. One participant was given a resource on mitochondrial replacement therapy, and the other stated she had heard a little bit about it. Both participants were asked about attitudes towards the possibility of mitochondrial replacement therapy should it become legal in the United States:

“So, I'm definitely a person of faith, and that impacts my thought process on some of that. I definitely think it's a cool thing. It's exciting, but I don't know if it'd be something I would personally do.” -1001

“I have the articles.. but I just didn't feel like it was an option. I mean, it's not legalized in the United States. There wasn't a lot of evidence about it. Do I really want to go to another country to get pregnant? How do I follow up?... I can't imagine the cost because we did look at IVF. So, we were all set to do IVF. I understand the cost. It is a very expensive process.” -1002

One participant explained that if she would have known about the diagnosis of MELAS sooner, it would have made childbearing decisions more difficult. She refers to her son as a miracle child because he came at the right time, just prior to learning about the diagnosis in the family:

“If I had known in advance before I found out, I think my childbearing decisions would be different. I think it would have been a much harder decision... Because I feel like that's why I call him a miracle child is he came at the right time... Because otherwise I probably wouldn't have kids. Or maybe I would have thought about adoption much earlier, or maybe doing mitochondrial donation because I don't know. I have no idea what I would do.”

-1002

3.4 Discussion

The goals of this study were to interview women who have MELAS and analyze transcripts to assess psychosocial implications following a diagnosis of MELAS as well as experiences with reproductive choices, family planning, and genetic counseling. Themes were identified from the qualitative data that may be useful for suggesting beneficial resources for MELAS families, identifying gaps in support, and assessing ways genetic counselors can help improve outcomes for families. This study identified four themes related to the impact of a MELAS diagnosis on women and their families: impact of the range of severity of MELAS, familial implications and concern for family, intellectual curiosity and desire for knowledge, and implications for family planning and reproductive decision making. Higher recruitment numbers were anticipated for this study than what was achieved; this would have possibly allowed for the identification of a broader range

of experiences of women with MELAS. In addition, there was a significant amount of congruence observed among the experiences of the two individuals who participated in this study, which limited the issues or concerns that were discussed in the results of this study. As such, the discussion of results represents preliminary findings from the two interviews that were analyzed using thematic analysis and lays the groundwork for ongoing development of these and perhaps other themes for this patient population. Data saturation was not achieved in this study due to insufficient sample size; a larger sample size could have led to exhaustion of ideas raised by participants, which is important to ensure that there are no additional themes or useful information to include in the research report and to integrate into development of material for patients and families. Identification of as many themes as possible is important for understanding the experiences of women with MELAS, which may be useful to better recognize the needs of women and their families following a diagnosis of MELAS.

3.4.1 Impact of the range of severity of MELAS

The participants in this study had different experiences with age and primary symptoms of the first person in the family who was diagnosed with MELAS and thus brought the family to attention. These experiences highlight the nature of mitochondrial disease, which can present with varying symptoms ranging in severity, even among members of the same family. The responses of the participants in this study are consistent with other studies in that prior to the initial diagnosis, individuals or family members may have been unaware that they were experiencing symptoms that are considered features of mitochondrial disease (Dimond, 2013). Once the initial diagnosis was confirmed, both participants were able to notice other health concerns in the family that could

be connected to MELAS. Further, one participant shared that there was no reason to suspect a mitochondrial condition because some symptoms (e.g., hearing loss, diabetes) were just common health conditions that tend to run in the family. A previous study has shown that many individuals diagnosed with a mitochondrial DNA-mediated condition are able to identify maternal family members who have been affected by symptoms of the condition, and that the range of symptoms associated with mitochondrial conditions can allow for some normalization of features among family members that are actually consistent with the mitochondrial disease in the family (Dimond, 2013). The study participants talked about their experiences with severe illness in a mother and child, and their experiences with a MELAS diagnosis seem to align with the experiences of other adults who have been diagnosed with a mitochondrial disease. For example, one individual in a study of adults with mitochondrial disease explained that seeing his brother fall seriously ill and pass away from mitochondrial disease raised concern for his own future (Dimond, 2013). Preliminary findings from this study concur that witnessing a close family member rapidly decline from severe mitochondrial disease impacts how one perceives the diagnosis and its implications.

3.4.2 Familial implications and concern for family

The participants in this study described how the range in severity of MELAS affected acceptance of the diagnosis among family members, particularly older family member who are mildly affected or seemingly unaffected. The responses of these participants are consistent with research on patient and family experiences with a diagnosis of a mitochondrial condition, which suggests that there could be a generational component to reactions to a new diagnosis within a family (Dimond, 2013). The participants in this study were both of reproductive age and discussed

a strong desire to have children. Research on individuals diagnosed with mitochondrial disease concludes that the generation of adults in the family who are relatively healthy and not actively considering reproductive choices may not perceive the diagnosis as problematic (Dimond, 2013). This appears to be consistent with the experiences of the participants in the study who were met with reactions of denial or lack of acceptance when discussing the diagnosis with family members, particularly individuals who were older or asymptomatic. Future studies could explore the experiences of additional individuals of reproductive age and gauge level of concern about the condition as compared to older family members. Both participants discussed an understanding that they needed to inform other family members of the genetic diagnosis, but they could not force family members to get genetic testing or seek care.

In addition, the participants in this study had a close relationship with a first-degree family member who had severe symptoms and received the initial diagnosis of MELAS within the family; one participant was involved with the initial diagnosis as a mother and the other was involved with the initial diagnosis as a child. The participants had close relationships with the family member who was diagnosed with MELAS and were actively involved in hospital stays and doctors' appointments that eventually led to the diagnosis of MELAS for their family member. These experiences elevated their concerns for others in the family who may be at risk for complications related to MELAS. Studies on individuals with mitochondrial disease indicate that the diagnosis may not be as shocking or concerning for individuals if the diagnosis has been known within the family (Dimond, 2013). The participants described significant concerns for themselves, and their families related to this new diagnosis. It is also possible that normalization of symptoms within the family may reduce concern about the diagnosis for some individuals in the family (Dimond, 2013).

3.4.3 Intellectual curiosity and desire for knowledge

In a study of adults who were diagnosed with a mitochondrial condition, the researchers found that the participants did not talk much about the technical or genetic aspects of the condition during their interviews (Dimond, 2013). However, the participants in this study discussed intellectual curiosity about MELAS and a strong desire for as much knowledge as possible about the condition. One participant has a medical background which may have been driving some of this need for didactic information about MELAS, but the other participant also shared wanting to learn as much as possible about the condition and described her desire for acquiring information as an approach to coping with the diagnosis. These findings are supported by a study of adults with mitochondrial disease that described a need for professional support as an important factor for coping with and learning to adjust to the diagnosis. Participants discussed limitations in the information that they received about mitochondrial disease and talked about the need for information pertaining to all aspects of living with a mitochondrial condition (Noorda et al., 2012). Both participants were provided information about a support group as part of their diagnosis, but they both described a need for better resources to start their own research on MELAS. This preliminary evidence may indicate an area for genetics providers to improve the ways in which they support and take care of MELAS families. The Mitochondrial Disease Community Registry collected survey data to characterize mitochondrial disease from the patient perspective and found that participants reported that they want healthcare providers to know that one of the most important factors for their care is effective clinician-patient communication and better knowledge about the disease among medical professionals. The author's highlight the availability of

educational resources as a way of facilitating and improving communication between clinician and patient (Zilber & Yeske, 2020).

3.4.4 Implications for family planning and reproductive decision making

The participants in this study described the significant impact of the diagnosis of MELAS on their family planning and reproductive decision making. The severity of the diagnosis, concern for their family, and their understanding of MELAS all contributed to a firm decision to not have additional children after the initial diagnosis of MELAS was made in the family. Due to a strong commitment to not pass down MELAS to more children, participants described the emotional and mental struggles they had with the loss of reproductive hope and longevity that accompanied a MELAS diagnosis. The responses from the participants in this study provide preliminary evidence that a diagnosis of MELAS can have significant impacts on reproductive planning for a family. These results are consistent with a study of adults with mitochondrial disease which found that there is an overall theme of loss in various areas of life following a diagnosis, including the loss of desire to have children or to have more children. In addition, these future losses were described by some participants as being the most prominent and difficult to process due to changes in the expectations they had for their life (Noorda et al., 2012). Healthcare providers should take perspectives such as these into consideration when discussing a diagnosis with families, particularly genetic counselors who have the training to facilitate conversations about reproductive planning for families with a genetic diagnosis.

Participants in this study discussed conversations they had with their reproductive partners about alternate ways to complete their families, such as adoption or surrogacy. In addition, Vento

& Pappa state that not much is known about the parental attitudes toward mitochondrial replacement therapy, so the participants in this study were asked about their thoughts on the use of mitochondrial replacement therapy for women who carry a mitochondrial mutation (Vento & Pappa, 2013). Both participants expressed intrigue and excitement at the possibility, but they also expressed some moral concerns related to faith as well as concerns about cost and legalization of the procedure. The responses from this study should be part of a larger conversation about parental attitudes related to mitochondrial replacement therapy as a possible reproductive option. In a study of 92 female carriers of a mtDNA mutation, 95% reported that the development of mitochondrial replacement therapy is “important and worthwhile,” and a majority of participants indicated support for the development of this assisted reproductive technology (Engelstad et al., 2016). Another study found that a participant sees an urgency for the FDA to approve the use of mitochondrial replacement therapy so that it can be taken advantage of before it is too late (Zilber & Yeske, 2020). A qualitative study in Canada conducted interviews with mitochondrial disease experts, patients, egg donors, and the public. Results indicated that while there was support from participants for mitochondrial replacement therapy, the responses varied about the possibility of implementing this into clinical practice considering ongoing debates and the current ban on mitochondrial replacement therapy in Canada (Noohi et al., 2021). In addition, this study found that patient motives for implementing mitochondrial replacement therapy included psychosocial considerations; patients reported emotional distress and profound impacts on mental health as a result of a mitochondrial disease diagnosis. Patients who were interviewed who had no children asserted that a diagnosis of a mitochondrial condition was the primary reason for changes to family planning (Noohi et al., 2021).

3.4.5 Limitations

3.4.5.1 Sample Size

A major limitation of this study is the small sample size; therefore, results may not be generalizable to the experiences of all women with MELAS. Additionally, the women who participated in this study were married in heterosexual relationships and had strong desire for children. The participants were of reproductive age and actively making decisions about childbearing, which limits the diversity of the study population. The views that appeared to be common between these women may reflect the commonalities between their particular perspectives on having children and the experiences they had with family planning given the diagnosis of MELAS. There was a significant amount of congruence observed among the experiences of the women in this study; this is important to consider because the study did not capture the experiences of all types of women with MELAS, including individuals who are not interested in having children or who are younger and not actively making family planning decisions. In addition, insufficient sample size did not allow for sufficient analysis of the qualitative data; further research with additional participants is necessary to reach data saturation. Data saturation is important to ensure that there are no additional themes or information to include in the research report and is necessary to develop a robust understanding about the reproductive choices, family planning, and genetic counseling experiences of women with MELAS.

3.4.5.2 Recruitment Methods

All the participants in this study were recruited from the United Mitochondrial Disease Foundation member lists. This is a support organization to which families with mitochondrial

disease are commonly referred; however, women with MELAS who are not members of the UMDF, not active with the UMDF, or active with another support organization would not have received notification about this research study. While there may be significant overlap in members of various support groups for mitochondrial conditions, outreach to other support organizations is a possible way to reach a broader audience for future studies with this population. It is also possible that reaching out to hospital systems with large mitochondrial disease centers would be a more effective way to reach potential participants.

3.4.6 Future Directions

The results of this study suggest that a diagnosis of MELAS may impact perspectives on family planning and reproductive decision making. Additionally, the results provide preliminary evidence about the perspectives of women after receiving a diagnosis of MELAS; however, further work needs to be done to generate additional data from a variety of experiences and to reach data saturation. Future studies may continue this work and focus on gathering more participants to create a more expansive data set. Possibilities for accessing a larger study population include reaching out to additional support organizations, such as MitoAction, or hospital systems with large mitochondrial disease centers, such as the Children's Hospital of Philadelphia. Allowing a longer recruitment time period might also yield additional interviews as only 8 weeks were available for interviews. Additional data may be useful in determining ways in which healthcare providers and genetic counselors can better support and take care of families with MELAS as well as determine comprehensive resource needs for this community.

3.5 Conclusions

A goal of this project was to explore the patient experience with a diagnosis of MELAS and how the diagnosis influenced personal and family decision making. The preliminary results from this study suggest that the range of severity of MELAS symptoms can create challenges in communicating with family members about the condition. In addition, participants demonstrated a high level of awareness and understanding about MELAS that came from a desire for knowledge about the condition. These participants indicated that they needed more resources and places to start research about the condition, which may indicate an area for healthcare providers to improve patient care around the time of an initial MELAS diagnosis. Further, this study showed supporting evidence that a diagnosis of MELAS can have significant impacts on the mental and emotional state of an individual, and both participants talked about how the diagnosis impacted their ability to have more children. They talked candidly about a sense of responsibility to not pass a severe condition on to any additional children, and this came along with a loss of reproductive hope that they had to learn to cope with as part of the diagnosis. This information can further define parental feelings around a diagnosis of MELAS and guide conversations about family planning considerations. This was a study that attempted to further explore the parental attitudes of mitochondrial replacement therapy with families who have MELAS; additional conversations need to take place to gain a deeper understanding regarding the considerations around the potential use of this assisted reproductive technology. Future studies may use a framework such as the one demonstrated in this study to further examine the impacts of a diagnosis of MELAS and ways to improve the care that is provided to MELAS families.

4.0 Research Significance to Genetic Counseling and Public Health

The goals of this study were to interview women who have MELAS and analyze transcripts to assess psychosocial implications following a diagnosis of MELAS as well as experiences with reproductive choices, family planning, and genetic counseling. Themes were identified from the qualitative data that may be useful for suggesting beneficial resources for MELAS families, identifying gaps in support, and assessing ways genetic counselors can help improve outcomes for families. The study was limited by low recruitment; however, preliminary results highlighted psychosocial concerns and family implications related to a diagnosis of MELAS. This information can guide future research on ways in which healthcare providers and genetic counselors can improve practices related to giving a diagnosis of MELAS as well as considerations for supporting and taking care of families with MELAS. This type of study could lead to survey development, which could be used to survey a wider population to elicit perceptions and experiences of those who have received a diagnosis of MELAS. In turn, this information could be used to develop materials specifically designed to support women and their families as they deal with the impact of this diagnosis.

The perspectives of the participants in this study are important for assessing genetic counseling practices. The participants in this study described significant impacts to their thinking about having more children following the diagnosis of MELAS, and they also discussed not having conversations with healthcare providers about reproductive options for mitochondrial conditions. Genetic counselors have the training necessary to facilitate conversations about family planning and should be an available resource to help individuals navigate difficult decisions that impact the

family. Future research that incorporates more patient experiences may enhance the genetic counseling practices as more is learned about the implications of a MELAS diagnosis on reproductive decision making.

During their interviews, participants were asked about their experiences with a diagnosis of MELAS, and they described some sources of support that were helpful as well as areas that could be improved. One consideration was a concern for other family members who may also be impacted by the genetic condition. Participants described encountering some difficulty having conversations with family members about this complex condition, especially if the family member was seemingly unaffected. Genetic counseling practices often include providing individuals with a family letter about the diagnosis; the goal of this practice is to have a summary of important considerations and next steps that individuals can provide to their family members who are interested in pursuing genetic testing or seeking additional care. The participants also described being provided with information about a support organization, which is consistent with genetic counseling practices of providing resources and support organizations to families who are given a genetic diagnosis. Based on the preliminary results of this study, current genetic counseling practices like family letters and information about support organization are likely to be helpful and valuable for MELAS families.

The preliminary results from this study can also influence public health practices. A core tenet of public health is effective communication and health education for all people in all communities. The participants in this study exhibited a high level of knowledge around the complexities of MELAS, but they expressed a need for accessible research and additional educational materials. This indicates an area in which healthcare providers and genetic counselors can improve the care that they are providing for MELAS families. Support organizations such as

the United Mitochondrial Disease Foundation (UMDF) work to develop patient friendly resources; providers can work with organizations such as these to identify the best educational materials to provide at the time of initial diagnosis. Future studies could also explore the specific types of information and resources patients are seeking or explore satisfaction with and effectiveness of the educational materials that are being provided to MELAS patients.

Another major component of public health practice is ensuring equitable access to services. It is important to consider socioeconomic disparities that exist in terms of access to expensive technologies such as assisted reproductive techniques. Even if reproductive options like mitochondrial replacement therapy are legalized in the United States, there are major concerns about realistic implementation of these services for families with mitochondrial disease. In fact, one participant commented on concerns about the high expense of assisted reproductive technologies and how that may be a barrier for families accessing services that may allow them to have a biological child. Further, one participant discussed not being offered genetic counseling services as part of her diagnosis of MELAS, and she explained that she could see the value of being able to talk through the diagnosis and implications for her family with a genetic counselor. Exploring the accessibility of genetic counselors and current barriers to genetic counseling services should be considered as a component of ensuring equitable care for individuals who are diagnosed with a genetic condition.

Appendix A IRB Approval Letter

IRB Application submitted 12/17/2021



EXEMPT DETERMINATION

Date:	January 4, 2022
IRB:	STUDY21100227
PI:	Maria Rhine
Title:	Exploring the Reproductive Choices and Family Planning Experiences of Women who have MELAS: A Qualitative Study
Funding:	None

The Institutional Review Board reviewed and determined the above referenced study meets the regulatory requirements for exempt research under 45 CFR 46.104.

Determination Documentation

Determination Date:	1/4/2022
Exempt Category:	(2)(ii) Tests, surveys, interviews, or observation (low risk)
Approved Documents:	<ul style="list-style-type: none">• Interview Guide, Category: Data Collection;• Participant Interview Intro, Category: Data Collection;• Exempt Application, Category: IRB Protocol;• FRIAR Form, Category: Data Collection;• Introductory Letter for Participants, Category: Recruitment Materials;• Recruitment Flyer, Category: Recruitment Materials;• UMDF Support Letter, Category: External Site Permission Letter;

UPMC LIFE
CHANGING
MEDICINE

Office of Sponsored Programs and Research Support (OSPARS)
Iroquois Building
3600 Forbes Avenue (@ Meyran Ave.) Suite #300
Pittsburgh, PA 15213
Phone: (412) 647-4461
E-mail: OSPARS@upmc.edu
[OSPARS SharePoint](#)

OSPARS USE ONLY

PittPRO#: STUDY21100227	OSIRIS:
Qualifying Clinical Trial: No	Reasons: observational
Healthy Controls: No	Mixed Billing:
Drug/Biologic: No	FDA issued IND #:
IDE/Device: No	FDA issued IDE #:

Institutional Accounts Issued:

FIA/Administrative FIA – No
PIA –
Plan Code – No

SECTION 1 - Study Information		
A) Study Title: Exploring the Reproductive Choices and Family Planning Experiences of Women who have MELAS: A Qualitative Study	C) Sponsor Name: Click here to enter name.	
B) Principal Investigator: Rhine, Maria	E) Protocol # Assigned by Funding Agency/Sponsor: Enter # Here	
D) Host Department and Division: Department of Human Genetics	F) Protocol Version: 1 Protocol Date: 12/14/2021	
F) Clinical Coordinator (name/email/phone): Maria Rhine/mr119@pitt.edu/814-410-5694	I) ClinicalTrials.gov NCT #	
H) Patient Type: Outpatient		
SECTION 2 - Billing/Institutional Account Request Information		
A) Charge Reconciliation Contact (name/email/phone): Jennifer Palaski/jpd1150@pitt.edu/412-614-1550	B) Address for Billing: 130 Desoto Street, A741 Crabtree Hall, Pittsburgh, PA 15260	
C) Name of Account (limit to 25 letters; NO NUMBERS): Click here to enter name.	D) Type of Account Needed: Choose an item.	
E) Type of Services: Choose an item.	F) Number of expected subjects (at your site): 10-15	
G) Payment will be made by: Choose an item.	H) Research funds located: Choose an item.	
I) Type of Research: Human	J) Division Chief/Department Chair (name/email/phone): Daniel E. Weeks/weeks@pitt.edu	
SECTION 3 - UPMC SITES where medical services and procedures will be performed (Use as a reference - list number(s) in Column E of Section 4 and/or Column B of Section 6)		
UPMC HOSPITALS	UPMC CANCER CENTERS	CTRC SPACES
1) BFD / Bedford Memorial	50) Altoona CC (ALT)	43) UPCI/Hillman CTRC (MWH)
2) CHP / Children's Hospital of Pittsburgh	27) Arnold Palmer/Mtn View CC (external)	37) Magee CTRC (MWH)
3) Eye and Ear Institute	23) Arnold Palmer/Mt. Pleasant CC (external)	38) Montefiore CTRC (UPH)
4) HRZ / Horizon	20) Arnold Palmer Norwin CC (external)	35) Children's PCTR (CHP)
5) MWH / Magee Women's Hospital	27) Beaver CC (MWH)	42) WPKC CTRC (UPH)
6) MCK / McKeesport	51) Bethel Park/Upper St. Clair CC (external)	
7) MCY / Mercy **ERD Required**	52) Butler CC (external)	44) OTHER (specify):
8) NWH / Northwest	72) Chautauqua CC (CHQ)	
9) PST / Passavant/Passavant-Cranberry	53) Erie CC (HMT)	
10) PHS / Presbyterian/ShadySide/Montefiore	54) Greenville (Shanango) CC (HRZ)	
11) SHS / St. Margaret	55) Hamot CC (HMT)	
12) RES / Western Psych (WPC)	15) Hillman @ SHV 2nd, 3rd & 4th fls CC (MWH)	
13) HMT / Hamot	56) Horizon CC (HRZ)	
14) EAST / UPMC East	26) Indiana CC (external)	
45) ALT / Altoona	57) Jamestown (Chautauqua) CC (CHQ)	
46) CHQ / Chautauqua/Jamestown	16) Johnston/Murtha CC (ALT)	
47) Pinnacle / Ortesense	58) Magee Women's CC (MWH)	
48) MCK / Pinnacle (York/Memorial)	23) McKeesport CC (MCK)	
49) SQWR / Susquehanna/Williamsport (Divine Providence)		
	60) Natrona Heights CC (MWH)	
	30) New Castle CC (HRZ)	
	61) Northwest CC (MWH)	
	38) Passavant-Cranberry CC (PST)	
	28) Passavant/McCandless CC (MWH)	
	70) Pinnacle (Camp Hill) CC (external)	
	62) Pinnacle (Carlisle) CC (external)	
	63) Pinnacle (Harrisburg) CC (external)	
	64) Pinnacle (Lancaster) CC (external)	
	65) Pinnacle (Littell) CC (external)	
	66) Pinnacle (Ortensense) CC (external)	
	66) Pinnacle (York) CC (MEM)	
	31) St. Margaret CC (SMH)	
	71) Sewickley CC (MWH)	
	29) Struvenville (Trinity) CC (external)	
	67) Susquehanna CC (SQWR)	
	19) Uniontown CC (MCK)	
	32) Washington CC (MWH)	
	68) West Mifflin/Jefferson CC (MWH)	

Reviewer Initials: J
Fiscal Revision #: 0

30) UPMC Western Maryland (external)		24) Mercy CC (MCY)		33) Windsor CC (external)					
SECTION 4 – Schedule of Events									
A) M O D I F I E R	B) L I N E #	C) Description of Services to be provided (Use same terminology as protocol)	D) CPT Codes For all billable activities	E) S I T E S	F) S I T E C	G) S O C	H) Visit Time Point (Use same terminology as protocol)	I) Payment Method (BILL STUDY OR INSURANCE)	J) Comments: If you have selected CC or UA as payment, please specify in this column: • who will obtain the sample/do the test • who provides supplies or owns equipment • who performs the test or reads results
								BILLED TO STUDY UTILIZING INSTITUTIONAL ACCOUNTS (FIA/PIA) OR COMPLETED BY STUDY TEAM (UA/CC) <div> <input type="checkbox"/> FIA <input type="checkbox"/> PIA <input type="checkbox"/> UA <input type="checkbox"/> CC <input type="checkbox"/> INS </div>	
N/A	1	<u>Data Collection</u> Record audio from interviews Utilize third-party transcription service			No	No	Zoom and phone call	<input type="checkbox"/> FIA <input type="checkbox"/> PIA <input checked="" type="checkbox"/> UA <input type="checkbox"/> CC <input type="checkbox"/> INS	De-identified research only interview. No UPMC patient billing
N/A				Yes or No	Yes or No			<input type="checkbox"/> FIA <input type="checkbox"/> PIA <input type="checkbox"/> UA <input type="checkbox"/> CC <input type="checkbox"/> INS	
N/A				Yes or No	Yes or No			<input type="checkbox"/> FIA <input type="checkbox"/> PIA <input type="checkbox"/> UA <input type="checkbox"/> CC <input type="checkbox"/> INS	
N/A				Yes or No	Yes or No			<input type="checkbox"/> FIA <input type="checkbox"/> PIA <input type="checkbox"/> UA <input type="checkbox"/> CC <input type="checkbox"/> INS	
N/A				Yes or No	Yes or No			<input type="checkbox"/> FIA <input type="checkbox"/> PIA <input type="checkbox"/> UA <input type="checkbox"/> CC <input type="checkbox"/> INS	
N/A				Yes or No	Yes or No			<input type="checkbox"/> FIA <input type="checkbox"/> PIA <input type="checkbox"/> UA <input type="checkbox"/> CC <input type="checkbox"/> INS	
N/A				Yes or No	Yes or No			<input type="checkbox"/> FIA <input type="checkbox"/> PIA <input type="checkbox"/> UA <input type="checkbox"/> CC <input type="checkbox"/> INS	
N/A				Yes or No	Yes or No			<input type="checkbox"/> FIA <input type="checkbox"/> PIA <input type="checkbox"/> UA <input type="checkbox"/> CC <input type="checkbox"/> INS	
N/A				Yes or No	Yes or No			<input type="checkbox"/> FIA <input type="checkbox"/> PIA <input type="checkbox"/> UA <input type="checkbox"/> CC <input type="checkbox"/> INS	

Fiscal Revision #: 0

Appendix C Recruitment Letter for the United Mitochondrial Disease Foundation

November 16, 2021

UPMC Children's Hospital of Pittsburgh
Division of Medical Genetics
c/o Maria Rhine
4401 Penn Ave
Pittsburgh, PA 15224

United Mitochondrial Disease Foundation
8085 Saltsburg Road
Suite 201
Pittsburgh, PA 15239

To Whom it May Concern,

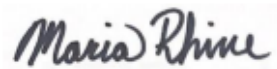
My name is Maria Rhine, and I am currently a graduate student at the University of Pittsburgh completing my master's degree in genetic counseling. During my time in the program, I have developed an interest in mitochondrial disorders and the genetic complexities associated with these conditions. I have decided to dedicate my required master's thesis project to a research study on MELAS, one of the most common conditions caused by alterations in the mitochondrial DNA. This letter serves to tell you more about my project and to request assistance in identifying individuals who may be suited to participate in my study.

I am specifically interested in the experiences of women over age 18 who have a mitochondrial alteration associated with MELAS. The project will give these women an opportunity to share their experiences with family planning, reproductive options, and genetic counseling through an interview with me as the main investigator. I will utilize a list of self-developed interview questions that are primarily open-ended in structure and designed to elicit their experiences. The long-term goals of this study will be to identify any additional beneficial resources for MELAS families with a specific focus on ways that genetic counselors can help improve outcomes for these families. I am supported in this project and will be collaborating with individuals at the Children's Hospital of Pittsburgh who work with the MELAS community as well as additional academic advisors from my program. I am requesting assistance in facilitating my recruitment to women who have MELAS through the website and mailing list of the United Mitochondrial Disease Foundation.

Enclosed you will find my summary of intent for this project and a description of the interview materials that will be used in this study.

Please feel free to contact me if you need any additional information about the plans for my thesis project. I look forward to working with you and all of the families you support.

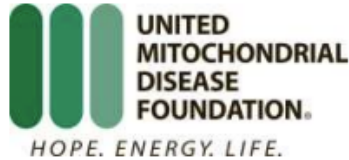
Sincerely,



Maria Rhine
MS Genetic Counseling Student
University of Pittsburgh
Department of Human Genetics

Graduate Student Researcher
Pediatric Medical Genetics
UPMC Children's Hospital of Pittsburgh

Appendix D United Mitochondrial Disease Foundation Letter of Support



December 10, 2021

To whom it may concern,

The Science and Alliance Officer of the United Mitochondrial Disease Foundation has reviewed the proposed project of Maria Rhine. It is understood that the proposed project will focus on women who have MELAS in regard to their experiences with family planning, reproductive choices, and genetic counseling.

We have agreed to help facilitate Ms. Rhine's recruitment by sharing her letter and flyer with foundation members who may be eligible to participate. Materials will be distributed through the UMDF's e-communications and/or member email lists and will include Ms. Rhine's contact information for any members interested in participating.

We look forward to assisting Ms. Rhine with the recruitment process for her proposed project.

Best,

A handwritten signature in dark ink, appearing to read "Kara Strittmatter", is written over a light blue horizontal line.

Kara Strittmatter, CMM, MA
The United Mitochondrial Disease Foundation (UMDF)
Director of Education and Support Services
Voice: 412-744-1062, Ext 116
National Office: 1-888-317-UMDF
Support Line: 1-888-900-MITO
Email: kara@umdf.org

www.umdf.org | 8085 Saltsburg Road, Suite 201 | Pittsburgh, PA 15239 | P 888.317.8633 | F 412.793.6477 | info@umdf.org

***Promoting research and education for the diagnosis, treatment and cure
of mitochondrial disorders and providing support to affected individuals and families.***

Appendix E Recruitment Letter

January 5, 2022

UPMC Children's Hospital of Pittsburgh
Division of Genetic and Genomic Medicine
c/o Maria Rhine
4401 Penn Ave
Pittsburgh, PA 15224

To whom it may concern,


My name is Maria Rhine, and I am currently a graduate student at the University of Pittsburgh completing my master's degree in genetic counseling. I am inviting you to participate in a research study that I have developed as part of the thesis requirements for my graduate program.

During my time in the program, I have developed an interest in mitochondrial disorders and the genetic complexities associated with these conditions. Given these interests, I would like to give women who have MELAS the opportunity to share their experiences. The long-term goal of this project is to identify additional beneficial resources for MELAS families and help genetic counselors and other healthcare providers better understand your needs.

I have been assisted by the United Mitochondrial Disease Foundation (UMDF) to get this invitation to you. If you are interested in learning more about the research study or in participating, please contact me at the number below. For the study, you will be asked to participate in a one-hour interview with me as the main investigator. This interview will focus on your experiences with family planning, reproductive choices, and genetic counseling. If you choose to participate, the audio from your interview will be recorded and stored anonymously on a secure, password-protected computer, and your responses will not be linked to you in any way. There are no direct benefits to participating in this research study, and minimal risks are anticipated, but may include negative feelings brought on by discussing difficult or stressful situations. Your participation is completely voluntary and can be withdrawn at any time.

This research study is being conducted by me, Maria Rhine, under the supervision of licensed, certified genetic counselors. I can be reached at **814-410-5694** or **mer119@pitt.edu** if you would like to participate or have any questions about the study. Thank you for considering participating in this study. I look forward to sharing information from this project to help add more knowledge about how to best support and care for individuals in the MELAS community.

Sincerely,



Maria Rhine
MS Genetic Counseling Student
University of Pittsburgh
Department of Human Genetics

Graduate Student Researcher
Pediatric Medical Genetics
UPMC Children's Hospital of Pittsburgh

Appendix F Recruitment Flyer

MELAS Thesis Project

Family Planning Experiences and Reproductive Choices of Women who have MELAS

Are you a woman who has MELAS due to a mitochondrial mutation?



My name is Maria Rhine, and I am a graduate student in the genetic counseling Masters degree program at the University of Pittsburgh. I would like to learn more about how a diagnosis of MELAS influences women and their families.



Those in the research study will be asked to participate in a one hour interview to explore implications of the diagnosis, specifically regarding family planning and reproductive choices. Individuals will also be asked about their experiences with genetic counseling, if applicable.



If you would like to participate or have any questions about this research study, please contact me at **814-410-5694** or **mer119@pitt.edu**



Children's
Hospital of Pittsburgh

Appendix G Participant Interview Introduction

Thank you for agreeing to speak with me today. We will be talking about your experience with MELAS. I'm not an expert on mitochondrial conditions, and I am new at conducting these interviews; however, I am here to listen and to give you the opportunity to express your thoughts and experiences. In this interview, there are no right or wrong answers, I am interested in hearing your thoughts because they may be similar to those of many other people who are also affected by MELAS. Your experiences are extremely important to me, and I am interested in any comments, opinions, or questions that you may have as we go along. If there are any questions you would prefer not to answer, please feel free not to respond to them.

There is certainly a lot to discuss in the one hour time frame that we have, so I may jump to a new topic from time to time. Please feel free to stop me if you wish to add anything else to the discussion. This interview will be recorded as I want to make sure no comments are missed. After the interview, I will be going through all your comments and those of others to prepare a report on the discussions I have had with a number of women who have MELAS. Please be assured that all of your comments are confidential and will be used only for the purposes of this study, and nothing you say will be connected to your name.

If you are interested in participating in the study, we will first review a verbal consent document and obtain your acknowledgement.

Appendix H Participant Verbal Consent Script

Participant Verbal Consent Script (Telephone or Zoom)

My name is Maria Rhine, and I am a genetic counseling student currently working on my thesis project, which is entitled "Exploring the Reproductive Choices and Family Planning Experiences of Women with MELAS: A Qualitative Study." The United Mitochondrial Disease Foundation (UMDF) reached out to you on my behalf regarding being a part of this study.

You are being asked to participate in this study because you have been identified as carrying a mitochondrial alteration causing MELAS. The purpose of this study is to better understand the experiences of women with MELAS to help genetic counselors and other healthcare providers improve the support and care we are providing to MELAS families.

Participants will be asked to complete an approximately 1 hour telephone or zoom interview with me. The interview will focus on your thoughts about diagnosis, family planning, reproductive options, and genetic counseling for MELAS.

Interviews will be recorded for the purpose of transcribing the audio for review of our conversation. Any identifying information will be removed from the transcript to preserve your confidentiality. All responses to interview questions are confidential and will be stored in a secure manner, primarily on a personal password-protected computer. If data is shared with any of my co-investigators of the study, they will not be able to link the data to the participants in any way. If any clarification is needed from the interview, I may recontact you during the study period.

There is no direct benefit to you from participation in this study; however, information gathered through the study will be used for improving genetic counseling practices for families with MELAS and other similarly inherited mitochondrial conditions. This knowledge may benefit how we take care of and support individuals with MELAS. There is no cost to participate in the study, and you will not receive any compensation for your participation.

In unusual cases, in response to a court order, the investigator may be required to release identifiable information (which may include your identifiable medical information) related to your participation in this research study. If investigators learn that you or someone with whom you are involved is in serious danger or at risk for potential harm, as required by Pennsylvania law, they will need to inform the appropriate agencies. Your participation is voluntary, and you may withdraw from this project at any time. Any answers recorded in the interview prior to your withdrawal will remain a part of the study. Your decision to participate or not participate will not affect your current or future relationship with the University of Pittsburgh Medical Center or the University of Pittsburgh.

You are encouraged to ask questions or voice concerns about any aspect of this research study by contacting the principal investigator, Maria Rhine, at 814-410-5694 or her mentor, Cate Walsh Vockley, MS, LCGC at 412-692-7349. You may contact the Human Subjects Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668) to discuss problems, concerns, questions, obtain information, offer input, or discuss situations in the event that the research team is unavailable.

Do you have any questions about the study or the information I just went over?

Are you willing to participate in this study?

Appendix I Participant Interview Guide

Date:

Study participant's ID:

Age:

Relationship Status:

Interview method: Phone _____ Zoom _____

Confirmation of Verbal Consent _____

Finding a Diagnosis

Please... Tell me about yourself and your family.

Tell me about the first person in the family who had symptoms of the condition.

Tell me about the experience from the onset of symptoms to finding a diagnosis.

- What was the age at diagnosis?
- How many other specialists had this person seen prior to or along with genetics?
- What sorts of other tests/treatments had already been performed prior to genetic testing?

Tell me about how you first found out about MELAS.

Do you have any symptoms of MELAS?

- If yes, what symptoms have you experienced?

How many children do you have?

- Are any children symptomatic?
- If yes, what was the age of onset, and what are the child's symptoms?

Is there any other family history of MELAS?

- Tell me about any conversations with family members.

Experience with Genetic Counseling

Have you ever met with a genetic counselor?

- If so, please describe your experience and what you learned.
- Is there anything else that you would have liked to learn?

What did you learn about mitochondrial disease and how it is passed in families?

- What is your understanding of the variability of the condition?

What questions or concerns did you have after receiving the diagnosis?

- What are one or two main concerns that you had?

Were any resources/support groups presented to you?

- Are there any other resources that you needed at the time of the diagnosis?

Family Planning (*tailored to each participant)

What was your personal perspective on having children? Did you always want to have children?

Tell me about any discussions with your reproductive partner about carrying a MELAS variant.

- How did your partner react or contribute to these conversations?

Did learning the diagnosis impact your decision about whether to have children?

- If so, how?

Do you feel that your mutation status impacted or would have impacted your decision to have children?

How has this experience affected your thoughts on future family planning?

- Or perhaps family planning for affected family members (ex. daughters)?

Reproductive Options

Preimplantation or prenatal testing/diagnosis may be an option for some genetic conditions but is not offered for mitochondrial variants because of inheritance and uncertain prediction of clinical features..

What challenges do you feel this presents for a woman with a known mitochondrial condition?

Have you heard about the concept of mitochondrial replacement therapy?

- Intended mother and father have a biological child with mitochondria from a female donor.. what are your thoughts on this option for families affected by mitochondrial conditions (currently not available in the United States)
- Do you feel that this is a therapy that should be accessible for families with MELAS?
- Would you consider this option if it were available to you?

What are your thoughts on assisted reproductive procedures for mitochondrial conditions like MELAS?

Do your thoughts differ on egg donation versus mitochondrial replacement therapy?
What do you think some risks and benefits might be for assisted reproductive procedures?

Psychosocial Implications

What were some of the emotions that you felt after learning you carry a mitochondrial variant for MELAS?

How are you coping with or adapting to the diagnosis of MELAS?

Tell me about the impact of knowing your MELAS variant will be passed on to children.

Uncertainty can be very uncomfortable; how did you handle any feelings of uncertainty around the diagnosis of MELAS?

- What things make you feel uncertain?

Reflections

When it comes to the experience of family planning, reproductive options, and genetic counseling, is there anything that I didn't ask about that you would like to share?

What would you say to a family who has just received a diagnosis? What have you learned that you wished you had known then?

Bibliography

- Alston, C. L., Rocha, M. C., Lax, N. Z., Turnbull, D. M., & Taylor, R. W. (2017). The genetics and pathology of mitochondrial disease. *J Pathol*, 241(2), 236-250. doi:10.1002/path.4809
- Braun, V., & Clarke, V. (2006). Using thematic analysis in psychology. *Qualitative Research in Psychology*, 3(2), 77-101. doi:10.1191/1478088706qp063oa
- Chiaratti, M. R., Macabelli, C. H., Augusto Neto, J. D., Grejo, M. P., Pandey, A. K., Perecin, F., & Collado, M. D. (2020). Maternal transmission of mitochondrial diseases. *Genet Mol Biol*, 43(1 suppl. 1), e20190095. doi:10.1590/1678-4685-gmb-2019-0095
- Craven, L., Alston, C. L., Taylor, R. W., & Turnbull, D. M. (2017). Recent Advances in Mitochondrial Disease. *Annu Rev Genomics Hum Genet*, 18, 257-275. doi:10.1146/annurev-genom-091416-035426
- de Laat, P., Fleuren, L. H., Bekker, M. N., Smeitink, J. A., & Janssen, M. C. (2015). Obstetric complications in carriers of the m.3243A>G mutation, a retrospective cohort study on maternal and fetal outcome. *Mitochondrion*, 25, 98-103. doi:10.1016/j.mito.2015.10.005
- de Laat, P., Koene, S., van den Heuvel, L. P., Rodenburg, R. J., Janssen, M. C., & Smeitink, J. A. (2012). Clinical features and heteroplasmy in blood, urine and saliva in 34 Dutch families carrying the m.3243A > G mutation. *J Inherit Metab Dis*, 35(6), 1059-1069. doi:10.1007/s10545-012-9465-2
- Dimond, R. (2013). Patient and family trajectories of mitochondrial disease: diversity, uncertainty and genetic risk. *Life Sciences, Society and Policy*, 9(1), 2. doi:10.1186/2195-7819-9-2
- El-Hattab, A. W., Adesina, A. M., Jones, J., & Scaglia, F. (2015). MELAS syndrome: Clinical manifestations, pathogenesis, and treatment options. *Mol Genet Metab*, 116(1-2), 4-12. doi:10.1016/j.ymgme.2015.06.004
- Engelstad, K., Sklerov, M., Kriger, J., Sanford, A., Grier, J., Ash, D., . . . Hirano, M. (2016). Attitudes toward prevention of mtDNA-related diseases through oocyte mitochondrial replacement therapy. *Hum Reprod*, 31(5), 1058-1065. doi:10.1093/humrep/dew033
- Fan, H. C., Lee, H. F., Yue, C. T., & Chi, C. S. (2021). Clinical Characteristics of Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-Like Episodes. *Life (Basel)*, 11(11). doi:10.3390/life11111111

- Farnezi, H. C. M., Goulart, A. C. X., Santos, A. D., Ramos, M. G., & Penna, M. L. F. (2020). Three-parent babies: Mitochondrial replacement therapies. *JBRA Assist Reprod*, 24(2), 189-196. doi:10.5935/1518-0557.20190086
- Gorman, G. S., Chinnery, P. F., DiMauro, S., Hirano, M., Koga, Y., McFarland, R., . . . Turnbull, D. M. (2016). Mitochondrial diseases. *Nat Rev Dis Primers*, 2, 16080. doi:10.1038/nrdp.2016.80
- Grady, J. P., Pickett, S. J., Ng, Y. S., Alston, C. L., Blakely, E. L., Hardy, S. A., . . . McFarland, R. (2018). mtDNA heteroplasmy level and copy number indicate disease burden in m.3243A>G mitochondrial disease. *EMBO Mol Med*, 10(6). doi:10.15252/emmm.201708262
- Ikawa, M., Povalko, N., & Koga, Y. (2020). Arginine therapy in mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes. *Curr Opin Clin Nutr Metab Care*, 23(1), 17-22. doi:10.1097/mco.0000000000000610
- Kaufmann, P., Engelstad, K., Wei, Y., Kulikova, R., Oskoui, M., Sproule, D. M., . . . De Vivo, D. C. (2011). Natural history of MELAS associated with mitochondrial DNA m.3243A>G genotype. *Neurology*, 77(22), 1965-1971. doi:10.1212/WNL.0b013e31823a0c7f
- Manwaring, N., Jones, M. M., Wang, J. J., Rochtchina, E., Howard, C., Mitchell, P., & Sue, C. M. (2007). Population prevalence of the MELAS A3243G mutation. *Mitochondrion*, 7(3), 230-233. doi:10.1016/j.mito.2006.12.004
- Ng, Y. S., & Turnbull, D. M. (2016). Mitochondrial disease: genetics and management. *J Neurol*, 263(1), 179-191. doi:10.1007/s00415-015-7884-3
- Noohi, F., Li, M., & Joly, Y. (2021). Clinical translation of mitochondrial replacement therapy in Canada: a qualitative study of stakeholders' attitudes. *FACETS*, 6, 449-464. doi:10.1139/facets-2020-0062
- Noorda, G., van Achterberg, T., van der Hooft, T., Smeitink, J., Schoonhoven, L., & van Engelen, B. (2012). Problems of adults with a mitochondrial disease - the patients' perspective: focus on loss. *JIMD Rep*, 6, 85-94. doi:10.1007/8904_2011_121
- Poulton, J., Finsterer, J., & Yu-Wai-Man, P. (2017). Genetic Counselling for Maternally Inherited Mitochondrial Disorders. *Mol Diagn Ther*, 21(4), 419-429. doi:10.1007/s40291-017-0279-7
- Sendra, L., García-Mares, A., Herrero, M. J., & Aliño, S. F. (2021). Mitochondrial DNA Replacement Techniques to Prevent Human Mitochondrial Diseases. *Int J Mol Sci*, 22(2). doi:10.3390/ijms22020551

- Stewart, J. B., & Chinnery, P. F. (2015). The dynamics of mitochondrial DNA heteroplasmy: implications for human health and disease. *Nat Rev Genet*, 16(9), 530-542. doi:10.1038/nrg3966
- Treff, N. R., Campos, J., Tao, X., Levy, B., Ferry, K. M., & Scott, R. T., Jr. (2012). Blastocyst preimplantation genetic diagnosis (PGD) of a mitochondrial DNA disorder. *Fertil Steril*, 98(5), 1236-1240. doi:10.1016/j.fertnstert.2012.07.1119
- van den Ameele, J., Li, A. Y. Z., Ma, H., & Chinnery, P. F. (2020). Mitochondrial heteroplasmy beyond the oocyte bottleneck. *Semin Cell Dev Biol*, 97, 156-166. doi:10.1016/j.semcdb.2019.10.001
- Vento, J. M., & Pappa, B. (2013). Genetic counseling in mitochondrial disease. *Neurotherapeutics*, 10(2), 243-250. doi:10.1007/s13311-012-0173-2
- Yatsuga, S., Povalko, N., Nishioka, J., Katayama, K., Kakimoto, N., Matsuishi, T., . . . Koga, Y. (2012). MELAS: a nationwide prospective cohort study of 96 patients in Japan. *Biochim Biophys Acta*, 1820(5), 619-624. doi:10.1016/j.bbagen.2011.03.015
- Zaidi, A. A., Wilton, P. R., Su, M. S., Paul, I. M., Arbeithuber, B., Anthony, K., . . . Makova, K. D. (2019). Bottleneck and selection in the germline and maternal age influence transmission of mitochondrial DNA in human pedigrees. *Proc Natl Acad Sci U S A*, 116(50), 25172-25178. doi:10.1073/pnas.1906331116
- Zhang, Z., Zhao, D., Zhang, X., Xiong, H., Bao, X., Yuan, Y., & Wang, Z. (2018). Survival analysis of a cohort of Chinese patients with mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS) based on clinical features. *J Neurol Sci*, 385, 151-155. doi:10.1016/j.jns.2017.12.033
- Zilber, S., & Yeske, P. E. (2020). Mitochondrial Disease Community Registry: First look at the data, perspectives from patients and families. *Mitochondrial and Metabolic Medicine* 2. doi:doi:10.9777/mmm.2020.10001
- Zou, W., Slone, J., Cao, Y., & Huang, T. (2020). Mitochondria and Their Role in Human Reproduction. *DNA Cell Biol*, 39(8), 1370-1378. doi:10.1089/dna.2019.4807