Mitochondrial 3243 A to G Mutation: Clinical Phenotypes on Different Stages of Life and Their Relevance to Heteroplasmy

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (B. Pharm.)

School of Pharmacy
Brac University
September 2024

Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis titled "Mitochondrial 3243 A to G Mutation: Clinical Phenotypes on Different Stages of Life and Their Relevance to Heteroplasmy" submitted by Md. Abu Saleh (20146014) of Spring, 2020 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (B.Pharm.) on September 2024.

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Ethics Statement

This project does not involve any kind of animal and human trial.

Abstract

Mitochondrial 3243 A to G mutation is a major mutation, genetically causing mitochondrial diseases. It was first found in 1990 in a MELAS patient. One of the most prevalent pathogenic mutations, mitochondrial 3243 A to G mutation in mitochondrial DNA (mtDNA), is frequently linked to a variety of clinical phenotypes. The degree and prevalence of these symptoms differ, depending on variables including heteroplasmy levels (the percentage of mutant mtDNA), and life stage. Clinical phenotypes mainly include lactic acidosis, mitochondrial encephalomyopathy, stroke-like episodes (MELAS), maternally inherited diabetes and deafness, MERRF, CPEO and asymptomatic carriers as well as severe multisystem diseases. Till now, a lot of cohort studies have been performed to identify phenotypes connected with this mutation and a lot of phenotypes were found. Additionally, heteroplasmy level related studies were also performed. Different levels of heteroplasmy showed different phenotypes at different stages of life. The purpose of this study is to identify the clinical characteristics of the mutation at various phases of life and to emphasize the significance of heteroplasmy in the development and prognosis of disease.

Keywords: Mutation; Mitochondrial DNA; Point mutation; MELAS; MIDD; MERRF; CPEO; Heteroplasmy; Clinical phenotype; Diabetes; Deafness; Leukocyte; Cardiomyopathy.

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Dedication

Dedicated to Syed Golamur Rahman Maizvandari

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List of Acronyms

DNA	Deoxyribonucleic acid
ATP	Adenosine triphosphate
RNA	Ribonucleic acid
MELAS	Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like episodes
MIDD	Maternally Inherited Diabetes and Deafness
MERRF	Myoclonic epilepsy with ragged red fibers
CPEO	Chronic progressive external ophthalmoplegia
DM	Diabetes Mellitus
BMI	Body Mass Index
FPG	Fasting Plasma Glucose
ESRD	End Stage Renal Disease
RPA	Retinal Pigmentary Abnormalities
MRI	Magnetic Resonance Imaging
DGGE	Denaturing Gradient Gel Electrophoresis
Anti-GAD	Antibodies to Glutamic Acid Decarboxylase

Chapter 1

Introduction

1.1 Mitochondria

Mitochondria, which are membrane-bound organelles within cells, generate most of the chemical energy needed for cellular metabolism. This energy is stored in a small molecule called adenosine triphosphate (ATP), produced by the mitochondria. These organelles also have their own small set of chromosomes. Typically, mitochondrial DNA is only inherited from the mother. The size of mitochondria is generally between 0.75 to 3 micrometers, and unless they are stained, they are invisible under microscope (Newman, 2018). The number of mitochondria differs among various cell types. While they are often shown as oval-shaped organelles, mitochondria are constantly undergoing division (fission) and merging (fusion). Consequently, these organelles form dynamic, interconnected networks.

1.2 Mitochondrial DNA

Unlike other organelles in mammalian cells, mitochondria contain a small amount of DNA known as mitochondrial DNA (mtDNA), which encodes several key proteins necessary for mitochondrial respiration. A range of proteins, including prohibitins, mitochondrial transcription factor A (TFAM), ATPase family AAA domain-containing protein 3 (ATAD3), and POLG (DNA polymerase gamma, catalytic subunit), package mtDNA. While distinct from nuclear DNA (nDNA), mtDNA resembles bacterial chromosomes. These proteins and mtDNA together form a complex known as a nucleoid (Yan et al., 2019). The mtDNA's sense and antisense strands are referred to as the heavy (H) and light (L) strands, respectively. 16,569 base pairs of mtDNA are

found in human cells which encode 37 different genes, including 13 polypeptides, 2 ribosomal RNAs, and 22 tRNAs (Nicholls & Gustafsson, 2018). The L strand contains 1 polypeptide (ND6) and 8 tRNAs, while the H strand encodes the remaining 12 polypeptides, 2 rRNAs, and 14 tRNAs. A noncoding element known as a displacement loop (D-loop), which is found in nearly all known cases of mtDNA transcription and replication, is also present in mtDNA (Sbisà et al., 1997).

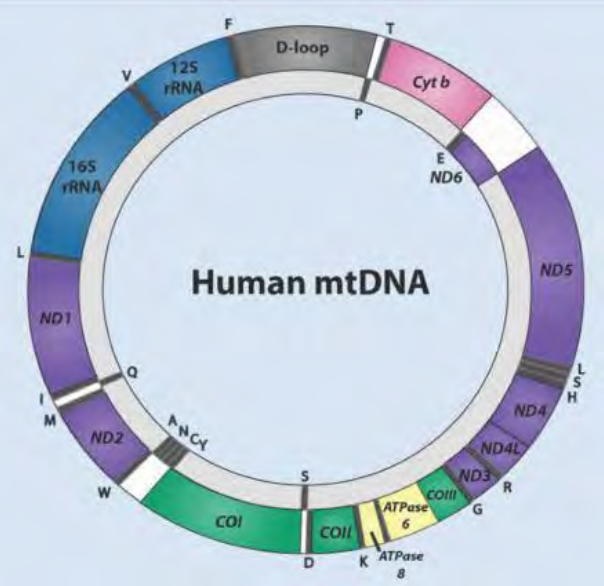

Figure 1: Human mitochondrial DNA

Note. This image was created to depict mitochondrial DNA. From "What is mitochondrial DNA?" by Home. (n.d.). Wellcome Trust Centre for Mitochondrial Research. https://www.newcastle-mitochondria.com/patient-and-public-home-page/what-is-mitochondrial-dna/

1.3 Mitochondrial Point Mutation

Point mutations in the mtDNA are generally inherited from mothers. They could be found in rRNA, tRNA, or protein genes. Yet mt-tRNA genes contain more than half of the documented

point mutations linked to disease. From a phenotypic perspective, point mutations in genes that code for proteins in the mitochondria directly impact the functioning of the RC complex that the corresponding protein is a part of, whereas mutations in mt-tRNAs can alter the availability of functional mt-tRNAs, which may affect the general efficacy of mitochondrial translation. Point mutations are thought to be largely recessive, with the majority being heteroplasmic and exhibiting significant clinical variability. On the other hand, more and more harmful homoplasmic mutations are being identified; these mutations frequently impact only one tissue and exhibit partial penetration (Tuppen et al., 2010). There is a significant chance that mitochondrial DNA will come into contact with oxidative chemicals in this atmosphere. Superoxide radicals, which may damage DNA, are mostly found in the mitochondrial respiratory chain. Moreover, the mitochondrial membrane's monoamine oxidase produces hydrogen peroxide, which has the potential to be a genotoxic substance. It means that the high rate of mutation in mitochondrial DNA and the accumulation of mutations with age are not surprising (Rustin et al., 2000). A lot of mitochondrial mutations are identified. Among them mitochondrial 3243 A to G mutation is a remarkable one.

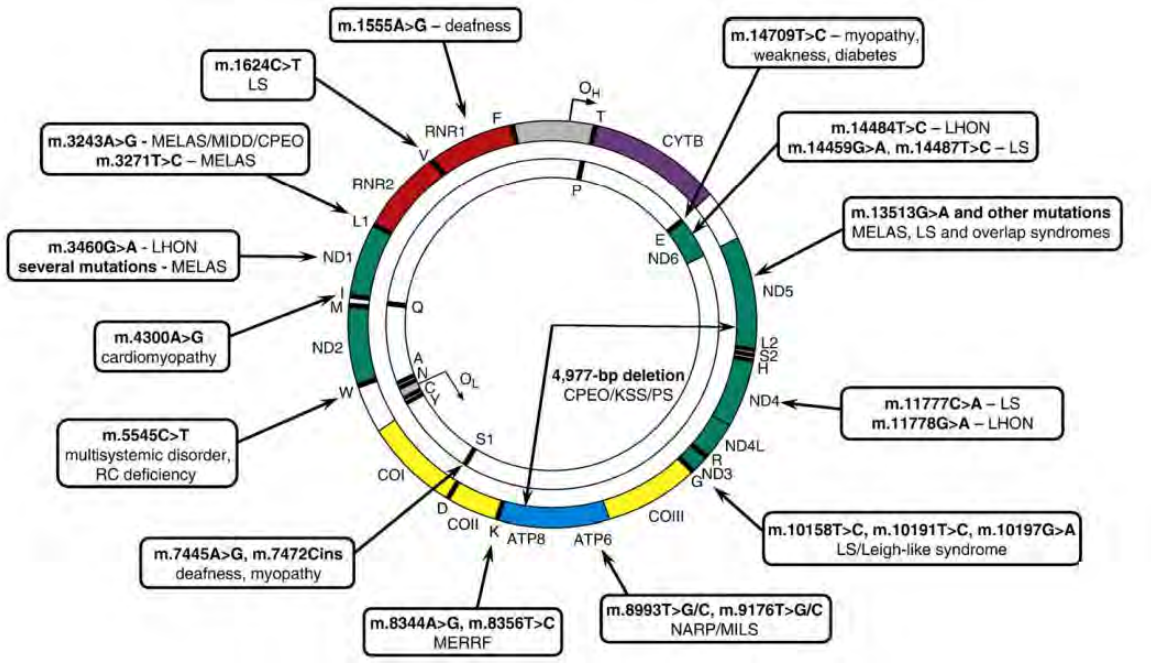

Figure 2: Mitochondrial point mutations.

Note. This image was created to depict mitochondrial point mutations. From "Mitochondrial DNA mutations and human disease" by Tuppen, H. A. L., Blakely, E. L., Turnbull, D. M., & Taylor, R. W. (2010). Mitochondrial DNA mutations and human disease. Biochimica et Biophysica Acta (BBA) - Bioenergetics, 1797(2), 113–128. https://doi.org/10.1016/j.bbabio.2009.09.005

1.4 Mitochondrial 3243 A to G Mutation

Mitochondrial 3243 A to G mutation is basically a nucleotide change from adenine to guanine at 3243 position in the mitochondrial tRNA leucine (UUR) gene (MT-TL1) of the mitochondrial genome. This is the most common point mutation which has been reported with Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like episodes (MELAS) syndrome(Gorman et al., 2015). The mutation was first identified by Goto and colleagues (Goto et al., 1990). The mutation is seen in heteroplasmic state mostly. Heteroplasmy is such a state where the mutations

are found in only a portion of the entire mtDNA population (Maassen, 2002). A cell that has both mutant and wild-type mtDNA genomes is said to be heteroplasmic. By means of somatic mutagenesis and continuous mtDNA replication, mutations can proliferate clonally by stochastic or selective processes, and manifest in different ratios or levels of heteroplasmy inside cells (Elson et al., 2001). Based on heteroplasmy level in different tissues, different types of phenotypes are seen. However, the mutation has significant molecular and functional consequences.

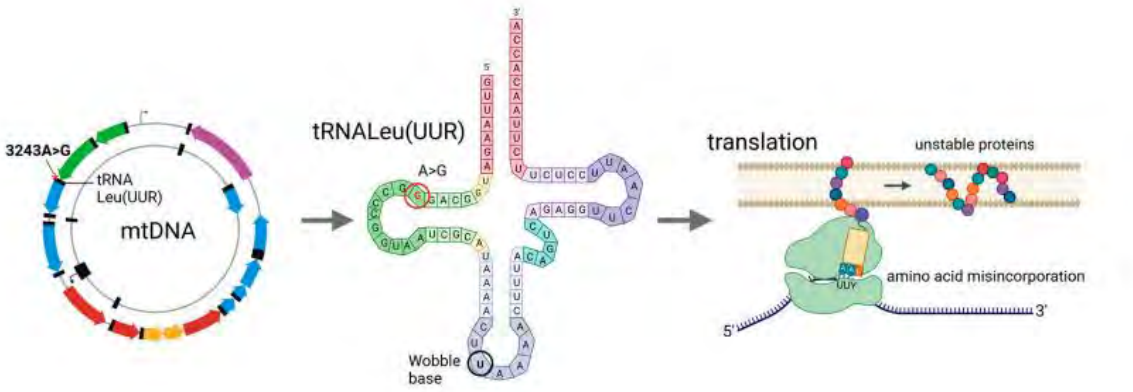

Figure 3: Mitochondrial 3243 A to G mutation.

Note. This image was created to depict mitochondrial 3243 A to G mutation. From "The Mitochondrial m.3243A>G Mutation on the Dish, Lessons from In Vitro Models," by Ryytty, S., & Hämäläinen, R. H. (2023). The Mitochondrial m.3243A>G Mutation on the Dish, Lessons from In Vitro Models. International Journal of Molecular Sciences, 24(17), 13478. https://doi.org/10.3390/ijms241713478

The MT-TL1 gene, which codes for the mitochondrial tRNA Leu(UUR), a 75 bp long tRNA for leucine, is impacted by the m.3243A>G mutation (Li et al., 2022a). The adenine-to-guanine mutation at position 3243 affects the D-loop of the tRNALeu(UUR) molecule, altering its stability, structure, and codon recognition. Specifically, the m.3243A>G mutation reduces UUG translation without significantly impacting UUA translation, causing amino acid misincorporation in all

mitochondrial proteins encoded by mtDNA (Chomyn et al., 2000). Additionally, this mutant lacks post-transcriptional taurine modifications at the wobble U base. The resulting unstable protein products lead to defects in the electron transport chain. The mutation primarily impacts proteins with abundant UUG codons, such as the ND6 subunit of complex I (Li et al., 2022b). As this mutation is heteroplasmic—that is, it coexists in cells with both mutant and non-mutated mtDNA—it is linked to a wide range of clinical outcomes. The amount of mutant to wild-type mtDNA in various tissues affects how severe the symptoms are. Fibroblast cells produced from patients offer a direct connection to the physiology of the patient; yet, their capacity to completely recapitulate disease phenotypes is limited, especially for complex tissues. In patient cultures, fibroblast proliferation is impacted. It has been observed that fibroblasts with a 30% mutation load proliferate at a slower rate (Chung et al., 2021), and that fibroblasts with a high mutation frequency (>95%) have much lower growth potential (Yokota et al., 2015). For the purpose of isolating effects specific to mitochondria, cybrid (cytoplasmic hybrid) cells are used, in which mutant mtDNA is inserted into a host cell with a different nuclear background. Also, highly proliferative cells can be utilized. However, these models might not accurately represent the interactions that take place in vivo between the nuclear and mitochondrial genomes. Additionally, the tolerance of various cell types to the m.3243A>G mutation varies (Ryytty & Hämäläinen, 2023).

1.5 Aim of the Study

The aim of the study is to find out prominent clinical phenotypes in individuals harboring mitochondrial 3243 A to G mutation during different stages of life and the correlation of the phenotype and heteroplasmy level. The study seeks most of the phenotypes and a statistical analysis of them. These phenotypes are found at different stages of life. So, building up a general concept about the onset of diseases is a goal. The study also aims to relate the phenotypes with the

heteroplasmy level of the mutation. Till date there is no established correlation found between heteroplasmy level and phenotype. So, the study intends to set a general correlation between heteroplasmy level and phenotype analyzing previous research in this field. The study contains information of 20 different studies conducted upon patients harboring the mutation. Next, it contains the phenotypes which are seen at different stages of life. Finally, it contains a relationship between phenotype and heteroplasmy level.

Chapter 2

Methodology

2.1 Search strategies

For collecting articles reputed and renowned sources were used. Sources were PubMed, Google Scholar, ELSEVIER, NCBI, Research gate, Springer and ScienceDirect. To get the desired articles, a search string was generated using the major keywords which were- mitochondrial 3243 A to G mutation, MELAS, MIDD, mitochondrial mutation, MERRF, CPEO and clinical phenotypes. Boolean logic using "and" and "or" was applied to combine the keywords, enhancing the accuracy of the search. As the mitochondrial 3243 A to G mutation was identified in 1990, there have been a few studies done. So, filters were not used.

2.2 Criteria for Selection of Articles

Certain inclusion and exclusion criteria were applied to select the journal articles. Below, table 1 represents the inclusion and exclusion criteria. The process of identifying articles through the database is illustrated with a PRISMA flow diagram in Figure 4.

Table 1: Inclusion and Exclusion Criteria

Parameters	Inclusion Criteria	Exclusion Criteria
Patient	Patients harboring mitochondrial 3243 A to G mutation. Or those patients who had phenotypes before and the mutation was found later.	Patients having phenotypes but not the mutation.
Relevance	Articles relevant to only mitochondrial 3243 A to G mutation.	Articles relevant to mitochondrial mutations other than 3243 A to G mutation.
Publication date range	Articles from 1990 to 2024.	Articles before 1990 as the mutation was first identified in 1990.
Availability	Articles that were available through different ways.	Articles that were not available at all.
Language	Articles written in English	Articles in languages other than English.

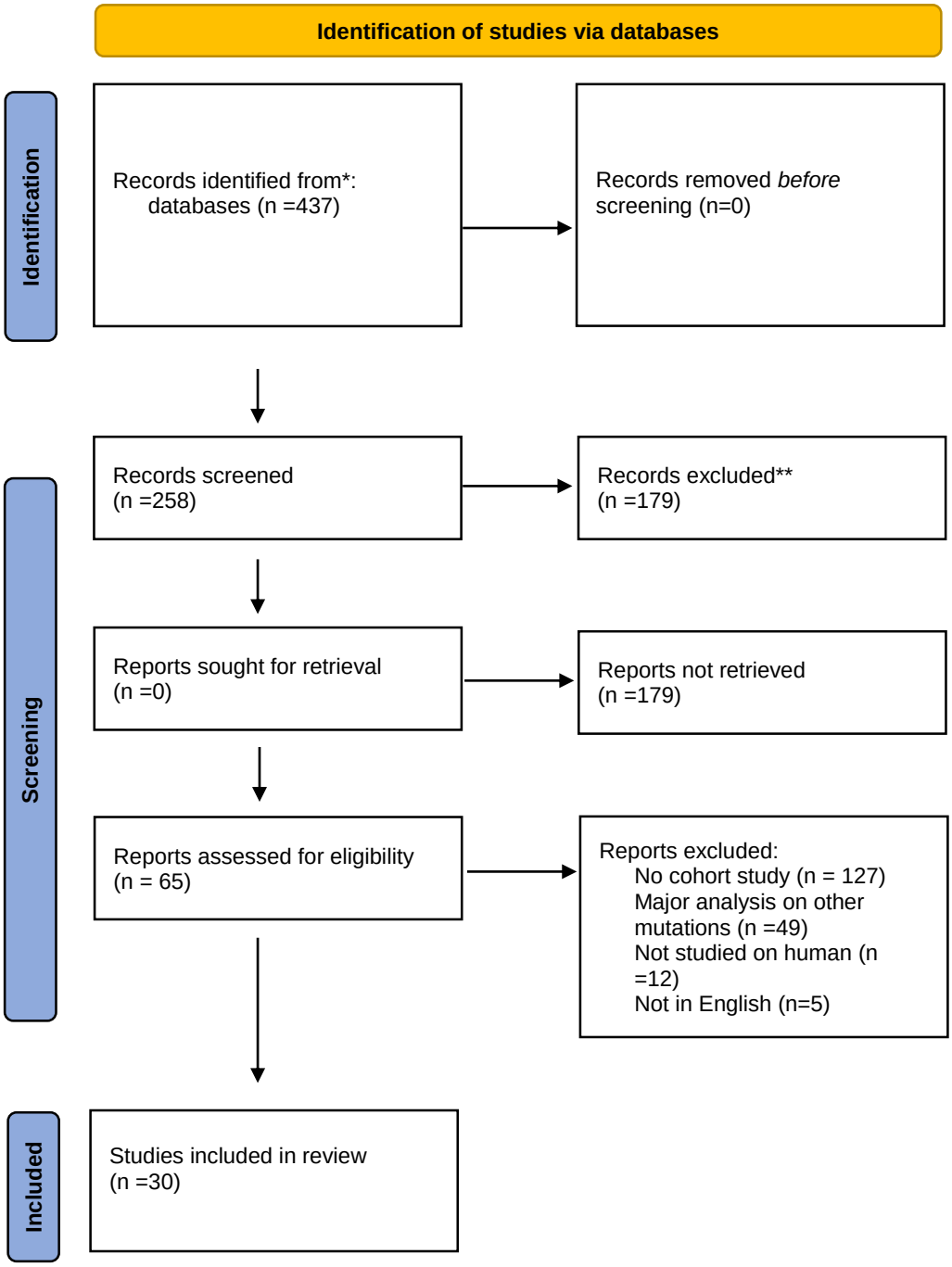

Figure 4: Prisma flow diagram of identification of articles via database

Chapter 3

Result

3.1 Tabulation of the selected studies

Table 2: Selected Studies

Serial No	Sample Number	Control Population Number	Tested Population Number	Primary Outcomes	Secondary Outcomes	Phenotypes	Citation
1	1079 subjects	309 healthy controls	770 diabetes mellitus (DM) patients	1.Mitochondrial 3243 A to G mutation was present in 13 patients. 2. The mitochondrial 3243 A to G mutation was more common in DM patients compared to healthy controls; this was correlated with DM patients' younger ages, lower BMIs, and higher FPG levels.	1. Among 309 healthy people, no mitochondrial 3243 A to G mutation was present. 2. Younger age was associated with a higher likelihood of DM patients with the mitochondrial 3243 A to G mutation.	1.Diabetes mellitus	(Wang et al., 2013)

2	113 patients harboring mitochondrial 3243 A to G mutation.	0	113 patients harboring mitochondrial 3243 A to G mutation.	1.32.8 years was the mean age of the diagnosis of diabetes. 2.12.9 years was the mean duration of diabetes. 3. Approximately 68% of the patients with the mutation who had diabetes had maternal inheritance, which was found to be substantially greater than paternal inheritance.	1. Maternal inheritance of glucose intolerance was linked to early middle-aged diabetes development, decreased insulin secretory capability, and early insulin therapy requirements. This was shown in 75% of mutant diabetic patients.	1.Materneally inherited diabetes and deafness. 2. High incidence of neurosensory deafness 3.Early middle aged onset of diabetes and deafness.	(Suzuki et al., 2003)

3	151 patients harboring mitochondrial 3243 A to G mutation	0	151 patients harboring mitochondrial 3243 A to G mutation	1.The number of symptom carrier patient is 124. 2. The degree of heteroplasmy in leucocytes and Urea Electrolytes and Creatinine (UEC) was only weakly correlated to the severity of the illness.	1.The Mitochondrial 3243 A to G mutation causes a slowly progressive disease.	1.Melas (7 patients) 2.MIDD (60 patients)	(de Laat et al., 2021)
4	15 patients harboring	0	15 patients harboring mitoch	1.The degree of heteroplasmy ranged from 3% to 37% and the mean was 23.9%. 2. The mean age at which hearing loss first appeared was 28.6 years; in	1.The heteroplasmy and age-corrected heteroplasmy levels were linked with the age at which hearing loss first appeared.	1.Hearing loss 2.Diabetes mellitus 3.Balance-gait disorder.	(Sakata et al., 2022)

	mitochondrial 3243 A to G mutation		ondrial 3243 A to G mutation	the earliest cases, acquired hearing loss was detected as early as age 10, and in more latest cases, it was detected as late as age 56. 3.In seven individuals, their hearing rapidly declined to total deafness between the ages of 40 and 63. 4. In all 15 patients the average rate of the development of hearing loss was 5.5db per year.	2.The mitochondrial 3243 A to G mutation results in gradual hearing loss.		
5	53 patients harboring mitoch	0	53 patients harboring mitochondrial	1.Most patients (41.5%) were categorized into the stable high fatigue trajectory, followed by the fluctuating fatigue group at 35.9%, and the stable low fatigue group at 22.6%.	1.Individuals with stable high fatigue had worse clinical performance and more (severe) organ system involvement than individuals with a consistent low fatigue trajectory.	1.Fatigue 2.Mental health issues	(Klein et al., 2022)

	ondrial 3243 A to G mutation		3243 A to G mutation	2.The stable high fatigue group had a higher percentage of female patients (91%) compared to the fluctuating fatigue group (58%). 3. Patients who had consistent high levels of fatigue had noticeably greater issues with their psychological and mental health.	2.Patients experiencing severe fatigue have a heavier burden, impacting various aspects of daily life such as mental health, functioning, and overall quality of life compared to those with mild fatigue.		
6	227 individuals	92 individuals	135 patients with diabetes and End stage renal	1.Among the 135 patients with diabetes and end-stage renal disease (ESRD), 8 patients had the mutation and it was in heteroplasmic form. 2. Among the 92 patients with ESRD alone, none had a mitochondrial 3243 a to g mutation.	1. Type II diabetes was the initial diagnosis of all participants carrying the mitochondrial 3243 A to G mutation.	1.Diabetes 2.Neuropathy 3.Retinopathy 4.Proteinuria 5.Deafness 6.MELAS	(Iwasaki et al., 2001)

			disease(ESRD).				
7	14 patients harboring mitochondrial 3243 A to G mutation	0	14 patients harboring mitochondrial 3243 A to G mutation	1.Retinal pigmentary abnormalities (RPAs) are highly prevalent in patients with the mitochondrial 3243 A to G mutation, occurring in 8 patients (57%) of these patients. 2. Among the patients with pigmentary retinal abnormalities, only one had corrected visual acuity that was moderately impaired (20/60); all other patients had corrected visual acuity that was 20/30 or better.	1. It is probably justified to classify retinal pigmentary abnormalities as a component of the MELAS syndrome clinical spectrum. 2. Concomitant optic atrophy was absent in all individuals.	1.Retinal Pigmentary abnormality 2.Deafness 3.Diabetes 4.Epilepsy 5.Stroke-like episodes 6.Proximal myopathy 7.External ophthalmoplegia	(Sue et al., n.d.)

8	91 patients with mitochondrial myopathies	0	91 patients with mitochondrial myopathies	1.The mitochondrial 3243 A to G mutation was found in 21 patients. 2.Progressive external ophthalmoplegia was present in 16 of the 21 patients (76%).	1.Detectable mtDNA deletions in muscle were present in none of the patients.	1.Progressive external ophthalmoplegia. 2.Ptosis 3.Proximal weakness 4.Stroke 5.Deafness 6.Seizures 7.Ataxia 8.Retinopathy 9.Endocrinopathy	(Moraes et al., 1993)
9	22 probands and	0	62 individuals	1.In the families of probands 14 patients were found harboring	1.In individuals with Fatigue compared to those with MELAS syndrome, the	1.Limb weakness 2.Deafness 3.Ataxia	(Hammans et al., n.d.)

	their families (62 patients total)			mitochondrial 3243 A to G mutation. 2.The mitochondrial 3243 A to G mutation was identified in one or more tissues from 22 unrelated index patients across 22 families. 3. Age of onset and clinical severity score were significantly correlated with the amount of mutant mtDNA in muscle and blood.	percentage of COX negative fibers were significantly higher, whereas the frequency of highly COX positive fibers was the opposite. 2. The blood's mutant percentage of mitochondrial 3243 A to G mutation is probably going decline as individuals being old. 3. There could be significant changes in heteroplasmy across generations.	4.Short stature 5.Seizures 6.Ophthalmoplegia or ptosis 7.Multiple stroke-like episodes 8.Dementia 9.Retinopathy 10.Diabetes 11.Neuropathy 12.Cardiomyopathy 13.Wolff-Parkinson-White syndrome	

						14.Gastrointestinal pseudo-obstruction	
10	41 subjects	25 healthy subjects	16 patients with type 2 diabetes	1.Mitochondrial 3243A to G mutation score was positive in seven plasma and serum samples from patients with type 2 diabetes while the 25 samples from healthy subjects did not display any mutant bands,also, the other nine samples from diabetic patients also showed no mutant bands.	1.Serum, plasma, and blood leukocyte samples were examined for their levels of mitochondrial 3243A to G mutation heteroplasmy. The seven affected patients had varying percentages of mitochondrial 3243 A to G mutation in their blood leukocytes (1.1%–13.5%), serum (1.5%–35.2%), and plasma (1.6%–36.5%).	1.Type 2 diabetes.	(Zhong, 2000)

11	6 children and their mothers (Total 12)	6 individuals	6 individuals	1. All of the patients and their mothers had a mitochondrial 3243 A to G mutation. 2. Six patients had mutation loads ranging from 43.6% to 58%. 3.Among their mothers, 14.1% to 28.6% did not exhibit any symptoms. 4. In all patients hyperlactemia was found. 5. All patients were found with mixed neurogenic and myopathic change and it was detected by electromyography.	1.The six patients' parents were healthy non-consanguineous individuals. 2. Four patients' brain magnetic resonance imaging (MRI) revealed hyperintense signals in the asymmetric parietal, temporal, and occipital lobes on T2-weighted scans.	1.Hypertrichosis 2.Headache 3.Vomiting 4.Blurred vision 5.Epilepsy 6.Stroke-like episode	(Ma et al., 2013)

12	212 individuals	90 individuals	122 diabetic patients	1.By DGGE analysis, only one patient was found harboring mitochondrial 3243 A to G mutation. 2.The affected patient was a woman who was 63 years old. She was diagnosed with diabetes at the age of 57.	1.In any of the healthy controls mitochondrial 3243 A to G mutation was not detected.	1.Diabetes mellitus type 1 2. Hearing loss 3.Diabetes mellitus type 2	(Klemm et al., 2001)
13	17 unrelated patients with mitochondrial	0	17 unrelated patients with mitochondrial	1.5 patients were found diagnosed with MELAS. 2.All of them 5 MELAS patients had a mitochondrial 3243 A to G mutation.	1. A MELAS patient died because he had severe concentric hypertrophy of the left ventricle which resulted in and heart failure 2. It was reported to have had 83% of mutant genomes in the heart.	1.MELAS 2.Left ventricular hypertrophy 3.Wall motion abnormalities 4.Occasional palpitation	(Anan et al., 1995)

	diseases		disease s		2.In MELAS patients, similarity was seen in the percentage of mutant genomes in clinically affected and unaffected tissues.		
14	6 patients harboring mitochondrial 3243 A to G mutation	0	6 patients harboring mitochondrial 3243 A to G mutation	1.Two were diagnosed with MIDD and four with MELAS. 2. In peripheral leukocytes, the heteroplasmy rate of the A3243G mutation varied from 9 to 32%. 3. During the initial examination with caloric testing, two patients (33%) exhibited normal responses in both ears, two patients (33%) had reduced responses in one ear, and	1.In patients with the mitochondrial 3243 A to G mutation, hearing loss was attributed to cochlear dysfunction rather than damage to the retrocochlear structures.	1.Vestibular symptoms 2.Diabetes 3.Hearing loss	(Inoue et al., 2019)

				two patients (33%) showed reduced responses in both ears. 4. Three individuals (50%) had a worsening of their caloric response between the first and second assessments.			
15	13 patients harboring mitochondrial 3243 A to G	0	13 patients harboring mitochondrial 3243 A to G mutation	1. Retinal pigment disease was evident in fundoscopic images in ten out of the thirteen individuals (77%). 2.In three participants (23%) who had visual impairments, the most significant changes were observed. 3. A funduscopic examination of seven participants (54%) indicated	1.There was no relationship seen between the severity of the retinal pigmentary abnormalities with age or duration of diabetes. 2.Diabetic retinopathy was present in two (22%) of the nine patients with diabetes.	1.Pigmentary Retinal Dystrophy 2.Patchy hyperpigmentation 3.Loss of retinal pigmentation 4.Diabetes	(Smith et al., 1999)

	mutation			minor pigmentary abnormalities that were asymptomatic. 4.All individuals with pigmentary retinal dystrophy and impaired electrophysiologic responses were older than 40 and had been suffering from diabetes over five years. 5. Among the ten participants with abnormal retinal pigmentation, seven (70%) had both diabetes and deafness, while three (30%), including the two youngest individuals, were either diabetic or deaf.			

16	3 patients	0	3 patients	1. All patients and their mothers had the mitochondrial tRNA-Leu gene mutation m.3243 A>G. 2. Every patient was born following uneventful pregnancy.	1. Results of biochemical studies showed that urine organic acid, ketone bodies, carnitine esters, and blood glucose were all within normal ranges.	1.Isolated respiratory chain complex III deficiency. 2.Hyperlactacidemia	(Jiang et al., 2015)
17	4 individuals	0	4 individuals	1.It was found that the original patient, her daughter, and son, as well as the sister with MELAS syndrome, all four tested family members had the A to G 3243 mt DNA mutation heteroplasmic with wild-type mtDNA.	1.One patient's best-corrected visual acuities were found to be 20/25 and 20/30 on an ocular examination.	1.Ophthalmoplegia 2.Neurosensory deafness 3.Myopathy 4.Macular retinal pigment epithelial atrophy	(Latkany et al., 1999)

18	1 patient harboring mitochondrial 3243 A to G mutation	0	1 patient harboring mitochondrial 3243 A to G mutation	1. The patient's serum creatinine was 70 umol/L, but his uric acid increased unexpectedly to 1011 umol/L when he was in the emergency room. 2. A thicker and more stiff glomerular basement membrane was seen.	1.While there was a small amount of malformed mitochondria in the renal tubules, there was no obvious accumulation of mitochondria in the podocytes.	1.Hyperlactatemia 2.Proteinuria 3.Membranous nephropathy	(Cai et al., 2022)
19	1 patient harboring mitochondrial	0	1 patient harboring mitochondrial	1.Mitochondrial 3243 A to G mutation was found in white blood cells. 2.Anti-GAD antibody was negative.	1.The primary cause of the onset of diabetes in this case was a mitochondrial DNA mutation-related diabetes mellitus, which is known to	1.Diabetes mellitus 2.Cardiomyopathy 3.Hypothyroidism.	(Yamamoto, 2003)

	3243 A to G mutation		3243 A to G mutation	3. The number of a-cells was growing while that of p-cells was decreasing.	produce a severe and rapid drop in insulin secretion.		
20	1 patient harboring mitochondrial 3243 A to G mutation	0	1 patient harboring mitochondrial 3243 A to G mutation	1. 1. The first biopsy of the deltoid muscle revealed 8%–10% of ragged red fibers. 2. In the second biopsy of the vastus lateralis, ragged red fibers were reduced to 1%–2%, yet the succinate dehydrogenase staining in many of these fibers was more intense than in normal fibers.	1.Quantification of the mitochondrial 3243 A to G mutation showed that 78% of the proband's muscle mtDNA was mutated.	1.MERRF	(Fabrizi et al., 1996)

3.2 Statistical presentation of phenotypes

Table 1 encompasses 20 different studies conducted on individuals harboring mitochondrial 3243 A to G mutation. This includes sample number, control population number, tested population number, primary outcomes, secondary outcomes and phenotypes. Various phenotypes have been found. Statistical presentation is shown in the chart below.

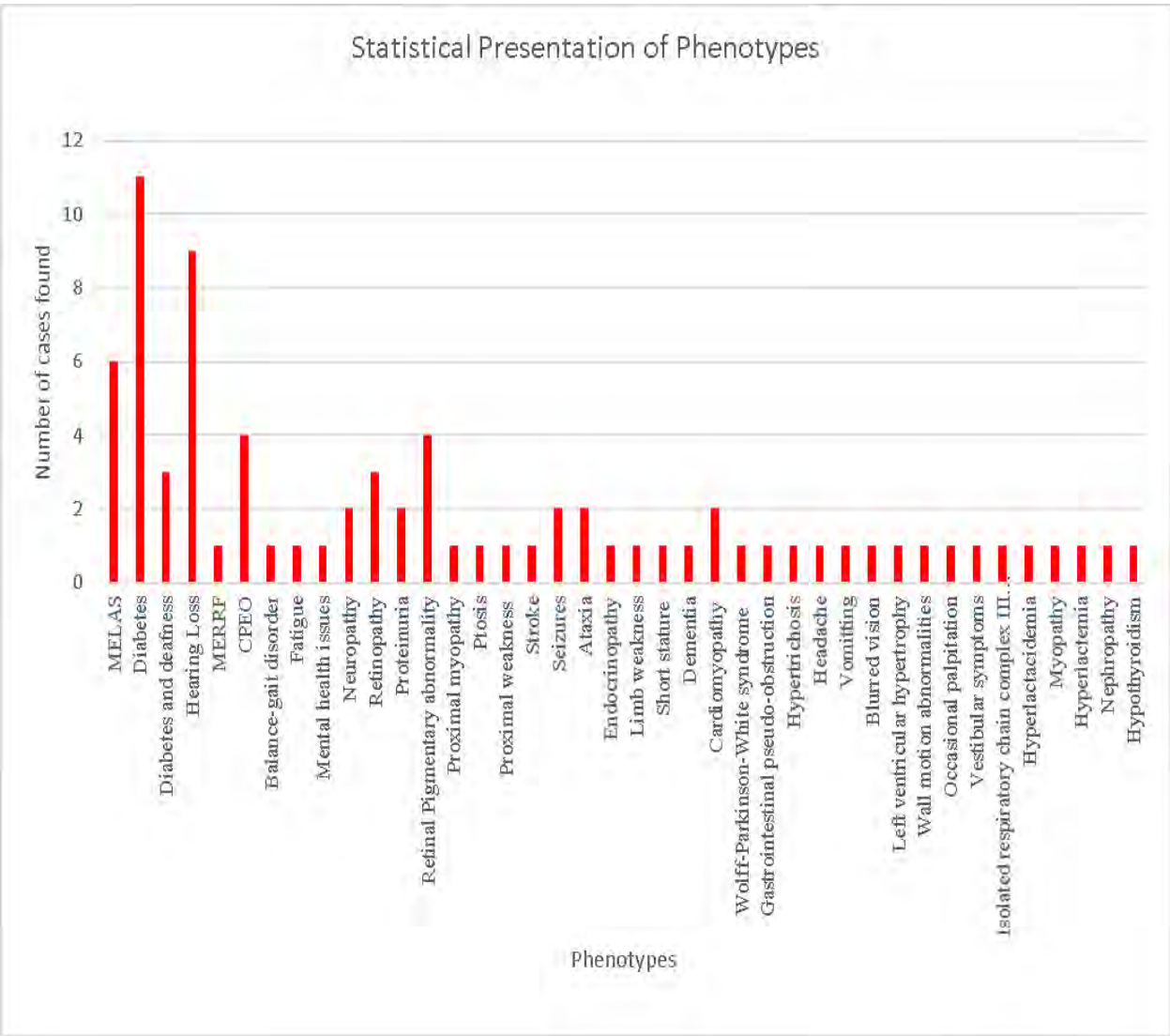

Figure 5: Statistical Presentation of phenotypes

3.3 Identified phenotypes and their prevalence

From the tabulated 20 different studies, 40 different phenotypes were identified. Among them, diabetes is found, the highest, 11 times. Second, hearing loss/deafness is found 9 times. Third, MELAS is found 6 times. Fourth, CPEO and retinal pigmentary abnormality are found 4 times. Fifth, diabetes and deafness as well as retinopathy are found 3 times. Diseases those are found 2 times are- neuropathy, proteinuria, seizure, ataxia and cardiomyopathy. Finally, the diseases those found only once are- MERRF, balance-gait disorder, fatigue, mental health issues, proximal myopathy, ptosis, proximal weakness, stroke, endocrinopathy, limb weakness, short stature, dementia, Wolff-Parkinson-White syndrome, gastrointestinal pseudo-obstruction, hypertrichosis, headache, vomiting, blurred vision, left ventricular hypertrophy, wall motion abnormalities, occasional palpitation, vestibular symptom, isolated respiratory chain complex III deficiency, hyperlactacidemia, myopathy, hyperlactemia, nephropathy and hypothyroidism.

3.4 Clinical phenotypes at different stages of life

The mitochondrial 3243 A to G mutation is the cause of various diseases which manifest at different stages of life. From previous studies it has been found that different clinical features, generally, are seen during different phases. So, a classification is made based on age which are early stage (age<20), middle stage (age 20-40) and end stage (age>40). The following chart represents phenotypes at different stages of life.

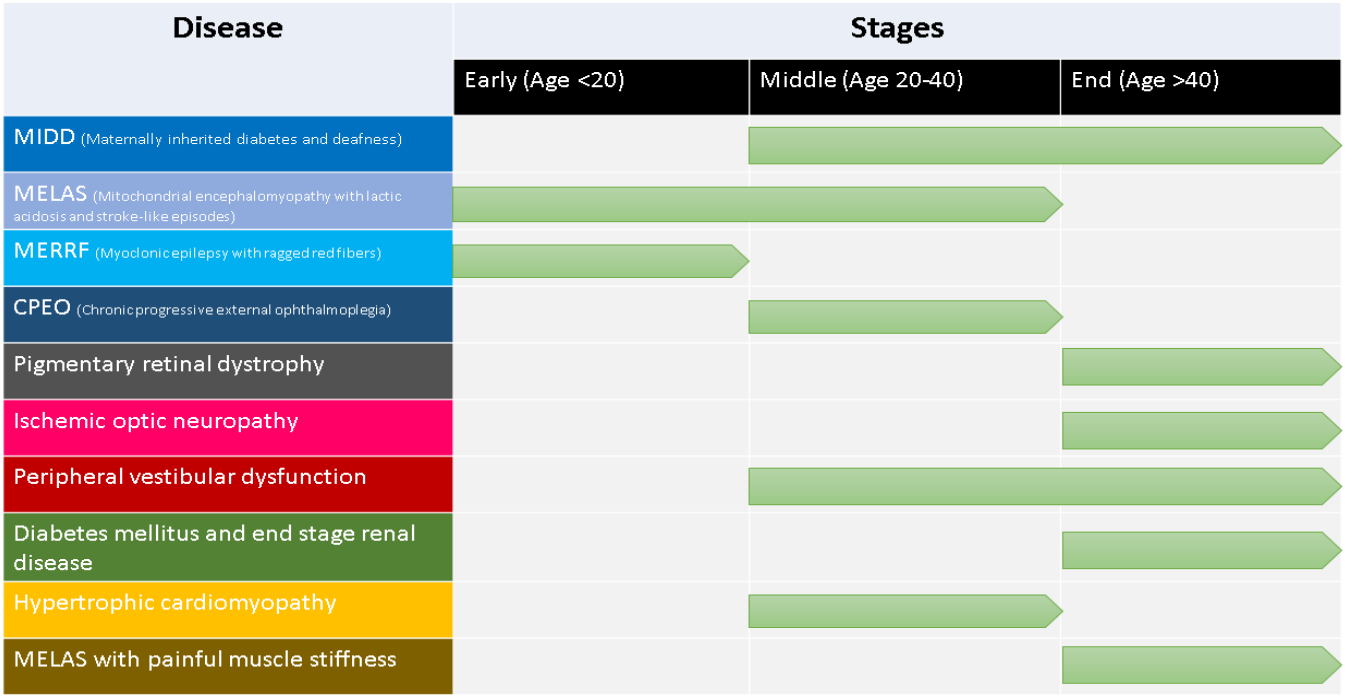

Figure 6: Phenotypes at different stages of life

One of the main clinical phenotypes of this mutation is maternally inherited diabetes and deafness(MIDD). From a study it is found that most patients harboring mitochondrial 3243 A to G mutation develop diabetes around age 35 (Maassen, 2002). From another study it is found that, the average age of developing diabetes due to mitochondrial 3243 A to G mutation is 40.23 ± 3.30 (Wang et al., 2013). The mean age of hearing loss was found 28.6 from another study (Sakata et al., 2022). Which ultimately results in deafness. So, generally MIDD is found at the middle stage and end stage of life in patients harboring the mutation. Another main phenotype of this disease is Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS). MELAS is usually developed between ages of 2 and 15 (Pia & Lui, 2024). In another study, from 42 MELAS patients the average age of diagnosis was found 33 (Cox et al., 2023). So, generally MELAS is found at the early stage and middle stage of life. Another notable phenotype of the mutation is myoclonic epilepsy with ragged red fibers (MERRF). Generally, it is hard to claim any

specific stage of life to develop MERRF. There has been too little information found. But, a study shows that MERRF is found in patients at an early stage of life (Fabrizi et al., 1996). Next, a disorder of the eye known as chronic progressive external ophthalmoplegia (CPEO) is characterized by a gradually worsening incapacity to move the eyebrows and eyes. In many cases, it is the only indication of mitochondrial disease. From a study, it is observed that the age of onset of CPEO in a patient harboring the mutation is 39 (Greaves et al., 2010). This indicates that CPEO is found in the middle stage of life. Another phenotype of the mutation is pigmentary retinal dystrophy. A study found that all patients with pigmentary retinal dystrophy and impaired electrophysiological responses were over 40 years old (Smith et al., 1999), suggesting that pigmentary retinal dystrophy occurs at the end stage of life. The condition known as ischemic optic neuropathy refers to optic nerve disease resulting from a temporary or permanent interruption of blood flow to any part of the optic nerve (Patil et al., 2022). From a case study, this disease was found in a patient harboring the mutation as first presentation. And, it is observed that ischemic optic neuropathy is found at the end stage of life (Scarcella et al., 2023). The mitochondrial A3243G mutation may influence the peripheral vestibular systems due to the strong connections between the cochlea and the peripheral vestibular end organs in terms of embryology, physiology, and morphology (Jin et al., 2006). Research indicates that patients with this mutation typically begin to experience peripheral vestibular symptoms around the age of 36.5 ± 13.2 years (Inoue et al., 2019), suggesting that peripheral vestibular dysfunction is prevalent in the middle to late stages of life. Recently, this mutation was identified in three diabetic individuals with progressive kidney disease, indicating a potential role in the development of renal disease in diabetic patients. Consequently, a study was conducted to assess the impact of this mutation on the progression to end-stage renal disease (ESRD) among diabetic patients. The study identified eight patients with

the mutation, diabetes, and ESRD, all of whom were over 40 years old (Iwasaki et al., 2001). So, ESRD is found in the end stage of life in patients harboring the mutation. Another phenotype found of the mutation is hypertrophic cardiomyopathy. This phenotype is found from a case study which involves a patient of 36 years old. The patient was harboring the mutation and had hypertrophic cardiomyopathy as phenotype (Hsu et al., 2008). This indicates hypertrophic cardiomyopathy is found at the middle stage of life. Lastly, MELAS with muscle stiffness is found in another case study as the phenotype of the mutation. According to the study, MELAS with muscle stiffness is found at the end stage of life (Deschauer et al., 1999).

3.5 Correlation between phenotypes and heteroplasmy level

Heteroplasmy plays a crucial role in mitochondrial diseases, which are caused by malfunctioning mitochondria (Gorman et al., 2016). The development of these diseases depends on the proportion of mutant mtDNA relative to wild-type mtDNA, known as the heteroplasmy level (Parakatselaki & Ladoukakis, 2021). Phenotypes are mostly heteroplasmy level dependent. The following chart represents the general heteroplasmy level of different phenotypes.

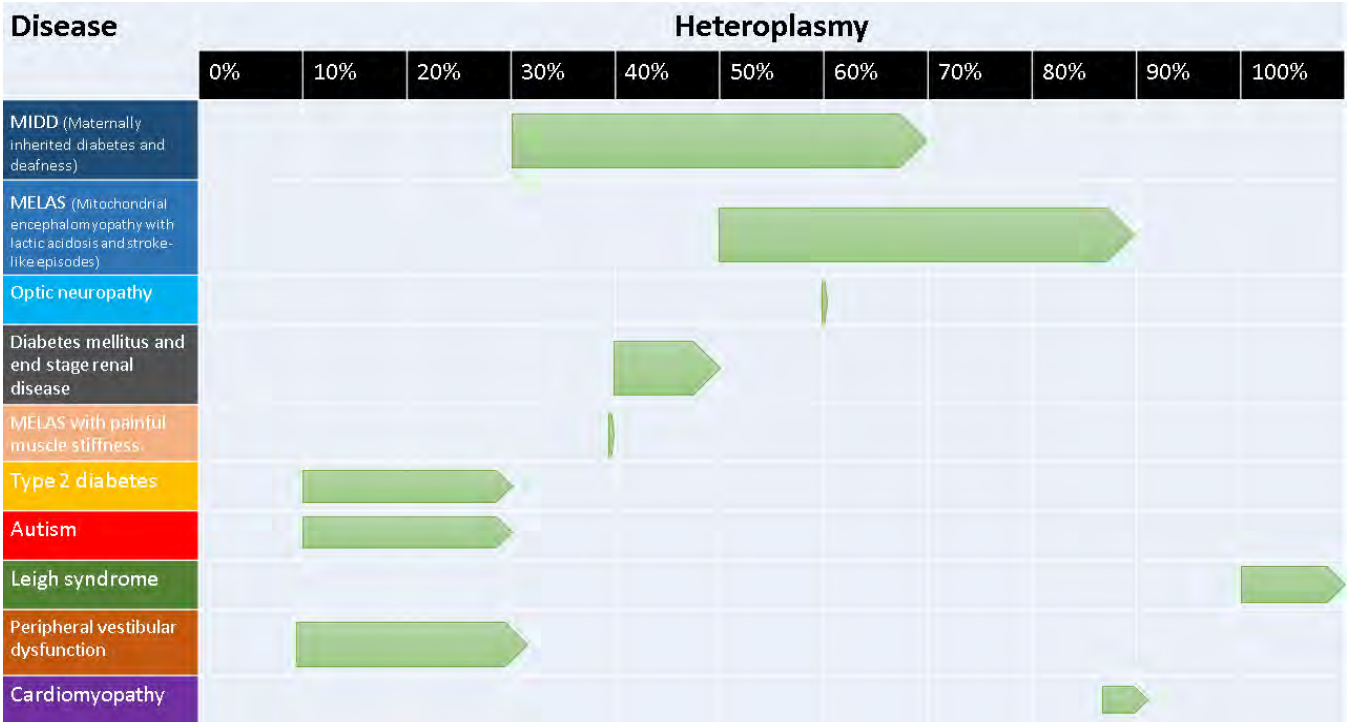

Figure 7: Correlation between phenotypes and heteroplasmy level

Here, discussed levels are not tissue specific as it varies depending upon tissues. Heteroplasmy level for MIDD is 30%-70% (Kyriakidou et al., 2023). Next, heteroplasmy level for MELAS is 50%-90% (Tranah et al., 2018). A study shows that, heteroplasmy level for optic neuropathy is 60% (Motlagh Scholle et al., 2020). Proceeding, heteroplasmy level for diabetes mellitus and end stage renal disease is 40%-50% (Iwasaki et al., 2001). Thereafter, heteroplasmy level for MELAS with painful muscle stiffness is 39% (Deschauer et al., 1999). Then, heteroplasmy level for type 2 diabetes is 10%-30% (van den Ouweland et al., 1992). Next, heteroplasmy level for autism is 10%-30% (Pons et al., 2004). Further, heteroplasmy level for leigh syndrome is (Picard et al., 2014). Next, heteroplasmy level for peripheral vestibular dysfunction is 9%-32% (Inoue et al., 2019). At last, the heteroplasmy level for cardiomyopathy is 87%-91% (Gallego-Delgado et al., 2015).

Chapter 4

Discussion

The mitochondrial 3243 A to G mutation is among the most common causes of neurogenetic disorders, with an estimated prevalence of about 1 in 15,000 individuals (Majamaa et al., 1998). Historically some of the clinical manifestations have been ascribed with this mutation which includes mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS), maternally inherited deafness and diabetes (MIDD) and chronic progressive external ophthalmoplegia (CPEO) (Nesbitt et al., 2013). The mutation was first identified by Goto and colleagues in 1990 and was identified as MELAS mutation (Goto et al., 1990). With the progression of time diseases associated with the mutation have increased. Many mitochondrial 3243 A to G mutation carriers, however, do not exhibit the whole MELAS syndrome. Rather than developing the strokelike episodes typical of MELAS, they may either be asymptomatic or exhibit a wide range of clinical symptoms which indicate multiorgan engagement but differ in their clinical severity from mild to severe involvement (Damian et al., 1995). The A3243G mutation appears to be more common than previously believed, and its clinical symptoms are likely underestimated(Manwaring et al., 2007).

The objective of this study was to better understand the clinical manifestations connected to this mutation across several phases of life and analyze the significance of heteroplasmy, or the percentage of mutant mitochondrial DNA (mtDNA), in defining these manifestations. The results illustrate the complexity of mitochondrial disease by emphasizing how age and heteroplasmy levels affect the clinical manifestations. The study showed that the 3243 A to G mutation is linked to a wide range of clinical characteristics that differ markedly during different phases of life.

Generally, common childhood symptoms include MELAS and MERRF. MELAS is generally diagnosed below age 20. The disease is diagnosed when heteroplasmy is generally 50%-90%. Also, MERRF is diagnosed at an early stage of life. With the progression of age MIDD is generally diagnosed with a heteroplasmy level of 30%-70%. Also, CPEO, Peripheral vestibular dysfunction and hypertrophic cardiomyopathy is generally diagnosed at the middle stage of life which is under 40. Lately, retinal pigmentary abnormalities, muscle stiffness and end stage renal diseases are diagnosed at the end stage of life which is basically after 40 years. These phenotypes show different levels of heteroplasmy. It is to mention that, age of onset of these diseases may vary as case of exception and heteroplasmy level is not tissue specific.

Our findings align with the majority of existing research on the 3243 A to G mutation, particularly regarding the wide range of clinical symptoms and the significant role heteroplasmy plays in determining disease severity. Additionally, the mutation has been linked in the past to a variety of illnesses, including maternally inherited diabetes and deafness (MIDD), MELAS syndrome, and others. Our research supports the idea that heteroplasmy is essential for this variability.

Research by Geng and colleagues demonstrates that the m.3243A>G mutation in mitochondrial DNA (mtDNA) impairs mitochondrial function, especially in peripheral blood mononuclear cells (PBMCs). Increased oxidative stress and decreased energy generation are associated with this disorder. More prominent clinical symptoms and more obvious functional impairment are correlated with higher heteroplasmy levels (Geng et al., 2019). It is seen that a heteroplasmy of 90% - 100% causes Leigh syndrome. This syndrome is a devastating neurodegenerative disease which is generally diagnosed at an early stage of life (Baertling et al., 2014). This sets an example of the consistency of our findings with previous studies.

It is possible to partially explain the variation in clinical symptoms linked to the 3243 A to G mutation by considering the molecular mechanisms behind mitochondrial malfunction, especially when considering different life stages. Due to the mutation's impact on mitochondrial tRNA, there will be a reduction in the production of mitochondrial proteins and consequent energy shortages. The brain, muscles, and pancreas are high-energy tissues that are disproportionately affected by this energy imbalance, which accounts for the early onset of symptoms in these organs (Goto et al., 1990). This image is further complicated by heteroplasmy. The concept of heteroplasmy suggests that cells contain a mix of normal and mutated mtDNA, with varying proportions of mutated mtDNA across different tissues. There is a good chance that this tissue-specific heterogeneity adds to the variety of clinical manifestations seen. For instance, tissues like neurons and muscle cells, which require more energy, might be more susceptible to the negative consequences of high heteroplasmy levels, which could result in severe symptoms like muscle weakness and stroke-like episodes. Conversely, organs that require less energy could be able to handle higher concentrations of mutant mtDNA, which could account for some patients' milder symptoms even though they have significant heteroplasmy (Wallace, 1999). In the study, these facts appeared as a generalized form. Heteroplasmy level is presented without specification of tissues so that a standard level can be formed. For these phenotypes, different levels of heteroplasmy are observed in different tissues. But, heteroplasmy in leukocyte generally shows a standard expression (Tranah et al., 2018).

The diagnosis and treatment of patients with the mitochondrial 3243 A to G mutation will have a notable benefit by the study's findings. Firstly, findings emphasize the importance of prompt and precise diagnosis, especially for patients with high heteroplasmy levels who can be susceptible to

severe phenotypes. When combined with heteroplasmy quantification, genetic testing for the mitochondrial 3243 A to G mutation can yield important prognostic data and direct therapeutic care.

From the study, the best course of treatment may involve a customized strategy that considers the patient's stage of life as well as their amount of heteroplasmy. For example, young children with high heteroplasmy levels require early management to avoid or lessen severe consequences like MELAS syndrome. Contrarily, patients at middle or end stage with medium heteroplasmy level may take treatment approaches for treating diseases like MIDD, cardiomyopathy and nephropathy(Parikh et al., 2015).

The result of the study enlists the identified clinical phenotypes which appear at different stages of life and their correlation to heteroplasmy level. Age of onset and age of diagnosis are not always the same. Because, disease is not diagnosed clinically just after the onset often. However, the idea of classification of age in the study may help to enforce the initiation of the diagnosis process. Also, the idea of heteroplasmy may predict the phenotype which may appear in the individual. To sum up, determination of heteroplasmy level at a certain age may predict the appearance of phenotype and can be beneficial in the treatment.

Chapter 5

Limitation of the study

Though the study provides important insights on the mutation and its phenotypes, there are some limitations which should be acknowledged. First, most of the the selected study reports lack control population number. Second, three studies had only 1 individual as tested population. Still, those studies were selected to ensure the listing of phenotypes. Third, the classification based on age can not be considered as standard. Because, those information were collected from only a few papers. Additionally, there are not so many studies available based on age. Fourth, heteroplasmy levels mentioned are not tissue specific. Because, till date there has no study taken place which ensures the constant level of heteroplasmy in certain tissues. In different tissues, different levels of heteroplasmy were seen. Moreover, they don't have any direct relation with each other. So, this study can't claim the mentioned heteroplasmy levels as standards.

Chapter 6

Conclusion

This study concludes by illustrating the complex relationship between heteroplasmy, clinical phenotypes and the mitochondrial 3243 A to G mutation. These findings are collected from different studies conducted on individuals harboring the mutation. There were limitations in those studies as well as in this study as a consequence. To establish a standard classification of age and heteroplasmy level more research is required. The results highlight the significance of taking the patient's life stage and heteroplasmy levels into account when diagnosing and treating mitochondrial disorders. Understanding of the variation in heteroplasmy levels among tissues and how these variations affect clinical variability may help the progression of targeted therapy. Finding nuclear genes that interact with the mitochondrial mutation may help explain the disease's pathophysiology and identify new targets for treatment. Even though there is still a lot to learn about the variables that affect clinical variability, this work lays the groundwork for further investigations targeted at enhancing the treatment and prognosis of individuals with mitochondrial illnesses.

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