Mutation in DNA Polymerase γ: Mitochondrial Disorders

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 (Hons.)

Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
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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.
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1 Approval

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Abstract

Together with key components of the mitochondrial DNA (mtDNA) replication machinery, DNA polymerase γ (POLG) replicates the human mitochondrial genome. Defects in mtDNA replication or nucleotide metabolism result in mtDNA deletions, point mutations, or depletion. The resulting loss of cellular respiration ultimately causes mitochondrial genetic diseases, such as mtDNA deletion disorders, progressive external ophthalmoplegia, ataxia-neuropathy, or mitochondrial neurogastrointestinal encephalomyopathy, as well as mtDNA depletion syndromes like Alpers or early infantile hepatocerebral syndromes. This review paper provides an overview of the most recent research on the role of POLG in the manifestation of mitochondrial diseases.

Keywords: Mitochondria, DNA polymerase, POLG, DNA replication, DNA repair.

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List of Acronyms

POLG- DNA polymerase subunit gamma

MMR- Mismatch repair

SNP- single nucleotide polymorphism

SSB- Single strand break

DSB-Double strand break

BBB- blood brain barrier

SiRNA-small interfereing RNA

ROS- radioactive oxygen species

PEO-Progressive external opthelmegia

ANS-Autonomic nervous system

MEMSA –myoclonic epilepsy myopathy sensory ataxia

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

ArPEO-autosomal recessive progressive external ophthalmoplegia

2 Introduction

2.1 Overview

In eukaryotic cells, the enzyme known as DNA polymerase (also known as POLG) is the one that is in charge of replicating the mitochondrial DNA. Various mitochondrial diseases, such as tissue degradation and neuromuscular disorders, can be caused by mutations in mitochondrial DNA (mtDNA), which are connected to the aging process. mtDNA mutations can also cause aging. (Copeland & Longley, 2003) Only POLG, one of the 16 different types of DNA polymerases found in eukaryotic cells, is known to have a function in mitochondria. A catalytic subunit and a homodimeric version of its accessory subunit are the primary components of the holoenzyme that represents human polymerase gamma. There is a high degree of homology between the catalytic component portions of different species. The other component, which is referred to as the accessory subunit, is essential for secure binding and continuous DNA synthesis. The year 2001 marked the discovery of the very first disease-causing mutations in POLG. Patients diagnosed with Alpers syndrome, ataxia neuropathy syndrome, idiopathic parkinsonism, nucleoside reverse transcriptase inhibitor toxicity, and other conditions have been shown to carry mutations in the POLG gene. These disorders are fundamentally brought on by deletions or shortages of mtDNA in the tissues where symptoms manifest. (Milone et al., 2008)

POLG is thought to be the replicative polymerase of mitochondrial DNA (mtDNA), and it may also play a role in the repair of mtDNA. Mutations in the POLG1 gene, which is responsible for coding for the catalytic subunit, are the root cause of a wide variety of clinical abnormalities. Progressive external ophthalmoplegia is characterized by the accumulation of mtDNA deletions in muscle, which can be brought on by either dominant or recessive mutations in the POLG1 gene. New research has shown that mutations in the gene POLG1, particularly those that affect its spacer domain, are a major cause of inherited neurodegenerative symptoms such as sensory ataxic neuropathy, dysarthria, myoclonus, seizures, ataxia, and parkinsonism. These symptoms can be passed down through generations. (Hakonen et al., 2005). To summarize, there have been approximately 150 disease mutations found so far in POLG, which indicates that it is a prominent locus for mitochondrial illness. This validates that POLG is a big locus. (Copeland et al., 2003).

2.2 Aim of the Study

The dysfunction of mitochondrial DNA replication brought on by mutations in the mitochondrial DNA polymerase has been investigated in this thesis. Even amid the numerous clinical signs and symptoms brought on by mitochondrial disease, dysfunction of mitochondrial DNA polymerase results in an even more varied collection of clinical manifestations.

3 Structure of the Mitochondria

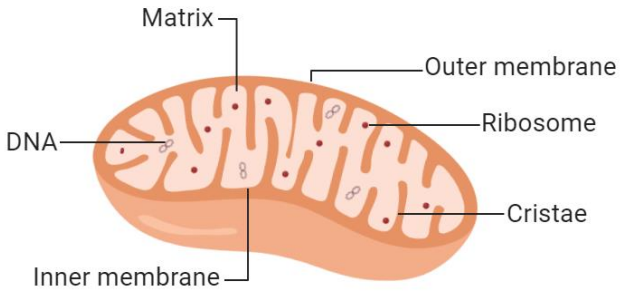

Figure 1:Structure of Mitochondria

- The mitochondrion is a rod-shaped structure that is double-membraned and may be found in both plant cells and animal cells.
- Its diameter might be anywhere from 0.5 and 1.0 micrometers, depending on its size.
- The structure is made up of an exterior membrane, an inner membrane, and a substance that is similar to gel and is referred to as the matrix.
- Layers of proteins and phospholipids make up both the outer membrane and the inner membrane of a cell, which are separated by a region called the intermembrane space.
- Porins are specialized proteins that are abundant in the mitochondrion's outer membrane, which covers the surface of the organelle and protects it from the outside environment.(DiMauro & Davidzon, 2005)

3.1.1 The Outer Membrane

The mitochondria are surrounded by two biological membranes. There are several important membrane proteins, protein complexes, and structures that are involved in the transport of proteins from the cytosol to the mitochondria. These components may be found in the mitochondrial outer membrane (Endo & Yamano, 2010). Smaller molecules, those with a

molecular weight of less than 5 kilodaltons (kDa), may easily pass through the membrane pores, but larger proteins need a transporter like the translocase of the mitochondrial outer membrane (TOM). In addition to this, it plays a role in apoptosis and the body's natural defensive mechanisms against viruses (Suhaili et al., 2017). (Gillies and Kuwana, 2014).

3.1.2 Space Within the Inner Membrane

Mitochondrial matrix and intermembranous space are two watery compartments. Intermembranous space plays a critical role in coordinating mitochondrial activities with other cellular processes, which was not previously known despite the fact that many of the matrix's physiological roles were previously widely documented. Such as : Matrix-cytoplasmic protein, lipid, or metal ion exchange, apoptosis cascade regulation, and signal transduction pathways (Herrmann & Riemer, 2010). Both breathing and metabolism are controlled by it. Exchange of ions, metabolites, and other small molecules between the cytosol and the matrix is first regulated by the outer and inner mitochondrial membranes. Throughout evolution, porin has remained mostly unchanged and now serves as the primary transport molecule across the outer membrane, allowing molecules as large as 5–6 kDa to pass freely (Herrmann & Riemer, 2010).

3.1.3 Mitochondrial Inner Membrane

The inner border membrane (IBM) and the cristae membrane (CM) of the mitochondrial inner membrane are two morphologically different domains that are joined by thin, tubular cristae junctions. The mitochondrial inner membrane is separated into these two domains. Cristae are the folds that can be seen in the inner membrane. These folds help to enhance the surface area of the membrane while also splitting it into a number of different parts that serve different functions. The inner border area contributes to the development of the respiratory chain complex as well as the transport of proteins and lipids because of its close contact to the outer membrane. This close proximity also plays a function in the process. Cristae are extensions of the matrix that include assembled respiratory complexes and function as a location for the replication of mitochondrial DNA. Cristae are found in all eukaryotic cells (mtDNA). (Vogel et al., 2006)

3.1.4 Matrix

The citric acid cycle can be completed using the enzymes found in the mitochondrial matrix. In addition, it serves as the primary Ca2+ storage site and includes mtDNA and ribosomes. (Srere, 1980).

4 Functions of Mitochondria

- Controls the metabolic processes that take place inside the cell.
- Encourages the formation of new cells and the proliferation of existing ones.
- Facilitates the elimination of ammonia from the cells of the liver.
- Participates significantly in the process of apoptosis, also known as programmed cell death.
- Contributes to the formation of some components of the blood as well as a number of hormones, including testosterone and oestrogen.
- Contributes to the upkeep of a calcium ion concentration that is appropriate across the various compartments of the cell.
- It also plays a role in a variety of other cellular processes, such as cellular differentiation, cell signaling, cell senescence, the regulation of the cell cycle, and cell proliferation.
- Role in neurotransmitter metabolism.
- Role on cholesterol metabolism.
- Role in heme synthesis(Ozawa et al., 1987)

5 mtDNA Related Diseases

The human mitochondrial genome is very tiny when compared to the nuclear genome, and the issues that are faced in clinical and experimental mitochondrial genetics are distinct from those that are faced in nuclear genetics. Mutations in mitochondrial DNA, often known as mtDNA, are a substantial contributor to hereditary illness, despite the relatively modest size of the mitochondrial genome. In the recent past, a significant amount of headway has been achieved in the areas of fundamental mitochondrial genetics, the association between hereditary mutations and disease phenotypes, as well as the discovery of acquired mtDNA mutations in both the aging process and cancer. (Taylor & Turnbull, 2005)

Prior to relatively recent times, mitochondrial genetics and disorders induced by abnormalities in mitochondrial DNA (mtDNA) were not thought to be part of conventional genetics. These perspectives were hampered by the facts that mtDNA is transmitted in a manner that is distinct from that of nuclear DNA, that individual cells contain a large number of copies of mtDNA, and that it is generally accepted that mtDNA illness is an exceptional condition. In addition to this, clinical syndromes that are brought on by mtDNA mutations can have a wide variety of symptoms and are frequently presented in a condensed fashion.

Diseases that originate in the mitochondria can also be brought on by issues with the genes found in the nucleus. This is due to the fact that nuclear genes are responsible for coding for the majority of the proteins that are involved in mitochondrial metabolism as well as all of the proteins that are involved in maintaining healthy mtDNA. Some mutations in these nuclear genes can induce symptoms that are similar to those that persons who have faults in their mtDNA have, and some genetic conditions that are caused by nuclear genes can also create issues with the mitochondrial genome.

Table 1: Disorders Caused by mtDNA Mutations

Disorders	Clinical Phenotype	MtDNA Genotype	Gene	Status	Inheritence
Kearns sayre syndrome	Cardiomyopathy	Large scale deletion	Several deleted genes	Heteroplasmic	Usualy sporadic
Chronic Progressive External Ophthalmoplegia	Opthalmoplegia	A single,large scale deletation	Several deleted genes	Heteroplasmic	Usually sporadic
Pearson syndrome	Pancytopenia	A single, large scale deletation	Several deleted genes	Heteroplasmic	Usually sporadic
Melas	Myopathy	Individual mutations	TRNL 1	Heteroplasmic	Maternal
Sensorineural hearing loss	Deafness	Individual mutations	TRNS1	Homoplasmic	Maternal

Exercise intolerance	Fatigue, muscle weakness	Individual mutations	RNR1, TRNS1	Homo or Heteroplasmic	Maternal
Myopathy and disorders	Myopathy, weakness, diabetes	Individual mutations	TRNE	Hetero or Homoplasmic	Maternal

5.MtDNA-Related Disease Prevalence

In recent years, there has been a rush of epidemiological research that has reinforced the assumption that mitochondrial illnesses are among the most prevalent genetic abnormalities and a significant burden on society. These studies have taken place in both developed and developing countries. The minimal prevalence is at least 1 in 5,000 individuals when research on both children and adults are included. A study of adult patients in northern England reported an overall frequency of 6.57/100,000, with LHON having a particularly high prevalence (3.22/100,000). The study focused on individuals who had mtDNA mutations. Sweden and Australia, two countries on opposite sides of the world, each conducted their own epidemiological study of children who were impacted. Both in terms of the overall prevalence of mitochondrial diseases (approximately 5/100,000) and the prevalence of mtDNA-related disorders, which accounted for about 15% of the total, the findings were eerily similar. The prevalence of mtDNA-related disorders accounted for approximately 15% of the total. (Taylor & Turnbull, 2005).

6.Sources of mtDNA Damage

The mitochondrial components are likely to be the first to be exposed and damaged by ROS because of their high reactivity and local production within the mitochondria. (Jarrett et al., 2008). Radiation, chemicals that are present in the environment, metabolites that are derived from the components of the food, and medicines that are used in therapeutic therapy are the primary contributors to environmentally induced damage to mtDNA. DNA adducts can be produced by cigarette smoke's benzopyrene and acrolein components, as well as by platinum-based chemotherapeutic drugs and ultraviolet radiation. These DNA adducts hinder mitochondrial transcription. (Cline, 2012)

7.Mechanism of MtDNA Repair

7.1Base Excision Repair

Repairing DNA that has been damaged by oxidation, deamination, or alkylation requires a process called base excision repair. The BER provides protection against a variety of diseases, including cancer, aging, and neurodegeneration (Krokan & Bjoras, 2013). DNA glycosylases are the enzymes responsible for the beginning of the BER pathway. DNA glycosylases are enzymes that identify a specific set of modified bases, such as 8-deoxyguanosine or thymine glycol (Bohr & Anson, 1999). BER is made up of two subpathways in the nucleus, which are called short patch BER and long patch BER respectively. The short patch BER that is present in mitochondria may replace a single damaged base. The mitochondrial extracts were shown to contain BER with a long route. These two go through a number of processes, including base excision, lesion identification, gap filling, end processing, and ligation. The presence of DNA damage stimulates the progression of these processes (Marn-Garca, 20Marn-Garca, 2011).

7.1.1Mechanism

There are some unwanted bases which causes problems in DNA. Example: Uracil causes transition mutation, hypoxanthine causes DNA misleading, 3 methyl adenosine stops replication process. To cut the bases from the DNA base, excision repair mechanism is used. So, from the damaged base DNA glycosylase used to cut just the damaged base without cutting phosphodiester backbone. It produces AP sites and furthermore AP endonuclease enzyme recruits which will recognize the AP site and cut Ap site into the backbone of DNA. After that, it recruits DNA polymerase 1 which uses 3’-5’exonuclease activity and fulfill gap formation by correct base pairs which is filled by DNA ligase enzyme.

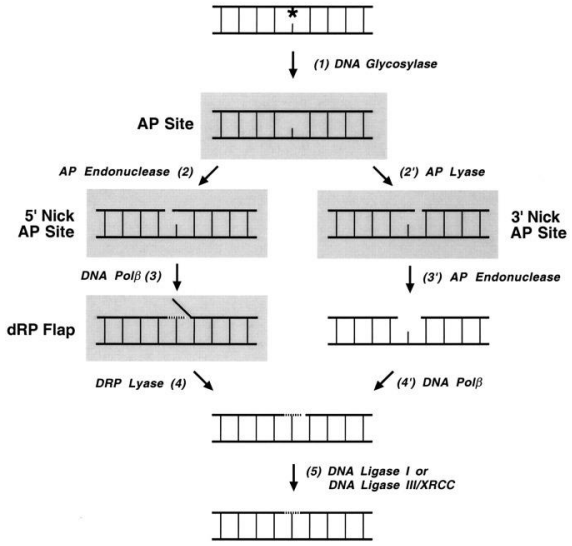

Figure 2: Mechanism of Base Excision Repair

7.2Nucleotide Break Excision Repair

The nucleotide excision repair (NER) pathway is often used to fix damage to DNA caused by ultraviolet (UV) light, environmental mutagens, and some chemotherapeutic drugs. (Larsen et al., 2005). Mammals primarily use nucleotide excision repair (NER) to eliminate large DNA lesions from DNA, such as those caused by UV light, environmental mutagens, and some cancer chemotherapy adducts. (Schärer, 2013)

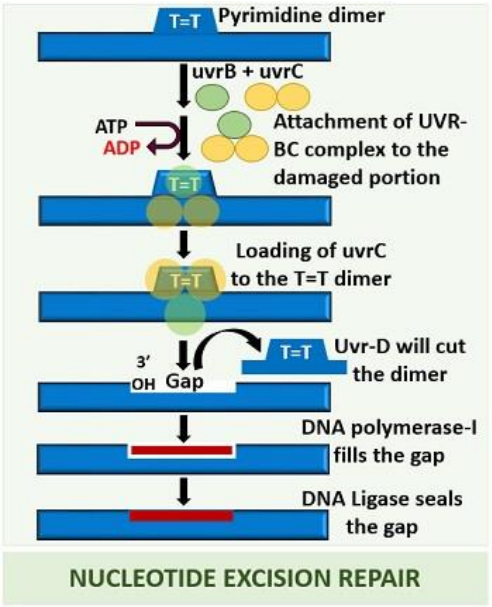

Figure 3: Mechanism of Nucleotide Excision Repair

7.2.1Mechanism

Nucleotide excision repair is done when there is UV radiation or any kind of physical or chemical mutagenic effect and it causes DNA lesion. By exposure to radiation DNA form pyrimidine dimer. UVRB and UVRC will form dimer and make complex with the help of ATP hydrolysis. Then, attachment of UVR-BC complex will occur in damaged protein. After that, UVRC will load to the T=T dimer and it causes a cleave in the backbone of the DNA. Then, UVRD, a functional helicase will simply separate the lesion containing DNA. It will simply remove thymine section. After that, DNA polymerase 1 fills the gap and DNA ligase seals the gap and complete nucleotide excision repair.(Zhang et al., 2017)

7.3Mismatch Repair

Mismatch repair exists in the nucleus and in mitochondria to repair mismatches caused by alkylation, oxidation, deamination of bases, erroneous insertions in DNA replication. (Zhang et al., 2017) Mismatch repair is a post replicative DNA repair system which corrects base mismatches and small lopes. (Boesch et al., 2011). Mismatch repair is done for excluding out unwanted base from the DNA. Uracil is an unwanted base. Normally, uracil is produced due to mutation of a base cytosine. Cytosine can be converted into uracil by a process called deamination. It is unwanted because cytosine pairs with guanine, when it is deaminated it is converted into uracil and uracil pairs with adenine. So, instead of G-C base pairing and shifts to A-T base pairing and causes transitional mutation. Again, deamination of adenine causes hypoxanthine. It is also unwanted.

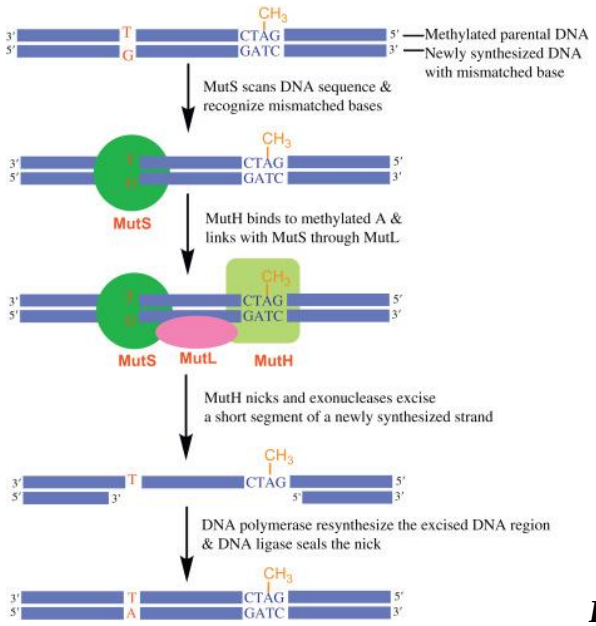

Figure 4:Mechanism of Mismatch repair

7.3.1Mechanism

There are three proteins which are required for mismatch repair. Those are called mutator gene conductor protein. MUT proteins are MutS, MutL and MutH. At first a wrong nucleotide is incorporated in the methylated parental DNA. MutS recognizes the particular area and the mismatched bases. It recruits MutL, which recognizes methyl group in the parent strand. Then, it brings the daughter strand to a position to cut and it is done by MutH and it recruits another enzyme named Uvr-d which is a helicase enzyme. It cleaves unwanted bases from the DNA. DNA polymerase resynthesized the excised DNA region and DNA legion seals the nick.

7.4Double Strand Break Repair

Nonhomologous end joining, often known as NHEJ, and homologous recombination are two methods that can be used in eukaryotic cells to repair double-strand breaks (Zhang et al., 2017). In contrast to the repair routes for more clearly delineated flaws, the enzymes responsible for NHEJ repair can function independently at each of the two DNA ends that are being joined. NHEJ is required for the repair of double-strand breaks (DSBs), regardless of whether the DSBs are physiological or pathological (Lieber, 2011).

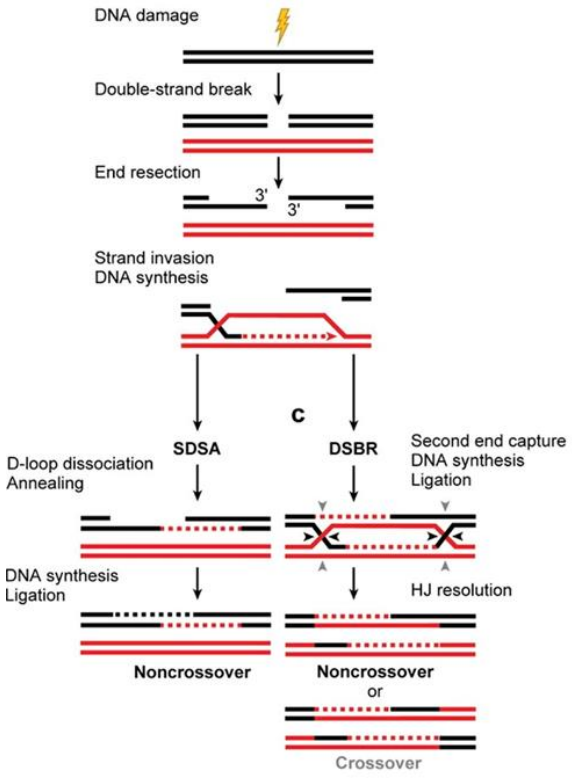

Figure 5: Mechanism of Double Strand Break Repair

7.4.1Mechanism

Double strand breaks can be repaired by SDSA and DSBR. After DSB formation, DNA ends are resected to give 3'single strand DNA. Strand invasion forms a D loop. In the SDSA pathway, the D loop is unwound, and the freed ssDNA strand anneals with the complementary ssDNA strand that is associated with the other DSB end. The reaction is completed by gap-filling DNA synthesis and ligation. Only non-crossover products are formed. Alternatively, the second DSB end can be captured to form an intermediate that harbors two Holliday Junctions (HJ)s, accompanied by gap-filling DNA synthesis and ligation. The resolution of HJs by a specialized endonuclease can result in either non-crossover (black triangles) or crossover products.

8.Diseases Caused by POLG Mutation

Some diseases with its mutational mechanism are given below:

Table 2: Patient History and POLG Gene Mutational Mechanism

Patient History	POLG Gene Mutational Mechanism
54-year-old woman had a history of distal lower limbs paresthesia and bilateral ptosis	Two mutations occurred: 1. G > A change at the nucleotide position 1399 2. C > T change at the nucleotide position 3412
A 34-year-old woman was evaluated for a 2.5-year history of progressive bilateral ptosis, limited eye movements, lower extremities paresthesia, and unsteadiness	It is found homozygous for the p.A467T, the most common POLG mutations. It gives its effect by decreasing polymerase activity and interferes with the interaction with the accessory POLG2 subunit.

33-year-old white male presented with a one-year history of progressive difficulties with ambulation, stiffness, cramping of the lower extremities, foot numbness, and poor balance	They are found to be homozygous for G>A in the 2243 positions and it is responsible for the amino acid change.
A 30-year-old woman, sis, presented with a 3-year history of progressive unsteadiness, limb paresthesia, dysarthria, dysphagia, and weakness	They are found to be homozygous for G>A in the 3428 positions and it is responsible for the amino acid changes.

8.1Mitochondrial Recessive Ataxia Syndrome

Mitochondrial recessive ataxia is a disease that causes movement problems that get worse over time. This is because nerve cells in the part of the brain that controls movement start to damage. After extensive genetic and metabolic investigation, a nucleotide substitution c.2207 A→G in the POLG gene results in amino acid change Asn 736Ser in exon 13 was demonstrated. This mutation was considered to be compatible with mitochondrial disorder and it thus implicated in the pathophysiology of the neuropsychiatric syndrome. (Verhoeven et al., 2011)

Major Symptoms:

- Epilepsy
- Headache
- Dysarthia
- Opthalmoplegia
- Peripheral neuropathy
- Intellectual disability
- Psychiatric symtoms

- Movement disorders

8.2Progressive External Ophthalmoplegia (PEO) with Parkinsonism

The term progressive external ophthalmoplegia refers to a diverse set of illnesses defined by chronic, progressive, bilateral, and usually symmetric ocular movement impairment and ptosis. Muscle biopsies with histology and ultrastructural alterations suggested a mitochondrial dysfunction. The primary mitochondrial abnormality in these individuals was later confirmed as the origin of the condition by the discovery of respiratory chain failure.(Bau & Zierz, 2005) POLG1 mutations cause autosomal dominant PEO with parkinsonism (POLG1-PD). Parkinsonian features develop slowly and show a good response to levodopa treatment. (Luoma et al., 2004). Additionally, two case reports involving PEO with parkinsonism caused by the C10ORF2 mutation c. 1121GA have been published. (Vandenberghe et al., 2009).

Major Symptoms:

- Abnormality of the eye
- Abnormality of the musculoskeletal system
- Constitutional symptom
- Ear malformation

8.3Infantile Hepatocerebral Syndrome (Alpers Syndrome)

Multiple homozygous or compound heterozygous POLG1 mutations, c. 1399GA, resulting in p.Ala467Thr amino acid alteration, are the cause of Alpers' syndrome, a recessive disorder. Psychomotor delay, uncontrollable seizures, and liver failure in newborns and early children are characteristics of Alpers syndrome. Typically, symptoms appear between the ages of 3 and newborn. Ataxia, hemiparesis, and hypotonia are common presentations; sensory neuropathy is a much less common occurrence. Other clinical symptoms that may develop are encephalopathy, ataxia, hypotonia and occasional elevation in serum lactate. Alpers' syndrome is a recessive disease, caused by several different homozygous or compound heterozygous POLG1 mutations. (Harding, 1990).

Major Symptoms:

- Psychomotor regression (dementia)

- Seizures
- cortical blindness
- Liver disease with micronodular cirrhosis

8.4Sensory Ataxic Neuropathy, Dysarthria and Ophthalmoparesis

Sensory ataxic neuropathy, dysarthria and ophthalmoparesis (SANDO) is a mitochondrial ataxia, caused by several different mutations in POLG1. c. 1399G-A and c. 2243G-C mutations in POLG1 are the most common mutations. The symptoms behind the disease are weakness in lower limbs or gait disturbances. Additionally, ataxia, dysarthria, neuropathy, external ophthalmoplegia, ptosis, lack of deep tendon reflexes, diminished vibration and position sense may also be present. (Schulte, 2011)

Major Symptoms:

- myopathy
- seizures
- hearing loss
- progressive gait unsteadiness
- absent deep tendon reflexes
- Romberg's sign
- decreased sense of vibration
- detection of ragged-red fibers on muscle biopsy

9Future Prospect

A dysfunctional POLG that results in the depletion or deletion of mtDNA is the primary pathogenic route in this group of mitochondrial illnesses. Our perspective on and strategy for treating primary mitochondrial diseases have evolved as a result of developments in the molecular research of mtDNA. However, more research is required to clearly relate the mutation to the clinical condition.

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