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# The Relevance of Mitochondrial DNA Mutation in Human Diseases and Forensic Sciences: Review Article

Ruaa Hussein Ali[1], Farhan Khaleel Hussein[2,*], Dmoaa Majid Nasar[3], Asmaa Abd Al salam Salem[4]

[1]Forensic DNA Center for Research and Training, Al-Nahrain University, Jadiriya, Baghdad, Iraq
[2]Department of Biology, College of Education for Pure Sciences, Kirkuk University, Kirkuk, Iraq
[3]College of Pharmacy, Thi-Qar University, Iraq
[4]College of Applied Science, University of Fallujah, Fallujah, Iraq.

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**Abstract**

Several studies have been carried out on mitochondrial DNA mutation in human diseases. Mitochondria are self-contained organelles with their DNA. The primary function of mitochondria is oxidative phosphorylation (OXPHOS), which is how the Electron Transport Chain (ETC) provides energy to the cell. Reactive oxygen species (ROS), which can oxidative destroy DNA, proteins, and macromolecules like lipids, are one of the process's potentially hazardous byproducts. Compared to mitochondrial DNA (mtDNA), nuclear DNA is better protective and has more repair mechanisms, making it more susceptible to oxidative damage that might result in mutations. This review focuses on the illnesses caused by mtDNA mutations known as "mitochondrial diseases. Numerous characteristics of (mtDNA), mainly those related to matrilineal heredity, a high duplicate number, and the absence of recombination, are advantageous for forensic study. Old bones, teeth, and hair are used as forensic samples for analysis, along with other biological samples with low levels of DNA. Different mtDNA haplogroups can affect longevity and risk of infection, in addition to being used to determine a person's geographic origin.

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*Corresponding author: farhankhaleel@uokirkuk.edu.iq


## 1. Introduction

Mitochondria are inimitable organelles that have evolved from the addition of endosymbiotic alphaproteobacteria into a host eukaryotic cell of the Archaea phylum [1]. These organelles have a symbiotic connection that makes them necessary for the proper operation of eukaryotic cells. They are essential for several physiological functions, including redox equilibrium, fatty acid oxidation, acetyl CoA production, and ATP generation [2]. These activities are made possible by their distinct structural features, which include double membrane structures with inner membrane folds known as cristae. For the creation of a proton motive force during electron transport for ATP synthesis, the dual membrane structure and positioning of the inner mitochondrial membrane's electron transport chain (ETC) components are essential[3].Inducing rate-dependent concentration gradients and acting as a diffusion barrier for molecules are two functions of the outer mitochondrial membrane [4]. Outer membrane proteins are essential for mitophagy, mitochondrial fusion, and fission [5]. The existence of mitochondrial DNA is another unique property of these organelles.

DNA is found in the organelle's matrix. The matrix contains many duplicates of this circular, double-stranded genome that codes for 13 ETC proteins, mitochondria-specific ribosomal RNA, and transfer RNA (tRNA) [6]. Identifying human genetic material for forensic applications relies on establishing genetic profiles, also known as genetic fingerprints. The European DNA Profiling Group (EDNAP) recommends using only autosomal short tandem repeats (STRs) for genetic fingerprinting. However, autosomal DNA is often absent or

damaged, leading to using (mtDNA) for human identification [2]. Furthermore, because mtDNA is transmitted from mothers, it may be used to determine links between individuals across several generations or to trace an individual's maternal history [7],unlike nuclear markers. Analysis of mitochondrial genome polymorphisms and SNPs related to ancestry and physical and psychological characteristics provide important information for forensic investigations (Figure 1).

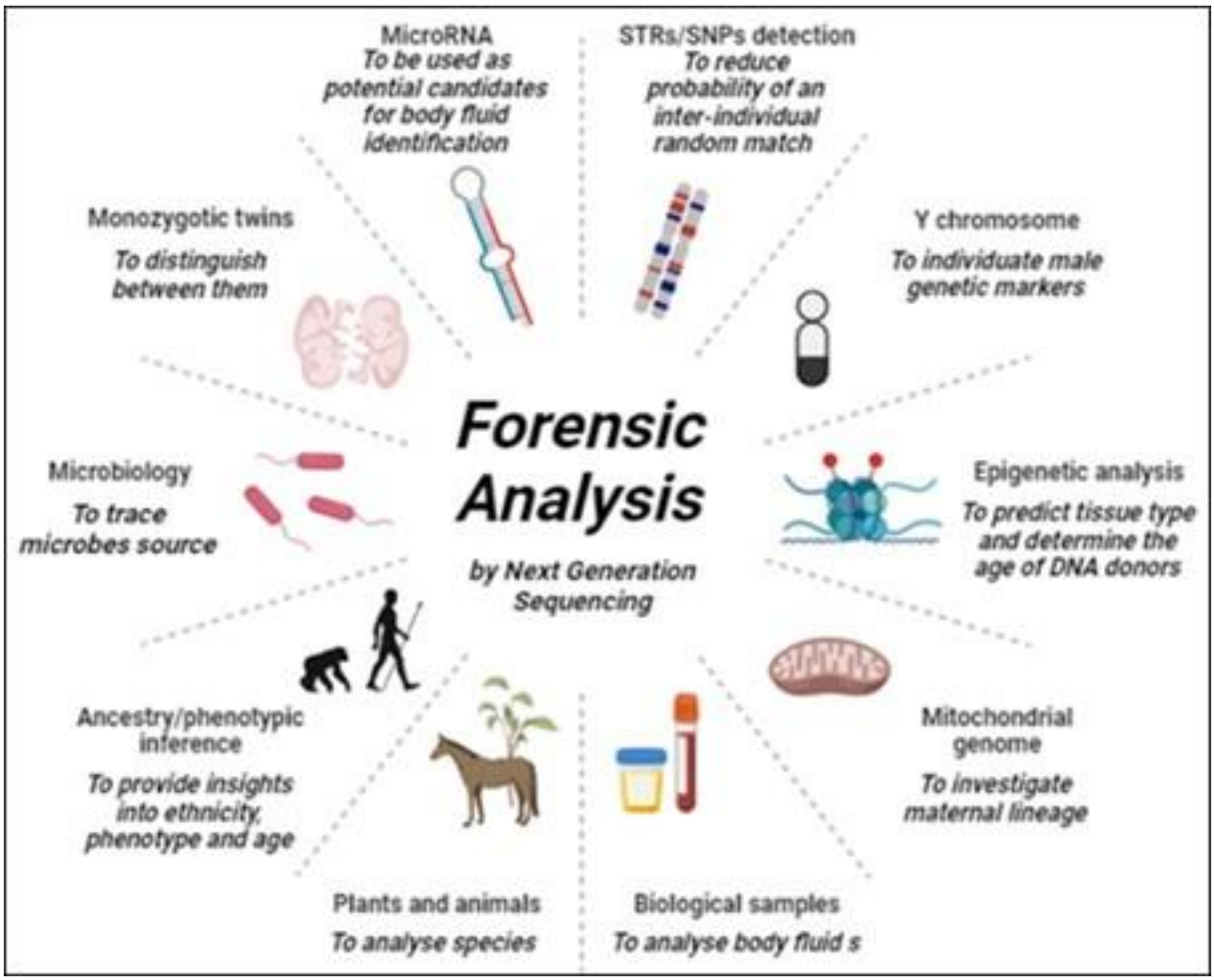


Figure 1: Forensic Analysis is assembled using dynamic created with BioRender.com.

## 2. Main body

In this section, we present some basic concepts and definitions that will be used in this article.

### 2.1. Biological and genetic aspects of mitochondrial DNA

Mitochondria are cellular organelles with an extra chromosomal genome that is different from and separate from the nuclear genome. The discovery of mitochondrial DNA (mtDNA) in 1963 by Margit Nass and Sylvan Nass led to the release of the first mtDNA sequence in 1981, but it took 18 years for the entire first mtDNA sequence to be released. MtDNA is a circular, double-stranded DNA molecule with no histones, measuring five millimeters in diameter, weighing 107 Daltons, and having about 16,569 base pairs. MtDNA Cambridge Reference Sequence(CRS) was released in 1981 [4]. MtDNA strands have diverse densities due to the varying G+T and base structure. The light (L) strand contains eight tRNA and one polypeptide, while the heavy (H) strand contains more details, including a pair of genes rRNA, twelve polypeptides, fourteen tRNA, and 12S and 16S rRNA. The

oxidative phosphorylation system's 13 protein-based goods are all included in the complexes of enzymes that make up this system. Other distinguishing characteristics of mtDNA include intron-less genes and little to no intragenic sequences, except one regulatory region [8]. One of the mtDNA genome's largest non-coding regions (NCR) is the mitochondrial D-loop, generated by the steady insertion of a third, 680-base DNA strand called 7S [9]. According to CRS numeration, the D-loop area, which has Base pairs of 1, 121 and is located at addresses 16,024 and 576, is where replication starts [7]. Two transcription promoters exist in the D-loop region, one for each strand. The locations of nucleotides in the mtDNA genome are numbered using the procedure for replacing CRS updated for RCRS is that the H strand is where each base pair's numeral designation begins, and it continues there for roughly 16,569 base pairs around the molecule [10]. The mitochondrial genome has an advanced mutation ratio to the nuclear genome due to the absence of mtDNA reparation pathways and the conformity of mtDNA polymerase. The mutation rate in people's mtDNA regulatory areas is estimated to be 0.32 x 10_6/site/year [11]. Compared to 0.5 x 10_9/site/year in the nuclear genome [12]. Hyper variable regions (HV1, coordinates 16,024 to 16, 365) and 2 (HV2, locations 73 to 340) of the control region contain the most sequence variation across people [8]. To resolve indistinguishable HV1/HV2 samples, additional polymorphism sites are present in the third hyper variable region (HV3, positions 438 to 574) and may be helpful too [1]. Forensic testing can benefit from the HV areas' modest size and significant inter-person variability. The mtDNA sequence and the dissimilar base pairs regarding the RCRS mtDNA sequence report define the individual haplotype. Haplogroups were created as a result of the successive addition of mutations through maternal lineage and are characterized by the collection of related haplotypes defined by the combination of Single Nucleotide Polymorphisms (SNPs) in mtDNA inherited from a common ancestor [13]. Apiece somatic cells can have up to 1,000 mitochondria, and each mitochondrion comprises 2 to 10 copies of the mtDNA [14, 15]. Typing the mtDNA is more likely to yield a result than typing polymorphic areas discovered in nuclear DNA when the amount of recovered DNA is small or damaged.

MtDNA, transmitted from the mother, can explain why siblings and other maternal relatives share identical mtDNA sequences unless a mutation occurs [16]. Reference samples can be obtained from known maternal relatives, which can be helpful in forensic situations like examining missing individuals' remains [16]. Most mtDNA is haploid and monoclonal, making DNA sequencing easier. However, Heteroplasm can be discovered in rare circumstances [17]. Heteroplasm has two types: Point substitutions and length variations. The latter is the only factor that matters for forensic human identification (FHID). Furthermore, forensic laboratories don't record length polymorphisms, and recommendations for using mtDNA to identify people do not specifically include them as information. Additionally, information about length polymorphisms does not affect the explanation of haplogroups [18]. Heteroplasm, a type of DNA found in human tissue, can manifest in various ways, such as heteroplasm in one tissue sample, homoplasm in another, or one form of mtDNA in one tissue and another type in another [19]. The likelihood of heteroplasm is the lowest when it is found in an individual's mtDNA. Heteroplasm is often seen with one base in HV1 or HV2 [20]. The mitochondrial genome is typically passed down from a mother; despite two or three mitochondria in the neck and sperm tail area, male mitochondrial genome degeneration occurs each through or immediately following conception. Early embryogenesis sees the selective destruction, deactivation, or dilution of sperm mitochondria [21]. However, recent research has shown rare mtDNA inheritance from both parents. Luo and colleagues reported direct or indirect biparental mtDNA inheritance in 17 individuals from three families with many generations [22]. Some cases also involve animals passing down their mitochondrial genomes from father to son [23]. Despite the lack of indication for the fatherly legacy of the human mitochondrial genome, this could encourage courts to minimize the use of mtDNA evidence in court cases. This raises questions about the potential for genetically modified organisms to inherit their mitochondrial DNA.

### 2.2. Mitochondrial DNA mutations

The first damaging mtDNA mutations were identified in 1988 [24]. Since then, more than 250 deleterious point mutations and mtDNA rearrangements have been found to cause various diseases with a broad spectrum of symptoms. The table shows that defects in any aspect of mitochondrial function, including nuclear or mitochondrial DNA abnormalities and Leber Hereditary Optic Neuropathy (LHON), are characteristics of mitochondrial disorders [25, 15]. Despite having few genes, mitochondrial DNA

(mtDNA) frequently experiences mutations. Molecular genetics diagnoses in various domains, including cancer, infectious diseases, and human genetics, make DNA analysis possible. Since these mutations directly influence mitochondrial metabolism and may contribute to multiple ailments, uncovering anomalies in patients' mitochondrial DNA is imperative [26].

Table 1: Mitochondrial disorders, the classification for mtDNA mutations, nuclear DNA errors, and mitochondrial gene mutations [27].

| Type | Subtype | Diseases |
|---|---|---|
| mtDNA mutations | Rearrangement mutations | Progressive external ophtalmoplegia (PEO) Kearns-Sayre Syndrome (KSS) Pearson Syndrome (PS) |
| | Point mutation tRNA or rRNA | Mitochondrial Encephalopathy lactic acidosis, and stroke-like episodes (MELAS) Myoclonus Epilepsy and Ragged-Red Fibers (MERRF) |
| | Point mutation proteins | Neurogenic muscle weakness, ataxia and retinitis pigmentosa (NARP) Leber Hereditary Optic Neuropathy (LHON) Leigh Syndrome |
| Nuclear DNA defects | Replication mutation proteins | Chromosome 15-linked Autosomal Dominant Progressive External Ophtalmoplegia (adPEO) Chromosome 10-linked adPEO Chromosome 4-linked adPEO |
| | Mutation in Thymidine kinase2 | mtDNA depletion Syndrome (MDS) |
| | Mutation in Thymidine phospholipase | Mitochondrial Neurogastroinstestinal Encephalomyopathy (MNGIE) |

The precise incidence of mtDNA illness is challenging to determine owing to the clinical variety of these disorders and the abundance of known causal mutations. Approximations suggest that 1 in 6000 persons may be at risk for developing mtDNA illness, and up to 1 in 10,000 may already have it [15]. The m.1555A > G MT-RNR1 mutation associated to aminoglycoside-induced sensorineural hearing loss has a mutation incidence of 0.2%, and the m.3243A > G mutation has a mutation incidence of 0.14%, according to recent research on birth pervasiveness [28]. These findings show that the apparent frequency of mtDNA mutations is currently overstated. A circular, double-stranded structure representing the human mitochondrial genome is displayed, along with prevalent mtDNA mutations. These diseases include retinitis pigments, neurogenic weakness, ataxia, Leber hereditary optic neuropathy encephalopathy, lactic acidosis, stroke-like episodes, myoclonic epilepsy, and ragged red fibers. Maternally inherited Leigh syndrome (MILS) is another of these illnesses. Pearson syndrome is another. [29]. The mitochondrial genome experiences 10- to 17-fold more mutations than nuclear DNA. However, there are mtDNA repair systems. The mitochondrial genome's proximity to the respiratory chain (RC) complexes in the inner mitochondrial membrane (IMM) and the reactive oxygen species(ROS) they produce make it unable to prevent the oxidative damage it suffers. Neutral polymorphisms, which comprise the majority of mtDNA variations, help trace human migrations [29,30]. The majority of mtDNA mutations found to be associated with human diseases are hetero plasma, which means that a cell or tissue coexists with both mutant and wild-type mtDNA [31]. mtDNA is susceptible to mutations, just like any other genetic material, and some of these mutations can cause a diverse range of human disorders known as mitochondrial syndromes or mitochondrial encephalo myopathies [32]. Mitochondrial diseases affect the brain, skeletal muscles, endocrine system, and other tissues with high energy requirements. Clinical signs include muscle weakness, blindness, mental retardation, dementia, progressive epilepsy, sensory neuropathies, ataxia and renal dysfunction. Other metabolic disorders like diabetes, obesity, cardiovascular disease, neurodegenerative syndromes, and cancer have also been linked to mitochondrial changes. Nuclear DNA mutations can also be the source of mitochondrial disorders, impacting the composition or structure of proteins that enter mitochondria. mtDNA flaws exist in multiple cell copies and can coexist with mutant and wild-type alleles [33].

A single disease-causing mtDNA mutation may result in altered mitochondrial morphology and reduced mtDNA transcription, simulating moderate mitochondrial malfunction in conditions like diabetes and autism. It may cause broad changes in the expression of genes involved in signal transduction, epigenetic control, and pathways linked to neurodegenerative disorders when present in relatively high concentrations. This presents a problem for mtDNA investigations, as different downstream phenotypes may result from different fractions and abundances of mutations and tissue sites [34]. mtDNA copy number, sometimes called mtDNA content or the total amount of mtDNA molecules present in a cell, can impact mitochondrial function and mtDNA sequence differences. Figure2

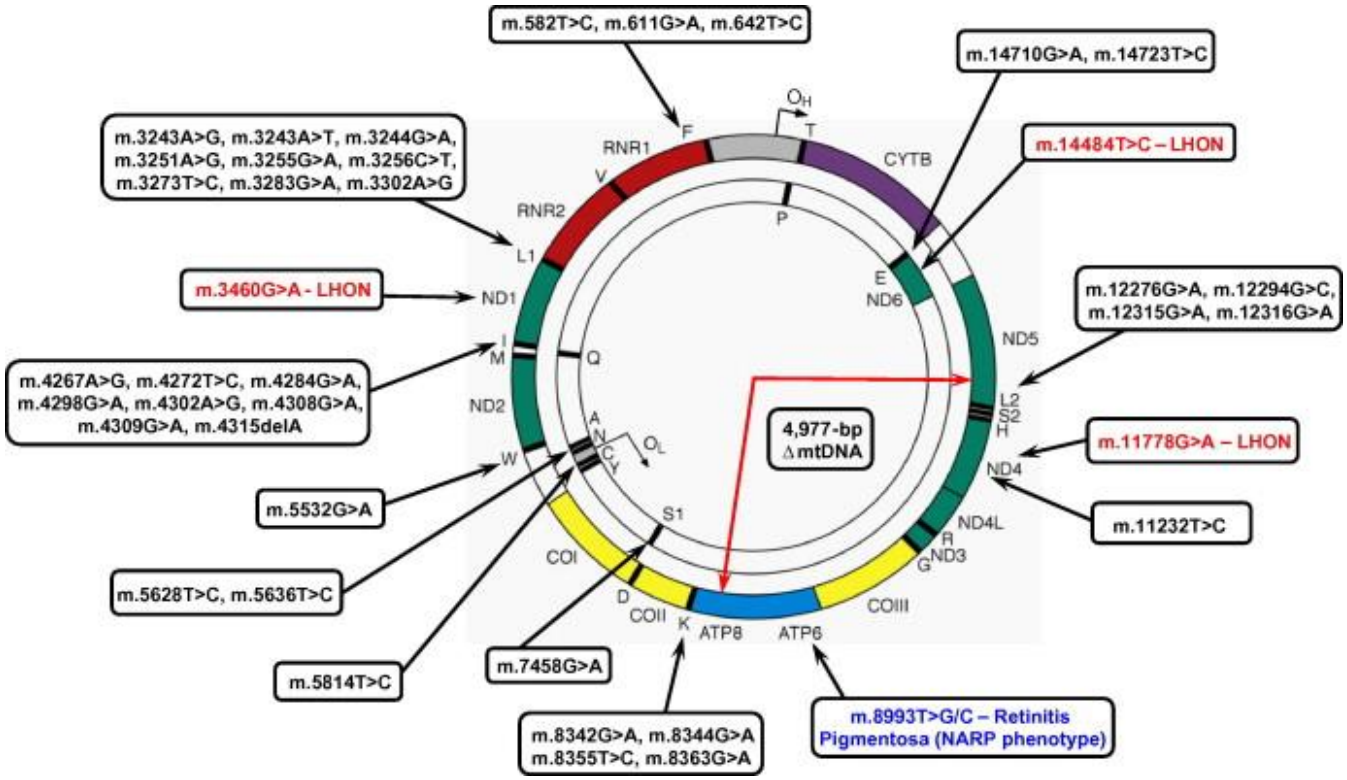


Figure 2. The role of the mitochondrial genome in energy generation [35].

### 2.3. Mutations of the mtDNA in forensic science estimation of the death age

Forensic inquiry aims to estimate a person's age at death to identify a victim. However, determining an adult's age can be challenging due to the natural aging process that alters organs and tissues on various biochemical levels [36]. One such change is mtDNA mutations; only a few authors have explored the link between mitochondrial mutations and age in forensics, research in skeletal muscle revealed a relationship between age and the prevalent mutation, providing a rough estimate of the age at death [24]. Additionally, tissue that has experienced significant putrefaction can be employed with this technology, which employs Polymerase Chain Reaction. Three methods were used to analyze A189G mutations: Automated DNA sequencing, blot hybridization with an oligonucleotide probe labeled with digoxigenin, and peptide nucleic acid (PNA)/real-time PCR. They found that mutation accumulates to extremely high levels in post-mitotic muscular tissue and mitotic buccal cells in people 60 or older. Similar methodologies were used by Zapico *et al.*, [28]. To demonstrate that this age-related somatic point mutation occurs in bone tissue. Similar to the transition mutation in the MERRF syndrome, other authors searched for disease mutations using PCR technology [37]. They discovered a correlation between this mutation and age in healthy persons' extra ocular and skeletal muscle postmortem samples.

Other researchers have also looked for deletions in mitochondrial DNA, using blood and bone samples from patients who had undergone orthopedic

surgery [38]. They found mtDNA deletions in bone but not in blood in patients with osteoporosis/rheumatoid arthritis up to 70 years old [39]. PCR amplification of the mitochondrial D-loop's hyper variable region 2 (HV2) demonstrated a reduction in mtDNA content in dentine with aging in wisdom teeth from healthy people. In bone and muscle samples, three different forms of mini-duplications were found [40]. They are using capillary electrophoresis. The oldest individuals never had evidence of the three duplications in their bones, but anyone over 38 can see at least one. On the other hand, duplicate fragments have been seen in people around 20 and accumulated in older people, carrying several duplicates. This kind of rearrangement has a strong tissue selectivity. Although this process may be straightforward, inexpensive, and valuable for forensic laboratories, and even though it can be applied to tissue that has rotted or putrefied, further research is required before it can be used in forensic cases.

### 2.4. Haplogroups their function in forensic sciences, illnesses, and aging

According to Sukser *et al.*, [41] identified haplogroups as mutations widely spread among human populations. mtDNA from various human populations has been studied using restriction fragment-length polymorphisms, revealing ancestor mutations that identify these haplogroups. These haplogroups have a common ancestor but evolve separately due to uniparental inheritance. The classification of mtDNA molecules within a particular population is made possible by the specific sets of associated mutations [42]. Human populations worldwide can be divided into over 20 mtDNA haplogroups, with the human mtDNA tree having roots in Africa and branches spreading across other geographical areas [43, 44]. mtDNA the most ancient and diverse form of DNA, originates from Africa and has four main haplogroups: L0, L1, L2, and L3. Migrations from Africa to Europe and Asia have led to the emergence of new haplogroups, enriching existing ones. 35% of mutations are confined to specific continents [45], making this phylogeographic distribution useful in forensic research. Studies have linked mtDNA variants to human longevity and aging [46], with C150T polymorphisms describing hereditary mtDNA haplogroups and longer longevity in Finland and Japan. Haplogroup J is overrepresented in northern Italian males who live long lives and reach centenarian age [47], while Irish nonagenarians, centenarians, and long-lived Finns are overrepresented in this haplogroup. Nonagenarian Chinese Uygurs are underrepresented in this haplogroup [48]. Disease risk is also correlated with haplogroups, with Japanese centenarians and supercentenarians being resistant to conditions like Parkinson's disease, type 2 diabetes, myocardial infarction, cerebrovascular infarction, and Alzheimer's dementia [49]. Male members of haplogroup U among Europeans had an increased danger of AD, whereas female members of these haplogroups had a decreased risk. Compared to people with the most prevalent H, haplogroup J had a lower risk of Parkinson's disease [50]. Several research studies have described the risk of cancer-related to various haplogroups. In a Japanese hospital, 30 different haplotypes were examined regarding their relationship to cancer [51]. They found that the haplogroup M7b2, a risk factor for hemopoietic malignancy, increased the likelihood of developing leukemia. According to Young et al. [52], the haplogroup U was linked to a higher risk of kidney cancer in Northern Americans. Indian populations are at increased risk for this cancer due to the polymorphism mtG10398A in haplogroups N and its sublineages [53]. This risk is also present in breast and esophageal cancers. Based on their discovery, the significant haplogroups have been named A through Z alphabetically. The most recent common ancestor, or "Mitochondrial Eve," is a hypothetical root that emerged between 120,000 and 156,000 years ago and represents an individual from whom all living things today have descended. However, she was not the "first" or "only" woman of the species. Haplogroup L was Mitochondrial Eve's group [7]. Figure 3 shows the significant haplogroups and probable migration paths over time.

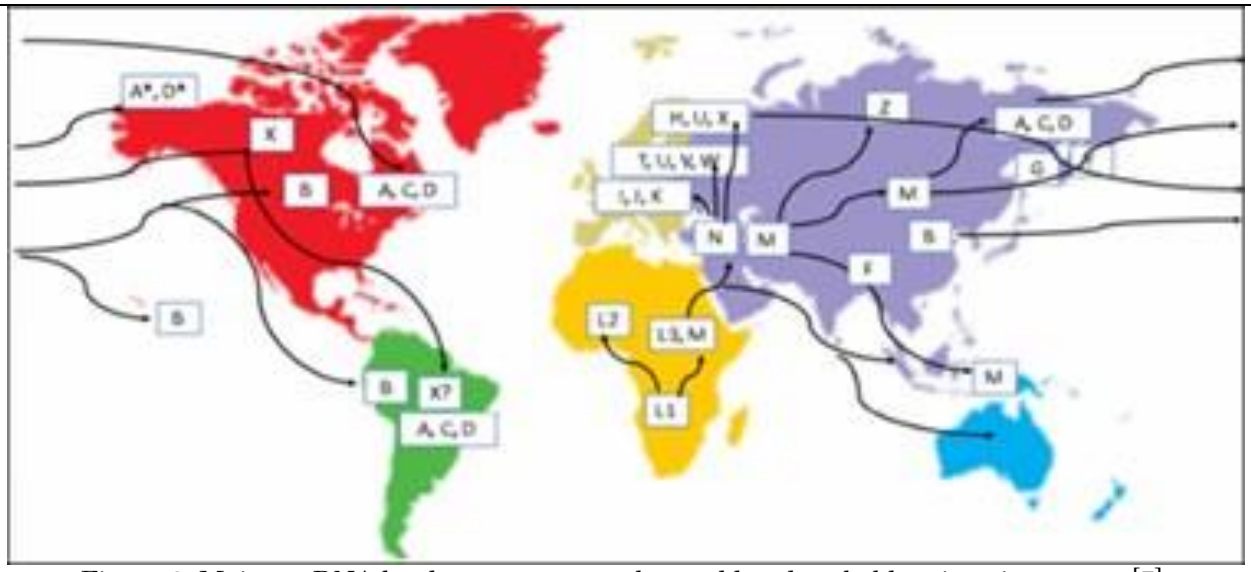


Figure 3. Main mt DNA haplogroups across the world and probable migration routes [7].

These research findings are consistent with the hypothesis that mtDNA variations and haplogroups have a population- and perhaps sex-dependent impact on longevity and disease risk.

### 2.5. Forensic application of mitochondrial DNA for person identification

The mtDNA sequences of an evidentiary sample(s) and a reference sample are compared to forensic analysis. The result is that they can be rejected as having originated from the same source when the sequences are distinct. A sample cannot be dismissed if the mtDNA sequences match since they must share a common ancestor or come from a common maternal lineage. When hetero plasma is present in both samples at the exact nucleotide locations, samples cannot be excluded. The specimens cannot be ruled out if one is homoplasmic and the other is heteroplasmic yet they both have at least one kind of mtDNA. This is because they could have the same precise origin. Several publications [18] have suggested that mtDNA samples differing by a single nucleotide should be investigated further, particularly regarding mutation rate [54]. The International Society of Forensic Genetics' suggested standards and principles were still adhered to in 2017 to identify the victims' bodies from the September 11, 2001 terrorist assault on the World Trade Center [55].

### 2.6. Recognizing and reducing the spread of pathogenic mtDNA variations

Most individuals with mtDNA illness experience increasing symptoms, which frequently result in severe morbidity or early mortality. The obvious priority is attempting to stop the transmission of harmful variations during pregnancy because there are no disease-modifying medications currently available. The alternatives available have improved due to the development of new in vitro fertilization (IVF) methods, which bring several obstacles. There are several different aspects to take into account when providing reproductive counselling for females with harmful mtDNA variants. Thus, families must get guidance on their options for having children from professionals aware of the associated hazards. Oocytes will inherit variant levels far below the disease threshold [56]. Preimplantation genetic diagnosis (PGD) presents much more of a difficulty for other hetero plasma types. For instance, women with high amounts of a variant and little germ line segregation might not exhibit any symptoms- embryos with levels of variation below those considered to carry a low risk of disease [57].

### 2.7. Treatment of disease

Significant strides have been made in our comprehension of mtDNA illness. Treatment progress for mtDNA is moving more slowly. The treatment of sick people has significantly improved in recent years. In recent years, there has been a noticeable improvement in patient care. The growth of significant clinical cohorts in numerous countries can now create clinical leaders. These include improved monitoring practices to identify clinical symptoms before they become severe and select the best treatments for mitochondrial illness. Numerous minor molecule treatments are being investigated through clinical studies [58]. However, mtDNA sickness currently has no effective treatment; people

have been interested in orthodontics for many years, eliminating genetic defects with gene therapy, and it looks simpler. Lack of nuclear gene therapy Because of misleading the mitochondrial genome's simplicity. However, it has been very challenging to study the inner membrane of the mitochondrion, which is highly specialized for OXPHOS and impenetrable to most molecules, including DNA. However, several strategies have been considered, and recent developments have been very significant. Using viral vectors, allotropic expression requires introducing wild-type copies of the altered mtDNA gene to the cytosol [59]. This would be translated into the cytosol, and the mitochondria would use a mitochondrial targeting sequence to import the protein. In this study, one patient's eye's damaged retinal ganglion cells received the MT-ND4 by a local viral vector injection. Throughout a 96-week follow-up, the authors noted consistent vision improvement in both eyes, demonstrating the possibility that the viral vector DNA has passed the ocular barrier. Targeting the pathogenic mtDNA variation is another strategy for manipulating variant levels. To stop the replication of altered genomes, peptide nucleic acid oligomers were initially used to test this [60]. Initial results employing in vitro replication systems were intriguing, but they could not demonstrate absorption into mitochondria, and cell responses were poor. Using endonucleases that can specifically eliminate harmful mutations by crossing mitochondrial membranes and targeting mitochondria is an alternative strategy [61].

## 3. Conclusions

Several diseases are linked to mtDNA mutations. Different mtDNA haplogroups can affect longevity and disease risk and can also be used to identify a person's geographic origin. To better understand the role mtDNA mutation plays in illness and how it applies to forensic science, further research is required. Regarding the mtDNA testing's legal admissibility, some questions remain, especially regarding the problem of hetero plasma fusion and, more recently, the potential for parental inheritance. The ability to predict the circumstances in which this is most likely to happen, the capacity to identify and define hetero plasma with great precision, and the comprehensive understanding of cellular mechanisms underlying parental inheritance of mtDNA are significant concerns that need to be addressed.

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**Author contributions**

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**References**

[1] Amorim, A.; Fernandes, T.; Taveira, N.; "Mitochondrial DNA in human identification" : a review. *Peer J.,* 7: 1-24, 2019.

[2] Dong, X. C.; Liu, C.; Zhuo, G. C.; Ding, Y.; "Potential Roles of mtDNA mutations in PCOS-IR" : a review". *Diabetes Metab Syndr Obes.*,16: 139-149, 2023.

[3] Glancy, B.; Kim, Y.; Katti, P.; Willingham, T. B.; "The functional impact of mitochondrial structure across subcellular scales". *Front. physiol.*, 11: 1-24,2020.

[4] Vadakedath, S.; Kandi, V.; Jayashankar, C. A.; Vijayan, S.; Achyut, K. C.; Uppuluri, S.; Kumar J. r, P. P.;" Mitochondrial Deoxyribonucleic Acid (mtDNA), Maternal Inheritance, and Their Role in the Development of Cancers": A Scoping Review. *Cureus J.*, 15(6):1-7, 2023.

[5] Xian, H.; Liou, Y. C.; "Functions of outer mitochondrial membrane proteins: mediating the crosstalk between mitochondrial dynamics and mitophagy". *Cell Death Differ.*, 28(3): 827-842, 2021.

[6] Fromenty, B.; "Alteration of mitochondrial DNA homeostasis in drug-induced liver injury". *Food Chem Toxicol.*, 135: 1-49, 2020.

[7] Al–Zubaidi, M. M.; Mahdi, A. A. L.; Al-Tabra, R. H.; Al-Sammaraie, H. K. I.; Abd el_Jabbar, B. A.; Jawad, A. A.; Hameed, S. N.; "Mitochondrial DNA in Fornescis: Principles, Applications, and Limitations". *Al-Nahrain J. Sci.*, 27(2):50-62,2024.

[8] Fourdrilis, S.; de Frias Martins, A. M.; Backeljau, T.; "Relation between mitochondrial DNA hyperdiversity, mutation rate and

mitochondrial genome evolution in Melarhaphe neritoides (Gastropoda: Littorinidae) and other Caenogastropoda" . *Sci. Rep.*, 8(1): 1-12, 2018.

[9] Wang, L.; Cheng, H. X.; Zhou, Y. H.; Ma, M.; "Clinical significance of the D-loop gene mutation in mitochondrial DNA in laryngeal cancer". *Onco Targets ther.*,14: 3461-3466, 2021.

[10] Peng, S.; Gao, J.; Stojkov, D.; Yousefi, S.; Simon, H. U.; "Established and emerging roles for mitochondria in neutrophils". *Immunol. Rev.*, 314(1): 413-426,2023.

[11] Dahal, S.; Raghavan, S. C.; "Mitochondrial genome stability in human: understanding the role of DNA repair pathways". *Biochem.J.,* 478(6): 1179-1197,2021.

[12] Begriche, K.; Penhoat, C.; Bernabeu-Gentey, P.; Massart, J.; Fromenty, B.; "Acetaminophen-Induced Hepatotoxicity in Obesity and Nonalcoholic Fatty Liver Disease" : *J. Crit. Rev.*, 3(1): 33-53,2023.

[13] Louvet, A.; Ntandja Wandji, L. C.; Lemaître, E.; Khaldi, M.; Lafforgue, C.; Artru, F.; Mathurin, P.; "Acute liver injury with therapeutic doses of acetaminophen": a prospective study. *Hepatol.*, 73(5): 1945-1955,2021.

[14] Nguyen, N. T.; Umbaugh, D. S.; Sanchez-Guerrero, G.; Ramachandran, A.; Jaeschke, H.; "Kupffer cells regulate liver recovery through induction of chemokine receptor CXCR2 on hepatocytes after acetaminophen overdose in mice". *Arch. Toxicol.*,96:305-320 ,2022.

[15] Ramachandran, A.;Jaeschke, H.; "Mitochondria in Acetaminophen-Induced Liver Injury and Recovery": A Concise Review. *Livers J.,* 3(2): 219-231, 2023.

[16] Yapa, N. M.; Lisnyak, V.; Reljic, B.; Ryan, M. T.; "Mitochondrial dynamics in health and disease". *FEBS letters*, 595(8): 1184-1204, 2021.

[17] Sato, K.; "Multiple roles of endocytosis and autophagy in intracellular remodeling during oocyte-to-embryo transition". *Pro. Japan Acad.*, Series B, 98(5): 207-221,2022.

[18] Duan, M.; Chen, L.; Ge, Q.; Lu, N.; Li, J.; Pan, X.; Lu, Z.; "Evaluating heteroplasmic variations of the mitochondrial genome from whole genome sequencing data". *Gene J.*, 699: 145-154,2019.

[19] Lin, Y.; Yang, B.; Huang, Y.; Zhang, Y.; Jiang, Y.;Ma, L.; Shen,Y.Q.; "Mitochondrial DNA-targeted therapy": A novel approach to combat cancer. *Cell Insight*, 100113,2023.

[20] Li, H.; Slone, J.; Fei, L.;Huang, T.; "Mitochondrial DNA variants and common diseases: a mathematical model for the diversity of age-related mtDNA mutations" . *Cells J.,* 8(6): 608,2019.

[21] Kozakiewicz, P.; Grzybowska-Szatkowska, L.; Ciesielka, M.; Rzymowska, J.; "The role of mitochondria in carcinogenesis". *Int. J. Mol. Sci.*, 22(10): 5100, 2021.

[22] Luo, S.; Valencia, C. A.; Zhang, J.; Lee, N. C.; Slone, J.; Gui, B.; Huang, T.; "Biparental inheritance of mitochondrial DNA in humans". *Pro. National Acad. Sci.,* 115(51): 13039-13044,2018.

[23] Tang, Z.; Lu, Z.; Chen, B.; Zhang, W.; Chang, H. Y.; Hu, Z.; Xu, J.; "A genetic bottleneck of mitochondrial DNA during human lymphocyte development". *Mol. Biol. Evol.*, 39(5):1-12,2022.

[24] Massart, J.; Begriche, K.; Hartman, J. H.; "Fromenty, B. "Role of mitochondrial cytochrome P450 2E1 in healthy and diseased liver". *Cells J.,* 11(2): 288,2022.

[25] Vazquez, J. H.; Clemens, M. M.; Allard, F. D.; Yee, E. U.; Kennon-McGill, S.; Mackintosh, S. G.;McGill, M. R.;"Identification of serum biomarkers to distinguish hazardous and benign aminotransferase elevations. *Toxicol. Sci.,* 173(2): 244-254, 2020.

[26] Hussein, M. A.; Abdulridha, R. H.; Sodani, I. J.; Al-Ward, M. A.; Jaafar, M. R.; Al-Sammarrie, H. K. I.; Safir, N. H.; "Mitochondrial DNA and Disease: Areview". *Al-Nahrain J. Sci.*, 27(2): 81-90, 2024.

[27] Jaberi, E.; Tresse, E.; Grønbæk, K.; Weischenfeldt, J.; Issazadeh-Navikas, S.; "Identification of unique and shared mitochondrial DNA mutations in neurodegeneration and cancer by single-cell mitochondrial DNA structural variation sequencing (MitoSV-seq)". *EBioMedicine*, 57 : 1-17, 2020.

[28] Zapico, S. C.; Ubelaker, D. H.; "mtDNA mutations and their role in aging, diseases and forensic sciences". *Aging dis.*, 4(6): 364, 2013.

[29] Ramachandran, A.; Umbaugh, D. S.; Jaeschke, H.; "Mitochondrial dynamics in drug-induced liver injury". *Livers*, 1(3): 102-115, 2021.

[30] Ahmed, Q. A.; Almubarak, B. M. M.; Salih ,A.A.; "The effect of oxidative stress on the kidneys. *Biol. Pharm .Sci.*, 28(2): 215-219, 2024.

[31] Miyazono, Y.; Hirashima, S.; Ishihara, N.; Kusukawa, J.; Nakamura, K. I.; Ohta, K.; "Uncoupled mitochondria quickly shorten along their long axis to form indented spheroids, instead of rings, in a fission-independent manner".*Sci. Rep.*, 8(1): 350, 2018.

[32] Nemani, N.; Carvalho, E.; Tomar, D.; Dong, Z.; Ketschek, A.; Breves, S. L.; Madesh, M.;

"MIRO-1 determines mitochondrial shape transition upon GPCR activation and Ca2+ stress" .*Cell Rep.*, 23(4): 1005-1019, 2018.

[33] Umbaugh, D. S.; Nguyen, N. T.; Jaeschke, H.; Ramachandran, A.; "Mitochondrial membrane potential drives early change in mitochondrial morphology after acetaminophen exposure". *Toxicol. Sci.,* 180(1): 186-195, 2021.

[34] Du, K.; Ramachandran, A.; Weemhoff, J. L.; Woolbright, B. L.; Jaeschke, A. H.; Chao, X.; Jaeschke, H.; "Mito-tempo protects against acute liver injury but induces limited secondary apoptosis during the late phase of acetaminophen hepatotoxicity". *Arch. Toxicol.*, 93: 163-178,2019.

[35] Connell, J. R.; Benton, M. C.; Lea, R. A.; Sutherland, H. G.; Haupt, L. M.; Wright, K. M.; Griffiths, L. R.; "Evaluating the suitability of current mitochondrial DNA interpretation guidelines for multigenerational whole mitochondrial genome comparisons". *J. Fore. Sci.,* 67(5): 1766-1775, 2022.

[36] Taylor, R. W.; Turnbull, D. M.; "Mitochondrial DNA mutations in human disease". *Nat. Revi. Gene.*, 6(5): 389-402, 2005.

[37] Palodhi, A.; Ghosh, S.; Biswas, N. K.; Basu, A.; Majumder, P. P.; Maitra, A.; "Profiling of genomic alterations of mitochondrial DNA in gingivobuccal oral squamous cell carcinoma: Implications for disease progress". *Mitochondrion*, 46: 361-369, 2019.

[38] Shu, H. Y.; Li, H. C.; Xie, W. Q.; Ni, B., Zhou, H. Y.; "Cell carcinoma". *Biomed. Rep.*, 10(1): 23-28, 2019.

[39] Russell, O. M.; Gorman, G. S.; Lightowlers, R. N.; Turnbull, D. M.; "Mitochondrial diseases" : hope for the future. *Cell J.*, 181(1): 168-188, 2020.

[40] Sultana, G. N. N.; Sultan, M. Z.; "Mitochondrial DNA and methods for forensic identification". *J. Fore. Sci. Crimin, Inves*, 9(1):1-5, 2018.

[41] Sukser, V.; Rokić, F.; Barbarić, L.; Korolija, M.; "Assessment of Illumina® Human mtDNA Genome assay: workflow evaluation with development of analysis and interpretation guidelines". *Int. J. Leg. Med.,* 135(4): 1161-1178, 2021.

[42] Gammage, P. A.; Viscomi, C.; Simard, M. L.; Costa, A. S.; Gaude, E.; Powell, C. A.; "Minczuk, M.; Genome editing in mitochondria corrects a pathogenic mtDNA mutation in vivo". *Nat. Med.*, 24(11): 1691-1695,2018.

[43] Cihlar, J. C.; Amory, C.; Lagacé, R.; Roth, C.; Parson, W.; Budowle, B.; "Developmental validation of a MPS workflow with a PCR-based short amplicon whole mitochondrial genome panel". *Genes J.*, 11(11): 1-31, 2020.

[44] Holt, C. L.; Stephens, K. M.; Walichiewicz, P.; Fleming, K. D.; Forouzmand, E.; Wu, S. F.; "Human mitochondrial control region and mtgenome: design and forensic validation of NGS multiplexes, sequencing and analytical software". *Genes J.*, 12(4): 599, 2021.

[45] Basu Ball, W.; Neff, J. K.; Gohil, V. M.; "The role of nonbilayer phospholipids in mitochondrial structure and function". *FEBS letters*, *592*(8): 1273-1290, 2018.

[46] Cuenca, D.; Battaglia, J.; Halsing, M.; Sheehan, S.; "Mitochondrial sequencing of missing persons DNA casework by implementing Thermo Fisher's precision ID mtDNA whole genome assay". *Genes J.*, 11(11):1-17, 2020.

[47] Annis, S.; Fleischmann, Z.; Khrapko, M.; Franco, M.; Wasko, K.; Woods, D.; Khrapko, K.; "Quasi-Mendelian paternal inheritance of mitochondrial DNA: A notorious artifact, or anticipated behavior" *Pro. National Acad. Sci.,* 116(30): 14797-14798, 2019.

[48] Lutz-Bonengel, S.; Parson, W.; "No further evidence for paternal leakage of mitochondrial DNA in humans yet". *Pro. National Acad. Sci.*, 116(6): 1821-1822, 2019.

[49] Salas, A.; Schönherr, S.; Bandelt, H. J.; Gómez-Carballa, A.; Weissensteiner, H.; "Extraordinary claims require extraordinary evidence in the case of asserted mtDNA biparental inheritance". *bioRxiv*, 47: 1-10, 2019.

[50] Wei, W.; Pagnamenta, A. T.; Gleadall, N.; Sanchis-Juan, A., Stephens, J., Broxholme, J.; Chinnery, P. F.; "Nuclear-mitochondrial DNA segments resemble paternally inherited mitochondrial DNA in humans". *Nat. Comm.,* 11(1): 1-11,2020.

[51] Huber, N.; Parson, W.; Dür, A.; "Next generation database search algorithm for forensic mitogenome analyses". Forensic Science *Int. Gene.*, 37: 204-214,2018.

[52] Williams, S. B.; Ye, Y.; Huang, M.; Chang, D. W.; Kamat, A. M.; Pu, X.; Wu, X.; "Mitochondrial DNA content as risk factor for bladder cancer and its association with mitochondrial DNA polymorphisms". *Cancer Prev. Res.*, 8(7): 607-613,2015.

[53] Gorden, E. M.; Sturk-Andreaggi, K.; Marshall, C.; "Repair of DNA damage caused by cytosine deamination in mitochondrial DNA of forensic case samples". *Fore. Sci. Inter Genet.*, 34: 257-264, 2018.

[54] Loreille, O.; Ratnayake, S.; Bazinet, A. L.; Stockwell, T. B.; Sommer, D. D.; Rohland, N.;

Irwin, J. A.; "Biological sexing of a 4000-year-old Egyptian mummy head to assess the potential of nuclear DNA recovery from the most damaged and limited forensic specimens". *Genes J.*, 9(3): 135,2018.

[55] Bruijns, B.; Tiggelaar, R.; Gardeniers, H.; "Massively parallel sequencing techniques for forensics": A review. *Electrophoresis J.,* 39(21): 2642-2654,2018.

[56] Churchill, J. D.; Stoljarova, M.; King, J. L.; Budowle, B.; "Massively parallel sequencing-enabled mixture analysis of mitochondrial DNA samples". *Int. J. leg. Med.,* 132: 1263-1272, 2018.

[57] Strobl, C.; Eduardoff, M.; Bus, M. M.; Allen, M.; Parson, W.; "Evaluation of the precision ID whole MtDNA genome panel for forensic analyses". *Fore. Sci. Int. Gene.*, 35: 21-25, 2018.

[58] Woerner, A. E.; Ambers, A.; Wendt, F. R.; King, J. L., Moura-Neto, R. S., Silva, R., & Budowle, B.; "Evaluation of the precision ID mtDNA whole genome panel on two massively parallel sequencing systems". *Fore.Sci. Int Gene.*, 36: 213-224, 2018.

[59] Cai, S.; Zhao, M.; Zhou, B.; Yoshii, A.; Bugg, D.; Villet, O.; Tian, R.; "Mitochondrial dysfunction in macrophages promotes inflammation and suppresses repair after myocardial infarction". *J. Clin. Investi.*, 133(4):1-14,2023.

[60] Young, B.; King, J. L.; Budowle, B., Armogida, L.; "A technique for setting analytical thresholds in massively parallel sequencing-based forensic DNA analysis". *PLoS One*, 12(5): 1-15, 2017.

[61] Poletto, M. M.; Malaghini, M.; Silva, J. S.; Bicalho, M. G.; Braun-Prado, K.; "Mitochondrial DNA control region diversity in a population from Parana state—increasing the Brazilian forensic database". *Int. J. Leg. Med.*, 133: 347-351, 2019.