The Research of Medical Science Review
ISSN: 3007-1208 & 3007-1216 Volume 4, Issue 2, 2026

REVIEW: THE ROLE OF MITOCHONDRIAL DNA IN HUMAN IDENTIFICATION

Hina Khanzada[*1], Dr Ishrat Bibi[2], Memoona Laghari[3], Savera Talpur[4], Gulsher Junejo[5], Uzain Khan[6], Abdul Rauf Khokhar[7]

[*1,2,3,4,5,6,7]*Department of Forensic Medicine and Toxicology, Liaquat University of Medical and Health Sciences Jamshoro*

DOI: https://doi.org/10.5281/zenodo.18721707

Keywords
Human identification, Mitochondrial DNA, Maternal lineage, Forensic genetics, Hypervariable regions

Article History
Received: 22 December 2025
Accepted: 06 February 2026
Published: 21 February 2026

Corresponding Author: *
Hina Khanzada

Abstract

Background

Mitochondrial DNA is a genetic material found within the mitochondria of a cell. It is a circular, double-stranded molecule that is exclusively inherited from mother. Mitochondrial DNA is helpful in human identification when nuclear DNA is insufficient due to its high copy number and resistance towards environmental degradation.

Objective

This review aims to synthesize the current understanding of mitochondrial DNA's structure, its specific role in forensic investigations and the technological advancements in sequencing that have enhanced its analytical sensitivity.

Methodology

To compose this review, more than twenty research and review articles published over the past two decades were examined. Literature was collected from reputable scientific databases, including PubMed, ResearchGate and Web of Science. Emphasis was placed on studies discussing the forensic applicability, sequencing techniques and limitations of mitochondrial DNA analysis.

Result

The literature reviewed indicates that mitochondrial DNA analysis has significantly improved identification in cases involving aged, degraded, or limited biological samples. The hypervariable regions (HVR1 and HVR2) are the most frequently analyzed portions using Polymerase chain reaction amplification followed by Sanger sequencing. Although mitochondrial DNA has lower discriminatory power than nuclear DNA, it remains a critical tool in maternal lineage analysis and mass disaster investigation.

Conclusion

Mitochondrial DNA has proven to be a robust and reliable method for human identification, particularly in degraded samples. The integration of Next-Generation Sequencing and the expansion of forensic mitochondrial DNA databases are expected to further enhance accuracy, sensitivity and global standardization in forensic genetics.

INTRODUCTION

Mitochondria are specialized cellular components that play a vital role in energy production through oxidative phosphorylation, metabolite processing, cellular signaling and apoptosis (1). To perform these essential functions, mitochondria depend on both nuclear and mitochondrial genomes (2). Mitochondrial DNA was first identified and isolated by Margit Nass and Sylvan Nass from rat liver cells in 1963 (3). It is small circular,

double-stranded molecule (Figure 1) located within the mitochondrial matrix (4). It contains 16,569 base pairs and encodes 37 genes, of which 13 genes are essential for mitochondrial oxidative phosphorylation (OXPHOS), along with 2 genes for ribosomal RNAs and 22 for transfer RNAs necessary for mitochondrial DNA gene expression (5, 6). Structurally, mitochondrial DNA is mainly composed of coding regions and a single non-coding region known as displacement Loop (D-Loop), comprising about 1123 base pairs. Due to its highly polymorphic nature, this region is also referred to as the hypervariable region (7). The non-coding D-loop serves as the control region for the replication and transcription of mitochondrial DNA (8). Each human cell contains 100-10,000 copies of mitochondrial DNA per cell making it a multicopy genome (1, 9). Mitochondrial DNA is strictly maternally inherited as the embryo inherits mitochondria exclusively from oocyte, while paternal mitochondria either fail to enter or destroyed after fertilization, making the maternal line the sole source of mitochondrial DNA (10, 11).

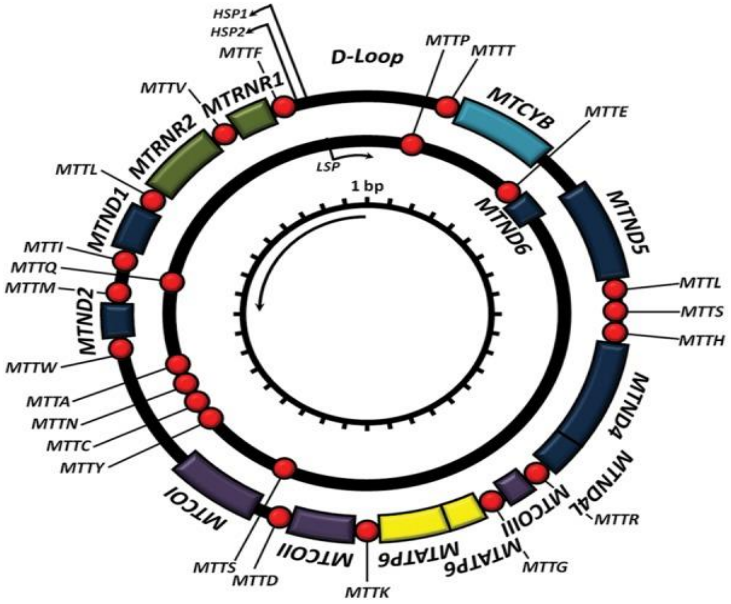

Figure 1. Schematic diagram of human mitochondrial DNA where the outer circle represents the heavy strand and the inner circle the light strand. Shown are the genes encoding the mitochondrial RC: MTND1–6, MTCOI–II, MTATP6 and 8 and MTCYB; the two ribosomal RNAs (green boxes) and each of the 22 tRNAs (red spheres). (Adapted from Chinnery & Hudson, 2013) (1).

In the field of forensic science, mitochondrial DNA has become a valuable genetic marker for the purpose of human identification, particularly in cases where nuclear DNA is absent, degraded or insufficient. Its high copy number and structural stability allow recovery from aged or trace biological samples such as bone, teeth and hair shaft (12).

The most frequently used portion of mitochondrial DNA is the hypervariable regions HVR1 and HVR2 (13). These regions exhibit high degree of sequence polymorphism among unrelated individuals, enabling effective differentiation based on maternal lineage (14). Sequencing of these regions produces an mitochondrial DNA profile that can be compared with that of maternal relatives to establish biological relationships or verify identity in challenging forensic cases (12).

Among the available analytical techniques, PCR amplification followed by Sanger Sequencing remains the gold standard method for mitochondrial DNA analysis due to its accuracy and compatibility with degraded samples (15). More recently, the advent of Next-Generation Sequencing (NGS) has allowed complete mitochondrial genome analysis, offering higher resolution, improved detection of heteroplasmy, and enhanced discriminatory power for forensic investigations (16).

Literature Review

Mitochondrial DNA has been extensively studied as a reliable marker in forensic identification due to its high copy number, maternal inheritance and resistance to degradation (3, 13, 17). Since its discovery in 1963, numerous studies have explored its structure, polymorphic region and forensic utility (1, 4, 18). The hypervariable regions (HVR1 and HVR2) within the control region are most frequently analyzed because of their high sequence variability among unrelated individuals (14, 19, 20). Earlier research established that mitochondrial DNA could be successfully extracted and sequenced from degraded samples such as bones, teeth and hair shafts, proving its advantage over nuclear DNA in compromised specimen (12, 21). Conventional PCR-based Sanger sequencing remains the gold standard for mitochondrial DNA analysis (15, 22), while recent advances in Next-Generation Sequencing (NGS) now allow complete mitochondrial genome profiling with improved sensitivity and discrimination (16, 20, 23). These developments have greatly enhanced the reliability and scope of mitochondrial DNA in modern forensic genetics (2, 5, 8).

Methodology

This review was conducted through a comprehensive search and analysis of published research and review articles related to mitochondrial DNA (mtDNA) and its forensic applications. Literature was collected from reputable scientific databases including PubMed, ResearchGate and Web of Science. key search terms included "mitochondrial DNA," "forensic identification," "maternal lineage," "hypervariable regions," "mtDNA sequencing," and "forensic genetics". More than twenty relevant articles published during the past two decades were reviewed, focusing on the structure, inheritance and analytical techniques of mtDNA in human identification. Preference was given to peer-reviewed studies and publications emphasizing recent technological advancements such as PCR-based sequencing and Next-Generation Sequencing (NGS).

Discussion

1) Forensic Significance of mitochondrial DNA

Building upon the research summarized above, it is evident that mitochondrial DNA has become a cornerstone of forensic genetics due to its resilience and maternal mode of inheritance (1, 3). While earlier studies primarily established its structural features and recovery potential from degraded biological material (12, 21), recent developments have focused on enhancing analytical precision and interpretation (22, 24). The following discussion critically examines how mitochondrial DNA contributes to human identification, its practical applications and the technological innovations that contribute to refine its forensic value. In forensic investigations, mitochondrial DNA analysis is especially valuable when nuclear DNA is unavailable or severely degraded (13, 17). Its multicopy nature allows successful profiling from minimal or damaged samples such as aged, burnt tissues and hair shafts (3, 19). These properties have enabled mitochondrial DNA to play a vital role in mass disaster identification, missing person investigations and historical reconstructions (14, 24). However, despite these strengths, its maternal inheritance limits the ability to differentiate between individuals from the same maternal lineage , requiring cautions interpretation alongside other forms of biological and circumstantial evidence (2, 23).

2) Hypervariable Regions and Maternal Lineage Analysis

The hypervariable regions (HVR1 and HVR2) of the mitochondrial DNA control region remain the primary targets for forensic sequencing due to their high degree of polymorphism among unrelated individuals (3, 19). Studies have shown that these regions provide sufficient variations to establish maternal relationship and exclude non-related individuals with high accuracy (14, 24). Nonetheless, when mitochondrial DNA profiles are identical within a population, the evidential value must be interpreted within a statistical and contextual framework, supported by additional reference databases (23, 24).

3) Advancements in Analytical Techniques

Technological advancements have significantly transformed mitochondrial DNA analysis. The PCR-based Sanger sequencing method remains the gold standard for routine forensic analysis due to its accuracy, reproducibility and suitability with degraded samples (15, 22). However recent introduction of Next-Generation Sequencing (NGS) has expanded analytical capability by enabling complete mitochondrial genome sequencing (16, 20). NGS enhances discriminatory power, improves the

detection of heteroplasmy (the coexistence of multiple mtDNA variants within an individual) and facilitates population-based comparisons (5, 8, 20). These improvements not only increase sensitivity and throughput but also strengthen the reliability of mitochondrial DNA profile in forensic casework (2, 24).

4) Limitations and Technical Challenges

Despite these achievements, several limitations and challenges persist. The strict maternal inheritance pattern of mitochondrial DNA restricts its discriminatory power among maternal relatives (3, 23). In addition, the high sensitivity of PCR-based amplifications increases the risk of contamination, which can lead to false or mixed profiles (21, 22). Interpretation of heteroplasmic variants also remains a technical challenge, requiring advanced bioinformatics tools and experienced analysts (8, 14). Moreover, limitations of global databases and the absence of standardized interpretation criteria can complicate cross laboratory comparisons (14, 20, 24).

5) Future Perspectives

To overcome these issues, researchers emphasize the need for population-specific mitochondrial DNA databases, improved standard operating procedures (SOPs) and enhanced bioinformatics pipelines to ensure consistent and reproducible results across forensic laboratories (8, 25). Integration of mitochondrial DNA profiling with nuclear DNA technologies such as autosomal STR and Y-STR analysis, can further enhance the overall discriminatory capacity and strengthen the evidential value in complex forensic cases (13, 23).

Conclusion

In summary, mitochondrial DNA analysis remains a powerful approach for identifying individuals from degraded or limited biological samples. Despite certain limitations, it has consistently proven valuable in cases such as missing persons, mass disaster identifications and historical investigations. For maximum reliability, mitochondrial DNA analysis should be used alongside nuclear DNA profiling whenever possible. Future advancements in sequencing technologies and the development of comprehensive forensic mitochondrial DNA databases are expected to further enhance its discriminatory capacity, analytical speed and global applicability in forensic genetics.

Conflict of interest

None

Funding source

None

Acknowledgements

None

List of abbreviations

mtDNA - Mitochondrial DNA.
DNA - Deoxyribonucleic acid.
HVR - Hypervariable region.
NGS - Next Generation Sequencing.
PCR - Polymerase chain reaction.
OXPHOS - Oxidative phosphorylation.

References

1. Chinnery PF, Hudson G. Mitochondrial genetics. Br Med Bull. 2013;106(1):135–59. DOI: 10.1093/bmb/ldt017
2. Basu U, Bostwick AM, Das K, Dittenhafer-Reed KE, Patel SS. Structure, mechanism, and regulation of mitochondrial DNA transcription initiation. J Biol Chem. 2020;295(52):18406–25. DOI: 10.1074/jbc.REV120.011202
3. Amorim A, Fernandes T, Taveira N. Mitochondrial DNA in human identification: a review. PeerJ. 2019;7:e7314. DOI: 10.7717/peerj.7314
4. Desalle R, Schierwater B, Hadrys H. MtDNA: The small workhorse of evolutionary studies. Front Biosci (Landmark Ed). 2017;22(5):873–87. DOI: 10.2741/4522
5. Bernardino Gomes TM, Vincent AE, Menger KE, Stewart JB, Nicholls TJ. Mechanisms and pathologies of human mitochondrial DNA replication and deletion formation. Biochem J. 2024;481(11):683–715. DOI: 10.1042/BCJ20230262

6. Garcia I, Jones E, Ramos M, Innis-Whitehouse W, Gilkerson R. The little big genome: the organization of mitochondrial DNA. Front Biosci (Landmark Ed). 2017;22(4):710–21. DOI: 10.2741/4511
7. Yusoff AAM, Abdullah WSW, Khair S, Radzak SMA. A comprehensive overview of mitochondrial DNA 4977-bp deletion in cancer studies. Oncol Rev. 2019;13(1):409. DOI: 10.4081/oncol.2019.409
8. Hernández CL. Mitochondrial DNA in Human Diversity and Health: From the Golden Age to the Omics Era. Genes (Basel). 2023;14(8). DOI: 10.3390/genes14081534
9. Gammage PA, Frezza C. Mitochondrial DNA: the overlooked oncogenome? BMC Biol. 2019;17(1):53. DOI: 10.1186/s12915-019-0668-y
10. Nakamura N. Ubiquitination regulates the morphogenesis and function of sperm organelles. Cells. 2013;2(4):732–50. DOI: 10.3390/cells2040732
11. Song WH, Yi YJ, Sutovsky M, Meyers S, Sutovsky P. Autophagy and ubiquitin-proteasome system contribute to sperm mitophagy after mammalian fertilization. Proc Natl Acad Sci U S A. 2016;113(36):E5261–70. DOI: 10.1073/pnas.1605844113
12. Parsons TJ, Coble MD. Increasing the forensic discrimination of mitochondrial DNA testing through analysis of the entire mitochondrial DNA genome. Croat Med J. 2001;42(3):304–9. PMID: **11387644**
13. Cavalcanti P, Nogueira TLS, Carvalho EF, Silva DAD. Forensic use of human mitochondrial DNA: A review. An Acad Bras Cienc. 2024;96(4):e20231179. DOI: 10.1590/0001-3765202420231179
14. Connell JR, Lea RA, Haupt LM, Griffiths LR. Mitochondrial DNA Analysis in Population Isolates: Challenges and Implications for Human Identification. Current Molecular Biology Reports. 2024;10(1):1–8. DOI:10.1007/s40610-023-00155-4
15. Eren K, Taktakoğlu N, Pirim I. DNA Sequencing Methods: From Past to Present. Eurasian J Med. 2022;54(Suppl1):47–56. DOI: 10.5152/eurasianjmed.2022.22280
16. Qin D. Next-generation sequencing and its clinical application. Cancer Biol Med. 2019;16(1):4–10. DOI: 10.20892/j.issn.2095-3941.2018.0055
17. Syndercombe Court D. Mitochondrial DNA in forensic use. Emerging Topics in Life Sciences. 2021;5(3):415–26. DOI: 10.1042/ETLS20210204
18. Garcia I, Jones E, Ramos M, Innis-Whitehouse W, Gilkerson R. The little big genome: The organization of mitochondrial DNA. Frontiers in bioscience (Landmark edition). 2017;22:710. DOI: 10.2741/4511
19. Morovvati S, Morovvati Z, Ranjbar R. Detecting rare triple heteroplasmic substitutions in the mitochondrial DNA control region: a potential concern for forensic DNA studies. Cell J. 2011;13(2):103–6. PMID: **23508063**
20. Just RS, Irwin JA, Parson W. Mitochondrial DNA heteroplasmy in the emerging field of massively parallel sequencing. Forensic Sci Int Genet. 2015;18:131–9. DOI: 10.1016/j.fsigen.2015.05.003
21. Sinha M, Rana M, Kushwaha P. Applications of Mitochondrial DNA in Forensic Science. 2020. p. 329–43. DOI:10.1007/978-981-15-6655-4_16
22. Strobl C, Churchill Cihlar J, Lagacé R, Wootton S, Roth C, Huber N, et al. Evaluation of mitogenome sequence concordance, heteroplasmy detection, and haplogrouping in a worldwide lineage study using the Precision ID mtDNA Whole Genome Panel. Forensic Sci Int Genet. 2019;42:244–51. DOI: 10.1016/j.fsigen.2019.07.013
23. Andersen MM, Balding DJ. Assessing the forensic value of DNA evidence from Y chromosomes and mitogenomes. Genes. 2021;12(8):1209. DOI: 10.3390/genes12081209

24. Irwin JA, Just RS, Parson W. Massively parallel mitochondrial DNA sequencing in forensic genetics: principles and opportunities. Handbook of Forensic Genetics: Biodiversity and Heredity in Civil and Criminal Investigation: World Scientific; 2017. p. 293–335.
25. Melton T, Holland C, Holland M. Forensic mitochondria DNA analysis: current practice and future potential. Forensic science review. 2012;24(2):101. PMID: **26244267**