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Review Research Paper

Mitochondrial DNA: A Reliable Tool in Forensic Odontology

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Abstract

Forensic odontology has emerged as one of the prime tool in Forensic investigations. Tooth being resistant to degrading environmental conditions, is a potential source in solving various criminal cases. Over the last few decades, DNA analysis has revolutionized the Forensic field. There have been several technical modifications and advancements in the DNA analysis. One such advance in the recent past is the use of mitochondrial DNA (mtDNA). Mitochondrial genome exhibit several unique characteristics such as multiple genome copies, heteroplasmy, variable expressivity, mitotic segregation and the threshold effect. These properties affect its inheritance pattern and even the Forensic analysis.

MtDNA differs from nDNA in many ways, including its location, its sequence, its quantity and its mode of inheritance with this technique; it is possible to analyze the DNA even in very small, damaged and degraded samples from where it is unable to analyze the nuclear DNA (nDNA). This article reviews the characteristics of mtDNA and its role in Forensic investigations.

Key Words: Odontology, Criminal cases, DNA analysis, mt DNA, nDNA

Introduction:

DNA is the chemical code and the genetic material found in the cells of the body. [1] It is present in all the cells including white blood cells, semen, hair roots, bone, teeth and other body tissue. DNA traces can be detected in body fluids as well, such as blood, saliva, semen, and perspiration. [1, 2] It is unique to each individual and because of this property it can be used in forensic investigations. [2, 3]

Since its introduction in the 1980s, the DNA analysis has enormously modified the pace of Forensic investigations. Though restriction fragment length polymorphism (RFLP) was the first technique introduced in 1985 by Dr. Alec Jeffreys, the technical advancements, especially the introduction of polymerase chain reaction (PCR) has revolutionized this field. [2, 4]

DNA analysis has several advantages over other techniques used for Forensic investigations. It can be applied to all the biological materials; it is resistant to environmental factors and high temperatures.

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It has tremendous discriminatory potential and a very high sensitivity. Even if DNA is reduced to low molecular weight through progressive fragmentation and degradation, smaller DNA fragments are present for considerable period, which can be obtained easily for DNA testing. [2, 5]

With advancements in technology, the time required for DNA testing has decreased to hours and this has hastened the process of Forensic investigations and judgement.

Mitochondria and Mitochondrial DNA:

Mitochondria are the ovoid or elongated thread like membrane bound organelles of great metabolic significance and form the principle source of chemical energy. [1, 6] The number of mitochondria in a cell differs in relation to their energy requirement. They are situated close to the parts of cell that shows highest energy requirement. [6] They are present in all human cells except mature erythrocytes. [7]

Mitochondria are self-replicating and they increase in number by division throughout interphase, and their division is not synchronized with the cell cycle. [1, 8]

Mitochondria are thought to have originated billions of years ago as primitive bacteria. They have developed from the order of proteobacteria as endosymbionts. This mostly likely has occurred as a result of phagocytosis.

Over thousands of generations, some of the genetic information from these bacteria has migrated into what have become human cell nuclei, while the mitochondria now exist

separately within the cell cytoplasm, retaining their own independently-replicating DNA. [2, 9]

Mitochondria's genetic system, the mtDNA was discovered in 1963. In 1981, the mitochondrial genome became the first complete sequence of a human genome to be published. [6] MtDNA differs from nDNA in many ways, including its location, its sequence, its quantity and its mode of inheritance. [3, 5] (Table 1) Mitochondrial genome contains a ~16.5 kb circular DNA, small sized rRNA and about 3000 genes (Table 2). [5, 9, 10] Only about 3% of the genes (100 of the 3000) are allocated for making ATP and more than 95% (2900 of 3000) are involved with other functions. [11]

Mitochondrial proteins originate mainly from two sources. While most mitochondrial proteins are translated on free cytosolic ribosomes, few other proteins are synthesized by their own genomes. Their membrane lipids are synthesized by endoplasmic reticulum. [12]

Mitochondria are inherited maternally and hence, all maternal relatives have the same mtDNA. [3, 5, 10, 11] Mitochondrial genome exhibit several unique characteristics such as multiple genome copies, heteroplasmy, variable expressivity, mitotic segregation and the threshold effect. [9-11]

These properties affect its inheritance pattern and even the Forensic analysis.

Cambridge Sequence is the standard sequence to which all the human mtDNA are compared. It was sequenced from several different human mtDNAs by a Medical Research Council Laboratory based at Cambridge, UK.

Variations in the mtDNA genome, from the Cambridge sequence are termed polymorphism. Control Region or D-loop is highly polymorphic and hence is used for Forensic purposes in providing a "DNA finger print" of suspects in criminal investigations. This loci spans 1100bp and contains two hyper variable regions, HVI and HVII. [3, 11]

Mitochondrial DNA in Forensics:

MtDNA is a good source of evidence in Forensic investigations and it has been used as a tool for Forensic identification since 1993. [12] The FBI Lab conducted studies to test the usefulness of mtDNA analysis for human identity testing in late 1980s. In 1992, the lab began research on the use of mtDNA in resolving criminal cases. In June 1996, the mtDNA was used as evidentiary sample in the case of State of Tennessee v. Paul Ware.

Since then mtDNA typing has become routine and is used in investigations of missing persons, mass disasters, and other Forensic investigations such as murder, rape, robbery and drug offences. [12, 13]

MtDNA can be analyzed from samples such as old bones, teeth, hair shafts, and other biological samples where nDNA content is low. [2, 3]

Characteristics of mtDNA:

- 16,569 base pairs
- Encodes - 37 genes
- Codes 13 protein subunits– mainly responsible for certain key components of oxidative phosphorylation pathway
- Genes for 12s and 16s rRNA
- Genes for 22 tRNA
- D loop containing DNA replication and transcriptional promoter sequence
- Maternal inheritance
- Multiple copies
- No effective DNA repair system

mtDNA Analysis Advantages over the nDNA: [5, 13-15]

- High copy number, rapid rate of evolution, haploid nature, lack of recombination and the maternal mode of inheritance, which make the mtDNA better choice in situations where nDNA cannot be used for the analysis.
- Due to the high copy number of mtDNA in the cell, it can be analysed even from the highly damaged, degraded or very small quantity of the samples, when nDNA testing produces no results.
- The subcellular location of mtDNA within the mitochondria offers extra protection through the mitochondrial membranes compared to those surrounding the nucleus.
- Its exonuclease-resistant circular nature also contributes to its molecular stability.
- Since mtDNA is maternally inherited, siblings and all the maternally related family members will have similar mtDNA sequence. Hence, comparisons can be made using a reference sample from any maternal relative, even if the unknown and reference sample are separated by many generations.
- Mitochondrial DNA analysis of hair, bone and teeth is particularly successful in part due to the encapsulation of DNA by the exterior of the tissue and protection of mtDNA within layers of keratin (hair) and hydroxyapatite (bone and teeth).

MtDNA Analysis:

MtDNA analysis as compared to standard DNA analysis is laborious and lengthy.

This analysis is mainly based upon the strategy of polymerase chain reaction (PCR) amplifications that focuses on the Control Region or smaller regions of interest within the Control Region, hyper variable region I (HVI), hyper variable region II (HVII) and hyper variable region III (HVIII), which contain a large majority of the polymorphisms.

The various steps of the mtDNA analysis include primary visual analysis, sample preparation, DNA extraction, polymerase chain reaction (PCR) amplification, post amplification, quantification of the DNA, automated DNA Sequencing and data Analysis. [3, 5]

Visual Analysis:

It is the first step in the analysis which is executed to determine if the sample needs be subjected to DNA analysis or not.

If the sample is hair, it is mounted on to a glass slide and examined under the microscope comparing it with that of the reference sample. If both the samples match, further confirmation is done through molecular level analysis of mtDNA.

If the sample is bone and teeth, the forensic anthropologists or Odontologist inspect the tissue to determine if it is of human origin and then followed by mtDNA analysis. [3, 5]

Sample Preparation:

This step involves cleaning the sample to remove contaminating materials surrounding or adhering to the sample so that the exogenous human DNA sample if any is removed.

Hair sample cleaning involves detergent treatment in an ultrasonic water bath. For bone and tooth sample, their exterior surface is sanded to remove any extraneous material that may adhere to the surface. [3, 5]

DNA Processing:

The prepared sample is mixed with various organic or alkaline chemicals that separate the DNA from other biological materials, such as proteins.

Then it is centrifuged, sedimented and filtered to obtain purified DNA sample. This sample is further subjected to Polymerase chain reaction, where the sample is amplified, quantified and sequenced. [3, 5]

Data Analysis:

The differences and similarities between sequences are read based on the set guidelines. Software can be used for the analysis. [3, 5, 16] PCR is the most common technique employed for mtDNA analysis. It has several advantages.

It is a faster method, it produces more discrete results and it can be performed on cadaveric tissue, formalin fixed tissue, and on blood that has been exposed to environment for long. [5] Other techniques have also been attempted. The analysis of multiplex mini-sequencing of mtDNA has shown high success rates. It is approximately 80% for shed hairs and in excess of 90% for fecal material. [12]

The corroboration of SNP analysis of mtDNA in the noncoding and coding regions has been found to be useful in Forensic investigations. [17] As hair samples degrade postmortem, levels of amplifiable mtDNA decrease. [18]

A simplified method for mtDNA extraction was described involving alkaline digestion to dissolve a hair in approximately 6 hours. [19] A 5-year retrospective review of mtDNA on 691 hairs from forensic casework found that a full or partial mtDNA profile could be obtained on >92% of the examined hairs. [20]

Denaturing high-performance liquid chromatography (DHPLC) has been tried for mtDNA analysis and it has been found to be advantageous. It is more accurate, rapid, and cost-effective. [13]

Since teeth are highly resistant to degradation from adverse environmental conditions, they form good source for forensic analysis. MtDNA can be analyzed from pulp and dentinal tissues. Dentin can be sampled using techniques such as splitting, crushing, scraping, and filing of the teeth, cryogenic grinding or horizontal sectioning technique. [21]

mtDNA analysis has few disadvantages. The procedure is sensitive and expensive, mtDNA shows heteroplasmy and the discriminatory power is 1:200. [5] Fragmentation and environmental degradation of the DNA and laboratory contamination can complicate analysis. Hence, contamination prevention should be strictly followed in the laboratory.

mtDNA analysis has been successfully used in cases of identifying Romanovs, Anna Anderson and Jesse James. [14, 22, 23]

Conclusion:

Due to its special characteristic features, the mtDNA offers several advantages over nDNA, due to which it can be used even in cases, where nDNA is unavailable for the analysis. It has been pivotal in solving few of the unresolved cases.

Tooth has been proved to be a good source of nDNA. The extraction of mtDNA from tooth has also been done, but needs further research and exploration.

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Table 1
Differentiating Features between Nuclear DNA (nDNA) and Mitochondrial DNA (mtDNA)

	Nuclear DNA	Mitochondrial DNA
Location	Found in nucleus of the cell	Found in mitochondria of the cell
Number	2 sets of 23 chromosomes	Each mitochondria may have several copies of the single mtDNA molecule
Shape	Double helix	Circular
Structure	Bounded by a nuclear envelope	Free of a nuclear envelope
Chromatin	DNA packed into chromatin	DNA is not packed into chromatin
Inheritance Pattern	Both Maternal and paternal	Inheritance - Maternal only
Discrimination	Can "discriminate between individuals of the same maternal lineage"	Cannot "discriminate between individuals of the same maternal lineage"
Mutation Rate	Susceptible for mutation	Susceptible for mutation and is 10 times higher than nDNA
Forensic Evidence	Used with evidence such as saliva, semen, blood	Used with evidence such as hair, bones, and teeth