

# Use of Mitochondrial DNA in Forensic Odontology: A Review

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## Abstract

The advent of DNA typing has revolutionized law enforcement. The identification of dead bodies in mass casualties has been a Herculean task since time immemorial. Fatal crash of civil or military airliners generally leaves numerous unidentified bodies. Despite the fact that the inner of the teeth is relatively protected against external influence, the DNA of the pulpal tissue can be harmed so much that the DNA analysis are made impossible. Studies have shown that the dentine is a good source of mitochondrial DNA (mt DNA) and mitochondrial DNA presents several advantages compared to nuclear DNA (nu DNA), when teeth are used for identification purposes. There are indications of presence of heteroplasmy in mitochondrial DNA. This may have an impact on the interpretation of the analysis of results.

This paper is aimed to review the applications of Mitochondrial DNA in Forensic Odontology.

## Background

All organisms are either multi-cellular or unicellular. A single eukaryotic (protista, fungus, plant, animal) cell is comprised of multiple membrane-bound organelles. The nucleus is an organelle in eukaryotes which houses the primary genetic material (DNA) and mitochondria are organelles which are responsible for cellular respiration (ATP production). Mitochondria have a double membrane, cristae (folds), a matrix, and their own DNA. Mitochondrial DNA (mtDNA) codes for proteins and enzymes used by the mitochondria. Nuclear DNA also codes for enzymes used in the mitochondria.

## Key words

Mitochondrial DNA, Nuclear DNA, Forensic

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Odontology, Heteroplasmy.

## Introduction

The DNA from every human except identical twins is unique because of presence of polymorphism. On an average, only one in thousand base pairs differs which explains why all human beings have two legs, two hands, one nose etc. But in three million base pair sequence, there are quite a few differences. These polymorphisms are divided into two types (i) Length polymorphism and (ii) Sequence polymorphism.<sup>4</sup>

1. Length based polymorphism are found in repetitive DNA. More than 90% of our DNA is superfluous. Approximately 20-30% of non-coding regions are composed of repetitive regions.

Variable non-tandem repeat loci in DNA possess a core sequence that is repeated in a string form. The number of core repeats varies from individual to individual.<sup>4</sup>

2. Sequence polymorphism is composed of different nucleotides at a particular location in the genome. These sequenced variations can be manifested as base substitutions, additions or deletions.<sup>4</sup>

DNA analysis makes use of

- Nuclear DNA (N-DNA)
- Mitochondrial DNA (mt-DNA)<sup>8</sup>

Mt DNA of siblings will match each others and that of their mother. mtDNA is found as a single, circular chromosome in the cell. Mitochondrion may contain multiple copies of mt DNA. A human cell may contain hundreds or thousands of mitochondria. mt DNA is useful when nuclear DNA is limited because of its abundance.

## Advantages of mtDNA over traditional methods

DNA testing using conventional STR systems may produce insufficient results, if the genomic

DNA in the specimen is either highly degraded or the available quantity is very small (e.g. skin particles, hair shafts or ancient bones).<sup>2</sup>

The first major advantage is its application to all biological source material. The second major advantage is that mtDNA testing has tremendous discriminatory potential and a very high sensitivity. The third advantage is its resistance to environmental factors. MtDNA is a robust molecule resistant to acids, alkalis, detergents, oil, gasoline, high temperatures etc.<sup>10</sup> MtDNA analysis the last resort when the recovered mtDNA fragment is quite small, or severely fragmented.<sup>10</sup>

### **MtDNA degradation and environmental damage**

MtDNA normally undergoes progressive fragmentation or degradation reducing the high molecular weight mtDNA to low molecular weight mtDNA; however the sequence information will be still present. Smaller mtDNA fragments are present for considerable period and this allows for some mtDNA testing.

There are major differences which makes Mitochondrial DNA(mt DNA) more advantageous over Nuclear DNA(nu DNA),

### **Mitochondrial DNA versus Nuclear DNA**

#### **1. Nuclear DNA**

- \* Found in nucleus of the cell.
- \* 2 sets of 23 chromosomes
- \* Used with evidence such as saliva, semen, blood.
- \* Can be Maternal and paternal in origin.
- \* Can discriminate between individuals of the same maternal lineage.
- \* Double helix
- \* Bounded by a nuclear envelope
- \* DNA packed into chromatin.

#### **2. Mitochondrial DNA**

- \* It is found in mitochondria of the cell.
- \* Each mitochondria may have several copies of the single mtDNA molecule.
- \* It is of maternal origin only.
- \* Cannot discriminate between individuals of the same maternal lineage

- \* Circular
- \* Free of a nuclear envelope
- \* DNA is not packed into chromatin.<sup>3</sup>

### **Key Facts about MtDNA<sup>2</sup>**

- \* MtDNA of siblings will match each others and that of their mother.
- \* MtDNA is found as a single, circular chromosome in the cell.<sup>3</sup>
- \* Mitochondrion may contain multiple copies of mtDNA.
- \* A human cell may contain hundreds or thousands of mitochondria.
- \* MtDNA may be useful when nuclear DNA is limited because of its abundance.

### **The Mitochondrial Genome<sup>4</sup>**

- \* Has 16,569 base pairs (bp) in length.
- \* It encodes 37 genes, 13 proteins, 22 tRNAs, and 2 rRNAs
- \* It consists of two general regions:

#### **Coding region**

Responsible for the production of various biological molecules involved in cellular respiration.<sup>5</sup>

#### **Control region**

Responsible for the regulation of the mtDNA molecule.

Two regions that are highly polymorphic (variable) in the human population are

- \* Hypervariable Region I (HV1): 342 bp
- \* Hypervariable Region II (HV2): 268 bp
- \* HV1 and HV2 are used in mtDNA examinations because of their polymorphism among individuals.
- \* It contains little non-coding DNA (junk DNA, or introns)<sup>10</sup>
- \* For the bone, teeth, and hair samples, a hot-start PCR (7) was used to amplify the first hypervariable segment (HV1) of the mtDNA control region in up to four overlapping fragments.

#### **Historical perspective**

- \* In 1973 an article was published by Sognaes and colleagues that showed that

the charred remains Adolf Hitler has been identified without doubt by remains of the teeth and dental bridges found after war.

- \* Late 1980s - FBI Lab conducted studies to test the usefulness of mtDNA analysis for human identity testing.
- \* 1992 - Lab research began for using mtDNA in criminalistics.
- \* June 1996 - Examinations on mtDNA evidentiary samples began in the case of State of Tennessee v. Paul Ware.(USA)<sup>6</sup>
- \* 2001 - Retrieval of Mitochondrial DNA to identify victims of World trade centre terrorist attack in USA.<sup>3</sup>
- \* 2004 - Retrieval of Mitochondrial DNA was used to identify individuals in Tsunami disaster.

#### Uses for mtDNA in Forensics

- \* MtDNA will be used when biological evidence may be degraded [i.e. charred remains] or in small quantity.
- \* Cases in which evidence consists only of:
  - \* Hairs
  - \* Bones
  - \* Teeth(pulp, dentine)
- \* Missing Persons Cases (use of skeletal remains)
- \* Establishing individuals as suspects (hair evidence)

Hard tissue is preferred over soft tissue as source of evidence because of the following reasons.<sup>10</sup>

- \* They can survive in extreme conditions.
- \* They do not undergo decomposition.
- \* There are lesser chances of contamination of the hard tissue.

#### Forensic mtDNA analysis<sup>10</sup>

The Steps of mtDNA analysis process to obtain a mtDNA sequence from a sample principally consists of

1. Primary Visual Analysis
2. Sample Preparation
3. DNA extraction
4. Polymerase Chain Reaction (PCR)

Amplification

5. Postamplification Quantification of the DNA
6. Automated DNA Sequencing
7. Data Analysis

#### Primary Visual Analysis

- \* Bones and Teeth are visualized.
- \* Forensic anthropologists or odontologists inspect the tissue to determine if it's of human origin.
- \* If it is of human origin, then mtDNA analysis can be used in conjunction with medical, anthropological, odontological examinations to assist in the identification process.

#### Sample Preparation

- \* Evidentiary samples are cleansed to remove any contaminants; this ensures that the mtDNA obtained is from the sample itself and not from exogenous human DNA
- \* Bone and Tooth Samples:
  - a. Are sanded to remove material adhering to the surface.
  - b. Have a tissue sample removed and ground into a fine powder (in teeth samples, the tissue may be obtained from the dentin and the pulp).
  - c. Are placed in an extraction solution.

#### DNA Extraction

1. Cellular homogenate is exposed to a mixture of organic chemicals that separate the DNA from other biological molecules, such as proteins.
2. Mixture is spun in a centrifuge
3. DNA settles in the top water-based layer.
4. Top layer is filtered and concentrated.
5. DNA sample is now purified.<sup>3</sup>

#### Polymerase Chain Reaction (PCR) Amplification<sup>1, 10</sup>

PCR is a technical breakthrough that has revolutionized DNA testing and indeed, the biological sciences. All current non RFLP DNA tests are PCR based methods including.<sup>2</sup>

- (i) Dot blots
- (ii) Amplified DNA fragment polymorphism

and short tandem repeats

(iii) Mitochondrial DNA sequencing.

The main advantages of PCR are

- (i) It is a faster method
- (ii) It produces more discrete results
- (iii) It can be performed on cadaveric tissue, formalin fixed tissue, and on blood that has been exposed to environment for long.

PCR is a procedure that makes many copies of a small amount of DNA.

- \* DNA is heated at 94° C to separate the two strands of the DNA double helix in the sample.
- \* New DNA strands are then made from the template (initially separated strands) of DNA by using DNA polymerase, primers, and free nucleotides.
- \* The process is repeated multiple times, doubling the amount of DNA after each cycle

#### **Postamplification Purification and Quantification**

- \* Purification is performed using filtration devices that remove the excess reagents used in the PCR from the sample.
- \* Quantification is performed using capillary electrophoresis (CE), which compares the amount of DNA in the PCR product to a known DNA standard to determine the concentration of the DNA in the PCR-amplified sample.<sup>1,3</sup>

#### **Automated DNA Sequencing**

Dideoxy Terminator (Sanger's) Method is used

- \* It is similar to the PCR amplification process.
- \* Terminator bases are added in addition to free nucleotides.
- \* Terminator bases lack a chemical group which allows DNA polymerase to place another base after it.
- \* Terminator bases are tagged with a fluorescent dye.

Normal bases compete with the [terminator bases] for incorporation into the growing DNA strand, resulting in a collection of

DNA products that differ in size by one base and have a fluorescently labeled base at the end position.

- \* Final sequence is compared with Anderson's sequence (610 bp sequence can be referred to as the Anderson Sequence, the Cambridge reference sequence, or the Oxford sequence)
- \* To denote deviations from this sequence, the assigned number of the different base pair is recorded with a letter (A, T, G, or C) designating the different base pair.

#### **Data Analysis**

MtDNA sequences are generated by a computer and edited by a DNA examiner to obtain the final sequence.

- \* The differences are recorded by comparing the finalized sequence to the Anderson reference sequence
- \* If sequence concordance ("the presence of the same base or a common base at every position analyzed") is observed, then both mtDNA samples could be considered as originating from the same source.<sup>8</sup>
- \* DHPLC methods provide the forensic scientist with a powerful tool to rapidly screen mtDNA and may result in standardized methods to resolve mtDNA mixtures.

These advances will allow mtDNA analysis in cases not previously examined by current sequencing-based approaches and could allow more forensic case samples to be entered into the proposed mtDNA Combined DNA Index System (CODIS trade mark) databank as a result of mtDNA mixture resolution.

#### **Studies related to Mitochondrial DNA analysis**

According to Stone et al<sup>5</sup> forensic case work involving mtDNA analysis we have observed that DNA tends to preserve better in teeth than in bones.

According to the studies conducted by Svensson et al<sup>7</sup> it was shown that dentine is a good source of mitochondrial DNA. The reason for these findings is projection that the border between the dentine and the pulp is covered with a layer of odontoblasts. The odontoblasts

have cytoplasmic processes, reaching covered with a layer of odontoblasts. The odontoblasts have cytoplasmic processes, reaching out in the pulpal 1/3<sup>rd</sup> of the dental canalicules. There are approximately 45000 canalicules and processes in the pulpal part of the dentine per square mm in the coronal part of the tooth and approximately 10,000 per square mm in the apical part. In these processes mitochondria can be found mt DNA.

With increasing age, the odontoblastic processes retract and calcification of dentine canalicules occurs, with a sclerotization of the dentine as a result. It can be suspected that this change could affect the possibility to retrieve mitochondrial DNA from dentine. Mornstad and colleagues showed that mitochondrial can be extracted from apical dentine in elderly individuals, although a clear decrease in the amount of mt DNA in dentine with age was found. It can therefore be suggested that some of the odontoblastic process or at least parts of the mitochondrial DNA are left in the dentine when the canaliculae calcify.

According to Alice et al<sup>10</sup> another possible reason for findings of mitochondrial DNA in dentine can be that almost every preparation and airbrushing of the tooth are causing aspiration of odontoblast into the dentine canaliculae, due to capillary attraction. The mitochondrial DNA from the odontoblast are then captured in the dentine and are very well protected there also against the type of effects on the inside of the tooth that harm the pulpal tissue and its DNA.

According to Stone et al<sup>5</sup> MtDNA analysis is, like any DNA analysis, a test for exclusion. If the mtDNA sequence of a case sample is different from a maternal reference mtDNA sequence, then the case sample cannot come from a maternal relative of the reference sample, and the theoretical probability of exclusion is 100% (in actual practice the probability of exclusion is less than 100 since sample mixups, laboratory errors, or contamination with extraneous DNA can lead to false exclusions).

According to Kinra et al.<sup>2</sup> the three major disadvantages of mtDNA analysis are (i) Discriminatory power of mt DNA is 1:200 (ii) The procedure is for of mt DNA expensive

and (iii) Heteroplasmy (few single base pair difference might be there in different cells of the same individual unlike the nuclear DNA. According to Swenson et al<sup>7</sup> mt DNA shows heteroplasmy at some level in all individuals. If more than one type of mt DNA exists in an individual eg. due to change or mutation in one or some base pairs in a mitochondrion that further on creates a new population of mitochondria with different DNA sequence than individual heteroplasmy is present. The number of divisions that the mitochondria has undergone increases with age. Therefore chances of heteroplasmy increases in the case of older individuals. Thus it is important to establish the prevalence of heteroplasmy in mitochondrial DNA in dentine. More studies should be undertaken in order to prove the significance of heteroplasmy in Mitochondrial DNA of an individual.

### Conclusion

The DNA- typing identification modality has proved to be a valuable component of the large arsenal of identification tools deployed in the aftermath of mass disaster. The DNA- typing identification modality has proved to be a valuable component of the large arsenal of identification tools deployed in the aftermath of mass disaster. Mitochondrial DNA analysis is preferred over N- DNA analysis.

While mtDNA analyses do not provide the discrimination potential of some nuclear DNA tests, mtDNA data often are the only information that examiners can gather from degraded evidence. This is either old or has been exposed. past decade have been helpful to solve many past cases and will continue to provide useful information to the law enforcement community in future to the environment for a significant period of time. The development of forensic mtDNA sequencing over the decade has been helpful to many past cases and will continue to provide useful information to the law enforcement community in the future. The characteristics of high copy number, maternal inheritance, and high degree of sequence variability make mtDNA a powerful tool for forensic identification. Now it is necessary to get as much as possible individual genetic information as quickly as possible in order to

enable individual identification. Soon it will be possible to create a new era in which forensic identification can be performed using microarray technology.<sup>10</sup>

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