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APPLICATION OF MITOCHONDRIAL DNA (mt DNA) IN FORENSICS

Biological Science

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ABSTRACT

The DNA profiling is an essential forensic technique in criminal investigations and civil cases. DNA acts as decisive evidence in forensic science. DNA profiling primarily focuses on genomic (nuclear) DNA but besides genomic DNA, mitochondrial DNA analysis also plays an important role in forensics. Mitochondria are the organelles responsible for producing cellular energy with respect to ATP and regarded as power House of cells. The mitochondrial DNA is found in the matrix of the mitochondria. The specific and interesting characteristic feature of mitochondrial DNA is that it is inherited from the mother only, as at the time of fertilization sperm only contributes its nucleus, not its mitochondria so the only available Mitochondria that of the mother's and the zygote contains the mother's mitochondria. so that it is helpful to solve maternal-related cases. We can analyze a variety of forensic samples of mt DNA such as hair shafts, old bones and teeth, teeth or other biological samples with low DNA content. A cell contains only one-two genomic DNA but contains many mitochondria as well as approximate 1000 mtDNA. So in the damaged or degraded DNA samples that have low nuclear DNA quantity, mtDNA is used. The use of mitochondrial DNA to trace family lineages, solve criminal cases, and decipher historical puzzles has now come to the forefront. In this article, we have focused on various case studies, once unanswerable, but now solved with the help of mitochondrial DNA analysis.

KEYWORDS

mitochondrial DNA (mtDNA), genomic DNA, DNA profiling, forensics, family lineages

INTRODUCTION

The importance of forensic mitochondrial analysis is becoming increasingly crucial as the new analysis method has evolved alongside science and technology. Identification of Nicolas Romanov II, the last Russian Tsar, the title of the Tomb of Vietnam's unknown soldier, the murder of Lacy Peterson, and the Nicole Brown Simpson murder trial have all brought mitochondrial DNA profiling to the forefront.

Most people know about nuclear DNA which is inherited from both parents because of recombination. However, in the cytoplasm, there is an organelle called the mitochondria, also known as the "powerhouse of the cell". which also has its own genetic material. Mitochondrial DNA is inherited from the mother and is passed on to the daughter, generation after generation. The mitochondrial DNA is inherited without any recombination and paternal inheritance. The mitochondrial DNA in the son is of no use because it will not be used in his children. Also, each cell contains only one copy of nuclear DNA, but it may contain multiple of copies of mitochondrial DNA (Borst, 1977).

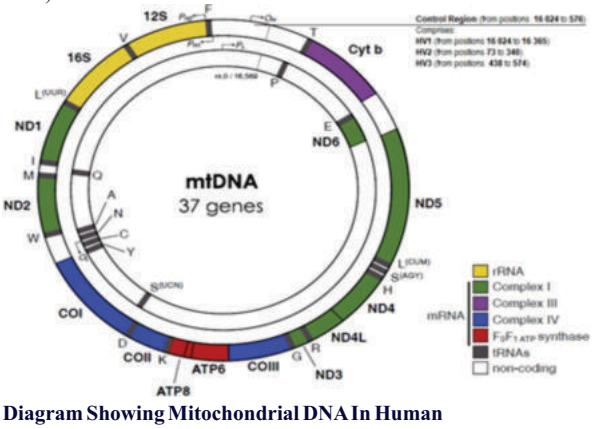

Diagram Showing Mitochondrial DNA In Human

Mitochondrial DNA is circular DNA about 16,566 base pairs long. It was first sequenced in 1981 by Anderson et al in the Sanger laboratory in Cambridge, and hence the sequence was named Cambridge Reference Sequence (CRS) (Anderson et al., 1981). Later, the sequence was confirmed by Andrew in 1999 and was named as revised CRS (rCRS). The sequence of mitochondrial DNA is highly conserved and has very little variation among individuals. It contains coding and non-coding regions. Coding regions contain 37 genes while the non-coding regions control mitochondrial DNA (Nelson & Cox, 2001). The 1000 base pair long noncoding region known as D- loop contains two hypervariable (HV) regions known as HV1 and HV2. The variation between HV1 and HV2 is very small and it's generally a single nucleotide polymorphism (SNP). This variation allows for differentiation between individuals and any biological sample; therefore, these regions are the target of forensic analysis. Also, the relatively high mutation rate in mitochondrial DNA causes variation between individuals.

In the forensic mitochondrial analysis, the mitochondrial DNA is extracted and subjected to PCR for amplification of HV1 and HV2 regions followed by the Sanger method of DNA sequencing. The sequenced DNA is then compared with the Cambridge Reference Sequence and the difference is noted down. The same method is followed for the suspect as well, and the two sequences are compared for any potential similarities. Since mitochondria are present in high copy numbers and are less susceptible to degradation because of their circular structure, they can be obtained from highly damaged, degraded, or even small biological samples like bone, skull, teeth, hair, etc. These features make mitochondrial DNA a potential candidate for cold scene investigation when compared with nuclear DNA. Mitochondrial DNA can play a significant role in the identification of the biological specimen collected from a crime scene and the identification of skeletal remains recovered from past military conflicts (Wilson et al., 2003).

In Britain, Mitochondrial DNA was used to identify a direct descendant of the oldest skeletal remains found. That of a 23-year-old male who had died from a blow to the head. The living descendant was a 42-year-old history teacher who lived a short distance away from him. These bones were first found in 1903. In the 9,000-year-old skeleton, Mitochondrial DNA is extracted from a tooth cavity and used to link the two distant relatives. The first criminal case in the history of sexual assault and murder of a four-year-old girl was to be solved using mitochondrial DNA in Tennessee, U.S.A., in September 1996. The foreign hair was found on the girl's throat and bed sheets and the test was performed using this material. Forensic DNA analysis was done by experts from the FBI performed mtDNA analysis to match mtDNA samples from the defendant.

Case Study-

1. Finding The Czar And His Family:

Since mitochondria are passed unchanged from mothers to their children, it is used to trace family history and relations. Romanovs ruled the country of Russia, for over 300 years. The last ruling Russian Tsar, Nicholas II, abdicated the throne at the end of the Bolshevik revolution in 1917. He, his wife, their 5 children, and four servants were kept under house arrest in the Ural Mountains. The entire group was executed in 1918 and was buried in a nearby unmarked grave. In

the late 1970s, a tomb was found with nine bodies. A DNA test confirmed that 5 of the bodies (Tsar, Tsarina, and three daughters) were related to one another and were undoubtedly the Tsar family and the other four were most likely the servants. Several years after the death of the family, Soviet Government acknowledged the death of all the Romanovs, but speculations grew that some of the family members escaped the execution. Anna Anderson was one of the most famous claimants, and she was Antassia, one of the missing children The children and the adult women all shared mtDNA with HRH Prince Philip who was related to Tsarina through his mother (Ivanov et al., 1996). . But in 2007, subsequent mitochondrial DNA testing from the grave confirmed that they were the missing Romanovs.

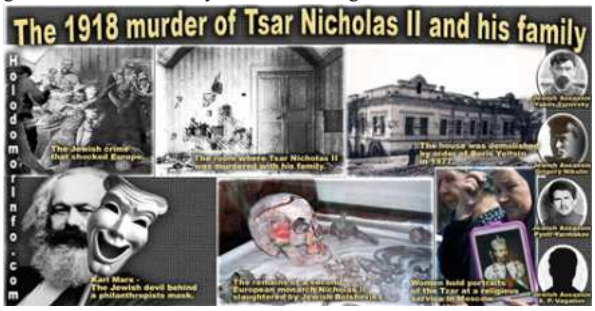

Image 1: The 1918 murder of Tsar Nicholas II and his family

2. Murder of LACI Peterson:

Laci Peterson, who was 8 months pregnant, was reported missing by her husband Scot Peterson on 23rd December 2002. Five months later on 13th April 2003, a male fetus was found on the shore of the San Francisco Bay.

Image 2: Poster showing Laci Peterson and her husband Scot Peterson

The next day, one mile down the shore, the body of the recently pregnant woman was found. After a thorough investigation, genetic materials matched with Laci's parents, and the fetus's genetic material also matched with Scot Peterson. Human hair was also found on the pliers of Scot Peterson's boat during an investigation. The cheek sample was taken from Laci's mother and after the mitochondrial DNA analysis, it was found that the mitochondrial DNA of the hair sample matched with her mother. Scot was found guilty and sentenced to a death penalty (Merheb et al., 2019).

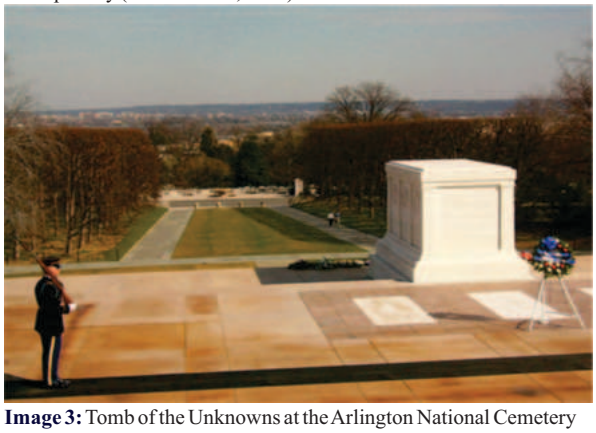

Image 3: Tomb of the Unknowns at the Arlington National Cemetery

3. Identification of the Tomb of Vietnam's Unknown Soldier:

Mitochondrial DNA is also used to include or exclude in a closed population by a process of elimination. The Tomb of the Unknowns in Arlington, Virginia, U.S.A., is a famous tourist destination. Mitochondrial DNA analysis was used to identify the remains of the Unknown soldiers from the remains of the Tomb in Arlington National Cemetry. There were ten reference specimens from seven families. Mitochondrial DNA sequence was obtained from the submitted sequence and was compared with each of the ten references. Six of the seven families were excluded from the analysis and only one family result matched that of Air Force lieutenant Michael J. Blassie. Armed force identification DNA laboratory (AFDIL) has used mitochondrial analysis to help identify more than 100 service members from the Vietnam War (*Tomb of the Unknown Soldier*, n.d.).

Mitochondrial DNA- a Tool To Trace Our Ancestors-

Ancestry refers to family history, roots, and ethnic origin or the place of birth of the individual. There are three methods by which genetic ancestry can be traced out, like a Y-chromosome DNA test, an autosomal DNA test, and a Mitochondrial DNA test. Y DNA is passed particularly along the patrilineal line while the X chromosome is passed along the matrilineal line through mitochondrial DNA transfer. A haplogroup is a genetic population of people who share a common ancestor based on a patrilineal or matrilineal line. Haplogroups are represented by the alphabet and refinement consists of more letters and numbers for elaborate description. Haplogroups are determined by Single Nuclear Polymorphism (SNP) tests where one nucleotide is mutated with a different nucleotide. All members of the haplogroup are descendants/progeny of a single man or woman who lived several years ago.

As mitochondrial DNA is uniparentally inherited, and it does not go under recombination, the mutation rate has divided the population into different haplogroups. Mitochondrial DNA haplogroups are generally represented by L, M, and N haplogroups. The ancient and distant African woman, who is now known as mitochondrial eve, is represented by the L haplogroup and consists of nearly all Africans. The M and N haplogroup also arose from the African haplogroup to populate the rest of the world through migration. The M haplogroup migrated to Asia and the N haplogroup was directed to Eurasia. At present the most common haplogroup in Europe is H.

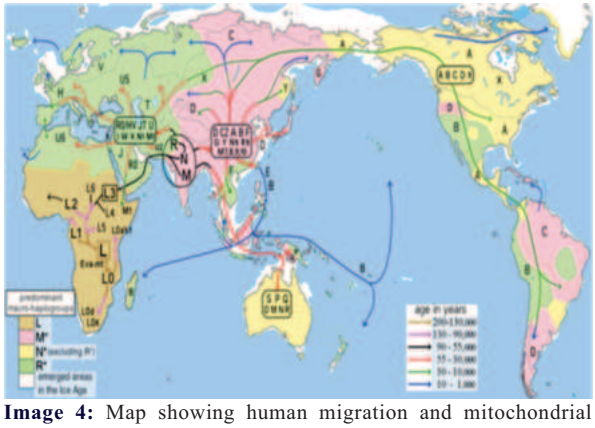

Image 4: Map showing human migration and mitochondrial haplogroup.

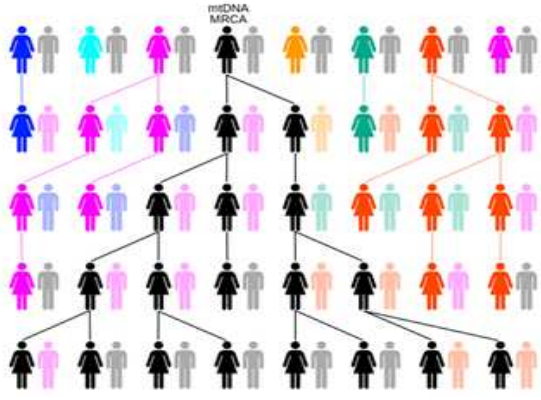

Image 5: A flow chart showing a Mitochondrial Eve. In this example, over five generations colors represent extinct matrilineal lines and black represent the matrilineal line descended from mtDNA MRCA.

Mitochondrial eve is the most recent common matrilineal ancestor (MRCA) of all current humans and was estimated to live between 99,000 to 100,000 years ago, most likely in East Africa.

Without DNA samples it is not possible to trace the matrilineal or the patrilineal lineages. To trace our ancestors, the mitochondrial DNA is extracted, sequenced, and then compared with the CRS. Through the analysis, the range of the DNA sequence matching with the CRS is obtained and looked for the SNP. There are many tools available online to check this SNP with the existing database and trace the origin of individual evolution and ancestors and finally, a family tree is drawn based on mitochondrial DNA analysis.

Here, we analyzed one sample (ID 19275) which was a Caucasian Swedish female. The extracted mitochondrial DNA was amplified, sequenced, and compared with the Cambridge Reference Sequence. The sequence range was 16152 to 16400. Within the range, there were two variations (SNPs) which were 16223T and 16278T. This set of information was then used to analyze and trace the ancestry of the sample. A very well-known forensic mitochondrial database is available known as EDNAP mitochondrial population database (EMPOP). EDNAP stands for European DNA profiling group which started in October 1988 in London to find a solution for crime investigation by using DNA technology. The frequency of this profile was 25 out of 30289. The sample was found to be originated maximally from America followed by Asia, Europe, and Africa. The haplogroup which was more prevalent was L followed by M and X (van Oven & Kayser, 2009).

Image 6: Map image showing the sampled populations within the query range (blue) and the matches in the sampled populations (pink).

The map given above shows the distribution of the sample in different parts of the world which is because of the migration of the very first mother. As migration increases, people with the matching mitochondrial sequence are found in different parts of the world.

DISCUSSION

But is it possible that every time the DNA test is correct? Let us see what DNA tests can tell us about our biological ancestors. As discussed earlier, a male inherits the Y-chromosome from the father and this is passed from generation to generation, which makes it possible to trace the grandchildren and the grandfather. The same is the case with female ancestry; the mother and the grandmothers and so on can be traced by mitochondrial DNA analysis. This was the way granddaughters were linked to their grandmothers in the aftermath of Argentina's Dirty War (1976-83). Several thousand parents disappeared because of the ruling Junta, and the orphaned children were given to the couple who wanted to adopt a child. It was through the mitochondrial DNA test the ancestry was resolved and the grandchildren were reunited with their actual grandmother (Knight, 1989; *(PDF) Forensic Mitochondrial DNA: Current Practice and Future Potential*, n.d.).

In a certain study, researchers attempted to trace the ancestry of North Americans with African ancestry to different parts of Africa. They studied 481 mtDNAs of recent African ancestry. While recent African ancestry traditionally refers to all existing human mtDNA, for example how Eurasian mtDNAs resulted from a haplogroup L3 from sub–Saharan Africa, in this context it refers to gene flow within known historical records, that is, the Atlantic Slave Trade (Salas et al., 2004). Surprisingly they found that their results were in tandem with historical research. Most of the people (62%) that arrived in America through the slave trade were from Western Africa. Similarly, another study found that Puerto Rico has substantial Native American ancestry (Martínez-Cruzado et al., 2001). So, mtDNA can be a useful tool for people to identify their ancestry and take pride in their origins. However, we must exercise caution while attempting to link sub-populations back to certain regions.

But apart from tracing the ancestry from the given sample, there are limitations as well for this analysis. A 42-year-old woman, Lorianne Rawson, who believed that she originated from Aleuts of Alaska, submitted her DNA sample to the Genographic project in North America. Surprisingly the result linked her to the enemy of the Aleuts of Alaska, Yup'Ik Eskimos which lead to political and personal trauma. Even the financial repercussions can be strong limitations for analyzing ancestry. The Black Seminoles are struggling to authenticate their relationship with Seminole Indian Tribe through DNA analysis but because of financial hindrances, they are not able to do so.

Another problem saw that the tests assess ancestry by comparing consumer DNA to a worldwide database of DNA samples. If a test-takers DNA is like a database sample from a particular population, it suggests that the test-taker has relatives in that population.

In forensics, the absence of recombination in mtDNA may pose a difficulty. This is because two individuals which seem unrelated at first may have identical mtDNA sequences because of a shared maternal ancestor. While it is a rare occurrence, the transmission of paternal mtDNA has also been observed in humans. A certain study observed biparental mtDNA inheritance in 17 individuals. These people belonged to three different multi-generational families (Luo et al., 2018). Combined with the high heteroplasmy of mitochondrial DNA-its ability to create multiple copies, it would pose a problem in forensics to track the lineage of a human with biparental inheritance. For example, scientists studied a 28-year-old man and found that he had a mutation that was paternal in origin and present in about ninety percent of his muscle DNA, causing him to suffer from mitochondrial myopathy (Arianne et al., 2002).

The presence of NUMT (Nuclear mitochondria DNA) segments may pose an interesting challenge to the accurate analysis of mtDNA lineages (Hazkani-Covo et al., 2010). Mitochondrial DNA has the uncanny ability to integrate itself into nuclear DNA and therefore may cause inaccuracies in the molecular clock (Zhang & Hewitt, 1996).NUMTs may contaminate mtDNA during PCR amplification. An extreme situation may occur in which human NUMTs may act as sources of contamination in research involved with forensic analysis and ancient DNA. In fact, the haphazard phylogenetic placement of dinosaur mtDNA discovered in 1994 was attributed to contamination by human nuclear DNA (Woodward et al., 1994).

CONCLUSION

Though mitochondrial DNA analysis is used in crime investigation in forensic sciences, and it is also used for creating the family tree through the matrilineal line, it has limitations also. So, great effort is needed for a better understanding of its implications and the public should be made aware of its pros and cons. The MtDNA has the privilege of degraded samples and having low or no genomic DNA. Additional research is needed for better marker selectivity and statistical analysis and the accuracy of the analysis of the sample from the present database.

The consequences of ancestry testing need to be assessed. The guidelines should be improved regarding ancestry estimation in consumers, in research areas, and in healthcare settings. Scientists should consult with scholars on the historical, socio-political, and cultural context to inform their research and commercial efforts. Mechanisms for greater accountability of the direct-to-consumer ancestry testing industry must be explored.

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