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HUMAN MITOCHONDRIAL DNA CHARACTERIZATION AND ITS APPLICATIONS

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ABSTRACT

Human mitochondrial DNA (mtDNA) has become a valuable tool in forensic studies. Its polymorphic nature and maternal inheritance are features that have supported investigators to identify missing persons, war losses and individuals tangled in mass disasters and criminal cases. Several unique properties of human mitochondrial DNA (mtDNA), including its high copy number, maternal inheritance, lack of recombination and high mutation rate have made it the molecule of choice for studies of human population history and evolution. Analysis of hypervariable segments of mitochondrial DNA (mtDNA) are presently being used for forensic analysis, human molecular genetics, evolutionary biology, human relocation studies and in identifying late persons. Mitochondrial DNA analysis offers a unique maternal ancestral view of an individual's molecular pin code, through typically examining the hypervariable segments and sampling the areas of hypervariable region 1 (HV1), hypervariable region 2 (HV2) and hypervariable region 3 (HV3). The objective of this study is to examine the utility of mtDNA typing in forensics through the analysis of a forensically related population.

KEYWORDS: hypervariable, haplogroup, recombination, maternal inheritance, mutation rate.

MITOCHONDRIA INTRODUCTION

Mitochondria are the organelles which are responsible for energy generation in the cells. Enzymes within the outer and inner membranes of the mitochondria support in converting materials into adenosine triphosphate (ATP) which is fuel for the metabolic activities of the cell.[1-2] The mitochondrion is the place in which the last stages of aerobic respiration take place. This network allows cells to use aerobic respiration to produce approximately 15 times more ATP than anaerobic respiration.[1]

All eukaryotic cells are believed to contain at least some mitochondria or a related organelle that developed from free living alpha proteobacteria.[3] These semi-autonomous organelles possess their own genetic material (mitochondrial mtDNA), encoding important proteins of the oxidative phosphorylation system and the necessary RNA machinery (rRNAs and tRNAs) for the translation of mtDNA transcripts.[4] Since almost all eukaryotic species have mitochondria, forensic investigations can benefit from the analysis of mtDNA in a wide range of species.

Human cells have a different number of mitochondria, depending on the metabolic requirements of the cells.[1] Typically, the number of mitochondrial mtDNA is 100-10000 copies/cell.[5] Some cells like that of ascidian spermatozoa contain one mitochondrion whereas in humans, heart and muscle cells can contain thousands of mitochondria.[6] The mitochondria possess their own genome and contain highly informative polymorphic sites.[7] These semi-autonomous organelles also possess the necessary RNA machinery for the translation of mtDNA transcripts.[8]

MITOCHONDRIA MORPHOLOGY

The mitochondrion's structure is complex due to its vital function in energy production (Figure 1). It possesses two membranes that separate it into four different sections each of them works to generate ATP.[9] The two membranes divide the organelle into inter membrane space and a large internal matrix.[1] The outer membrane of the mitochondrion contains channel proteins. The inner mitochondrial membrane consists of extensive folding called cristae.[1] The inward folds of the cristae increases the surface area for enzymes involved in cellular respiration.[1]

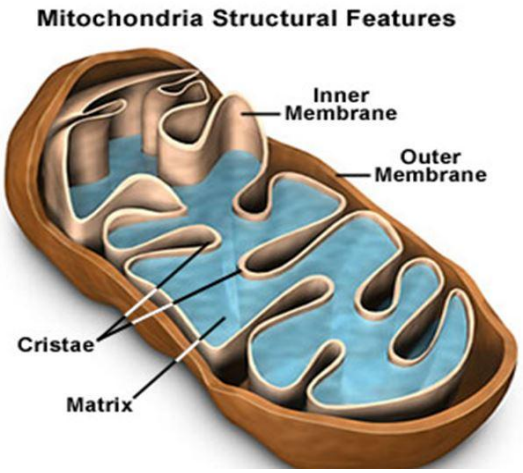

Figure1: Internal Structural Features of the Mitochondria.[1]

Mitochondria can be found in a variety of different morphologies in mammals.[10] They range in shape from long tubules connected to one another to small separate spheres.[10] Mitochondria also range in size between approximately 1 to 10 micrometers but vary in both size and morphology regularly.[1]

DNA Biology

Deoxyribonucleic acid (DNA) has rationalized forensic science. Forensic biology is a field of study that uses DNA to identify victims. The most important development in forensic human identification was the discovery by Alec Jeffreys, that one could distinguish individuals by targeting a specific set of genes and comparing the number repeated DNA sequences at a particular locus between individuals.[11] While performing research on gene evolution in sparrows, Jeffreys exposed repetitive DNA sequences in the human myoglobin gene and ultimately found that the human genome contained many regions of hypervariable DNA. Jeffreys exposed that these hypervariable regions were fairly distinctive and imagined to serve as unique identifiers. The first method of detection used was Restriction Fragment Length Polymorphism (RFLP).[12]

The typing of DNA markers arose as a result of the development of the polymerase chain reaction (PCR) in 1983. PCR is a fast and cheap technique used in molecular biology to exponentially amplify small DNA segments. PCR can generate millions of copies of a DNA segment in less than 4 hours. The development of PCR amplification had deep effects for forensic applications since amounts of evidentiary templates can be very small. Studies of DNA fragments are nearly impossible without PCR amplification.[13]

Mitochondrial and Nuclear DNA

Multicellular organisms rise by a quite slow process of progressive change that begins with the fusion of very specialized cells an egg and a sperm. The result of this union a zygote develops containing genetic information inherited from both parents that direct fetus development [14]. As the zygote divides mitotically to produce all cells of the body, nuclear genes are not lost or altered and the genome of each cell is equal to that of every other cell. The mtDNA molecule carries hundreds to thousands of genes which are the units of inheritance.

Mammalian mitochondrial DNAs (mtDNA**)** have two separate origins of replication. The origin of the heavy strand is within displacement loop (D-loop) and the light strand originates within a bunch of five tRNA genes nearly opposite of the D-loop. The D-loop consists of approximately 1100 base pairs of noncoding DNA and is generally known as the hypervariable region. The hypervariable region is further divided into three segments. Hypervariable region I (HV1) spans nucleotide positions 16024-16365, hypervariable region II (HV2) span nucleotide positions 73-340 and hypervariable region III (HV3) spans nucleotide positions 438-574. HVI and HV2 are usually targeted whereas HV3 is hardly examined in forensic sites. The hypervariable region has been described to mutate at a rate of 10 to 17 times more normally than that of the nuclear genome.[16]

The mtDNA, because of its circular structure and location inside the cell is more stable and strong than that of nuclear DNA. It is shown that mtDNA is secure from degradation even when exposed to prolonged environmental conditions which is not the case with nDNA. That is why mtDNA is present even in older and degraded specimens whose nDNA is already degraded.

Mitochondrial DNA is similar to nuclear DNA in a few ways but different in some way. One difference between autosomal nDNA and mtDNA is their inheritance pattern within families. The genome of an individual consists of equal contributions of chromosomes from both the maternal and paternal germ lines while mtDNA is inherited solely from the maternal line.[17] Mitochondrial DNA is a closed, circular molecule which is composed of 16,569 nucleotides.

Human mtDNA Characteristics

Human mitochondrial DNA is commonly used as tool in many fields including evolutionary anthropology, population history, medical genetics, genetic genealogy and forensic science. Interest in genetic testing to determine ancestry has increased rapidly since its introduction. Many individuals are willing to learn more about their ancestors and believe that genetic testing will be able to provide information where other traditional hereditary methods have failed.

Analysis of complete mitochondrial genome sequences is becoming common in genetic studies. The availability of full genome datasets empowers an analysis of the information throughout the mitochondrial genome in order to optimize the research design of future

evolutionary studies. The goal of this study is to identify informative regions of the human mitochondrial genome.

The important characteristics of human mtDNA are as follows.

- MtDNA is a small molecule, encoding a limited number of proteins and RNAs that are essential for the function of the mitochondrion.
- It is double stranded molecule containing 37 genes coding for 13 proteins, two rRNAs and 22 tRNAs which are essential components of four respiratory enzyme complexes.
- mtDNA is maternally inherited.
- mtDNA is a semiautonomous molecule that does not undergo recombination. It replicates rapidly without efficient proof reading and DNA repair mechanisms.
- The peculiar structure and unique replication system of mtDNA and the highly oxidative environment in which it is located have caused it to mutate at a rate 10-20 times higher than that of nuclear DNA.

The main characteristic of mitochondria relevant to this study is that they possess their own genome. High concentrations of mtDNA are found in bones, teeth and hair which are very useful for forensic analysis. The mitochondrial genome is 16,569 nucleotides in length compared to 3.2 billion nucleotides for haploid human nDNA, so the target size for degradation is much smaller for mtDNA. Similarly, mtDNA is circular in shape which means there are no free ends for exonuclease activity to degrade the genome.

A non-coding region of mtDNA called the displacement loop or the D-Loop has been universally studied for its potential use in forensic DNA applications. The D-Loop is also known as the control region because it regulates the gene products produced by the coding regions of the mitochondrial genome.[19]

The D-Loop is also a region of polymorphism within the mitochondrial genome due to a high frequency of base changes.[20] These highly polymorphic regions have been called hypervariable regions and have been the focus of forensic DNA typing for identification purposes.[21] Since mtDNA is inherited matrilinearly, specific mtDNA haplotypes have also been useful in determining an individual's ethnic origins.[22]

Mitochondrial DNA and Human Evolution

Employment of humans on the earth is one of the extreme delightful story in the history of human race.[23] Mitochondrial DNA (mtDNA) has been a basic line of evidence in developing the current understanding of our genetic prehistory. Phylogenetic studies of human mtDNA variation support a late Pleistocene extension of modern humans from Africa.[24-25] More work recommends the southern migration route from sub-Saharan Africa along the Indian Ocean coast into Eurasia[26-27] and a later migration from the Levant into North Africa and Europe.[28] However, in spite of these advances we lack a clear understanding the timing of human population developments.

Modern humans have gone through various processes of genetic variations when their ancestors left Africa about 100,000 years ago.[25] Hominines with morphology similar to present day humans appear in the fossil record across Eurasia between 40,000 and 50,000 years ago.[29] The environmental stresses and the social evolutions have been acting as the major selective forces remodeling the genetic make-up of human populations. Genetic adaptations have occurred in many aspects of human life. The molecular studies in recent years have detected many genetic variants. During the expansion of modern humans from Africa to the other parts of the world, they have threatened from different environmental changes. Recently, genetic analysis in East Asian populations has indicated that a considerable number of genes have changed significantly.[30]

East African populations may provide important evidences toward understanding modern human origins. Both palebiological and archeological data indicate that modern humans may have originated in Eastern Africa[31-32] perhaps as early as 196,000 years ago.[33] In addition, the earliest migrations of modern humans out of Africa are thought to have originated from Eastern Africa.[34-35]

Nucleated mammalian cells contain thousands of copies of the mitochondrial genome. It has been generally accepted that in the majority of humans, all mitochondrial DNA (mtDNA) molecules are identical at birth.[36] Each mitochondrion contains an average of five copies of its genomic DNA. There are hundreds to thousands of mtDNA molecules within a single cell.[37]

All modern humans have a certain type of mtDNA. These types of mtDNA are associated with the SNP pattern within the mitochondrial genome and are called haplogroups. Each person belongs to a certain haplogroup and a particular haplogroup can display a person's common female specific place of origin.[38] Haplogroup analysis is also used to trace population migration patterns.[39]

Mitochondrial DNA is the principal tool in the exploration of recent evolutionary history and consists mostly of coding DNA with the exception of 1100-bp long fragment that has mainly regulatory functions and is therefore named the control region. It has become widely used for studies of human evolution, migration and population histories.[40] Being entirely maternally inherited, mtDNA undergoes negligible recombination shown at the population level. As a result, homoplasmic variation of mtDNA has played a key role in determining population migrations on a global scale.[38]

The human mitochondrial DNA (mtDNA) is a circular double stranded molecule and was first fully sequenced in 1981 by Anderson.[41] Most human cells contain

hundreds of mitochondria and thousands of mitochondrial DNA copies.[42] mtDNA has 16,569 base pairs in length[43] and is present in high copy number in human cells with high mutation rate, haplogroup, without recombination and maternal inheritance.[44-45] These specifications make mtDNA easier to obtain for analysis and also make it the molecule of choice for analyzing ancient DNA and for certain forensic DNA applications.[45-46] The analysis of mtDNA can be used effectively even when a gap of several generations exists between an ancestor and a living person to determine maternal family relationship.[47-48]

Genetic analysis of mitochondrial DNA has been an important tool in understanding human evolution due to characteristics of mtDNA, such as high copy number, lack of recombination, high substitution rate and a maternal mode of inheritance. However, most studies of human evolution that have included mtDNA sequences have been restricted to the d-loop, which occupies less than 7% of the mtDNA genome.[49-56]

Sequencing of mtDNA offers many advantages in forensic. One advantage is the abundance of mtDNA within the cell. There are 500 to 2,000 copies of mtDNA in each human cell as compared to only two copies of nuclear DNA in most cells and some cells do not contain nuclear DNA at all.[19] Another important advantage of using mtDNA for forensic analysis is sequences availability in public databases from population genetic.[57] This data can help, either to estimate the significance of mtDNA haplotype in forensic casework [58] or to use as a tool for species identification.[59] Thus, for a small biological sample recovered from a crime scene, there will be excess of mtDNA for analysis as compared to nDNA. So, mtDNA is beneficial for those cases in which the obtained extracted DNA sample is very small.[53] Moreover, some tissues in the body that do not contain significant amounts of nDNA but contain mtDNA useful for typing. For example, hair shaft essentially lacks nDNA but contains considerable amounts of mtDNA. Hair is a common type of forensic evidence; in addition, finger and toe nails typically lack nDNA but contain mtDNA[60]

The maternal inheritance of animal's mtDNA is almost universal and highly concentrated.[61-62] Thus, permitting distant maternal relatives to be compared to the analyzed samples for relationship hypothesis testing or when the original depositor of the sample is not available. The genome of an individual consists of equal contributions of chromosomes from both the maternal and paternal germ lines, while mtDNA is inherited solely from the maternal line.[17]

The mitochondrial genome can be divided into two sections, a large coding region, which is responsible for the production of various biological molecules involved in the process of energy production in the cell and a smaller 1.2 kilobase pair fragment, called the control region. The control region is found to be highly polymorphic and has hypervariable regions. These hypervariable regions have been used extensively in practical forensic investigations, because mtDNA is stable during long storage and its circular form makes it less susceptible to exonuclease degradation.[63]

The control region is polymorphic within the mitochondrial genome due to high frequency of base substitutions[20] and mtgenome in it are enriched in sequence variation due to a higher mutation rate which allows researchers to create mtDNA profile [64]. This control region is also responsible for the particular binding of several nuclear encoded proteins that regulate mtDNA replication and transcription.[65]

The hyper variable regions are very useful in forensic for identification purposes [31]. Polymorphisms of the hypervariable regions in the D-Loop structure have also been valuable in anthropological studies on the historic origins and migratory patterns of early human populations.[66] Since mtDNA is inherited matrilinearly, specific mtDNA haplotypes have also been useful in determining an individual's ethnic origins. So, mitochondrial control region is very important for forensic casework and population studies.[67]

Mitochondrial DNA (mtDNA) typing in forensic case work has generally focused on the two hypervariable segments (HVS) of the non-coding control region (CR).[68-69]

Mitochondrial DNA Polymorphisms

Polymorphisms are changes in the nucleotide sequence of control region of DNA. Causes of polymorphisms in mtDNA include the lack of protective histone proteins. DNA databases in forensic genetics rely on the fact that there are portions of our DNA that differ from one individual to another, generally called polymorphisms. Among other properties, it is important that these polymorphisms differ generally between individuals. Various markers are combined with the objective of creating unique profiles. The most common polymorphisms studied in forensic genetics are Short Tandem Repeats (STRs) and Single Nucleotide Polymorphisms (SNPs). STRs are smaller in size, easily genotyped and can be multiplexed, making this the preferred marker for human identification. SNPs represent the most abundant class of human polymorphisms. SNPs have been revolutionary in the field of medicine for decades because they can be used as markers to identify genes that underlie complex diseases [70].

STR markers are units of 2–6 bp in length repeated in tandem widespread throughout autosomal and sex chromosomes and account for approximately 3% of the total human genome.[71] They have a relatively high mutation rate which is the reason for their high degree of polymorphism. Nevertheless, STR mutation rates are still

relatively low which is important when testing for paternity situations where segregations could be due to mutational events.[72] Another great advantage of STRs is that they are easily amplifiable by multiplex PCR. The combination of various autosomal STR loci increases deeply the power of discrimination.[73] STRs are still the polymorphisms of choice in terms of genetic identification and similarity analysis. However, in some cases its use may be limited namely in highly degraded DNA analysis.

A SNP is a DNA sequence variation occurring within a single nucleotide. SNP mutation rate is very low when compared to STRs and are often found to be population specific[74] which can be helpful when predicting the ethnic origin/geographic ancestry of a profile.[75] Another advantage of SNP typing is when samples present a high level of DNA degradation because the size of the amplified products is significantly lower making its detection more effective when compared to STR analyses.[76] The use of SNPs in forensic genetics has become very common in the analysis of mtDNA due to its particular characteristics. Single Nucleotide Polymorphisms (SNPs) are likely in the near future to have a fundamental role in forensics, both in human identification and description.

In support of mtDNA typing for forensic analysis, extensive population research has shown that the haplotype of single nucleotide polymorphisms (SNPs) in the D-Loop are well correlated with an individual's specific ethnicity.[66] The highly polymorphic nature and high amplification efficiency of mitochondrial DNA (mtDNA) is valuable for the analysis of biological evidence in forensic casework, such as the identification of individuals and task of ethnicity.[77] Mitochondrial DNA polymorphism proved to be very effective for obtaining molecular genetic sketches of the world populations, as well as for the explanation of the human evolutionary history and past migrations.[78]

Mitochondrial DNA Applications

Human mitochondrial DNA has become a useful tool in forensic investigations. Its polymorphic nature and maternal inheritance are characteristics that have enabled investigators to identify missing persons, war casualties and individuals involved in mass disasters and criminal case. Relatively mitochondrial DNA sequence analysis seems to be a reliable and powerful resource for human identification [79].

The polyploid nature of the mitochondrial genome has an important value in forensic mtDNA profiling, there is the possibility of two or more different mtDNA sequences existing in the same cell or organism. Unlike nuclear DNA, the replication of mtDNA in somatic cells occurs independently of the cell cycle by a process of relaxed replication. This means that different tissues from the same individual may exhibit different mtDNA haplotypes and that their frequency can change with age. In practical terms, the frequency of heteroplasmy is usually very low because acquired mutations do not necessarily occur at the same site and are distributed in a highly heterogeneous manner among different tissues and within the same tissue, meaning that they remain largely undetected by standard sequencing techniques.[80]

The sequencing of mtDNA is now a standard laboratory procedure for the examination of degraded casework samples of human origin with unique advantages over nuclear DNA profiling systems. Mitochondrial DNA (mtDNA) has many remarkable features that make it an important tool in forensic science. The circular shape of most mtDNA molecules also prevents its degradation by exonucleases, a characteristic that increases the possibility of obtaining results from critical samples.

Mitochondria are quite protected inside the cell and the genetic material appears to survive where nuclear DNA becomes degraded easily. The protection offered by a two walled organelle increases the probability of obtaining results from degraded DNA samples for which nuclear DNA fails to give a result. Scientists and investigators can compare two hypervariable sections of an mtDNA sample with the hypervariable sections of another. Human mitochondrial DNA polymorphic nature and maternal inheritance are characteristics that have combined with its sequence information, enabled investigators to identify missing persons, war sufferers and criminal cases. Different methods are used for this purpose which includes restriction fragment length polymorphism typing of variable number tandem repeat (VNTR) loci[81-82], polymerase chain reaction (PCR) based systems to analyze single nucleotide polymorphisms (SNPs)[83-85] and direct sequencing of mitochondrial DNA (mtDNA).[86-88]

The mitochondrial hypervariable regions I and II have proven to be a useful target for analysis of forensic materials, in which the amount of DNA is limited or highly degraded. Conventional mitochondrial DNA sequencing can be time consuming and expensive.[89] Amplification and automated sequencing of the hypervariable regions (HV1 and HV2) of the non-coding displacement region (D-loop) or control region (CR) in human mitochondrial DNA has been proposed as a technique that could provide evidence about the identity of crime victims, especially skeletal remains, as well as perpetrators of crime who leave biological material at a crime scene not suited for standard DNA typing, especially hair shafts without roots.[90] Information often would be useful in criminal investigations and subsequent court proceedings and so it would be a forensically useful technique.

CONCLUSION

The nature of the mitochondrial genome, its high copy number and small size makes it more resistant to degradation and more stable than nuclear DNA. Due to this reason mitochondrial DNA is frequently the only

possible option for the forensic analysis of environmentally compromised samples. At present the forensic analysis of the mtgenome is restricted to the hypervariable regions.

Mitochondrial DNA is the key tool in the investigation of recent evolutionary history, particularly for recognition human origins and expansion. Maternal inheritance of mitochondria enables models of population history to be much simpler that needed for the analysis of nuclear DNA. The effectiveness of mtDNA in forensic identifications is limited by its low power of discrimination and the absence of high quality mtDNA databases. Single nucleotide polymorphisms (SNPs) in the control region outside of hypervariable regions I and II (HVI/HVII) and in the coding region of the mtDNA genome can provide additional discrimination in mtDNA testing.

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