Xournals

Academic Journal of Anthropological Studies

ISSN 2581-4966 | Volume 02 | Issue 02 | October-2019

Identification of Human Remains through Molecular Genetics: A Review

Prachi Janardhan Kathane[1], Garima Jindal[2], Dr. Ranjeet Kumar Singh[2]

Available online at: www.xournals.com

Received 14th August 2019 | Revised 10th September 2019 | Accepted 8th October 2019

Abstract:

Molecular genetic identification of an individual from the human skeleton or remains plays a very important role in forensic field. There is need to extract, amplify and purify the sample before the analysis for getting the accurate result. This review paper discuss about the different genetic markers such as Short Tandem Repeats (STRs), Single Nucleotide Polymorphism (SNPs), Y-chromosome and Mitochondrial DNA (mtDNA) through which an individual can be identified from their skeletal remains. For this analysis, the comparison is made between the remains (evidence sample) and reference sample. After the collection of evidence sample, the reference sample is collected from their belonging or their family members, relatives. The time for extraction and analysis of the DNA depends on the availability of biological materials like blood, soft tissues, bone, teeth, nails and hair. The analysis of DNA from bones and teeth are most demanding and time consuming method. This molecular genetic identification are very helpful in case of missing person, burnt bodies, absence of sample of an individual for comparison. This paper also discuss about the estimation of sex from these genetic markers.

Keywords: *Genetic Markers, Short Tandem Repeat, Mitochondrial DNA, Single Nucleotide Polymorphism, Y-Chromosome*

Authors:

1. *Government Institute of Forensic Science, Nagpur, Maharashtra, India*

Introduction

The molecular genetics play a vital role in the identification of individuals from the human remains. During the disaster, the victims are identified in a mass graves through the DNA and their genetic markers. So, the genetic marker become a valuable tool for the identification purpose. The use of DNA for the identification purpose has been popularized worldwide as a unique evidence in criminal and civil legal proceedings. Molecular genetic methods are also used to identify the biological traces found in the case of criminal activity. Also used to identify the charred, hanged or drowned persons, road traffic or train accident, recovered from fire and explosion and found after long time of death. Before the identification of an individual on the basis of highly polymorphic DNA markers, traditional forensic technique, anthropological approaches are used for identification purpose. As we discussed the DNA evidence can be used after a long time of death, this is because of the unchanged property as it remains same throughout the life except contamination (mixing of another DNA during handling, or processing).

When the analysis of DNA is done with old bones, the problems like low quantity of starting molecules, presence of inhibitors of polymerase chain reaction, and degradation of DNA are faced. The mtDNA analysis is used the forensic context for the identification purpose of an individual but it is not sufficient to provide the complete result. So, nuclear short tandem repeat (STR) loci are used along with mtDNA analysis. In mtDNA, the circular structure DNA protect it from vulnerability and not degraded easily. The mtDNA also present in multiple copies of the cell.

In the history, the molecular genetic identification from 50 years old bones of pilot James B. McGovern called as a good example of the analysis because the discriminatory power of mtDNA data are limited by the presence of common mtDNA control region type and this occur after the examination of Y-chromosomal STR loci and autosomal STR loci which have the likelihood ratio (LR) that supported the premise as the bone belongs to an individual who relates to the family references. The success of solving the mystery depends up on the quality and quantity of the isolated DNA and what method was used to obtain the DNA from human remains.

In case of absence of any close relative, more distant relatives can be helpful to identify an individual or victim in mass graves because the gathering of genetic markers may give the satisfactory probabilities of identity. In the identification of victims or soldiers during and after Second World War (1945), the molecular genetic methods were used. There are many different genetic markers were used in the studies because of the usage of different DNA preservatives by which the chemical properties and the climatic affected **(Pajnic, Pogorelc and Balazic, 2010; Bajzelj and Zupanic, 2017)**.

There are four phases in DNA analysis used for the identification of missing person or from human skeletal remains.

1. Collection of samples that contain DNA material from human remains i.e., Bones

2. Collection of references samples for comparison purpose (Relative DNA, personal belonging etc.)

3. Analysis of DNA of post mortem remains and reference samples containing may steps such as extraction of DNA, determination, quantification, amplification and genetic profiling.

4. Comparison of genetic outcomes from human remains with the reference samples **(Bajzelj and Zupanic, 2017)**.

Study of Different Genetic Markers

There are many genetic markers which are used to identify the individuals because they remain unchanged throughout the life. In these genetic markers, autosomal STRs, Y-STRs, and mtDNA are analyzed after the extraction of DNA from human remains. These genetic markers provide the valuable information about an individuals or victims of disaster, accident and also include the ancient human remains.

- **Study of Nuclear DNA or Autosomal STRs**

Approximately six billion base pairs per somatic cell are comprised in nuclear genome. Inside the cell nucleus, the nuclear DNA is packed into the chromosomes. 46 chromosomes are present in the human somatic cells, inherited from parents, 23 from mother and 23 from father. A set of autosomal short tandem repeat loci is used in the forensic analysis. STRs are non-coding DNA which have tandem repeats about 2-6 nucleotides long. These STRs are polymorphic in nature, so their repeats vary from person to person. For the forensic analysis, 13 STRs loci are used for DNA profiling at the time of Federal Bureau of Investigation (FBI). These 13 STRs loci introduce the foundation of Combined DNA Index System (CODIS) National Database in 1998. These 13 STRs loci are placed on autosomal chromosome as 2, 3, 4, 5, 7, 8, 11, 12, 13, 16, 18, and 21.

Various commercial kits are available for the PCR amplification of STRs loci to produce DNAA profile. While the ability of 13 STR loci is to produce the random match probabilities as on in trillion individuals in the population. Different kits use different number of STR loci for example AmpFlSTR Identifier Plus PCR Amplification kit works on 15 STRs loci, while Applied Biosystems GlobalFiler PCR Amplification kit works on 21 STR loci. The extra loci help to push the random match probabilities into that ranges where many individuals cannot understand.

The case where the DNA is degraded due to which the STR amplicon size is reduced and not able to produce a DNA profile. In such condition, single nucleotide polymorphisms (SNPs) with amplicons ranging from 60-80 base pairs may be used. During the human identification from skeletonized remains or disaster victims, DNA material is fragmented almost and SNPs can provide more information rather than STRs. SNPs also provide information regarding the geographic ancestry of the individual. These SNPs markers are called ancestry informative markers (AIMs) **(Latham and Miller, 2019).**

- **Study of Y-chromosome Microsatellites**

Along with the STRs autosomal microsatellites, the sex chromosomes can be used in the identification of DNA. Y-chromosome has smaller size compare to the autosomes and X-chromosome, also contain some microsatellites. It is second smallest human chromosome which contain s 60 million base pairs. It does not show recombination because of that Y-chromosome microsatellite markers are used in tracing the parental lineages. All individual from same father have same Y-chromosome haplotypes, a DNA fragment which is inherited from one parent only. Due to Y-chromosome analysis, it is possible to identify the missing person and human remains by comparing the sample with the reference sample of relatives and family on the father's side. This technique has a drawback as itcan not be used in the identification of female victim **(Bajzelj and Zupanic, 2017).**

- **Study of Mitochondrial DNA (mtDNA)**

Mitochondria is an organelles of the cell that contains number of copies of DNA called Mitochondrial DNA (mtDNA) or extrachromosomal genome. It is different from nuclear DNA or nuclear genome. In 1963, Margit Nass and Sylvan Nass identified and isolated the mitochondrial DNA (mtDNA) for the first time. mtDNA is histone free double stranded DNA in circular form containing 16569 base pairs with 10^7 Daltons **(Amorim, Fernandes and Taveira, 2019)**. Hundreds to thousands of mitochondria is present in each cell. Because of this, old bones, single hair shaft, and charred remains from which the nuclear DNA analysis is not possible, provide the enough and intact mtDNA for analysis. Most of variation in mtDNA is present in non-coding region, called hypervariable regions I and II (HVI and HVII). So, the identification of human remains is based on these two regions.

The reference sample and evidence sample (victim's sample) are compared for the mtDNA sequences. When the sequences of both samples are unequivocally different, the conclusion is referred to as excluded means the origin of both sample are from different source. If the sequence of the mtDNA is same of both sample, the conclusion is not excluded and called as derived from same source (maternal lineage). In the same manner, sample cannot be excluded if one sample heteroplasmic and another is homoplasmic but both samples share one mtDNA species **(Amorim, Fernandes and Taveira, 2019)**.

Sex Determination from Molecular Genetics

Pelvic bone and their defined metric criteria are used to estimate morphological sex of an individual.

There is another method to determine the sex, called molecular genetic sex-typing which is based on the DNA extraction through the use of Genderplex PCR system. This system work co-amplified two areas of amelogenin gene (AMELX/Y); sex determining area on Y-chromosome (SRY) and four short tandem repeat (STR) loci, DXS6803, DXS8378, DXS7424 and GATA172D05. The samples from males show the SRY peak while in case of females, the result is shown in multiple heterozygous X-STR genotypes **(Bauer et al, 2013)**.

The isolation and amplification of gene or genes of sex chromosomes (X and Y) are done for the genetic sex identification. The determination of sex through the genes through the region Y (SRY locus), amelogenin (AMEL) genes, and zinc finger protein (ZF). The sex determination of teeth from the ZF gene is a new method presented by Pillay and Kramer. This ZF gene is located don both sex chromosome and co-amplified in one PCR reaction. The extraction method of DNA is through the phenol-chloroform extraction. While due to the PCR inhibition, this method is not suitable for archaeological, forensic and ancient sample.

In molecular sex-typing system, AMEL gene has been used in many studies for the identification from the skeletal material. Because of the structure and properties of this gene, it is considered as a good gene for sex determination from the highly fragmented, burnt bones, juvenile and foetal remains in where the traditional methods are not preferable for sex estimation **(Bidmos, Gibbon and Strkalj, 2010)**.

Review of Literature

Iwamura, Vieira and Munoz (2004), stated that the qualitative and quantitative difference are occurred in the human remains because of the environmental factors and storage condition. Due to storage, quantity is not affect, only quality does. In the environmental factors like low temperature, low humidity and microorganism's absence are in favor of DNA preservation. According to them, the detection of DNA polymorphism of human bones has not gained the good success. In their research, they analyzed the difficulty in reproducing the outcomes, as well they found the inconsistency with the result of DNA analysis from human bones.

Ambers et al, (2016) studied the human skeleton or their remains at the site of Deadwood through the massively parallel sequencing (MPS) and tried to identify the individuals. At that site the individuals were buried in unmarked graves and no investigative were taken place before that on their identity. They conducted the Y-chromosome and mitochondrial DNA profiles on these remains. After the analysis, they concluded that the human remains belong to the European background. They also applied the SNPs technique by which they identified the hair color and eye color that was red hair and brown eyes.

Amer, et.al (2017), discussed about the advanced techniques of DNA extraction and purification from bones. From old skeletal remains, STRs (Short Tandem Repeats) typing technique has been used to extract DNA and analysis of mitochondrial DNA for species and individual identification. In this paper, collected the 36 bone samples from human remains and extracted the DNA using organic method after the preparations of special sample and quantified the sample using RT-PCR (Real-Time Polymerase Chain Reaction). By using the PCR Amplification Kit plus Identifiler, PCR technique is done.

Cunero, et.al (2012), in this paper, discussed about the forensic DNA typing method through which genetic data was obtained from an individual and a variety of materials. Through this method, also identity the missing persons and develop investigative leads to assistance law enforcement. In this paper, collected the DNA databases from different sources such as forensic evidence, direct and family reference sample, convicted felons and human remains of human remains. Also discussed about that how the governmental, scientific and private communities worked together on genetic markers for making the effective database.

Bajzelj and Pajnic (2017), proposed the technique to identify the missing person through genetic markers. They compared the post mortem remains and reference samples of missing persons and concluded that genetic identification is the modern technique and complements to the other forensic and anthropological techniques. This method provides the most accurate and reliable results and also allows to analyze unrecognized and heavy degraded, skeletonized and fragmented human remains.

Conclusion

Molecular genetic identification is a main technique of forensic science as it helps to catch the suspect or criminal by comparing the suspect sample with the crime scene sample. Along with the suspect identification, the molecular genetic identification technique is used in the identification of missing person, disaster victims and ancient people form their human remains. This papers discussed about different genetic markers and on the basis of review, it is concluded that these techniques can work individually to identify an individual. As Y-chromosome gives an idea of the parental lineage and in the same manner, the mtDNA provides the meternal lineage. STR loci (nuclear DNA) are very vulnerable due to which the DNA is damaged in that case, SNP and mtDNA provide the information about an individual. In spite of having the vulnerable property, STR is most important than other as it gives the direct linkage with an individual.

References:

Alvarez-Cubero, M. J., et al. "Genetic Identification of Missing Persons: DNA Analysis of Human Remains and Compromised Samples." *Pathobiology*, 21 June 2012, pp. 228–238.

Ambers, Angie D., et al. "More Comprehensive Forensic Genetic Marker Analyses for Accurate Human Remains Identification Using Massively Parallel DNA Sequencing." *BMC Genomics*, vol. 17, 2016, pp. 21–30.

Amer, Sayed AM, et al. "Identification of Human Bone Remains by Autosomal STRs and Mitochondrial DNA SNPs." *Journal of Hard Tissue Biology*, vol. 26, no. 2, 2017, pp. 111–118.

Amorim, António, et al. "Mitochondrial DNA in Human Identification: a Review." *PeerJ*, 2019, doi:10.7287/peerj.preprints.27500v1.

Bajzelj, Matija, and Irena Zupanic Pajnic. "Missing Persons Genetic Identification." Zdrav Vestn, vol. 86, 2017, pp. 18–29.

Bauer, Christiane Maria, et al. "Comparison of Morphological and Molecular Genetic Sex-Typing on Mediaeval Human Skeletal Remains." *Forensic Science International: Genetics*, vol. 7, no. 6, 2013, pp. 581–586., doi:10.1016/j.fsigen.2013.05.005.

Bidmos, Mubarak A., et al. "Recent Advances in Sex Identification of Human Skeletal Remains in South Africa." *South African Journal of Science*, vol. 106, no. 11/12, 2010, doi:10.4102/sajs.v106i11/12.238.

Davison, Sean, et al. "Molecular Genetic Identification of Skeletal Remains of Apartheid Activists in South Africa." *African Journal of Biotechnology*. vol. 7, no. 25, 29 Dec. 2008, pp. 4750–4757.

Iwamura, Edna Sadayo Miazato, et al. "Human Identification and Analysis of DNA in Bones." *Rev Hosp Clin Fac Med Sao Paulo*, vol. 56, 2004, pp. 383–388.

Latham, Krista E., and Jessica J. Miller. "DNA Recovery and Analysis from Skeletal Material in Modern Forensic Contexts." *Taylor and Francis*, vol. 4, no. 1, 2019, pp. 51–59.

Pajnic, Irena Zupanic, et al. "Molecular Genetic Identification of Skeletal Remains from the Second World War Konfin i Mass Grave in Solvenia." *International Journal of Legal Medicine*, vol. 124, 2010, pp. 307–317.