

Role of deoxyribonucleic acid technology in forensic dentistry

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Introduction

Forensic dentistry plays an important role in human identification in mass disasters, especially in fires, explosions, decomposing bodies or skeletonized bodies, when there is little material remaining to perform visual identification. It has led dentists working with forensic investigation teams to become more familiar with the new molecular biology technologies.^[1]

Disaster victim identification traditionally relies on the efforts where ante-mortem information from the missing persons are compared with the postmortem data of the dead persons.^[2]

If ante-mortem data is unavailable then the exact identification becomes difficult and only the DNA profiling

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Abstract

In the last few years, Deoxyribonucleic Acid (DNA) analysis methods have been applied to forensic cases. Forensic dental record comparison has been used for human identification in cases where destruction of bodily tissues or prolonged exposure to the environment has made other means of identification impractical, that is, after fire exposure or mass disaster. Teeth play an important role in identification and criminology, due to their unique characteristics and relatively high degree of physical and chemical resistance. The use of a DNA profile test in forensic dentistry offers a new perspective in human identification. The DNA is responsible for storing all the genetic material and is unique to each individual. The currently available DNA tests have high reliability and are accepted as legal proofs in courts. This article gives an overview of the evolution of DNA technology in the last few years, highlighting its importance in cases of forensic investigation.

Key words: DNA profiling, forensic dentistry, human identification, teeth

systems can reveal the exact identity of a person. Matching of the DNA extracted from the teeth of an unidentified individual with DNA isolated from known ante-mortem samples, such as, stored blood, tooth brush, hairbrush, clothing, cervical smear, biopsy, to a parent or sibling is the usual procedure in DNA analysis.^[3]

This article presents a literature review on DNA analysis for human identification, and makes an overview of the evolution of this technology, highlighting the importance of molecular biology in cases of forensic investigation.

Methodology

For this review, articles were identified by searches on electronic databases such as the Pubmed database and EMBASE, from 1985 through June 2011. The following search terms were used: 'DNA finger printing in forensic dentistry', 'Teeth and DNA analysis', 'Dental pulp and DNA analysis', 'DNA isolation and amplification methods', 'Forensic DNA Typing'.

Background

Human beings can be identified by examination of DNA sequences. Every cell of an individual carries a copy of

the DNA. Every human being is characterized based on the unique DNA sequence, due to hypervariable regions of DNA, which are specific for an individual. The order of the base pairs (bp) in the DNA of every individual is different except in identical twins. The uniqueness is due to the intron regions of the DNA, which contain sequences that are 20 – 100 bp in length, and are repeated at different locations (loci) along the chromosome, like AGACTAGACATT – AGATTAGGCATT, which are called sequence polymorphisms.^[4] The length polymorphism like (AATG) (AATG) (two repeats) and (AATG) (AATG) (AATG) (three repeats) are termed as 'Short tandem repeats' (STRs), which are used in forensic identification.^[4,5]

Jeffreys *et al.*,^[6] in 1985, created radioactive molecular probes that could recognize highly variable regions of the DNA (minisatellites in the human genome) and thus determine the specific patterns of each individual. These hypervariable loci were constituted by tandem repeat of oligonucleotides sequences (from 2 to 80 bp). Depending on their size, these loci were nominated as variable number of tandem repeats (VNTR) or minisatellites, 9 to 80 pb, and STR (short tandem repeats) or microsatellites, 2 to 7 bp.^[1]

The most common forensic method to characterize VNTRs was first analyzed using Southern hybridization. STR analysis was performed by extracting nuclear DNA from the cells of interest. The DNA was amplified using the polymerase chain reaction (PCR) and tested by gel electrophoresis or capillary electrophoresis.

These repeated sequences are named DNA fingerprints or DNA typing (profiling) as it is now known. DNA profiling is a standard forensic DNA system used in human identification, criminal case work, as well as paternity testing, worldwide.^[7]

Initially, the forensic community used VNTR testing; but this method required a large amount of material and had low quality results, especially when only small amounts of biological material samples were available.

Currently, in most forensic samples, the study of DNA is usually performed by STR analysis. The most valuable STRs for human identification are those that present a greater polymorphism (greater number of alleles), smaller size (in base pairs), higher frequency of heterozygotes (higher than 90%) and a low frequency of mutations.

However, STR mutation can be due to various mechanisms like polymerase slippage, primer mismatch or a deletion of the FGA locus.^[8]

Deoxyribonucleic Acid and Forensic Dentistry

Due to the resistant nature of dental tissues to environmental assaults, such as, incineration, immersion, trauma,

mutilation, decomposition, and microbial action, teeth represent an excellent source of DNA material.

In the tooth, dentin and pulp are rich sources of DNA, which can be successfully extracted.^[9] The total production of genomic DNA obtained from the fresh dental sample may range from 6 to 50 µg DNA.^[10] Sweet stated that the PCR method enables differentiation of one individual from another, with a high level of reliability, and with about 1 ng (one billionth of a gram) of the target DNA.^[11] Thus, abundance of quality DNA can be extracted from a tooth, which is an important advantage in DNA analysis.^[12]

Deoxyribonucleic acid is preserved in the teeth and bones for a very long period and thus is a valuable source of information. Ancient DNA (aDNA) analysis can be carried out in samples that are hundreds to tens of thousands of years old.^[13]

Sampling of the Tooth and Deoxyribonucleic Acid Extraction

Efficient DNA extraction procedures as well as accurate DNA quantification methods are critical steps involved in the process of successful DNA analysis of such samples.

Different methods have been reported regarding the extraction of DNA from the tooth, which includes sectioning of teeth horizontally at the cemento-enamel junction or vertically up to the root tip, scraping, and aspiration.^[14]

Other methods include crushing of the teeth or cryogenic grinding or conventional access cavity preparation and retrieval of dental pulp. The advantages of the access cavity preparation technique are, its simplicity, relatively low cost, and preservation of tooth integrity, which can be considered in forensic investigations.^[15]

A small amount of DNA material extracted from the teeth in most situations is always under high risk of contamination due to environmental factors, which may also include PCR inhibitors. Environmental factors leading to the degradation of DNA include time, temperature, humidity (facilitating the growth of microorganisms), light (both sunlight and ultraviolet (UV) light) and exposure to various chemical substances.^[16]

The DNA extraction process comprises of three different stages: Cell rupture or lysis, protein denaturation and inactivation, and finally the DNA extraction itself.^[5]

Accurate DNA quantification methods are critical for a successful DNA analysis of the samples. The techniques of DNA extraction most often employed in Forensic Dentistry are the organic method (composed of phenol–chloroform, which is used for high molecular weight DNA, and is

laborious and time consuming, with a higher likelihood of errors, given the use of multiple tubes, and can only be done if abundance of the sample is available; FTA Paper (composed of absorbent cellulose paper with chemical substances, which speed up its use); isopropyl alcohol (containing ammonium and isopropanol, which is less expensive and also an alternative to the organic method); and^[1] Chelex 100 (the fastest; simple with the lowest risk of contamination, yet very expensive) are used to extract DNA from samples to be used with PCR. Chelax 100 based DNA extraction, amplification, and typing are possible in incinerated teeth; Commercial DNA extraction kits are available to facilitate the reversible binding of DNA with the magnetic particles, resulting in a high DNA recovery from the samples; Silica-based methods are suitable for extraction DNA from ancient bones and teeth (aDNA). This method is better for nuclear STR typing from degraded samples than the phenol–chloroform method. Silica based DNA extracts using ion exchange columns considerably improve PCR amplification and can be useful in poorly preserved, PCR resistant, ancient samples.^[4]

Types of Deoxyribonucleic Acid

Genomic and mitochondrial are two types of DNA that are used in forensic sciences. The genomic DNA is found in the nucleus of each cell in the human body and represents a DNA source for most forensic applications. The teeth are an excellent source of genomic DNA.

Mitochondrial Deoxyribonucleic acid is another type of material that can be used when the extracted DNA samples are too small or degraded, such as those obtained from skeletonized tissues. The likelihood of obtaining a DNA profile from mtDNA is higher than that with any marker found in a genomic DNA.^[17] Various biological samples such as hair, bones, and teeth that lack nucleated cellular material can be analyzed with mtDNA and it is very useful.

Applications of Deoxyribonucleic Acid Profiling in Forensic Dentistry

The currently performed DNA profile tests are totally reliable and give details about an individual's physical characteristics, ethnicity, place of origin, and sex. These tests are also accepted as legal proofs in courts, for investigation of paternity and human identification.

1. Restriction Fragment Length Polymorphism (RFLP) it is used for analyzing the variable lengths of the DNA fragments that result from digesting a DNA sample with a special kind of restriction enzyme called 'restriction endonuclease,' which sections DNA at a specific sequence pattern known as a restriction endonuclease recognition site. It may be difficult in samples degraded by environmental factors and also takes a longer time to get the results.^[18]

2. Short tandem repeat typing. It is described as a short stretch of DNA that is repeated at various locations throughout the human genome and this technology is used to evaluate the specific regions (loci) within the nuclear DNA (m). Each person has some STRs that are inherited from the father and some from the mother, however, no person has STRs that are identical to those of either parent. The uniqueness of an individual's STRs provides the scientific marker of identity, and hence, is helpful in forensic identification and paternity testing.^[19]

A short tandem repeat can be used for the identification of bodies in mass disasters and old skeletal remains.^[20] Even though the DNA present in the ancient remains appear to be very degraded, it is conserved better in the tooth than in the bone samples.^[21] The highest success rates for human identification using STR analysis were observed in samples from the dense cortical bone of the weight bearing leg bones (femur 86.9%), and intact teeth also exhibited high success rates (teeth 82.7%).^[22] On the basis of STR, the Combined DNA Index System (CODIS) was established by the Federal Bureau of Investigation (FBI).^[23] It was developed specifically for enabling the public forensic DNA laboratories to create searchable DNA databases of authorized DNA profiles. The odd chance that two individuals will have the same 13-loci DNA profile is about one in a billion. The United States maintains the largest DNA database in the world. The British data base for STR loci identification is the UK National DNA Database (NDNAD). The British system uses 10 loci, rather than the American 13 loci.

3. At times it is difficult to perform genetic identification with nuclear DNA due to the long time interval between the time of death and examination of tissues. Usually in such cases only bone and teeth may be available for analysis. Teeth provide an excellent source for high molecular weight mtDNA, which offers several unique advantages for the identification of human remains.^[24]

Mitochondrial Deoxyribonucleic acid is a powerful tool for forensic identification as it possesses high copy number, maternal inheritance, and high degree of sequence variability. Each offspring has the same mtDNA as their mothers, as the mitochondrion of each new embryo comes from the mother's egg cell and the nuclear DNA is contributed by the father's sperm. In investigations involving missing persons, comparing the mtDNA profile of the unidentified remains with the profile of a potential maternal relative can be an important technique.^[23]

However, mtDNA analysis is a slightly time-consuming technique and is exclusively matrilineal, and hence, less informative. Thus, this analysis is not usual in all forensic laboratories directed at resolution of crimes and identification of persons.

4. Y-Chromosome analysis

Deoxyribonucleic acid polymorphisms on the human Y chromosome are valuable tools for understanding human evolution, migration, and for tracing relationships among males.^[25] The majority of the length of the human Y chromosome is inherited as a single block in linkage from father to male offspring, as a haploid entity. Hence, Y chromosomal DNA variation has been mainly used for investigations on human evolution and for forensic purposes or paternity analysis.^[26]

Y-chromosome STR (Y-STR) polymorphisms are used in deficiency paternity testing, cases of physical assault, murders, sexual assault, and child abuse, where bite marks are frequently found on the skin.

5. X-Chromosome short tandem repeat

The chromosome X-specific STR is used for the identification and genomic studies of various ethnic groups in the world.^[27] As the size of the X-chromosome STR alleles is small, generally including 100 – 350 nucleotides, it is relatively easy to be amplified and is detected with high sensitivity.^[28] X-chromosome STR (X-STR) markers form a powerful complementary system, especially in deficiency paternity testing.

6. Single nucleotide polymorphism

Single nucleotide polymorphisms (SNPs) are DNA sequence variations that occur when a single nucleotide (A, T, C or G) in the genome sequence is altered. For example an SNP might change the DNA sequence AAGGCTAA to ATGGCTAA.^[29]

Single nucleotide polymorphisms have emerged as markers of interest to Forensic Medicine because of their small amplicon size, which is useful in analyzing degraded samples, lower mutation rate compared to STRs, amenable to high throughput analysis (automation), abundant in the human genome, can provide specific information about ancestry, lineage, evolution, identity or phenotype, and also determine sex.

Limitations of SNPs include, no widely established core loci and requirement of large multiplexing assays. Efforts are being made to investigate whether it can replace STR, nevertheless SNPs are the DNA technology of the future.

7. Gender typing

The enamel proteins that are required for the development of normal tooth enamel are encoded by the amelogenin genes (they are part of a small group of genes that are active on both sex chromosomes).^[30]

The amelogenin gene is a single copy gene, homologs of which are located on Xp22.1 – Xp22.3 and Yp 11.2.^[31] The variation of length in the X-Y homologous amelogenin gene (AMELX and AMELY), are used for gender identification.^[32] The gender may also be identified from the dental pulp DNA through the analysis of the peaks of X and Y loci by capillary gel electrophoresis.^[33,34]

Conclusion

Applied aspects of DNA technology have revolutionized forensic identification procedures. Teeth represent an excellent source of DNA, and are protected by epithelial, connective, muscular and bone tissues in case of incineration. Additionally, the dental pulp cells are protected by enamel, dentin and cementum hard dental tissues. Therefore, dental professionals working in the field of Forensic Dentistry should incorporate these new technologies in their studies, as several methods are available for DNA extraction from biological materials; yet standardization of the protocols adopted for such purposes has not been reached so far. Nevertheless, the field is developing at a fast pace, to reach new frontiers and solve many riddles hidden in the human genome.

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