DNA Profiling in Forensic Dentistry

Abstract

In last few years, DNA analysis methods are 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, i.e., 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 DNA profile test in forensic dentistry offers a new perspective in human identification. 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: Forensic dentistry; DNA profiling; Teeth; Human identification

Introduction: The established importance of Forensic Dentistry for human identification in mass disaster, mainly when there is little remaining material to perform visual identification (e.g. in fires, explosions, decomposing bodies or skeletonized bodies), has led dentists working with forensic investigation to become more familiar with the new molecular biology technologies. Disaster victim identification traditionally relies on the efforts where ante mortem information from the missing persons is compared with post mortem data of the dead persons. [1] If ante mortem data is unavailable then the exact identification becomes difficult and only the DNA profiling 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. [2]

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 data bases such as Pub med database and EMBASE from 1975 through March 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:

Any type of organism can be identified by examination of DNA sequences unique to that species. Every cell of an individual carries a copy of the DNA. Order of base pairs (bp) in the DNA of every individual is different except identical twins. The uniqueness is due to the intron regions of DNA which contain sequences that are 20 - 100 bp in length that are repeated at different locations (loci) along the chromosome like AGACTAGACATT—AGATTAGGCATT which are called sequence polymorphism.

recognize highly variable regions of DNA (minisatellites in human genome) and thus determine the specific patterns of each individual. These hyper variable loci were constituted by tandem repeat of oligonucleotides sequences (from 2 to 80 bp). Depending on their size, these loci were nominated as VNTR (variable number of tandem repeat) or minisatellites, 9 to 80 pb, and STR (short tandem repeats) or microsatellites, 2 to 7 bp.

These repeated sequences were 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. [4]

Initially, the forensic community used VNTR testing; but this method requires a large amount of material and has low quality results, especially when only little biological material samples are 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 greater polymorphism (greater number of alleles), smaller size (in base pairs), higher frequency of heterozygotes (higher than 90%) and low frequency of mutations.

**DNA 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. [5] Total production of genomic DNA obtained from dental sample may range from 6 μg to 50 μg DNA. [6] Sweet stated that the PCR (polymer chain reaction) method enables differentiation of an individual from another, with a high level of reliability and with about 1 ng (one one-billionth of a gram) of the target DNA. [7] Thus, abundance of quality DNA can be extracted from a tooth which is an important advantage in DNA analysis. [8]

DNA is preserved in the teeth and bones for a very long period and thus are 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. [9]

**Sampling of the Tooth and DNA 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.

Various 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 root tip, scraping and aspiration.

Other methods include crushing of the teeth or cryogenic grinding or conventional access cavity preparation and retrieval of dental pulp. The advantages of access cavity preparation technique are its simplicity, relatively low cost and preservation of the tooth integrity which can be considered in forensic investigations. [10]

Researchers must carefully evaluate the conditions of the material to be examined, in which there is a greater risk of sample contamination and influence of environmental factors, in addition to a small amount of DNA material available in most situations, 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 UV light) and exposure to various chemical substances. [11]

DNA extraction process is composed of 3 different stages: cell rupture or lysis (which allows use of several techniques for effective rupture of the cell membranes), protein denaturation and inactivation (by chelating agents and proteinases in order to inactive elements, such as proteins), and finally DNA extraction itself. [12]

The techniques of DNA extraction most often employed in Forensic Dentistry are the organic method (composed of phenol-chloroform and used for high molecular weight DNA, laborious, time consuming, with a higher likelihood of errors, given the use of multiple tubes and can only be done if abundance of sample is available); Chelex 100 (the fastest with the lowest risk of contamination, yet very expensive); FTA Paper (composed of absorbent cellulose paper with chemical substances, which speed up its use); and isopropyl alcohol (containing ammonium and isopropanol, which is less expensive and also an alternative to the organic method).

**Types of DNA:**

Genomic and mitochondrial are two types of DNA which 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 DNA (mtDNA) 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 genomic DNA. [13] Various biological samples such as hair, bones, and teeth that lack nucleated cellular material can be analyzed with mtDNA and is very useful.

Applications of DNA 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, such as for investigation of paternity and human identification.

1. Restriction Fragment Length Polymorphism (RFLP) Typing:

It is used for analyzing the variable lengths of 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. RFLP requires relatively large amounts of DNA. Hence, cannot be performed with the samples degraded by environmental factors and also takes longer time to get the results. [14]

2. STRs Typing:

These are described as short stretches of DNA that are repeated at various locations throughout the human genome and this technology is used to evaluate specific regions (loci) within nuclear DNA. Each person has some STRs that were inherited from father and some from mother but 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. [15]

STR can be used for identification of bodies in the mass disasters and old skeletal remains. [16] Even though the DNA present in the ancient remains appeared much degraded, it was better conserved in tooth than in bone samples.[17] Highest success rates for human identification using STR analysis were observed with samples from dense cortical bone of weight-bearing leg bones (femur 86.9%) and intact teeth also exhibited high success rates (teeth 82.7%). [18] Based on STR, Combined DNA Index System CODIS was established by the Federal Bureau of Investigation (FBI).

It was developed specifically for enabling public forensic DNA laboratories to create searchable DNA databases of authorized DNA profiles. The odd 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.

3. Mitochondrial DNA (mtDNA) Analysis:

Long intervals between the time of death and examination of tissues complicate the genetic identification with nuclear DNA and sometimes only bone and teeth may be available for analysis. Teeth provide an excellent source for high molecular weight mtDNA that offer several unique advantages for the identification of human remains. [19]

mtDNA is a powerful tool for forensic identification as it possesses high copy number, maternal inheritance, and high degree of sequence variability. Each offspring have the same mtDNA as their mothers since the mitochondrion of each new embryo comes from the mother’s egg cell and the nuclear DNA is contributed by father’s sperm. In investigations involving missing persons, comparing the mtDNA profile of unidentified remains with the profile of a potential maternal relative can be an important technique. [20] However, mtDNA analysis is a very expensive 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:

DNA-polymorphisms on the human Y chromosome are valuable tools for understanding human evolution, migration and for tracing relationships among males. [21] 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. [22]

5. X-Chromosome STR:

Chromosome X specific STR is used in the identification and the genomic studies of various ethnic groups in the World. [23] Since the size of X-chromosome STR alleles is small, generally including 100-350 nucleotides, it is relatively easy to be amplified and detected with high sensitivity. [24] X-chromosome STR (X-
STR) markers are a powerful complimentary system especially in deficiency paternity testing.

6. Single Nucleotide Polymorphism (SNPs):

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. [25] SNPs are emerging as new markers of interest to the forensic medicine because of their small amplicon size which is useful in analyzing degraded samples, lower mutation rate compared with 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 such as no widely established core loci, and requirement of large multiplexing assays.

Gender Typing:

The enamel proteins that is required for the development of normal tooth enamel is encoded by the amelogenin genes. The amelogenin gene is a single copy gene, homologues of which are located on Xp22.1-Xp22.3 and Yp 11.2. [26] The variation of length in the X-Y homologous amelogenin gene (AMELX and AMELY), are used for gender identification. [27] Dental pulp is a valuable source of DNA for sex determination. Komuro T et al have identified the sex from the dental pulp source of DNA for sex determination. Komuro T et al.

Conclusion:

The application of DNA technology has revolutionized forensic identification procedures. Teeth represent an excellent source of DNA, which is 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 on the field of Forensic Dentistry should incorporate these new technologies in their work, as several methods are available for DNA extraction from biological materials, yet standardization of the protocols adopted for such purpose has not been reached so far. Nevertheless, the field is developing at fast pace to reach new frontiers and solve many riddles hidden in the human genome.

References:

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