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The Role of DNA in Forensic Odontology (Part I) DNA Analysis Methods

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Abstract: During the last years, DNA analysis methods are applied to forensic cases. Also, 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, aircraft inflammation or mass disasters. Dental DNA represent an excellent source of genomic DNA. The interest in using dental tissues as a DNA-source of individual identification falls within the particular character of resistance of this organ towards physical or chemical exterior aggressions. DNA can be used for determination of the found remains identity. The identification of individuals is not the only use for DNA. The technique has allowed criminal investigators to link victims to crime scenes once the body has been removed and incinerated. Therefore, it is prudent for the forensic odontologist to become familiar with the DNA analysis methods. The purpose of the Part I of this report is to review of the DNA structure and explain of some common terms which are used for the description of current methods of DNA analysis. Furthermore, the importance of mitochondrial DNA is reported because of its difference from the nuclear or chromosomal DNA in a number of ways that make it an attractive alternative for forensic analysis.

Key words: Dental DNA, DNA analysis, forensic odontology, bite marks, salivary DNA, human identification

INTRODUCTION

Forensic odontology is a specialty of dentistry recognized in a few countries (USA, China, Scandinavian countries) and is defined as the proper handling, examination and evaluation of dental evidence which will be then presented in the interest of justice by using dental records or ante-mortem photographs. Purely it is the overlap between the dental and the legal professions. It derives from the latin word forum which is the place where legal matters are discussed.

The first forensic dentist was Paul Revere (USA) who was occupied with the identification of fallen revolutionary soldiers (Cottone, 1982; Stavrianos, 2009). Forensic dentistry is occupied with a wide range of fields including:

- Identification of the living or the deceased which is the most common role of forensic odontology
- Identification, analysis and comparison of bitemark
- Identification, analysis and comparison of lip and rugae print and patterned injury
- Identification of dental specimens at crime scene or elsewhere for instance in mass fatalities
- Evaluation of oro-facial trauma
- Malpractice and negligence claims
- Age estimation (Stavrianos, 2009; Spencer, 2004)

The maxillofacial complex and all forensic evidence derived from its structures are within the purview of the forensic odontologist to evaluate (human identification) (Smith *et al*., 1995). Traditionally, 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 (Stavrianos, 2009) or aircraft inflammation (Sweet and Sweet, 1995).

DNA contained in teeth, oral tissues and saliva can be extracted and typed (Smith *et al*., 1995). Therefore, it is prudent for the forensic odontologist to become familiar with the fundamentals for obtaining and analyzing DNA from the oral tissues. The following review of DNA structure and an explanation of some common terms associated with DNA analysis are presented to prepare the reader for the description of current methods of DNA analysis. The potential role of the odontologist is managing DNA evidence and a brief review of some innovative research conducted with salivary DNA (Sweet and Shutler, 1999) dental and bite mark material is

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also included for review. A list of cases involving the typing of DNA from oral sources is intended to provide relevant examples in which the odontologist played an important role in the analysis of DNA (Smith *et al*., 1995).

DNA (Chemical composition): The three fundamental macromolecules in cells are polysaccharides, proteins and nuclein acids. Polysaccharides are composed of linked sugars and their role is to serve energy when it is needed. Proteins are made up of various combinations of amino acids and are involved in structural, regulatory and enzymatic roles. The genetic recipe is provided by the two nucleic acids: Deoxyribonucleic Acid (DNA) and Ribonucleic Acid (RNA) (Smith *et al*., 1995).

Genomic DNA is a polymer (s) composed of four different nucleotides (thymine, cytosine, adenine and guanine) and whose polymorphic sequence accounts for the genetic diversity of all living things. It is found in the nucleus of each cell and represents the DNA source for most forensic applications (there are no nuclei and hence there is no DNA in red blood cells) (Pretty and Sweet, 2001). Each of the four nucleotides constituting DNA consists of a sugar (deoxyribose), a phosphate group and a nitrogen-containing base (Smith *et al*., 1995).

The nucleotides form a continuous strand with the adjacent sugars bound by the intervening phosphate group (Smith *et al*., 1995). The phosphodiester linkage always occurs between the 3 carbon of 1 sugar and the 5 carbon of the adjacent nucleotide giving the molecule polarity or direction (Smith *et al*., 1995). By convention, the sequence of DNA is discussed in the 5-3 direction. In its natural form, single strands of DNA are cross linked by hydrogen bonds occurring between the nitrogen bases of paired strands (Smith *et al*., 1995). This configuration results in a railroad track of double-stranded DNA that twists to form the well recognized double helix (Smith *et al*., 1995).

The pairing of bases (Smith *et al*., 1995) between the two strands is very specific with hydrogen bonds occurring only between adenine and thymine and between guanine and cytocine. With one, strand running in the 5'-3' direction and the perfectly matched, yet opposite strand running in the 3'-5' direction, the strands are said to be anti-parallel and complementary.

Mitochondrial DNA (mtDNA): In addition to genomic DNA, cells contain mitochondrial DNA, the sequence of building blocks of which can be determined to assist in identification (Pretty and Sweet, 2001). Mitochondrial DNA (Cann *et al*., 1987) differs from nuclear or chromosomal DNA in a number of ways that make it an attractive alternative for forensic analysis. Unlike nuclear DNA, mitochondrial DNA is distributed throughout the cytoplasm of cells. It is confined to the mitochondria (or power house) of the cell which is responsible for the synthesis of Adenosine Triphosphate (ATP) (Smith *et al*., 1995). Whereas nuclear DNA is double-stranded and linear, mitochondrial DNA is double-stranded and circular (Smith *et al*., 1995). The main advantage of mtDNA is that there is a high copy number in each cell caused by the high number of mitochondria present in most cells (Pretty and Sweet, 2001).

The mtDNA contains only 16,569 base pairs that code for 2 ribosomal RNAs (rRNA), 22 transfer RNAs (tRNA) and 13 proteins (Nuclear DNA is huge by comparison with 6×109 base pairs) (Smith *et al*., 1995). The two strands of the mtDNA molecule are designated light (L-strand) and heavy (H-strand) based upon buoyant density. By convention, base pairs of the mtDNA molecule are numbered in a clockwise direction starting at its origin of replication (D loop or control region). The control region contains hypervariable segments (highly diverse) and is the area around which most forensic work has centered (Smith *et al*., 1995). The importance of mtDNA is underlying with:

- Of particular forensic significance is the hereditary character of mtDNA. Chromosomal DNA is inherited from both the mother and father whereas mtDNA is strictly maternally inherited (Hutchison *et al*., 1974). This means there is no mixing of sequence types from one generation to the next so that distant maternal relatives should have the identical mtDNA sequence (Smith *et al*., 1995). Thus when identifying a set of human remains, if close relatives are not available, distant maternal relatives can be used as a reference source to support identification. This has important implications for the identification of individuals for which there is no antemortem comparison sample (Pretty and Sweet, 2001)
- Furthermore, there are hundreds of copies of mtDNA in each cell compared to two copies of chromosomal DNA (Smith *et al*., 1995). Even cells without a nucleous, red blood cells found in whole blood or urine for example will have 3 end mitochondria (Pretty and Sweet, 2001)
- In many forensic cases, limited quantities of DNA are recovered from evidentiary material (i.e., skeletal remains and hair shafts) (Smith *et al*., 1995; Zaiats and Ivanov, 1997). Therefore, mtDNA testing may be successful when nuclear DNA testing fails or when genomic DNA cannot be analyzed, possibly because it is too degraded (Pretty and Sweet, 2001)

MATERIALS AND METHODS

Forensic scientists have a number of DNA testing methods to choose. These testing methods will reveal either sequence-specific or length-specific variations in DNA (Smith *et al*., 1995). The choice of which method or methods to use will depend on the quality and quality of the DNA removed from the evidence specimen and the reference source available at the time of analysis. Prior to testing, review of the evidence and references may dictate the type of testing to be performed. Through this type of pretesting triage, the success of the analysis and the speed of completing the testing will be greatly enhanced (Smith *et al*., 1995).

When a biological specimen (semen, blood, saliva, tooth, etc.) is recovered for forensic analysis, the DNA profile from this specimen can be directly compared to a suspect's or victim's DNA profile (Smith *et al*., 1995). The most widely used procedure to accomplish this is the RFLP method (Smith *et al*., 1995; Reynolds *et al*., 1991). The ability to differentiate between individuals using RFLP analysis was first described in 1980. The application of RFLP testing to obtain a conviction in a criminal investigation began in the USA in 1986.

RFLP (Botstein *et al*., 1980) is defined as restriction fragment length polymorphism analysis which uses endonucleases to cut the DNA at specific locations resulting in fragments of different lengths. RFLP analysis involves the following procedures:

- Removal and purification of DNA from the biological source
- Cutting of the purified DNA into relatively small fragments using restriction enzymes at specific sites (endonuleases used as biological scissors that hydrolyze double stranded DNA at specific sites, resulting in DNA fragments of various sizes)
- Separation of the fragments in an agarose gel electrophoresis according to size
- Transfer and immobilize all DNA separated fragments in the gel onto a nylon membrane
- Detection of specific DNA fragments using oligolucleotides labeled with radioisotopes (small fragments of single-stranded radioactive DNA)
- Autoradiography, in which an X-ray film is placed over the membrane for several days resulting in exposure of the film at the point of the probe (Smith *et al*., 1995)

RESULTS AND DISCUSSION

The labeled oligonucleotide will recognize one pair among the thousands of DNA fragments immobilized on the membrane (Smith *et al*., 1995). The size of these fragments (2,000-10,000 nucleotide base pairs) will vary

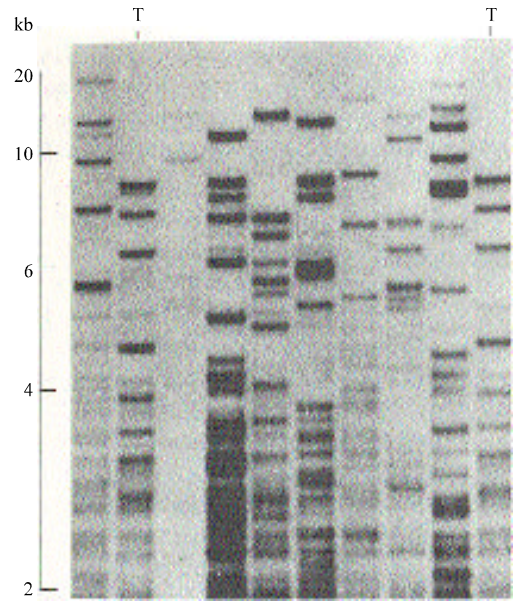

Fig. 1: Genetic fingerprints of 10 individuals (ten columns). Two of them are monozygotic. The scale shows the size of DNA-fragments in kb (Mourelatos, 1990)

from one individual to the next based on a VNTR (variable number tandem repeats are short segments of DNA which are repeated in tandem) found within each fragment (Smith *et al*., 1995; Hochmeister *et al*., 1991). By looking at a series of different VNTR locations (loci), a DNA profile is generated (Budowle *et al*., 1991). The number of VNTR locations will differ between individuals. For example, Fig. 1 shows as DNA fingerprinting technique and shows a genetic fingerprints of ten individuals, ten columns. Two of them are monozygotic (Fig. 1).

Another interesting document (Sasaki *et al*., 1997) reports of three cases of human identification by genotyping of personal studies by comparison of several Short Tandem Repeat (STR) loci in samples from these corpses in various stages of decomposition with samples from personal studies of the respective individuals. The first victim has been found dead in a burned car. The second victim was found in a forest after >5 years. In the last case the victim was found in the sea after >7 months. The researchers succeeded in identification of the three cases by comparison of several VNTR and STR loci in the corpses with those in personal studies.

A match of the suspect and evidence DNA profiles at four or more of these VNTR loci will infer identification of the perpetrator (Smith *et al*., 1995). The RFLP method can also be used to identify the parent of a child (Fig. 2) or the victim of a crime or disaster.

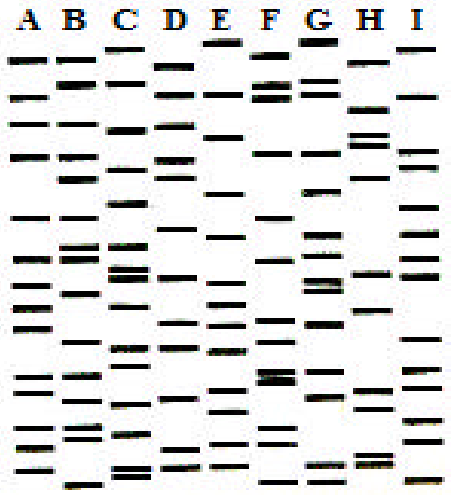

Fig. 2: Genetic fingerprints of a girl (A) of her parents and other persons, two of them are brothers without any relativity with the above family. According to the DNA analysis method, the identification of the parents of this girl (A) of her sister and of the two brothers can be easily performed. By the observation of DNA fingerprints B and E, it can be noted that they include all together the girl's DNA fingerprints. Thus, B and E are her parents (7 of the 14 zones of A are included in B and the rest 7 in E). Furthermore, it can noticed that all zones of H exists in DNA fingerprints of B and E (7 of each one), so H is daughter of B and E and sister of the girl (A). The individuals Z and I have 9 similar zones (they are brothers)

Fig. 3: Applied Biosystems, Step Ove Plus™ real time PCR system (Laboratory of Experimental Pharmacology, Medical School, Aristotle University of Thessaloniki)

Unfortunately, RFLP analysis requires a large quantities of high molecular weight DNA. DNA degradation, insufficient quantities of DNA (<100 ng) or both could result in an incomplete RFLP profile. With the development of the PCR (Fig. 3) in 1985 fragmented and minute quantities of DNA can be analyzed (Smith *et al*., 1995).

PCR (Polymerase Chain Reaction) is a laboratory technique in which a target section of DNA is duplicated (Smith *et al*., 1995). In this manner, a few copies of a specific DNA sequence can be amplified into millions of copies sufficient for analysis. During the PCR (Fig. 3), copies of a specific location (locus) are made using the same basic DNA replication machinery that a cell uses prior to mitosis. The ensuing amplification results in millions of copies of the original locus. Consequently, PCR amplification allows for analysis of DNA quantities as low as 100 pg and below. Given this elevated level sensitivity, analysis of DNA from a stamp or skeletal remains has become more common. In order to address the problems associated with DNA degradation, PCR methods have focused on smaller VNTR loci (Smith *et al*., 1995). Microsatellite and minisatellites VNTR loci ranging from 100-1500 nucleotide base pairs in length have been evaluated for forensic purpose (Smith *et al*., 1995; Misawa, 1994). The microsatellite loci or STRs are 100-350 base pairs in length with a core repeat unit of 2-5 base pairs. The minisatellite loci or LTRs range in length from 400-1500 base pairs with a core repeat unit of 16-70 base pairs (DNA-based molecular characterization of human remains caused by the explosion of the Israeli Embassy in Buenos Aires, March, 1992; Corach *et al*., 1995). The British and Canadian crime labs are moving towards using STR systems exclusively.

The evaluation of STR and LTR loci using the PCR is referred to as AmpFLP analysis, amplified fragment lenght polymorphisms (Smith *et al*., 1995). The PCR products generated during AmpFLP analysis can be separated using conventional PAGE methods (Budowle *et al*., 1991). Following or during the PAGE detection of DNA fragments can be achieved using non-isotopic (non-radioactive) methods such as silver staining and fluorescence. Discontinuous vertical PAGE and silver stain detection is being used routinely in a number of forensic laboratories.

Fluorescence-based detection methods (Smith *et al*., 1995) are an attractive alternative to conventional silver staining methods for many reasons. Multiple fluorophors allow for internal lane standards and for multiplexing many

STR or LTR systems together in the same lane (each locus is labeled with a different colored fluorophor). Fluorescently labeled DNA fragments are detected in real time using laser technologies. Thus, no further steps are required following electrophoresis to visualize the alleles (different sized fragments at the same locus).

Finally, data analysis is computerized eliminating the need for additional imaging systems for example with the applied Biosystems 373A DNA sequencer (Smith *et al*., 1995). Using either detection method, evaluation of 4-8 AmpFLP loci will provide a power of discrimination approaching that of RFLP analysis.

The PCR has also been utilized to help determine sequence specific differences at various genetic loci. For PCR based analyses, the AmplitypeO HLA DQ alpha and PolyMarker (PM) typing kits are used (Smith *et al*., 1995). Six different loci are analyzed between the two kits. These are listed in order of decreasing size:

- Human leukocyte antigen DQalpha locus for the HLA DQ alpha kit
- Low Density Lipoprotein Receptor (LDLR)
- Lycophorin A (GYPA)
- Hemoglobin G Gammaglobin (HBGG)
- D7S8
- Group specific component (GC) loci for the PM kit (Smith *et al*., 1995; Pai *et al*., 1995; Hochmeister *et al*., 1991)

Typing of all six loci is performed by incubating the PCR products with a membrane strip (one strip for HLA DQ and one for PolyMarker) (Smith *et al*., 1995). Small oligonucleotides are immobilized on to the strip. The oligonoucleotides are specific for sequence differences in the PCR product. Therefore, only PCR product which is a perfect match will bind to the membrane. Specifically bound PCR product is detected using a bound enzyme conjugate and the conversion of a colorless substrate to a colored precipitate. The combination of colored spots on the two membranes results in a genetic profile. The combined power of discrimination for these six loci is 2 or more orders of magnitude lower than RFLP or AmpFLP testing. Concluding, the AmplitypeO kits are simple, efficient and an excellent method for moderate level DNA typing (Smith *et al*., 1995).

Another method of sequence-specific DNA typing is the direct, full length sequence analysis of PCR product, i.e., the mitochondrial DNA testing (Cann *et al*., 1987; Smith *et al*., 1995). The control region of the mtDNA genome is the most polymorphic, evolving 5-10 times faster than a single copy nuclear markers. Therefore, this is the target for the majority of mtDNA analyses. Portions of the control region are amplified using the PCR and the primary sequence of the PCR product is determined. The mtDNA sequence generated from a maternal relative can then be compared directly to the sequence obtained from human remains in order ro identify the remains. Similarly, the mtDNA sequence generated from biological evidence can be compared directly to the sequence obtained from a suspect in order to imply association of the suspect to a crime. On the other hand, mtDNA sequence analysis is moderated informative. The discriminatory power of mtDNA testing is better than many single locus nuclear tests but less informative than the combined AmplitypeO kits and multi-locus RFLP or AmpFLP testing (Smith *et al*., 1995).

CONCLUSION

DNA analysis has provided a significant advance in identification efforts and is routinely used in criminal investigations, family investigations and mass disasters. The relative absence of general population DNA identification databanks against which putative specimens can be compared complicates identification efforts of unknown remains especially when there is no presumptive identification of the decedent. If a presumptive identification is available however, then it is often possible by using either nuclear or mitochondrial DNA profiling analysis to confirm the identification of the unknown. In mass disasters, a common approach is to identify fragmentary remains by traditional means such as dental comparisons, fingerprints or radiographic criteria, type recovered DNA from these fragments and use that profile to reassociate other fragmentary remains producing the same DNA profile pattern. Current DNA profiling or fingerprinting techniques are either Restriction Fragment Length Polymorphisms (RFLPs) on Variable Number Tandem Repeats (VNTRs) or Polymerase Chain Reaction (PCR) analysis.

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