Review Article

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Forensic Dentistry: Now and in Future

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ABSTRACT

Background: There are few scientific approaches to human identification that are more effective than a well-trained forensic dentist armed with a set of high-quality dental records and radiographs. Forensic science has embraced the use of DNA molecular biology tools for diagnostic purposes more than any other scientific field.by uncovering the code that exists within a biological sample, we have a quantifiable and unique basis for individualization. Because of the generational constancy of the mt DNA genome, a reference from a living individual today can be used to help identify a maternal relative missing from decades earlier. Now oral fluids have become "informative fluids" that can be used for diagnostic purpose the management of drug therapy, and a number of forensic applications. Indeed, it is incumbent on the forensic scientist to be vigilant and embrace new technologies that will benefit society by their ability to analyze more challenging samples in an effort to continue to exonerate the innocent, to enhance abilities to solve crime, and to identify missing persons. With this new technology comes an increased risk to personal privacy that actually crosses generations, as well as the fear of genetic discrimination in employment and insurance sectors.

Keywords: Forensic, molecular biology, PCR, automation, whole blood analysis.

INTRODUCTION

There are few scientific approaches to human identification that are more effective than a well trained forensic dentist armed with a set of high-quality dental records and radiographs. Fingerprinting is probably the only other technique used with greater frequency, but as we know, the soft tissue of the extremities does not resist the ravages of time and environment like the enamel and dentin of human teeth. So, in terms of rapidity, degree of certainty, cost-effectiveness, and applicability to a wide range of intact, decomposing, or skeletonized remains, forensic odontology has been the identification method of choice.[1]

Forensic science has embraced the use of DNA molecular biology tools for diagnostic purposes more than any other scientific field. The discipline has been driven by the need for high resolution human identity testing techniques. Over the past 20–25 years, forensic science has developed and implemented various robust and reliable DNA typing technologies.[2-4] Miniscule amounts of biological evidence can be individualized and the results quantified using statistics so staggering that the courts and the public have come to expect the same sort of return on all types of forensic analyses. However, the process of DNA analysis is very slow in comparison to other forensic examinations, extremely

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expensive, and with few exceptions, must be conducted in highly specialized and fixed facilities.[5]

To fully appreciate the power of discrimination offered by the DNA molecule, one must first take a brief moment to grasp its simple elegance. Although earlier investigators initially suspected that proteins were actually the carriers of hereditary information, William Thomas Astbury demonstrated in 1944 that DNA is the sole genetic material of life.[6,7] Although he received only minor recognition for his discovery, his successors became much more famous.James Watson, Francis Crick, and Maurice Wilkins received the Nobel Prize for Physiology and Medicine in 1962 for their elucidation of the structure of DNA.[8] With the understanding of the molecular structure came a series of techniques in which DNA could be manipulated, including splicing, cloning, sequencing, and even replicating the molecule. These steps led to a new phase of molecular biology called recombinant DNA technology.[9-12] Now, scientists could mimic and exploit in the laboratory some of the same changes in the DNA molecule that occur routinely in the natural environment. The impact of Astbury's discovery on forensic science was the realization that we inherit the code to produce our characteristics but, strictly speaking, not the characteristics themselves. Thus, by uncovering the code that existswithin a biological sample, we have a quantifiable and unique basis for individualization. And by focusing on the fundamental code, we remove the subjectivity that arises from analyzing the characteristics, which are the end product of the code and may be significantly impacted by unpredictable environmental forces.Because of the generational constancy of the mtDNA genome, a reference from a living individual today can be used to help identify a maternal relative missing from decades earlier.[13]

Now oral fluids have become "informative fluids" that can be used for diagnostic purpose, the management of drug therapy, and a number of forensic applications.[14] The science and technology of miniaturization (nanotechnology) now enables a full clinical laboratory to be compressed upon a miniature chip and this "lab-on-a-chip" technology is being applied to rapid and sensitive analysis using saliva as a diagnostic fluid.[15]

Rather than using restriction length polymorphism analysis by Southern blot–based hybridization methods,[16-18] scientists in the field are now routinely using PCR-based methods coupled with automated fluorescent detection technologies.[19-27]

Alteration of the Pathogenicity of Oro-Dental Infectious Agents

Researchers at the Joint Genome Institute operated by the University of California and the University of Oklahoma Health Science Center announced the completion of the sequencing of the pathogen *Streptococcus pyogenes*, the bacteria responsible for a wide variety of human ailments, including streptococcal sore throat, rheumatic fever, septicemia, and necrotizing fasciitis or flesh-eating disease.[28-29]

Complete genome sequence of an M1 strain of Streptococcus pyogenes. Proceedings of National Academy of Sciences USA, 98: 4658-63 Discovery of the specific bacterial SNPs and their products that cause dental disease will lead to the development of measures to counter or mitigate their untoward effects.[15]

Renaturalization of Dental Tissues

As genetic researchers continue to study the specific genes that control the development and maintenance of teeth and their surrounding structures, implementing proteins will be located, and their tissue-building functions defined. For example, during endodontic therapy, dentists will be able to seed genetically developed pulpal tissue into the canal to grow and fill the chamber. A layer of epithelial cells then could be triggered to form dentin and enamel to complete the biological restoration of the tooth.[15] In a report published in The New England Journal of Medicine, Tsai and colleagues presented the results of a study in which they cultured corneal epithelial cells from healthy eyes of patients with severe unilateral corneal disease and subsequently transplanted the cells to the diseased eyes to restore vision.[30]

Bioengineered or cultured tissue products to replace other tissues "indicates that such products are likely to revolutionize the treatment of many epithelial diseases"[31] and replacement of

mineralized tissues.[32] Research scientist Robert Freitas explained that nanotechnology, which involves the use of precise, molecular-sized robotic devices to control matter at the atomic and molecular level, could result in the manufacturing of instruments that are able to build and install "a biologically autologous whole-replacement tooth that includes both mineral and cellular components",[33] alteration of the germline affects the embryonic formation of the dental complex and creates disease resistance in people, who then pass this immunity on to their children and to all succeeding generations. Intercession in the dental formative process would become feasible, leading to infants who are free of caries and periodontal disease for a lifetime.[15]

PCR

The success and widespread acceptance of DNA typing in forensic science is due partly to its sensitivity of detection (by the use of PCR) and an ability to analyze minute samples.[34] Forensic samples can contain contaminants from the environment that inhibit PCR amplification.[35]

Improvements to typing low-quality samples

Mini-STRs Highly degraded samples that contain DNA fragments that are too short in length cannot be used to generate amplicons that are longer in length. All STR kits be reconfigured into mini-STR kits for routine analysis of forensic evidence.[36]

Gill et al[37] described the acceptance of three new mini-STRs (D10S1248, D14S1434, D22S1045) into the European standard Interpol loci which now comprise 10 STR loci.[37] Extremely minute quantities of DNA, with a resolving power such that, in many cases, the number of evidence-sample contributors can be reduced to a few individuals, if not just one source.[38]

Single-nucleotide polymorphisms (SNPs)

Since ~ 85% of human genomic variation is based on SNPs, there is an abundance of SNPs for human forensic identity testing.4. categorized SNPs into (*i*) identity-testing SNPs for individualization, requiring high heterozygosity and low population heterogeneity; (*ii*) lineage-informative SNPs, sets of tightly-linked SNPs that function as haplotype markers to identify missing persons through kinship analyses; (*iii*) ancestry-informative SNPs for establishing high probability of an individual's biogeographical ancestry to indirectly infer some phenotypic characteristics for investigative lead value, requiring low heterozygosity and high population heterogeneity; and (*iv*) phenotype-informative SNPs for establishing high probability that an individual has a particular phenotypic characteristic such as skin color, hair color, or eye color, for investigative lead value. A fifth class of SNPs is those for pharmocogenetic investigations for determining the cause of death.[39,40] Analysis of missing persons and unidentified human remains utilize genetic markers residing on the maternally-inherited mitochondrial genome and the paternally-inherited Y chromosome which is known as Lineage-based genetic markers.

Investigative information

Phenotypic information from a DNA sample

When there is no suspect, SNPs that describe phenotypic traits would enable a genetic prediction of appearance for investigative leads to identify the perpetrator of a crime.[4] Once a suspect is identified, his or her reference DNA profile can be compared with the evidence profile using standard DNA markers for inculpation or exculpation. The same phenotypic SNPs could be used to facilitate facial reconstructions for identifying missing persons.[36]

Automation

The demands of generating, entering, and maintaining DNA profiles in a national DNA database have driven developments in Automation. Most automation has focused on the extraction of DNA from standard reference samples and some have extended the application to casework samples such as bone, hair, teeth, cigarette butts, and sperm. The robotic platforms vary and include the Tecan Genesis RSP 150/8 robotic workstation and the Tecan Freedom EVO liquid handling stations (Tecan, Mannedorf, Switzerland), the Biomek 2000 automation workstation (Beckman Coulter, Fullerton, CA, USA), the Plato 3000 robotic system (Rosys/Anthos AG, Hombrechtikon, Switzerland), and the BioRobot EZ1 System and BioRobot 8000 workstation (Qiagen, Dusseldorf, Germany), to name a few.[41-46]

Forensic analysis of mitochondrial DNA (mtDNA) often provides results for samples where nuclear autosomal marker analyses are difficult or impossible (such as old bones, teeth, and hair shafts).[47] Multiplex PCR followed by electrospray ionization time-offlight mass spectrometry (ESI-TOF-MS) was demonstrated to be applicable for typing the hypervariable regions of human mtDNA, expanding the discriminating potential of an assay beyond that of specific SNP targeting.[48] The back end of the process is the interpretation of results. Expert systems are being developed to replace one if not both of the scientists for typing STR reference samples entered into DNA databanks.[49,50]

These robotic systems are macroscale approaches that have yet to capitalize on the potential benefits of microscale technologies ("In-field testing").[36] In-field testing the ability to perform DNA diagnostics at the crime scene. There are advocates for a portable field testing microfluidic device for performing human identification DNA typing at the crime scene to rapidly identify suspects. If DNA typing were performed at the crime scene, then qualified practitioners would have to be deployed, since expertise is required for DNA typing and interpretation of the generated DNA profiles. This deployment would reduce the throughput of an already backlogged laboratory: scientists would be occupied going to and from crime scenes and could only work one case at a time, and there would not be enough qualified personnel to analyze DNA at multiple, simultaneous crime scenes. One solution is for scientists to remain remote and the profiles transmitted electronically. Crimescene samples present themselves in myriad manners and these macro-samples may not be readily amenable to microfluidic processing. However, a microdevice may be useful at the crime scene for collection and sample storage.[36] By gathering phenotypic data and DNA from twins, it is now possible to use 'genome-wide' association studies and the monozygotic co-twin design to identify important genes in odontogenesis and also to clarify how epigenetic and environmental factors can affect this process. Given that many of the common dental anomalies affecting the human dentition are interrelated, apparently reflecting pleiotropic genetic effects.[51]

The use of aspartic acid racemization (AAR) in human dentine provides a simple, cost-effective solution and the method can achieve accuracies of Â±3 years at best.[52] The ability to obtain forensic DNA data from salivary evidence was well described by 1992, but it was not until Sweet et al. published the double swabbing technique in 1997 that the standard of practice for salivary DNA collection from skin was truly established.[53,54] The double swab procedure takes into consideration the need to moisten and lift the cellular component of an adherent saliva stain as well as addresses the risk that the bitemark victim's skin will be a likely contributor to the DNA in the sample.[55]

Whole blood samples drawn through a finger stick or veinapuncture procedure are the traditional methods in obtaining a DNA reference. Well-meaning investigators occasionally have collected head hair believing that they were procuring a simple noninvasive source of DNA. They did not realize that the laboratory processing requirements for hair samples is resource-intensive, compared to the more predictable blood sample. The taking of head hair as a DNA reference should be limited only to those cases where the questioned samples consist of shed hairs and the use of mitochondrial DNA testing is likely.For routine casework and long-term storage, whole blood applied to a filter paper card provides the greatest amount of reference DNA by volume with the least likelihood of contamination. However, the use of a buccal swab as a rapid, noninvasive alternative to blood collection is increasingly commonplace.[56,57]

Because of the anatomical specificity of the forensic odontologist's expertise, he or she may be consulted in the collection, storage, and transportation of the buccal swab. Targets of this collection technique are the stratified squamous epithelial cells that can be rubbed from the inner cheek,although a certain amount of salivary DNA is naturally collected as well and is beneficial.[58]

CONCLUSION

The substantial resource outlay to validate molecular biology analytical systems, to equip a laboratory, and to educate and make proficient practitioners; as well as the efforts undertaken to

gain admissibility in the courtroom,[47] forensic scientists tend not to change sound methodologies quickly. Such a view, however, would be myopic because there are several areas where molecular biology could offer improvements to the capabilities of the forensic scientist. Indeed, it is incumbent on the forensic scientist to be vigilant and embrace new technologies that will benefit society by their ability to analyze more challenging samples in an effort to continue to exonerate the innocent, to enhance abilities to solve crime, and to identify missing persons.

The very power of DNA and the ease with which large population databases can be developed have generated their own set of social problems and ethical concerns. With this new technology comes an increased risk to personal privacy that actually crosses generations, as well as the fear of genetic discrimination in employment and insurance sectors.[13]

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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