Forensic Identification of Missing Persons using DNA from Surviving Relatives and Femur Bone Retrieved from Salty Environment

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Abstract

Human identification using forensic DNA profiling has made enormous advancement over the past two-and-half decades. Forensic DNA profiling provides enormous genetic data from a variety of biological materials and individuals to help solve many important criminal and civil cases that confront society. Under certain environmental conditions, the total deterioration of soft-tissue leaves skeletal remains as the only available sample for DNA testing to identify missing persons, victims of natural disasters, or exonerate suspect(s) in a criminal case. We report the findings of a case involving the human remains of a missing person submitted to the Forensic Science Laboratory of the Ghana Police Service for forensic DNA profiling in comparison to an alleged living relative of the deceased. DNA from the femur bone and buccal swabs of alleged relative of the deceased were extracted, quantified, and short tandem repeat (STR) profiled using Qiagen’s Investigator kit, Applied Biosystem’s Quantifiler trio, and GlobalFiler kits. Full STR profiles were generated for both the femur bone from the salty environment and the buccal swabs from the alleged relative. The femur bone was genetically identified to be that of the missing person. The remains were thus handed over to the relatives for final funeral rites and burial to bring closure to the long search for the missing person.

Keywords: Electropherogram, genomic, profiling, short tandem repeat, skull

Introduction

The concept of human identification has made enormous progress over the past 25 years due to the advent of DNA profiling. Forensic DNA profiling provides enormous genetic data from a variety of biological materials and individuals to help solve many important criminal and civil cases that confront society. The development of DNA databases has contributed to the identification of missing persons and the development of investigative leads to assist law enforcement agencies. Forensic DNA databases consist of DNA profiles from convicted persons (and in some jurisdictions arrestees), forensic DNA evidence, human remains from unidentified missing persons, and direct family reference DNA samples of missing persons.¹

The scientific community, governmental agencies, and private research institutions continue to work together to standardize forensic DNA markers for effective worldwide data sharing, to develop and validate more robust DNA typing kits and software that contain the reagents and algorithms effective for typing core identity genetic markers. These forensic DNA technologies help to facilitate the speedy resolution of criminal, civil, and cases of victims of mass disasters.

The key objectives of forensic DNA profiling are to identify individuals who could be the source of biological evidence

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recovered from crime scenes, including associations of individuals due to some alleged relationship, and excluding individuals wrongly linked to the evidence from crime scenes.\[2\] The generation of reliable short tandem repeat (STR) profiles from unknown and reference samples, systematic and objective interpretation of results, and provision of statistical evaluation of results are subject to a stringent forensic DNA identification program.\[2\] Moreover, the standards of practice used for forensic DNA typing are dramatically impacting on the standards of criminalistics in a positive way.

The standard DNA markers used in most forensic DNA profiling laboratories in the world are autosomal STR loci.\[2,3\] The standard operating procedures for most of these laboratories include a set of 16–24 STR loci, which provide a high level of diversity and resolution for relationship testing.\[1,4,5\] Commercially available kits, such as the global filer polymerase chain reaction (PCR) amplification kit (Applied Biosystems, Foster City, California, USA) or the PowerPlex 24 system (Promega, Corporation, Madison, Wisconsin, USA), make it possible to analyze high-quality biological and forensic samples. These commercially available kits with the 16–24 STR genetic markers have been extensively used for DNA profiling of human remains as well as in kinship studies, such as paternity testing and family reconstructions.

We report on how DNA extracted from femur bones of human remains retrieved from a crime scene helped police detectives solve a case of an individual gone missing person for 9 months.

**Materials and Methods**

**Sampling**
The following sealed samples were submitted for forensic DNA profiling:

i. Left femur mid bone of the unknown decomposed body

ii. Buccal swab of deceased’s alleged son.

Ethical clearance was obtained from the Ethics committee of Kwame Nkrumah University of Science and Technology, and informed consent was obtained from the study individuals.

**DNA extraction**
Genomic DNA was extracted from 100 mg of powdered femur bone samples from the deceased as well as buccal swab samples from the deceased’s alleged son using the QiAamp DNA Investigator Kit following Manufacturer’s instructions, for genetic testing.

**DNA quantification**
DNA extracted from the samples were quantified with the 7500 Real-time PCR machine using the Quantifiler™ Trio DNA amplification kit (Applied Biosystems, Foster City, CA, USA) following manufacturer’s protocol.

**Short tandem repeat amplification and capillary electrophoresis**
The extracted DNA from the samples was then amplified with the 9700 PCR machine using Globalfiler™ STR amplification kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer’s protocol. The amplified STR targets were electrophoresed in the 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), and the generated STR profiles were analyzed using GeneMapper IDx version 1.5 software, following manufacturer’s protocol.

**Results**
The obtained results are shown in Tables 1, 2 and Figures 1, 2.

Figures 1 and 2 show the electropherograms of the alleged child and femur bone after STR profiling.

**Discussion and Conclusion**
DNA profiling is very helpful in identifying the human remains and in criminal investigations. This stems from the uniqueness

| Table 1: DNA concentrations and internal polymerase chain reaction control computed tomography values from real-time quantification |
|-----------------|-----------------|-----------------|
| Sample name     | Concentration (ng/µl) | IPC CT value    |
|-----------------|-----------------|-----------------|
| S2-17 B (bone)  | 0.261           | 27.144          |
| S2-17A (alleged son) | 13.404      | 27.210          |

PCR: Polymerase chain reaction, IPC: Internal PCR control, CT: Cycle threshold

| Table 2: The typing results of the analyzed samples |
|-----------------|-----------------|-----------------|
| STR locus       | Deceased (femur bone) | Alleged child   |
|-----------------|-----------------|-----------------|
| D3S1358         | 15, 16           | 15, 17          |
| vWA             | 16, 17           | 16, 17          |
| D16S539         | 10               | 10              |
| CSF1PO          | 10               | 10, 11          |
| TPOX            | 8, 11            | 11              |
| Y indel         | 2                | 2               |
| Amelogenin      | X, Y             | X, Y            |
| D8S1179         | 13, 16           | 13, 15          |
| D21S111         | 27, 28           | 28, 31.2        |
| D18S51          | 19, 21           | 18, 19          |
| DYS391          | 10               | 10              |
| D2S441          | 11, 12           | 11, 12          |
| D19S433         | 12, 13           | 11, 12          |
| TH01            | 6, 9             | 6, 7            |
| FGA             | 19, 24           | 19, 19.2        |
| D22S1045        | 15, 17           | 15, 16          |
| D5S818          | 11, 13           | 11              |
| D13S317         | 11, 14           | 11, 14          |
| D7S820          | 9, 11            | 9, 11           |
| SE33            | 15, 19           | 15, 18          |
| D10S1248        | 12, 14           | 12, 13          |
| D15S665         | 15, 17.3         | 15, 17.3        |
| D12S391         | 19, 20           | 17, 20          |
| D2S1338         | 19, 22           | 22, 27          |

STR: Short tandem repeat
of the DNA molecule to an individual, and also remains highly conserved in a person’s lifetime. Each individual’s DNA is composed of equal parts of his or her parents’ DNA and can be analyzed to produce genetic profiles comparable to other genetic profiles of close relatives. DNA molecule is a resilient molecule, degrades slowly in hard tissues, such as bones and teeth, and can be recovered and analyzed from small crime scene biological samples, such as bloodstains, saliva, or single hair with root. These unique characteristics of DNA molecule allow it to be recovered from old crime scene biological samples even when environmental conditions are unfavorable.\[^6\]

The use of human bone sampled from forensic cases, and a variety of taphonomic conditions results in a limited availability of extracted DNA for forensic analysis.

The Forensic Science Laboratory at the Criminal Investigation Department of the Ghana Police Service received samples of femur bone from investigators after the exhibits were recovered from a crime scene. Buccal swabs of an alleged relative (son) of the deceased were taken at the laboratory for DNA profiling and comparison purposes.

DNA was successfully extracted from the femur bone, and the buccal swab received, yielding 0.261 and 13.404 ng/\(\mu\)l, respectively. These values were enough to generate full DNA profiles for relationship testing. The successful recovery of DNA from femur bone agrees with the findings of Gaudio \textit{et al.},\[^7\] who demonstrated that it was viable to obtain substantial levels of endogenous DNA for forensic cases when targeting the cochlear region of the petrous bone, for cases in which there is extensive degradation to hard tissues degraded skeletal remains.

DNA profiles were also successfully generated for the alleged surviving relative (son) of the deceased (skeletal remains) for relationship testing. Assuming a prior probability of 0.5 (or 50%),

\[\text{Figure 1: Electropherogram generated from short tandem repeat profiling of the alleged child}\]
the combined paternity index was calculated to be $1.047 \times 10^8$, using published population data and DNA View software. This means the observed profile was $1.047 \times 10^8$ times more likely to occur under the scenario that the deceased is the true biological father of surviving relative (son), as opposed to the scenario that the deceased is an unrelated person of the African American population to the surviving relative (son). With a confidence probability of 99.99999904% conclusion is based on the calculated frequency of the DNA profile being rarer in unrelated individuals of the African American population.

Based on the findings of the genetic profiling and the relationship testing, the police detectives were able to unravel the mystery surrounding the disappearance of the deceased, returned the remains to the family for burial, and bring closure to the case to the family.

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Conflicts of interest

There are no conflicts of interest.

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