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A skeletal remains unrevealed the murder mystery of missing lady through DNA technology

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Abstract

In 1985, Alec Jeffreys first discovered and described the absolute identification technique through genomic DNA, which leads to open a new arena of forensic science. Since then, it has been adopted for crime-solving as well as various forensic applications across the globe. After the discovery of DNA technology, it passes through several continuous developmental steps concerning sensitivity, reduction in turnaround time, cost effect, minimizing multiple handling of the sample, and suitable challenging samples. After three and half decades of this DNA technology, Short Tandem Repeat (STR) based DNA technology established gold standard and irreplaceable technique in the field of forensic science, which is entirely different from its inception.

In the present study, with help of DNA technology, a murder mystery of missing women was solved. In this case, tooth and bone pieces of deceased and blood samples of probable mother and son of deceased were received for DNA test in the laboratory. STR based 20 autosomal STR markers and one sex-determining Amelogenin marker included in PowerPlex® 21 systems kit was used for identification of unknown deceased. Based on the genetic marker unknown deceased was identified as biologically related to the probable mother and son of the deceased.

Keywords: DNA Technology; STR; Forensic; Autosomal; Statistical Evaluation; Identification

1. Introduction

The structure of genetic material DNA was discovered in 1953 by Watson and Crick. This discovery led to open the new aspect of research and technological advancement to use genetic information for human being's welfare[1]. Continuous technological advancements in the field of molecular biology, Alec Jeffreys first discovered and described absolute identification technique through genomic DNA, which leads to open a new arena of forensic science [2],[3].

Presently, most popular short tandem repeats (STR's) based DNA analysis is widely used in forensic DNA application as well as genealogical, medical research on human populations[4], [5], [6], [7], [8], [9]and wildlife forensics. Short tandem repeats (STR's) based DNA analysis is well established, the irreplaceable and gold standard in present time, which entirely different from the inception of DNA technology before three and half decades [10]. Short tandem repeats (STR's) based DNA analysis is most popular in use due to high repeat numbers approximately once in every 10,000 nucleotides i.e., 3% of the total human genome[11]. The STR-based DNA technology is used in forensics to solve various criminal cases such as homicide, sexual assault, identification of unknown, mass disaster cases [12].

In the present case, a lady was disappeared from her home in October 2018 and the case was registered and investigated by the police. After 4 months, skeletal remains were found at River bank. Apparently, through skeletal remains, it was not decided that these skeletal remains belong to males or females. At the crime spot, there were bangles and clothes

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near the skeletal remains, which lead to predicting that the skeletal remains might be that missing lady. Finally, the investigation leads this case in the direction of the murder of that missing lady but the first identity of the missing lady was to be confirmed. The skeletal remains and blood samples of probable mother and son of that missing lady were sent to the laboratory for the DNA test. All the standard ethical guideline of the laboratory was followed from sample collection to laboratory examination.

2. Material and methods

In this case, the following samples were sent to State FSL, Jaipur for DNA examination.

- Tooth, rib bone, and femur bone of deceased.
- Blood sample of a probable mother of deceased.
- Blood sample of a probable son of deceased.

DNA was extracted from the tooth of the deceased and control blood samples of probable mother and son of deceased by automated DNA extraction system "Automate Express" (Thermo Fisher Scientific, USA). Real-Time PCR ABI 7500 (Thermo Fisher Scientific, USA) was used for quantification of the isolated DNA using the Quantifiler trio DNA Quantification Kit (Thermo Fisher Scientific, USA) as per the recommended protocol by the manufacturer. 1 ηg of DNA template was used for downstream processing by Amplification of 20 STR locus i.e., D3S1358, D1S1656, D6S1043, D13S317, D16S539, D18S51, D2S1338, CSF1PO, TH01, vWA, D21S11, D7S820, D5S818, TPOX,D8S1179, D12S391, D19S433 and FGA; and one sex-determining locus Amelogenin, included in Powerplex® 21 system (Promega, USA) as per the recommended protocol by the manufacturer except for half-reaction volume. Genotyping was done by capillary electrophoresis of the amplicon using Polymer POP-4 on Genetic Analyzer 3500XL(Thermo Fisher Scientific, USA) as per the recommended protocol.

3. Results and discussion

The DNA profile of 20 autosomal and one sex-determining genetic marker Amelogenin was obtained from tooth and blood samples of probable mother and son of deceased. The alleles of DNA profile obtained from the tooth of the deceased are accounted in the DNA profile obtained from the blood sample of probable mother and son of deceased as per mendelian inheritance law [13]. Thus the source of DNA profile obtained from the tooth of the deceased is biologically related to the sources of blood sample of probable mother and son (Table 1).

Table 1 DNA profile of 21 genetic marker

Locus	DNA profile of deceased	DNA profile of Mother of deceased	DNA profile of son of deceased
AMELOGENIN	X,X	X,X	X,Y
D3S1358	15,16	15,18	15,16
D1S1656	12,17.3	11,12	8,12
D6S1043	11,12	11,19	11,18
D13S317	12,15	11,12	8,12
PENTA-E	11,13	11,11	11,14
D16S539	11,11	11,12	11,11
D18S51	14,20	14,14	13,14
D2S1338	17,19	19,23	17,19
CSF1PO	10,12	10,10	10,11
PENTA-D	11,12	8,11	11,14
TH01	6,7	6,7	6,7
vWA	17,18	16,17	16,18
D21S11	28,30	29,30	30,31.2
D7S820	9,12	9,11	9,10
D5S818	11,12	12,12	11,12
TPOX	8,11	8,11	8,8
D8S1179	10,14	14,14	10,13
D12S391	17,18	18,19	17,18
D19S433	12,15	15,17	14,15
FGA	20,24	20,22	20,21

To support our result, the genetic data of skeletal remains, probable mother and son, were statistically evaluated. Interestingly in this case paternity was evaluated with the deceased and probable mother of the deceased, and with a probable son by comparing the locus-wise contribution of alleles in the DNA profile of the deceased. Allele frequencies for the population of Rajasthan reported previously on 21 markers[5] were used in the statistical evaluation of this parentage analysis. The parentage was established based on exclusion that no other woman could be the mother of this deceased lady and son. The probability of this deceased was calculated to be a daughter of probable mother and mother of a probable son. Paternity Index (PI) [14] which is a comparison of the relative chance of transmitting the obligate allele from probable mother, son, and any other random individual of the population, was calculated using likelihood ratio (LR) [14].

The final calculation of statistical evaluation of parentage is Probability of paternity, which is calculated using the following formula

Probability of paternity =1/1+ (1/the value of combined paternity index)

From Table 2 and Table 3 using 21 autosomal markers in Powerplex® 21 system, the resultant Probability of paternity between skeletal remains and probable son; and probable mother is 0.998102706 and 0.999714958999874 respectively.

Table 2 Calculation of complete paternity examination using genetic data of PowerPlex® 21 system kit

Locus	**DNA profile of deceased**	**DNA profile of son of deceased**	**obligate Allele**		**AF**		**Combined AF**		**PI**
AMELOGENIN	X,X	X,Y	X						
D3S1358	15,16	15,16	15	16	0.308	0.327	0.634	0.207024	0.765696784
D1S1656	12,17.3	8,12	12		0.139				1.801152738
D6S1043	11,12	11,18	11		0.331				0.754762552
D13S317	12,15	8,12	12		0.314				0.796482732
PENTA-E	11,13	11,14	11		0.145				1.722830956
D16S539	11	11	11		0.361				2.76854928
D18S51	14,20	13,14	14		0.308				0.812823097
D2S1338	17,19	17,19	17	19	0.058	0.174	0.232	0.040228	1.44092219
CSF1PO	10,12	10,11	10		0.196				1.27824931
PENTA-D	11,12	11,14	11		0.222				1.124100719
TH01	6,7	6,7	6	7	0.238	0.131	0.369	0.048316	1.90970896
vWA	17,18	16,18	18		0.186				1.343219428
D21S11	28,30	30,31.2	30		0.218				1.148527588
D7S820	9,12	9,10	9		0.060				4.170837504
D5S818	11,12	11,12	11	12	0.374	0.279	0.653	0.182305	0.895479619
TPOX	8,11	8	8		0.349				1.434390958
D8S1179	10,14	10,13	10		0.162				1.53884033
D12S391	17,18	17,18	17	18	0.114	0.274	0.388	0.106489	0.910912735
D19S433	12,15	14,15	15		0.140				1.780880467
FGA	20,24	20,21	20		0.125				2.00625953
								CPI	526.0665291
								CPI+1	527.0665291
								POP	0.998102706

PI= Paternity Index, CPI-Combined Paternity index, POP= Probability of Paternity, AF-Allele Frequency

Table 3 Calculation of complete paternity examination using genetic data of PowerPlex® 21 system kit

Locus	DNA profile of Mother of deceased	DNA profile of deceased	obligate Allele		AF		PI
AMELOGENIN	X,X	X,X	X				
D3S1358	15,18	15,16	15		0.308		0.812823097
D1S1656	11,12	12,17.3	12		0.139		1.801152738
D6S1043	11,19	11,12	11		0.331		0.754762552
D13S317	11,12	12,15	12		0.314		0.796482732
PENTA-E	11	11,13	11		0.145		3.445661912
D16S539	11,12	11	11		0.361		1.38427464
D18S51	14	14,20	14		0.308		1.625646194
D2S1338	19,23	17,19	19		0.174		1.44092219
CSF1PO	10	10,12	10		0.196		2.556498619
PENTA-D	8,11	11,12	11		0.222		1.124100719
TH01	6,7	6,7	6	7	0.238	0.131	1.049670403
vWA	16,17	17,18	17		0.270		0.926887142
D21S11	29,30	28,30	30		0.218		1.148527588
D7S820	9,11	9,12	9		0.060		4.170837504
D5S818	12	11,12	12		0.279		1.790959238
TPOX	8,11	8,11	8	11	0.349	0.360	1.412373723
D8S1179	14	10,14	14		0.155		3.234780358
D12S391	18,19	17,18	18		0.274		0.910912735
D19S433	15,17	12,15	15		0.140		1.780880467
FGA	20,22	20,24	20		0.125		2.00625953
						CPI	3507.26723
						CPI+1	3508.26723
						POP	0.999714958999874

PI= Paternity Index, CPI-Combined Paternity index, POP= Probability of Paternity, AF-Allele Frequency

Thus statistical results support towards the skeleton remains was belong to that missing lady.

4. Conclusion

Conclusively, DNA technology is the gold standard in forensics and a useful technique for absolute human identification. The present paper shall be highly useful for the working scientists engaged in forensic DNA examination.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declared that they have no conflict of interest.

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