Fatherhood Established from Skeletal Remains using Highly Variable 21 Autosomal STRs

R.K. Kumawat[1], Rajesh Singh[2], Ajay Sharma[3], Garima Singh[4], Dheeraj Singh Khiria[5], Pratima Srivastava[6], Sharik Akhtar Husen[7]

Abstract

STR markers are widely distributed throughout the human genome and are the main reason for the success of DNA-based genotyping technique owing to their amplification potential with the degraded forensic samples. As per Mendel's law of inheritance, every child/biological offspring inherits half of its alleles from the mother and the rest half from the father. Thus the paternity is examined by observing presence of alleles inherited from the parents in the child. In the forensic DNA typing and statistical approach leads towards absolute human identification. Also, there would be no allele in the DNA profile of the child which is not present in either of its parents. The degree of certainty of parentage establishment totally relies on the polymorphism of the tested loci and also on the number of loci included in the DNA test. Here we present a case study, where fatherhood was determined from DNA profile obtained from the skeletal remains and reference blood sample of probable son of missing person using 21 hyper variable STR markers. The combined paternity was observed as 1.3x108 for the 21 autosomal STR markers. The alleles of DNA profile obtained from skeletal remains are accounted in the DNA profile obtained from reference blood sample of probable son of missing person and statistically probability of paternity was calculated as 0.999999992056871. On the basis of these observations, the source of DNA profile obtained from skeletal remains was 99.9999992056871% father to the source of DNA profile obtained from blood sample probable son of missing person.

Key words: Fatherhood; DNA Profile; STR Markers; Skeletal Remains; Statistical approach.

Introduction

DNA is the hereditary material which inherits from parents to offspring. Paternity refers to fatherhood which is a very important part of DNA profiling to determine whether a man is the son's biological father or not. capillary electrophoresis and continuous development and further incremental advancements in the technique gave birth to the new era of absolute human identification[1-2]. STR markers are widely distributed throughout the human genome and are the main reason for the success of DNA-based genotyping technique owing to their amplification potential with the degraded forensic samples[3]. The paternity is examined by observing presence of alleles inherited from the parents to the child. As per Mendel's law of inheritance, every child/biological offspring inherits half of its alleles from the mother and the rest half from the father[4]. Also, there would be no allele in the DNA profile of the child which is not present in either of its parents. The degree of certainty of parentage establishment totally relies

Author's Affiliation: [1]Senior Scientific Officer, DNA Division, [2]Assistant Director, Biology Division, [3]Director, [4]Forensic Assistant, [5]Junior Scientific Assistant, Biology Division, [6-7]Forensic Assistant, State Forensic Science Laboratory Rajasthan, Jaipur 302016, India.

Correspondence: R K Kumawat, Senior Scientific Officer, DNA Division, State Forensic Science Laboratory Rajasthan, Jaipur 302016, India.

E-mail: ramkishankmwt@gmail.com

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on the polymorphism of the tested loci and also on the number of loci included in the DNA test.

Here we present a case of absolute identification of a missing person from skeletal remains using autosomal STR markers and statistical approach. In this case, skeletal remains were recovered from crime scene and its identification was not established on the basis of primary investigation. Further investigation leads to link with a middle aged person who was suddenly missed from his home. Even after searching many places, nothing was found. After eight days of the incident, a case of missing person was lodged in the concerned police station. After a few days there was reported to be found something similar to that person like glasses,shoes, tobacco, empty bottle of wine, cloth sheet, a piece of cloth and blanket. On the basis of these cluesinvestigating officerhad greater chance of these skeletal remains of missing person but not sure. Even though the skeletal remains were not identified as belongs to male or female. Therefore, investigating officer was send these skeletal remains and reference blood sample of probable son of missing person for DNA test at State Forensic Science Laboratory, Jaipur, Rajasthan, India.

Materials and Methods

Skeletal remains of deceased person and reference blood sample of probable son of missing person on FTA card were received in properly sealed condition with seal sample for maintain the integrity of samples. The written informed consent was obtained following the declaration of Helensiki5 and as per the sample collection and forwarding guidelines of the laboratory. Skeletal remains were subjected to DNA isolation using bone DNA extraction kit (Promega, CA, USA) for processing and DNA IQTM chemistry for purification of DNA on Maxwell® RSC 48 instrument (Promega, CA, USA) according recommendations of the manufacturer. The quantity of extracted DNA from bone sample was evaluated using QuantiFluor® ONE dsDNA system (Promrga, CA, USA) on QuantusTMFluorometer (Promrga, CA, USA) as per recommendations of manufacturer.

24 STR locus which included 20 CODIS (D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, D10S1248, D1S1656, D12S391, D2S1338) with another loci from USS (European Standard SET) i.e. SE33 and 3 gender-determining loci (Amelogenin, Y indel and DYS391) (Fig. 1) using GlobalfilerTMPCR amplification Kit (Thermo Fisher Scientific, CA,

USA) as per recommendations of manufacturer except half reaction volume. FTA card (Whatman, GE Health Care) was directly amplified without DNA extraction and quantitation. The VeritiTM Fast thermal cycler (Thermo Fisher Scientific, CA, USA) was used for amplification process. 1 μl of amplified fragment was diluted with 8.5 μl HiDiTM formamide (Thermo Fisher Scientific, CA, USA) and 0.5μl of GeneScan™ 600 LIZ™ Size Standard v2.0, (Thermo Fisher Scientific, CA, USA), respectively. Separation and detection of amplified fragments were performed with ABI 3500XL Genetic Analyzer (Thermo Fisher Scientific, CA, USA) using POP-4 polymer and 36 cm capillary array. Alleles were designated by using allelic ladder provided along with kit. DNA profiles were analyzed from the raw data using Gene Mapper IDX v 1.6 software (Thermo Fisher Scientific, CA, USA).

Quality Control

Laboratory standards were followed to ensure the quality control at each level of DNA test. Positive and negative controls were used along with the samples for accuracy of the quality results. Additionally, the corresponding author has been passed the international quality control DNA proficiency test conducted by GITAD, Spain http://gitad.ugr.es/principal.htm).

Result and Discussion

The allelic description of DNA profile obtained from the skeletal remains and blood sample of probable son was presented in Table 1. The gender-determining loci viz., Amelogenin, Y indel and DYS391 showed alleles XY, 2 and 10 respectively, form skeletal remains which showed the source of DNA profile obtained from skeletal remains belongs to male individual. The alleles obtained from blood sample of probable son showed match on all the loci of GlobalFilerTM PCR amplification kit (Thermo Fisher Scientific, CA, USA). The concordance in allele call was also observed in all the tested multiplex systems. The DNA profile obtained from skeletal remains showed heterozygous alleles at all the studied loci except loci CSF1PO, TH01, D7S820, D10S1248 and D12S391. The DNA profile from skeletal remains showed heterozygous alleles at all the studied loci except loci D8S1179, D19S433, TH01 and SE33. Parentage testing was performed by comparing locus-wise contribution of alleles in the DNA profile obtained from skeletal remains and blood samples of probable son. The paternity was confirmed by using statistical evaluation of the DNA profiles of the case. Allele frequencies of

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population of Rajasthan from the unpublished data of authors were used in statistical evaluation of this parentage analysis.

In statistical evaluation of paternity, the first step is the selection of obligate allele. Obligate allele is the allele inherited by the child from the father[6]. There are two alleles available on every marker in the DNA profile of any individual hence if the father is homozygous as observed on locus D8S1179 (10,10), and the probable son was heterozygous observed allele 10,14 (Table 1). In the DNA profile of the probable son, at this locus, allele 10 inherited from father hence it is obligate allele and the chance of transmitting allele 10 in the child from father would be 1/2 which is 0.5; at this locus, Likelihood Ratio (LR) is 0.5. After the selection of obligate allele and Likelihood Ratio, the second step would be calculating paternity index (PI). PI for a particular locus is calculated by dividing the Likelihood Ratio (LR) with the frequency of the obligate allele in the population. This is clear now that either the PI on any given locus can be 1/frequency of obligate allele or 0.5/frequency of obligate allele.

Another important aspect of this calculation is the fact that if there are two obligate alleles as in the case of the locus D16S539, then the PI will be calculated as 1/frequency of obligate allele 1 + frequency of obligate allele 2 or 0.5/frequency of obligate allele 1 + frequency of obligate allele 2. Simple multiplication of all the individual PI values

Table 1: Paternity calculation for the 21 autosomal STR markers.

Loci	DNA profile obtained from skeletal remains	DNA profile obtained from reference blood sample of probable son	obligate alleles		Frequency of obligate alleles		Paternity Index
D3S1358	15,16	15,16	15	16	0.295	0.332	1.598802215
vWA	16,17	16,17	16	17	0.241	0.288	1.903503322
D16S539	11,12	11,12	11	12	0.318	0.201	2.029486963
CSF1PO	11,12	12	12	-	0.387	-	1.292724546
TPOX	10,12	11,12	12	-	0.036	-	6.971556051
D8S1179	10	10,14	10	-	0.181	-	2.766557849
D21S11	30,32.2	28,30	30	-	0.187	-	1.338974881
D18S51	14,15	14,16	14	-	0.275	-	0.90922316
D2S441	10,11	10,14	10	-	0.298	-	1.68055929
D19S433	16	12,16	16	-	0.054	-	9.293680297
TH01	9	9	9	-	0.270	-	3.703155088
FGA	21,23	21,23	21	23	0.121	0.187	3.399810756
D22S1045	10,15	15,16	15	-	0.407	-	0.614220431
D5S818	11,12	11,12	11	12	0.356	0.323	1.476221426
D13S317	8,13	10,13	13	-	0.079	-	3.145841198
D7S820	8,11	8	8	-	0.224	-	2.235835979
SE33	20	19,20	20	-	0.071	-	6.999860003
D10S1248	14,15	15	15	-	0.285	-	1.753647587
D1S1656	12,16	12,16	12	16	0.140	0.140	3.581918476
D12S391	17,19	19	19	-	0.140	-	3.581918476
D2S1338	20,23	20,23	20	23	0.117	0.199	3.399942541
			Combined Paternity Index				1.3×10^8
			Probability of Paternity				99.9999992056871%

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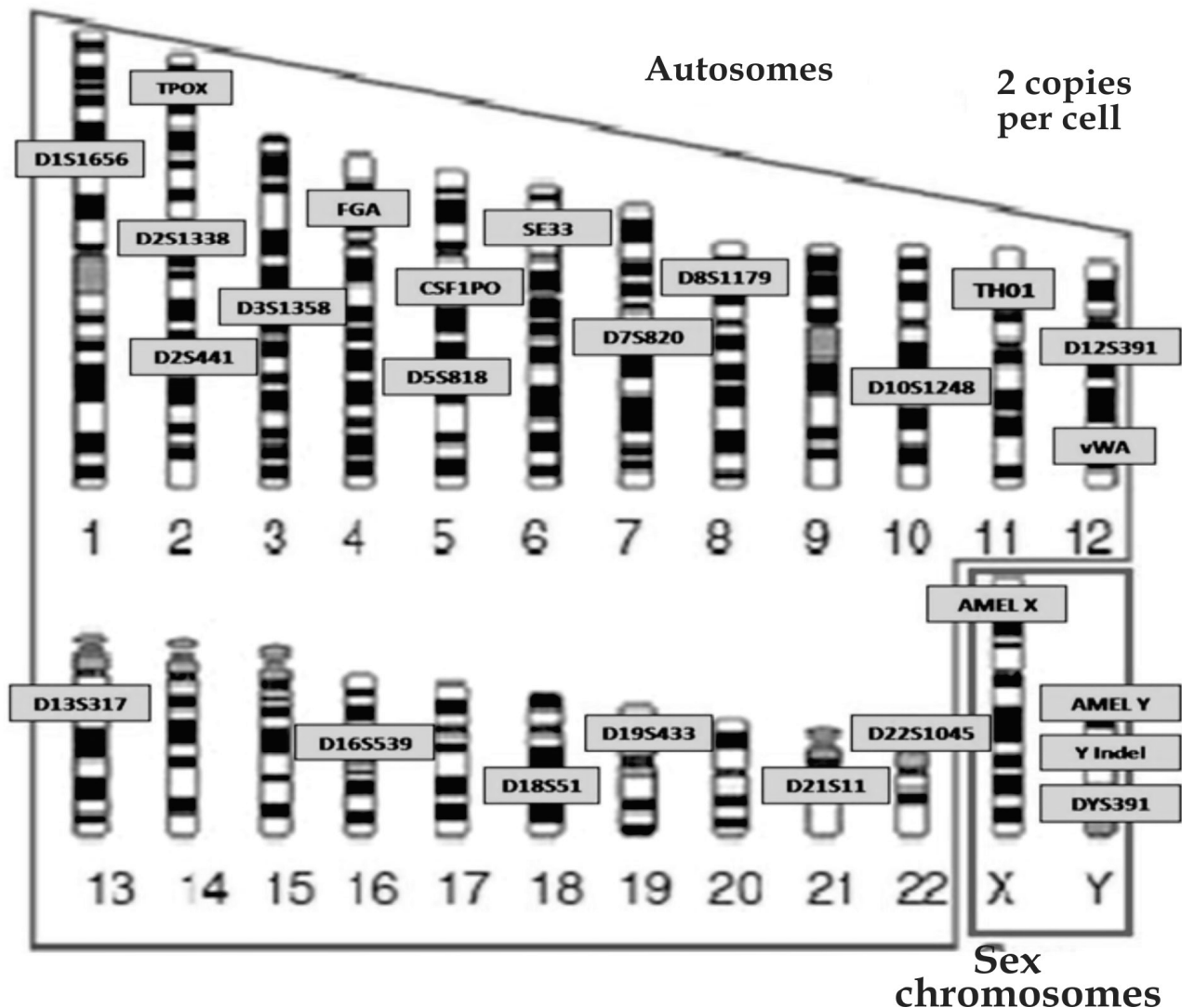Remains using Highly Variable 21 Autosomal STRs

Fig.1: Chromosomal position of 24 STR markers included in GlobalFilerTM PCR amplification kit.

gives Combined Paternity Index (CPI). From Table 1 using 21 autosomal markers in GlobalFiler™ PCR amplification kit (Thermo Fisher Scientific, CA, USA), obtained CPI value is 1.3×10^8

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)

For 21 autosomal markers in GlobalFiler™ PCR amplification kit (Thermo Fisher Scientific, CA, USA), resultant Probability of paternity is 0.999999992056871. The higher values obtained confirmed the fact that all the obligate alleles were inherited in the son from father.

Conclusion

In conclusion, the source of DNA profile obtained from skeletal remains was biological father of source of DNA profile obtained from blood sample of probable son with probability of paternity at 99.9999992056871%. The combined paternity index for 21 autosomal STR markers was observed as 1.3×10^8 which supports high degree of forensic relevance of STRs of GlobalFiler™ PCR amplification kit.

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Declaration of Interest Statement

Author (S) declares they have no conflict of interest.

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