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A CASE STUDY OF FORENSIC EVIDENCE IN MANIPUR: UTILIZING MITOCHONDRIAL DNA ANALYSIS TO RESOLVE A PATERNITY DISPUTE

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Abstract

This case study explores the application of mitochondrial DNA (Mt DNA) analysis in resolving a challenging paternity dispute in Manipur, India. The case involved a claim from one man asserting paternity over a child, while the mother contended that another man was the biological father. Amid the complexity of the dispute, Mt DNA analysis provided a critical alternative to conventional DNA testing methods. Samples from the child, mother, and both alleged fathers were subjected to thorough analysis, focusing on hypervariable regions to identify rare genetic markers. The findings confirmed the maternal lineage and established a distinct genetic link to one of the alleged fathers through shared Mt DNA patterns. This outcome highlights the effectiveness of Mt DNA analysis in forensic science, particularly when presented with ambiguous or rare genetic evidence. Furthermore, the case emphasizes the importance of collaboration between forensic specialists and the legal system in accurately interpreting DNA evidence to support judicial decisions. The results of this investigation advocate for the broader integration of mitochondrial DNA analysis in paternity testing and forensic investigations.

This case study investigates the role of mitochondrial DNA (Mt DNA) analysis in resolving a complex paternity dispute in Manipur, India. The case arose when a man claimed paternity over a child, while the mother maintained that another man was the biological father. Given the contentious nature of the claims, the court mandated genetic testing to clarify the child's biological connections. Traditional autosomal DNA profiling was deemed inconclusive due to the presence of unique and rare genetic markers among the alleged fathers. Consequently, the forensic team opted for comprehensive Mt DNA analysis, focusing on hypervariable regions (HVRs) known for their variability among individuals. Samples from the child, mother, and both alleged fathers underwent meticulous testing to identify and compare Mt DNA sequences.

Keywords: Forensic Science, Mitochondrial DNA, Genetic Interpretation, Judicial Decision.

INTRODUCTION

Alec Jeffreys in 1984 developed the DNA fingerprinting technique and in 1988 first time it was used for paternity testing. Earlier blood grouping was the most common procedure considered in human paternity testing. Till date DNA profiling is the universally accepted gold standard technique for the human identification purpose in forensics. It is being routinely used with a high degree of confidence to decipher accurate inferences in cases of paternity disputes, for the identification of human remains and in complicated criminal casework analysis, including sexual assault cases in forensics[1,2].

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DNA is an excellent biological marker to decide individual identity. Everyone has different DNA obtained from both parents, except for the case of identical twins. The principle of identification through DNA in forensics is based on the process of allelic comparison between the allele of the victim or perpetrator and the allele of the family line, especially parents in reference to Mendel's Law. Generally, paternity testing follows Mendelian law of inheritance, according to which child receives one allele from the mother and the other allele from the father[3].

In present scenario, with the advent of advanced and numerous DNA sequencing, amplification and profiling techniques, paternity testing has evolved even further than predicted. Indeed, present-day accuracy of genetic testing has attained an accuracy rate of up to 99.99%. The exact level of accuracy depends on the number and quality of the genetic markers being considered for testing. It is important to emphasize that during DNA testing scientists consider only specific regions of genome (markers) rather than entire genomes. Analysis of these specific genomic regions facilitates a great deal to save time and expense to the process with significantly improving the accuracy of the results. Thus, DNA-based methods of paternity testing have advantages over earlier methods. Moreover, higher throughput, better sensitivity and automation provide facility for DNA testing to be performed on even-smaller and sometimes degraded DNA samples of forensic importance with greater speed and excellent accuracy[4].

The whole concept of paternity testing is based on comparing genotypes and in case of differences in the alleles at same STR loci between the potential father and the questioned child, relationship between them can be assigned as non-biological paternity, which leads to exclusion of biological paternity.

Short tandem repeat loci in that manner have been accepted as perfect biological tool in forensics because that are highly polymorphic and variable[5].

Since inception the throughout the past decades, short tandem repeat (STR) loci have become the most accepted and important genetic markers in forensics. STRs can be analyzed at a reasonable cost/time ratio and provide high enough statistical discrimination power to identify individuals in the majority of crime and human identification cases[6]. STRs are made of tandemly repeated DNA sequences, that consists of short repetitive units from 2 to 7 base pairs in length. Number of repeats is designated as allele that varies among individuals. Besides the high robustness and reproducibility, another advantage of these markers that makes them most suitable for forensic analysis is simplicity of the detection process in the form of automation. There is lowest theoretical probability that two persons share identical allelic variants on all 15 or more STR loci i.e. up to 1 in 10^{13} for a population[8]. To reach up to 99.99% probability of paternity (PP) the International Society for Forensic Genetics (ISFG) suggested a minimum of 12 autosomal STR markers located on 10 different chromosomes to be analyzed.

Observations and analysis of mutations occurred at STRs in forensic genetics are very important in paternity testing. Therefore, precise elucidation of obtained genetic profiles is of much attention. However, alleles are inherited as per Mendelian inheritance patterns but in some cases, spontaneous mutations lead to allelic mismatch on particular locus, making paternity or maternity testing case complicated.

CSF1PO stands as one of the twenty core loci used for the CODIS database and its allelic range for this short tandem repeat (STR) locus has been reported as 6 to 15 repeats of the tetra-nucleotide AGAT[9]. Mutational rate for STR loci has been reported and estimated around 10^{-2} to 10^{-4} per generation by Vigouroux et al[10]. They explained it by two different mechanisms: unequal crossing-over during recombination or inaccurate pairing during replication because of DNA slippage. Fan and Chu[11] highlighted the strand-slippage replication as a main pattern

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of STR mutation among several different mechanisms described by them for STR mutations. Many researchers observed single-step mutations most of the times as compared to rarely occurred multi-step mutational events in routine parentage testing cases[12-15]. Unusual parentage cases of allelic mismatches cannot be easily solved by routine autosomal STR analysis, should be confirmed or excluded by employing an additional analysis[13]. In such cases the test process becomes tangled and requires analysis of additional genetic markers to confirm the exclusion[16]. Mutations arise mainly in meiosis. There are always great chances of autosomal chromosomal mutations in meiosis as compared to sex chromosomes. All types of chromosomal rearrangements, deletions, insertions, inversions, duplications, translocations, etc., occur in the pachytene phase of the meiosis. Mutation rates for microsatellite repeat sequences have been reported higher than that of single nucleotide polymorphism[12]. Many of the researchers evaluated a number of different Y-chromosome markers in the past[8] and gained a significant role of the Y-chromosome markers in paternity testing for male children[7]. Comparison of Y-haplotype may help to determine the paternal lineage. In case if an identical Y-haplotype profile is obtained for child and alleged father, obviously the alleged father and the child belong to the same paternal lineage.

As STR typing is based on DNA length polymorphism, it is limited to define only the deletion/ insertion and duplication type chromosomal rearrangements or mutations. The main mechanism behind length polymorphism in microsatellites or STRs is thought to be the polymerase template slippage[9]. Eckert & Hile[11] concluded that DNA strand slippage may transiently occur during DNA synthesis, which may result in mutant products where repeat units are added or deleted within the microsatellite. Most obvious explanation for a mutation in an STR locus was explained by Klint Schar et al.[13] as a contraction of the repeat stretch due to polymerase slippage and these mutations are almost invariably confined to a single repeat.

M. A. Jobling[6] preferred other strand-slippage replication mechanism of mutation (Figure-1). In general, larger contractions or expansions are considered extremely rare, and to be the consequence of recombination rather than slippage. Single-step mutations involve the gain or loss of a single repeat in the transmitted parental allele. Single-step mutations are assumed to take place more frequent over multistep mutations that involve the gain or loss of more than one repeat[12].

Kimura & Ohta[27] also inferred so-called stepwise mutation model (SMM) as the most accepted mutational model which considers single-step mutations as the most frequent when compared to multistep mutations.

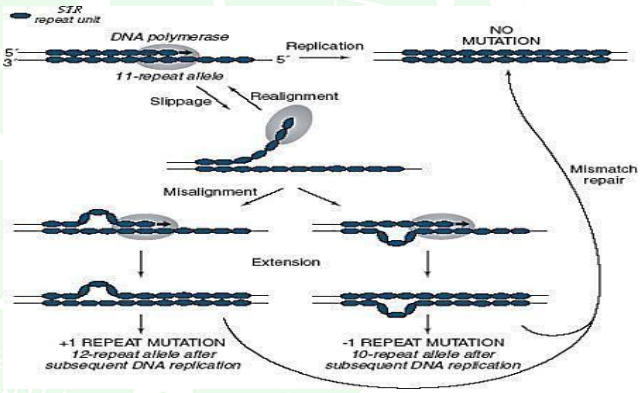

Figure-1: Schematic illustration of the strand-slippage replication at STR[6].

It is evident by the above cited explanations that the study of mutations at the level of practical applications of STR-markers is of great importance. The present study reports a mutation in either parent as both parents were homozygous for allele12 on locus CSF1PO. The case was received for a routine forensic examination of sexual assault combined with paternity trio. The samples of the women victim, male child and the putative father were examined with the multiplexes of 25 autosomal STRs, 25 Y-STRs and 12 X-STRs systems.

Materials and Methods

Sample collection: The samples were received

for a routine case work at State Forensic Science Laboratory, Jaipur, Rajasthan, India. The blood samples of women victim, child and putative or alleged father were collected on FTA Mini card as per standard guidelines.

DNA extraction: DNA was isolated and purified from 1.2 mm punch of blood-stained FTA papers using FTA purification reagent and TE buffer (10mM Tris-HCl, 0.1 mM EDTA, PH 8.0) with the manufacturer's (Whatman) recommendations.

PCR amplification: Microsatellite loci of Promega's Power plex® Fusion 6C kit, Power plex®-21 system kit and Applied Biosystem's Global Filer™ PCR Amplification kit were used for autosomal STR typing, Applied Biosystem's Y-Filer™ Plus PCR Amplification Kit was used for Y-STR typing. Likewise, Qiagen's Investigator® Argus X-12 QS Kit was used for X-STR typing. Data collection and data analysis was performed using Genetic Analyzer 3500 Series Data Collection v4.0 Software and Gene Mapper ID-X® Software v1.6 respectively. The purified and dried punches from FTA cards having blood sample, were subjected to PCR amplification using the STR amplification kits following the manufacturer's protocol.

Electrophoresis and Genotyping: All PCR products were electrophoresed on Genetic Analyzer 3500 Series Data collection v4.0 (Applied Biosystems). The electropherograms to determine the genotypes, were obtained using Gene Mapper ID-X® Software v1.6 (Applied Biosystems) following manufacture's recommendations.

Quality assurance: Human Identification Professional Services (HIPS) by Thermo Fisher Scientific CA, USA has validated the DNA division of State Forensic Science laboratory. The extraction, amplification and genotype of the samples were cross-checked to verify the results on different days. Positive and negative controls in different steps were investigated to exclude the occurrence of contaminations.

Statistical analysis: According to Schanfield et al.[3] probability of Paternity represents the probability that the alleged father is a biological father of the child. Probability of Paternity (PP) was calculated using the formula PP=CPI/CPI+1.

To deduce the Combined Paternity Index (CPI) PI values for all examined loci were multiplied. Paternity Index (PI) was calculated separately for each STR locus (PI=Likelihood ratio/frequency of obligate allele). Likelihood ratio is generated by comparing probability that the alleged father contributed the obligate allele with probability that randomly chosen man contributed the allele.

Another statistical parameter to evaluate the paternity is RMNE

{RMNE=1-(1-frequency of allele)^2}, which is represented as the proportion of any population which can share the same obligate alleles on the tested loci. For any case of paternity testing Combined Random Man Not Excluded (CRMNE) may be find out by multiplying RMNEs for all tested loci. CRMNE is used to decipher the Power of Exclusion (PE) with the formula: PE= (1-Value of CRMNE).

Results and Discussion

Paternity testing in the discussed case was conducted using Promega's Power plex® Fusion 6C kit which contains one sex determination marker i.e. Amylogenic, 23 autosomal markers and 3 Y-chromosomal markers. Genotype of child for all the tested loci was observed in accordance of Mendelian laws of inheritance but it was observed deviated from the standard law of inheritance at the locus CSF1PO. Obtained genotype at the CSF1PO for father, child and mother was 12/12, 12/13 and 12/12 respectively (Table-1). Each time the same genotypes were observed at the locus CSF1PO, even when testing was repeated with different PCR Amplification kits.

As per the Mendelian inheritance expected genotype for the child is12/12. The appearance of allele 13 in child's genotype was observed as a result of mutation at the locus CSF1PO. Mutations at STR loci are identified as the alleles that not inherit as per Mendelian law of

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inheritance. In paternity testing existence of one or two allelic mismatches in genotypes are considered to align the paternity inclusion as "two exclusion" paternity rule is well established by work of many researchers. In such situations much attention must be dedicated to analyze spontaneous mutations which may lead to interpret an inaccurate exclusion[14]. Findings of the work carried out by Brinkmann et al., Thangaraj et al. and Deepak et al. also support the standard practice to exclude paternity only if more than two mismatches have been observed at all tested loci. Carboni et. and Negi et al. suggested the addition of extra STRs as the main alternative if examiners find results blended with mutational occurrence in paternity testing.

In such a way examiners can improve the probability of paternity or they can conclude unambiguous paternity exclusions. As per findings of Li et al.[15] the number of analyzed loci, in cases when a mutation is identified, must be increased in order to increase and validate paternity index.

Table-1: Investigator® Argus X-12 QS Kit allelic data.

LOCUS ↓	Mother	Child	Alleged Father
QS1	Q	Q	Q
Amel.	X,X	X,Y	X,Y
DXS10103	16,20	20	16
DXS8378	11,12	12	11
DXS10101	29.2,32	29.2	31
DXS10134	34,36	34	39
DXS10074	17,18	17	18
DXS7132	13,13	13	12
DXS10135	23,29	29	29
DXS7423	15,16	15	14
DXS10146	29,30	30	28
DXS10079	20,21	20	18
HPRTB	12,12	12	11
DXS10148	18,25.1	18	18
D21S11	31.2,32.2	31.2,31.2	31.2,32.2

To strengthen the paternity inclusion Applied Biosystem's Y- Filer™ Plus PCR Amplification Kit was used for Y-STR typing and Qiagen's Investigator® Argus X-12 QS Kit was used for X-STR typing (Table-1).

Advance alternatives as DNA-SNP Array, MPS or NGS techniques may resolve the mutational abeyances in forensic DNA examinations in the cases like this one. The source of mutation in this case remains inconclusive due to unavailability of high-end techniques in the laboratory but definitely showed a way to adopt latest and advanced technologies[16].

Conclusion

In paternity testing if exclusion at one or two loci is observed, alternative PCR amplification system is always suggestive to increase the number of tested loci. Haplotype PCR amplification systems may also strengthen the results. Two exclusion theory is a remarkable tool to confer the paternity in cases of observed mutations. To resolve the mutational abeyances with a research-oriented approach, forensic DNA laboratories in Manipur need technical and instrumental advancement in routine case work too.

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