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Research Article

Investigation of Paternally Inherited Allele Mutation at Short Tandem Repeat (STR) Locus D7S820 Leading to Parent-Child Mismatch

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Abstract: During paternity investigation with Identifiler™ set of autosomal Short Tandem Repeats (STRs), a genetic mismatch was observed with D7S820 between the disputed father and child. The genotype at this locus in the disputed father, mother and child was 10/10, 11/12 and 11/11, respectively. The combined paternity index and probability of paternity after including the mutation in the calculation were 7.6×10^7 and 0.9998, respectively. Both values supported the suspicious father as the biological father of the child. Further analysis of Y-STRs revealed matching of all the alleles of the child with that of the suspicious father. It suggested that the mismatch at the D7S820 locus might be a case of mutation. DNA sequencing of D7S820 PCR products of the child and both the parents helped in determining that the child inherited the expanded repeat of the paternal allele 10, which was transmitted as allele 11 in the child from the suspicious father.

Keywords: Paternity Index, Y-STRs, DNA Typing, Paternity Test, Forensic Science.

1. INTRODUCTION

The human genome is densely packed with repetitive DNA sequences. The length of the core repeat units, the number of adjacent repeat units, and/or the overall length of the repeat area are used to categorize these repeating sequences, which occur in various sizes. Short Tandem Repeats (STRs), often known as microsatellite markers, are DNA sections with brief repeat units typically 2–6 bp in length [1]. The benefits of STRs have been demonstrated, and this makes them particularly ideal for human identification. A population's variation in the number of repeat units leads to numerous alleles at the STR locus. An STR marker is transmitted from each parent, resulting in repeat sizes that can vary between the two alleles. Due to the significant variability in the number of repeats found in STR markers among individuals, these loci serve as effective tools for human identification [2]. STRs have become popular DNA markers because these can easily be amplified by polymerase chain reaction (PCR) without the problem of differential amplification and typed by gel electrophoresis. For the amplification of STRs used in human identity testing, multiplex PCR with fluorescently labeled primers has been an essential approach. Multiplex PCR is frequently identified as the bottleneck in the STR workflow of extraction, quantification, amplification, separation, and detection [3]. Polymorphism of STR markers has been utilized in a range of case studies, such as determining parentage and various forensic applications. STRs are classified into three types based on the structure of their repeat units: simple repeats, compound repeats, and complex repeats [2]. When compared to other genetic markers, STRs exhibit a significantly high mutation rate, which is viewed

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as a limiting factor for their application in forensic investigations [4-6]. Mutations occurring during meiosis can affect the interpretation of paternity tests. A meiotic mutation could produce inconsistent outcomes at a location [7-9]. A repeat unit could be inserted or deleted from the DNA strand. The gain or loss of repetitive units in alleles mainly caused by replication slippage at one or more loci results in an allelic mismatch in the questioned child and complicates the interpretation of the analytical results [10]. This type of allele mismatch, which diverges from the Mendelian inheritance pattern due to size differences from the parental allele, may influence the paternity or maternity of a child. According to recommendations from the Forensic Society, a single allele mismatch generally does not provide sufficient grounds for exclusion unless there are more than two mismatches [11]. However, this should be validated by analyzing additional markers to confirm an inclusion or exclusion determination [7].

A recent study demonstrated that the AmpFlSTR® Identifiler® Plus Kit™ was unable to amplify the child's allele at the D7S820 locus during standard paternity testing. This resulted in an inconsistency between the parent and child due to a single-step mutation [12]. The D7S820 STR loci display a wide range of allele numbers, making them particularly valuable for forensic investigations and paternity determinations [13].

In this study, we present a case related to a paternity test that revealed a paternal allele mismatch at the D7S820 locus in the child. We obtained DNA profiles for the child, the mother, and the alleged father, which confirmed the allele mismatch at this particular locus. Additionally, we analyzed Y-chromosome STR markers from both the child and the alleged father. The results showed identical DNA profiles for the two. Consequently, we conclude that the mismatch at the D7S820 locus likely indicates a mutation in the paternal allele, which has been inherited by the child.

2. MATERIALS AND METHODS

2.1. Sample Collection

To determine the paternity of a child with the suspected father, initially, six samples were collected at the Institute of Biomedical and Genetic Engineering (IBGE) from the suspected father, the child, and the child's mother. To validate the initial findings, an additional six samples were obtained from the same individuals after obtaining informed consent, resulting in a total of 12 samples. The samples included both blood and buccal swabs, with the blood samples being preserved in Vacutainer™ tubes containing Acid Citrate Dextrose (ACD).

2.2. DNA Extraction and DNA Profiling

The QIAamp® DNA Blood Mini Kit, produced by QIAGEN GmbH in Germany, was utilized for DNA extraction from blood samples, adhering strictly to the manufacturer's guidelines. A modified DNA isolation protocol was applied to extract DNA from buccal swabs using the same QIAamp® DNA Blood Mini Kit. The concentration of the extracted DNA was assessed using NanoDrop 2000/2000c Spectrophotometers from Thermo Scientific. Following the manufacturer's instructions, DNA samples were amplified using the AmpFl STR Identifiler™, AmpFl STR Profiler™, AmpFl STR Cofiler™, and AmpFl STR Y-filer™ kits from Applied Biosystems, Foster City, USA. The PCR amplified products from the AmpFl STR kits were separated via capillary electrophoresis and analyzed with appropriate internal size standards and allelic ladders on the ABI3130 Genetic Analyzer, employing Data Collection software. Allele sizing and genotyping were conducted using GeneMapper ID version 3.1 software [14].

2.3. Sequencing of D7S820 Alleles

DNA samples from the presumed father, mother, and child were amplified at the D7S820 gene using a specific primer pair from the AmpFlSTR Identifiler™ kit [15]. The resulting PCR product was purified with the AccuPrep® PCR Purification Kit from Pioneer, a Korean company, and subsequently sequenced using the BigDye Version 3.1 terminator-ready reaction kit, employing both forward and reverse D7S820 primers (Perkin Elmer, Foster City, USA). Samples were genotyped using the ABI Prism® 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA).

2.4. Statistical Analysis

The Paternity Index (PI) is calculated using the formula PI = X/Y, where X represents the

likelihood that the alleged father could pass on the required allele, and Y represents the chance that a different male of the same ethnicity might transmit that allele. This measure indicates whether the suspect is the biological father of the child. Following the guidelines set forth by the Paternity Testing Commission of the International Society for Forensic Genetics, we also computed the Combined Paternity Index (CPI) and the Probability of Paternity (W) [16, 17]. The allelic frequency was also computed to calculate the CPI and W.

3. RESULTS AND DISCUSSION

A routine DNA paternity test was conducted and the samples were first analyzed using the Identifiler™ set of 16 STR markers. A mismatch was identified at the D7S820 locus between the child and the disputed father (Figure 1). At this locus, the disputed father is homozygous for allele10, the mother is heterozygous with alleles 11 and 12, and the child is homozygous for allele 11. The DNA profiling was repeated using the Identifiler™ set of STR markers, which again revealed that the D7S820 locus was the only site of mismatch (Table 1). Further analysis was performed using a 10 loci STR Profiler™ kit, which consistently confirmed the mismatch at the D7S820 locus alone, showing perfect matches at all other loci. If both tested individuals were the child's biological parents, the genotype of the child would be 10/11; however, only allele 11 was detected in the child's electropherogram (Figure 1). A child can be considered the biological offspring of the presumed father if all STR loci match during comparison. Conversely, a child is not the biological child of the suspected father if there are two or more STR loci that indicate exclusion. This situation suggests that the probability of paternity would fall below 90%, indicating a definitive lack of blood relation between the child and the suspected parent. Thus, it remains plausible that the alleged father is not the biological parent, or that a mutation may account for the discrepancies [18]. Mutations can occur at the STR loci, similar to any

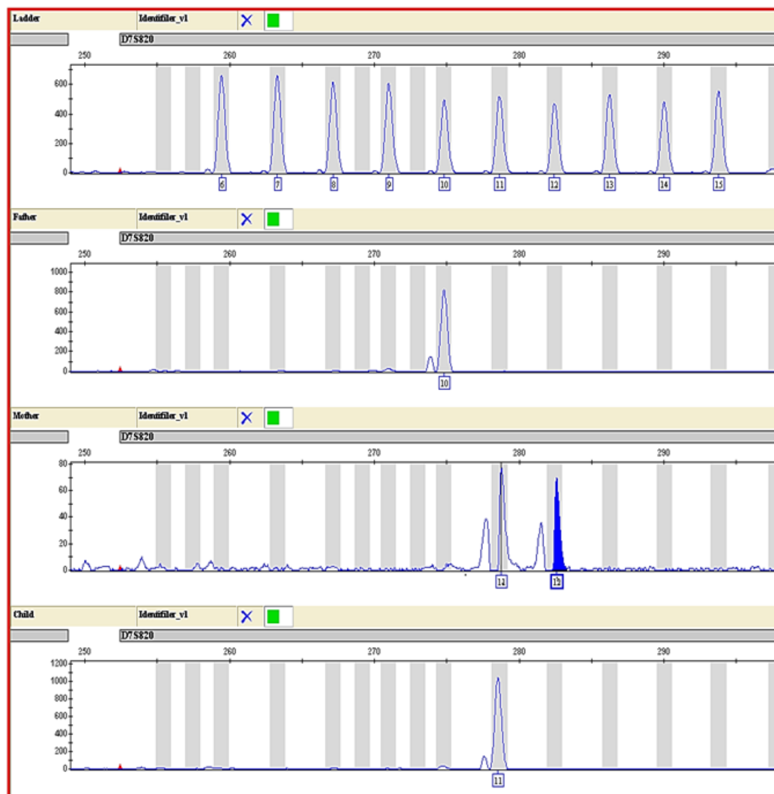

Fig. 1. Screenshots of genotype of the locus D7S820 of the Father, mother and child. Amplicons generated using Identifiler set of STR markers were analysed in ABI3130 Genetic Analyser and electropherograms of alleles were obtained using GeneMapper® ID Software v3.2. The genotyping results at D7S820 locus showing father homozygous for allele 10, mother heterozygous 11/12 and child homozygous for allele 11. Child sharing allele 11 only from his mother and father lack this allele in his profile at D7S820 locus.

Table 1. The DNA profiles of parents and child generated with 15 autosomal STR Identifiler™ markers. Only for the marker, D7S820, no match was observed between the suspected father's allele with the child (Highlighted as red).

STR Panel	Marker	Dye	Father		Mother		Child	
			Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
Identifiler_v1	D8S1179	B	11	11	10	16	11	16
Identifiler_v1	D21S11	B	31.2	31.2	27	31.2	31.2	31.2
Identifiler_v1	**D7S820**	**B**	**10**	**10**	**11**	**12**	**11**	**11**
Identifiler_v1	CSF1PO	B	10	13	11	12	10	12
Identifiler_v1	D3S1358	G	16	17	16	17	16	17
Identifiler_v1	TH01	G	7	9	8	9.3	7	8
Identifiler_v1	D13S317	G	8	11	11	12	11	11
Identifiler_v1	D16S539	G	12	13	11	12	12	12
Identifiler_v1	D2S1338	G	19	20	18	25	19	25
Identifiler_v1	D19S433	Y	14	15	12	13	12	14
Identifiler_v1	vWA	Y	18	18	15	15	15	18
Identifiler_v1	TPOX	Y	8	8	8	11	8	11
Identifiler_v1	D18S51	Y	15	16	14	16	15	16
Identifiler_v1	AMEL	R	X	Y	X	X	X	Y
Identifiler_v1	D5S818	R	10	12	11	12	10	11
Identifiler_v1	FGA	R	24	24	21	23	23	24

region of DNA, and the STR alleles may change over time. Each STR locus currently possesses known alleles derived from prior individuals [19]. For instance, a study by Youngest *et al*. [20] found that all paternal fragments from the child were identical to those of the alleged father, except for one locus, CSF1PO, which exhibited a mutation. While mutations in the STR locus can lower the paternity index, it may still be concluded that the child is indeed the biological child of the alleged father [20]. Similarly, a mismatch was reported at the locus D13S317 in a study conducted by Singh *et al.* [21]. The estimated combined paternity index (CPI) and probability of paternity (W) for 15 loci after including mutation in the calculation were 7.6×10^{7} and 0.9998. Our results indicate that there was a strong likelihood that the suspected father was also a biological father. The CPI and W values in this instance were so high that additional analysis was not necessary.

We conducted a further examination of the DNA from both the infant and the father, focusing on 16 Y chromosomal STR markers specific to the male lineage. The profiles revealed that all 16 Y chromosomal locations of the child matched those of the father (Table 2). It is important to note that the Y chromosome is passed down paternally without recombination, which raises the possibility that other males on the paternal side, such as the grandfather, uncles, and their male descendants, could share a similar Y-STR profile with the child. Nonetheless, this possibility was ruled out after thorough interviews with family members and the child's mother. The findings from the DNA profiling and additional evidence strongly indicate that the mismatch of paternal alleles at the D7S820 locus likely stems from a mutation event. Somatic mutations can occur at STR loci used in forensic analysis, and it is conceivable that the DNA profile derived from a buccal swab may differ from that obtained from a hair or blood sample. If such a mutation takes place early in the embryo's development, it is more likely to be uniformly present across all tissues. To further eliminate the possibility of somatic cell mutation, we also analyzed blood samples for the same set of autosomal and Y-STR markers. The results consistently aligned with those obtained from the buccal swab samples.

The alleles of the mother, child, and suspected father were directly sequenced to further characterize the D7S820 mutant allele. The suspicious father

Table 2. A comparison of Y-STR profiles between the alleged father and son reveals complete similarity at every analyzed locus.

STR Panel	Marker	Dye	Father		Child	
			Allele 1	Allele 2	Allele 1	Allele 2
Yfiler_v2	B_DYS456	B	15		15	
Yfiler_v2	B_DYS389I	B	14		14	
Yfiler_v2	B_DYS390	B	22		22	
Yfiler_v2	B_DYS389II	B	30		30	
Yfiler_v2	G_DYS458	G	15		15	
Yfiler_v2	G_DYS19	G	14		14	
Yfiler_v2	G_DYS385	G	14	18	14	18
Yfiler_v2	Y_DYS393	Y	14		14	
Yfiler_v2	Y_DYS391	Y	10		10	
Yfiler_v2	Y_DYS439	Y	10		10	
Yfiler_v2	Y_DYS635	Y	25		25	
Yfiler_v2	Y_DYS392	Y	10		10	
Yfiler_v2	R_Y_GATA_H4	R	12		12	
Yfiler_v2	R_DYS437	R	16		16	
Yfiler_v2	R_DYS438	R	11		11	
Yfiler_v2	R_DYS448	R	19		19	

only had 10 GATA repeats, the mother had 11 and 12 GATA repeats, and the child had 11 GATA repeats in homozygosity (Figure 2). Results show a repeated motif (GATA) mutation, which may have happened in the paternal germ cells. As was previously mentioned, STRs are generally subject to mutations, with replication slippage being the primary cause of these mutations [5, 9, 12, 22, 23]. The average autosomal STR mutation rate is thought to be less than 0.1%, and it would take about 1000 instances of parent-offspring allele transmission to detect one mutation in STR markers. The average mutation rate ranges from 0.0-0.7% in commonly used STR markers and the rate of mutation has been observed four times high in tetra-nucleotide repeats than the dinucleotide repeats [23]. The D7S820 STR marker is located on chromosome 7q21.11. The core repeat unit is GATA and the number of alleles observed at this locus ranges from allele number 5 to 16, with a mutation rate

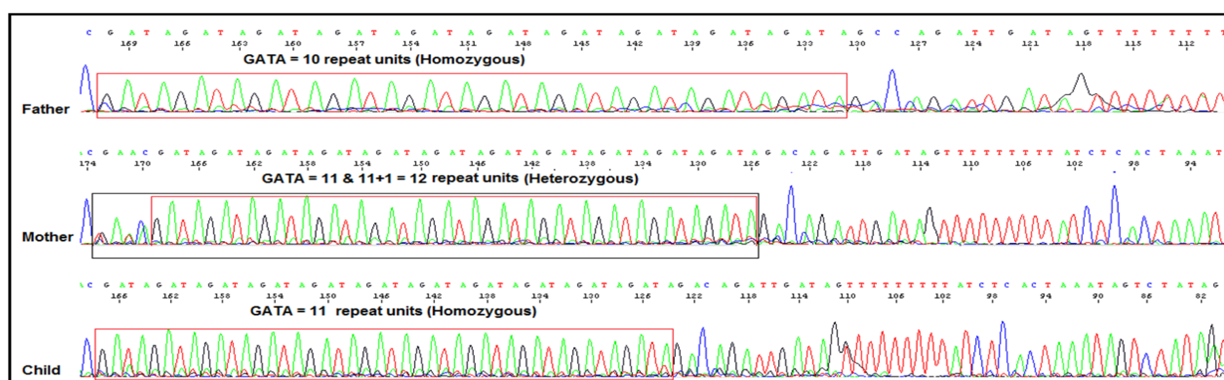

Fig. 2. DNA sequence electropherogram of the D7S820 locus. The top panel showing the DNA sequence obtained from father's DNA. The DNA sequencing results, indicating the presence of only 10 GATA repeats (allele 10) in father. The middle panel showing DNA sequence obtained from the Mother. The mother's first GATA repeats showing mixed peaks and rest of the 11 GATA repeats are homozygous indicating the DNA sequences of allele 12 and 11 respectively. The lower panel shows a DNA sequence of the child with 11 GATA repeat units (homozygous for allele 11). It indicates that the normal allele 11 of child is inherited from the mother and father's allele get mutated and loosed a repeat unit during gamete formation.

of 0.1%. In this particular case of paternity, there is a possibility of one mutation event for the allele mismatch at the D7S820 locus. The allele 10 of the father is expanded by a complete GATA repeat unit and transmitted to the child as allele 11.

Another explanation would be the presence of a null allele in the father or child. A null allele can be produced via a primer binding site mutation, which prevents the amplification of the original allele. An individual would type as a homozygote if they are heterozygous and have a primer binding site mutation for one of the alleles. Null alleles, which are brought on by mutations in the primer binding site, might cause discrepant DNA types at a certain locus when comparing DNA typing results from various kits. Although null alleles are uncommon, it's crucial to realise that they must be taken into account when interpreting prospective matches. Using an unlabeled primer pair from a separate kit, D7S820 was amplified and sequenced to further rule out the possibility of a null allele (Research Genetics MapPair kit, ver 8.0).

4. CONCLUSIONS

The study indicates a genetic mismatch involving the D7S820 locus between the child and the disputed father. Specifically, the alleged father is homozygous for allele 10, while the mother exhibits a heterozygous profile with alleles 11 and 12. In contrast, the child is homozygous for allele 11. This mismatch may be attributed to a potential mutation, as STRs are known to be susceptible to such genetic changes over time.

5. ETHICAL STATEMENT

The study was approved by the ethical committee of Institute of Biomedical and Genetic Engineering (IBGE), Islamabad, Pakistan, and Quaid-i-Azam University, Islamabad, Pakistan.

6. ACKNOWLEDGMENTS

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