Austin Journal of Forensic Science and Criminology

Open Access

Austin Publishing Group

Case Report

The Importance of Y DNA Profiling in Detecting Uniparental Maternal Heterodisomy at Locus D8S1179 in Case of Paternity Testing

Anshu Priya[1*]; Nisha Bara[2]; Aparajita Neena Soren[2]

[1]Forensic Finger Printing Lab, Centre for Advanced Research, KGMU Lucknow, India

[2]Directorate of Forensic Science Laboratory, Ranchi, India

***Corresponding author:** **Anshu Priya**

Assistant Professor, Forensic Finger Printing Lab, Centre for Advanced Research, KGMU Lucknow, Uttar Pradesh-226003, India.

Email: anshupriya@kgmcindia.edu

Received: May 29, 2024

Accepted: June 25, 2024

Published: July 02, 2024

Abstract

An unusual genetic disorder called uniparental maternal diasomy may disclose specific challenges in paternity testing, sometimes resulting in erroneous interpretations of familial relationships. We analysed a three-person family (the disputed child, the alleged father, and the mother) for purposes of disputed paternity testing by utilising autosomal short tandem repeat markers (15 STR loci) and a gender locus using the AmpFlSTR®Identifiler®PCR amplification kit. We discovered that the chromosome 8 marker at locus D8S1179 wasn't passed down between the alleged father and the disputed son in a mendelian fashion. However, each of the relevant locations included the maternal allele. The allelic distribution of this locus in the alleged father, disputed child and mother was 10/16, 13/17, and 13/17 respectively. This situation can potentially complicate the interpretation of paternity testing results using this specific STR marker. Y-DNA profiling focuses on the Y chromosome, which is inherited exclusively from the father to the son and we observed the same haplotype was inherited in the son. This case study explores the importance of utilizing Y DNA profiling to accurately detect and differentiate uniparental maternal heterodisomy at the D8S1179 locus during paternity testing. By employing Y DNA profiling techniques alongside traditional short tandem repeat (STR) markers, the research highlights the distinct advantages of incorporating Y-chromosomal data in such scenarios. Here the Y DNA profiling not only provides a robust tool for distinguishing uniparental maternal heterodisomy but also offers a more comprehensive understanding of the paternal lineage. To the best of our knowledge, this is the first-time maternally transmitted heterodisomy at this particular locus during paternity testing has been documented.

Keywords: Paternity testing; Short tandem repeat profiling; Uniparental heterodisomy

Introduction

A paternity test is a genetic examination done to determine who the biological father of a child. It entails examining certain genetic markers (such STRs) between the child, the alleged father, and the mother (if available) in order to assess the likelihood of a biological tie except for the sex chromosomes, a person normally inherits one copy of each chromosome from their mother and the other copy from their father. A person with "uniparental disomy," a genetic condition, they have two copies of one chromosome instead of one from each parent. Paternity testing may be made more challenging by uniparental disomy because the child may get both alleles of a STR marker from one parent, giving the impression that the child is unrelated.

Uniparental disomy, or inheriting both homologous chromosomes from one parent and none from the other, was first conceptualised by Engel E (1980) [1]. After seven years, Creau-Goldberg et al. (1987) pioneered the use of molecular methods to validate maternal UPD which can come from the mother (UPD mat) or the father (UPD pat) [2]. There are two further classifications for UPD: isodisomy and heterodisomy. In heterodisomy, one parent contributes two distinct alleles, whereas in isodisomy, one parent contributes two identical copies of a single allele. Important new information about UPD as a mechanism causing a variety of human genetic illnesses was revealed by Spence et al. (1991) [3]. It mostly results from mistakes made during cell division in either meiosis or mitosis, the processes that create gametes like sperm and eggs.

Citation: Priya A, Bara N, Soren AN. The Importance of Y DNA Profiling in Detecting Uniparental Maternal Heterodisomy at Locus D8S1179 in Case of Paternity Testing. Austin J Forensic Sci Criminol. 2024; 10(2): 1100.

Depending on which chromosomes are involved and which genes are impacted, it may result in different genetic illnesses. The results that were provided are related to three individuals' genetic investigations that were done for contested paternity testing. One of the 15 STR-type loci that were analysed showed a deviation from inheritance according to Mendel's Law. Laboratories that conduct paternity testing using STR analysis are generally aware of the possibility of uniparental disomy, however, it's important to note that uniparental disomy is relatively rare, and in most cases, traditional STR analysis can accurately determine paternity. However, if the child is male and there is genetic inconsistency at any autosomal genetic marker locus, Y DNA profiling is significant and should be used in conjunction with traditional STR analysis, particularly in situations where uniparental maternal heterodisomy might otherwise produce unclear or incorrect results. This case study emphasises the significance of a multidimensional approach in paternity testing, stressing the requirement to take into account both Y-chromosomal markers and autosomal markers to provide the highest level of accuracy and reliability in establishing family links.

Case Presentation

Here we present the case which had received in the forensic science laboratory to establish the paternity testing. Here a man is undergoing paternity testing to determine is he is the biological father of a disputed male child or not. In order to establish paternity, samples from the mother (source-blood sample on gauze piece), the disputed child (source-blood sample on gauze piece), and the alleged father (source-blood sample on gauze piece) were then analysed using an Identifiler plus kit in accordance with standard laboratory procedures.

Materials and Methods

Sample Collection

Blood samples on the gauze piece of the alleged father (exhibit marked-A), mother (exhibit marked-B) and disputed male child (exhibit marked-C) were received at the forensic science laboratory, Ranchi, Jharkhand, India to establish the paternity of the disputed child.

DNA Extraction

DNA were extracted from all the exhibits such as the alleged father (exhibit marked-A), mother (exhibit-marked-B), and disputed child (exhibit-marked-C) by using 400 µl of extraction buffer (10 mM Tris-Cl, pH 8.0, 0.1M EDTA, pH 8.0, 20 µg/ml RNase A, 0.5% SDS) having Proteinase K and the sample was kept for at least three hours at 56°C under shaking water bath. Then centrifuged the samples and supernatant was taken in which the equal volume of Tris-equilibrated phenol (pH 8.0) was added and mix the ingredients. After mixing, the tube was centrifuged at 5000 X g for 10 minutes at ambient temperature. The supernatant was then transferred to a fresh tube, and DNA was precipitated by adding 2X volume of 100% ethanol, centrifuging the tubes, and keeping them at -20°C for 30 minutes. After a 70% ethanol wash, the resulting pellet was permitted to air dry. The DNA pellet was reconstituted in the required amount of TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0), and it was subsequently stored at 4°C.

DNA Quantification

The DNA was quantified by means of the Quantifiler® Human DNA Quantification kit (Life Technologies Inc.) employing Real-Time Polymerase Chain Reaction (RT-PCR) [4]. A DNA standard solution (200 ng/µl), Quantifiler PCR Reaction Mix, and Quantifiler Human Primer mix were included. These were combined, and 10.5 µl/sample human primer mix and 12.5 µl/sample PCR reaction mix were then dispensed into 96 well plates (23 µl each). Two microliters of the sample or standard DNA of known concentration were added to each well, resulting in a 25 µl PCR reaction mixture. The content of DNA was estimated by the real-time PCR machine (Applied Biosystems).

Autosomal STR Amplification

The Identifiler Plus® kit (Applied Biosystem, USA) [5] is used to amplify for 15 autosomal STR loci such as D8S1179, D21S11, D7S820, CSF1PO, THO1, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA, and Amelogenin a gender locus for each sample 15 µl of PCR reaction mix, 10 µl of Identifiler Plus® Primer Set and 1 µl of DNA template was used.

Y-STR Amplification

To genotype the sample from alleged father's and the questioned child AmpFlSTR®Yfiler™ PCR amplification kit was used (Applied Biosystem, USA) according to the manufacturer user's instruction, Briefly, the Y filer enables the simultaneous amplification of 17 loci on the Y chromosome namely DYS456, DYS389I, DYS390, DYS389II, DYS458, DYS19, DYS385a/b, DYS393, DYS391, DYS439, DYS635, DYS392, Y GATA H4, DYS437, DYS438 and DYS448.

DNA Profiling

1 µl of the PCR product or the AmpFlSTR® Identifiler Plus® and AmpFlSTR®Yfiler TM allelic ladder, 0.3 µl of Gene Scan TM 500 LIZ®, a size standard dye, and 8.7 µl of Hi-Di TM formamide using POP-4 polymer by ABI-3130 Genetic Analyzer (Applied Biosystems) were used to evaluate the PCR product using electrophoresis equipment.

Data Analysis

Data were analysed in respect to the Gene Scan™ 500 LIZ® Size Standard using Gene Mapper ID software version 3.2.

Results and Discussion

The results of the aforementioned paternity test, which was conducted using 15 standard STR markers from a commercially available kit using the AmpFlSTR Identifiler Plus kit and the findings revealed that the mother, the disputed child, and the alleged father had all well genotyped STR markers; however, the D8S1179 locus did not follow Mendelian inheritance, indicating that the father and the disputed child did not have a biological relationship (Figure 1 & Table 1).The disputed child's genotype was 13/17, but the mothers and alleged fathers were 13/17 and 10/16, respectively (Figure 2). These findings suggested that the uniparental disomy occurred in a certain area of chromosome 8, and they showed that the child might inherit both alleles from her mother at the D18S1179 locus (Figure 3). As far as we are aware, this is the first time maternal uniparental heterodisomy for paternity testing-related human DNA identification has been found. In our earlier study we also noticed the Maternal Uniparental Isodisomy at Locus D13S317 during a paternity testing [6]. However, LOH or UPD were more prevalent in cancer cells than in healthy cells [7-10]. The modification of genomic imprinting conditions is one potential biological mechanism for illnesses caused by UPD or LOH. DNA methylation and other imprinting information, as well as both homologs, are inherited from the same parent in children with LOH or UPD. For

Table 1: Comparative chart of allele distribution (genotype) of 15 different loci of the autosomal DNA tested. * Both alleles of child inherited from the mother.

S.No	Loci	Alleged Father (A)	Mother (B)	Disputed son (C)
1.	**D8S1179**	**10, 16**	**13*, 17***	**13*, 17***
2.	D21S11	30, 33.2	32.2, 33.2	30, 33.2
3.	D7S820	10, 10	8, 12	10, 12
4.	CSF1PO	12, 13	10, 10	10, 12
5.	D3S1358	16, 18	15, 15	15, 18
6.	THO1	6, 9	6, 9.3	6, 6
7.	D13S317	10, 13	9, 12	12, 13
8.	D16S539	12, 13	9, 11	9, 12
9.	D2S1338	22, 23	18, 23	23, 23
10.	D19S433	12, 15	13, 13.2	13.2, 15
11.	vWA	17, 18	14, 17	14, 18
12.	TPOX	8, 11	9, 11	11, 11
13.	D18S51	18, 23	16, 17	16, 23
14.	D5S818	11, 12	9, 12	11, 12
15.	FGA	22, 23	23, 24	23, 24
16.	Amelo-genin	X, Y	X, X	X, Y

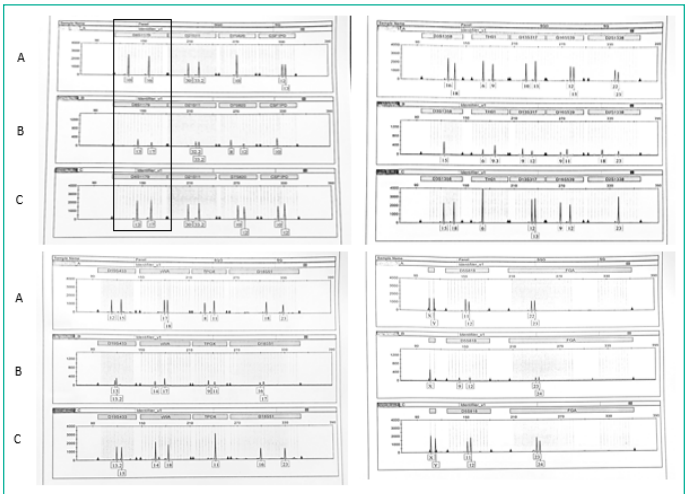

Figure 1: Electropherogram of genotypes of the alleged father (A), mother (B) and the disputed son (C) at 15 different loci of the autosomal DNA.

imprinted genes and illnesses with identifiable clinical features, this may lead to functional nullisomy [11]. It was noted that in Prader-Willi/Angelman syndrome, LOH or UPD changed the genomic imprinting condition [12]. Beckwith-Wiedemann syndrome [13] and transient neonatal diabetes mellitus. Present study suggests the Uniparental maternal heterodisomy at locus D8S1179 in which the both copies of the chromosome containing the locus D8S1179 marker were inherited from the mother, and these copies have different alleles due to heterodisomy. This situation can potentially complicate the interpretation of paternity testing results using this specific STR marker. Additionally, since the child was a boy, we opted to investigate the Y chromosomal markers of the alleged father's STR profile and the disputed child, all of which produced results that were consistent with the alleged father's paternity (Figure 4 & Table 2). Y-DNA profiling focuses on the Y chromosome, which is inherited exclusively from the father to the son. Since females don't have a Y chromosome, Y-DNA is only relevant in male inheritance. Y-DNA testing can provide information about the paternal lineage, including information about direct paternal ancestry and the transmission of specific Y chromosome markers across generations. The case study discussed here highlights the significance of Y DNA profiling in paternity testing, particularly in situations involving uniparental maternal heterodisomy at locus D8S1179. While traditional STR analysis may suggest a lack of paternity due to a mismatch at this locus, Y DNA profiling can provide crucial evidence by confirming the paternal relationship

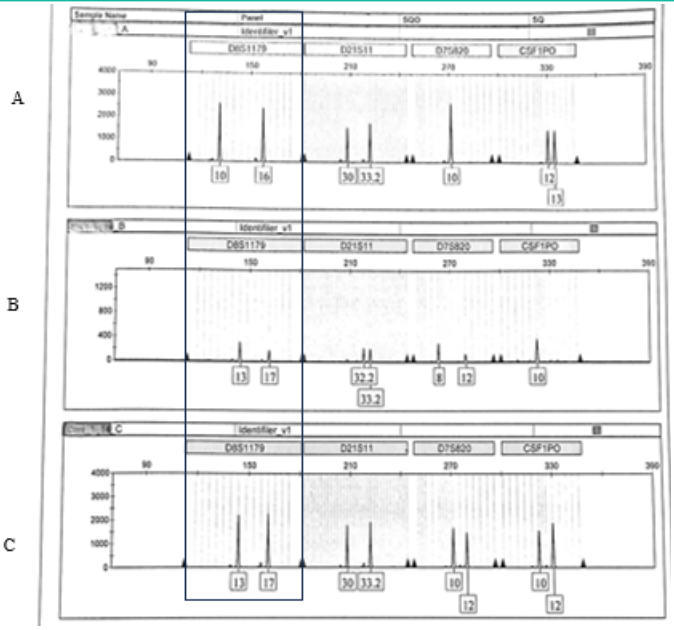

Figure 2: Electropherogram of genotypes of the alleged father (A), mother (B) and the disputed son (C) at test locus D8S1179.

Table 2: Comparative chart of allele distribution (genotype) of different loci of the 17 Y-STR Markers of father (A) and the disputed son (C).

S. No	Y FILER LOCI	Alleged Father (A)	Disputed son (C)
1.	DYS456	13	13
2.	DYS389I	13	13
3.	DYS390	24	24
4.	DYS389II	29	29
5.	DYS458	16	16
6.	DYS19	15	15
7.	DYS385a/b	13, 17	13, 17
8.	DYS393	13	13
9.	DYS391	11	11
10.	DYS439	12	12
11.	DYS635	21	21
12.	DYS392	11	11
13.	Y GATA H4	11	11
14.	DYS437	15	15
15.	DYS438	9	9
16.	DYS448	18	18

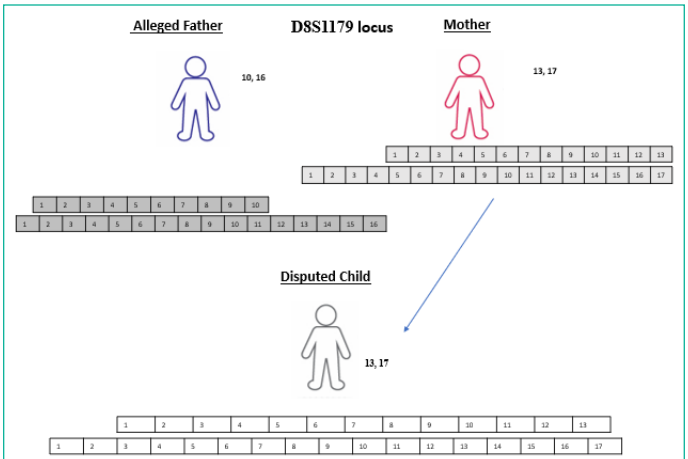

Figure 3: Pictorial representation of maternal UPD at locus D8S1179.

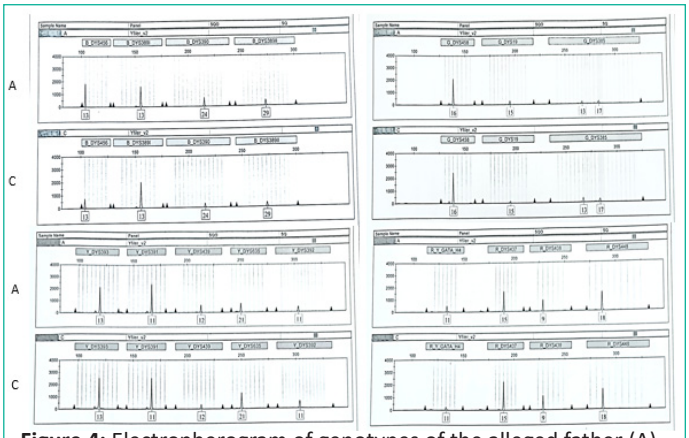

Figure 4: Electropherogram of genotypes of the alleged father (A) and disputed son (C) by 17 Y- STR Markers.

through the Y chromosome, which is not affected by maternal heterodisomy. This demonstrates the importance of incorporating advanced genetic techniques and considering the complexities of inheritance patterns to achieve more accurate and just results in paternity testing cases. It's important to note that the interpretation of genetic testing results, especially in complex cases like uniparental disomy, requires a deep understanding of genetics and access to up-to-date information. In the context of uniparental maternal heterodisomy at locus D8S1179, the implication is that the child has inherited both the allele of the D8S1179 STR locus from their mother, but the other allele is not inherited from their alleged father. This situation could be due to various genetic factors, such as mutations or chromosomal abnormalities, which can lead to such discrepancies in the genetic profile. The identification of such a scenario through paternity testing using STRs suggests that the genetic markers used in the testing process have revealed an inconsistency that might require further investigation or clarification to understand the genetic relationship between the individuals involved.

Conclusion

A key improvement in paternity testing is Uniparental Disomy (UPD) analysis, which offers improved accuracy in resolving complicated cases and offers a more thorough insight of a person's genetic ancestry. UPD analysis is a useful technique in the pursuit of precise and trustworthy paternity conclusions as genetic testing progresses. The efficiency of UPD analysis in difficult paternity situations is one of its most important advantages. While STR markers can help establish parent-child relationships and distinguish alleles inherited from different parents, they might not provide the detailed information. Detecting UPD often involves techniques like Single Nucleotide Polymorphism (SNP) arrays, Fluorescence In Situ Hybridization (FISH), and other advanced molecular methods that can directly assess the parental origin of the chromosomal material. In conclusion, the integration of Y DNA profiling into paternity testing protocols can significantly enhance the precision of results, particularly in cases involving uniparental diasomy. This research contributes to a broader understanding of the diagnostic potential of genetic profiling techniques and their critical role in resolving complex paternity disputes with confidence and clarity. Our study suggests that WGS, enhance the accuracy and reliability of parentage testing, can provide a powerful method to detect an UPD.

References

1. E Engel. A new genetic concept: uniparental disomy and its potential effect, isodisomy. Am J Med Genet. 1980; 6 :132–143.

2. Créau-Goldberg N, Gegonne A, Delabar J, Cochet C, Cabanis MO, Stehelin D, Turleau C, de Grouchy J: Maternal origin of a de novo balanced t(21q21q) identified by ets-2 polymorphism. Hum Genet. 1987; 76: 396–398.

3. Spence JE, Perciaccante RG, Greig GM, Willard HF, Ledbetter DH, Hejtmancik JF, et al. Uniparental disomy as a mechanism for human genetic disease. Am J Hum Genet. 1988;42: 217–226.

4. Robert L Green, Ines C Roinestad, Cherisse Boland, Lori K Hennessy. Developmental validation of the quantifiler real-time PCR kits for the quantification of human nuclear DNA samples. J Forensic Sci. 2005; 50: 809-25.

5. Qiu-Ling Liu 1, De-Jian Lu, Li Quan, Ye-Fei Chen, Min Shen, Hu Zhao. Development of multiplex PCR system with 15 X-STR loci and genetic analysis in three nationality populations from China Electrophoresis. 2012; 33: 1299-305.

6. Priya A, Rana AK, Kumar A, Bara N, Soren AN. Genetic Incompatibility between Father and Child Due to Maternal Uniparental Isodisomy at Locus D13S317 in A Paternity Testing: A Case Report. J Forensic Leg Investig Sci. 2023; 9: 074.

7. B Mccue, J Wisecarver, K Shepard, K Duffy, R Rubocki, S Shepherd. Loss of Heterozygosity Detected in a Short Tandem Repeat (STR) Locus Commonly Used for Human DNA Identification. 2000.

8. L Krskovahonzatkova, J Cermak, J Sajdova, J Stary, P Sedlacek, Z Sieglova. Loss of heterozygosity and heterogeneity of its appearance and persisting in the course of acute myeloid leukemia and myelodysplastic syndromes. Leuk Res. 2001; 25: 45–53.

9. J Edelmann, R Lessig, S Hering, L Horn. Loss of heterozygosity and microsatellite instability of forensically used STR markers in human cervical carcinoma. Int Congr Ser. 2004; 1261: 499–501.

10. J Powierskaczarny, D Mis´cickas´liwka, J Czarny, T Grzybowski, M Woz´niak, G Drewa, et al. Analysis of microsatellite instability and loss of heterozygosity in breast cancer with the use of a well characterized multiplex system. Acta Biochim Pol. 2003; 50: 1195–1203.

11. R Joshi, P Garg, N Zaitlen, T Lappalainen, C Watson, N Azam, et al. DNA methylation profiling of uniparental disomy subjects provides a map of parental epigenetic bias in the human genome. Am J Hum Genet. 2016; 99: 555–566.

12. B Horsthemke, J Wagstaff. Mechanisms of imprinting of the Prader-Willi/ Angelman region. Am J Med Genet. 2008; 146A: 2041–2052.

13. BA Market-Velker, L Zhang, LS Magri, AC Bonvissuto, MRW Mann. Dual effects of superovulation: loss of maternal and paternal imprinted methylation in a dose-dependent manner. Hum Mol Genet. 2010; 19: 36–51.