Forensic Sciences Research, 2024, Vol. 9, Issue 2
https://doi.org/10.1093/fsr/owae027
Case Report

Case reports on uniparental disomy of chromosomes 6 and 3 in paternity testing

He Ren[1,2,†], Zhiyong Liu[3,†], Chong Chen[4], Yan Shi[4], Jiarong Zhang[5], Ying Chen[6], Li Jia[4], Yacheng Liu[4], Jiangwei Yan[5,*]

[1]Shanghai Key Lab of Forensic Medicine, Key Lab of Forensic Science, Ministry of Justice, Academy of Forensic Science, Shanghai, China
[2]Beijing Police College, Beijing, China
[3]Faculty of Forensic Medicine, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, China
[4]Beijing Tongda Shoucheng Institute of Forensic Science, Beijing, China
[5]School of Forensic Medicine, Shanxi Medical University, Jinzhong, China
[6]Fangshan Branch of Beijing Public Security Bureau, Beijing, China

*Corresponding author. E-mail: yanjw@sxmu.edu.cn

†He Ren and Zhiyong Liu contributed equally to this work.

Abstract

In paternity testing, when there are Mendelian errors in the alleles between the child and the parents, a slippage mutation, or silent allele may not fully explain the phenomenon. Sometimes, it is attributed to chromosomal abnormalities, such as uniparental disomy (UPD). Here, we present the investigation of two cases of suspected UPD in paternity testing based on short tandem repeat (STR) detection (capillary electrophoresis platform). Case 1 involves a trio, where all genotypes detected on chromosome 6 in the child are homozygous and found in the father. Case 2 is a duo (mother and child), where all genotypes on chromosome 3 in the child are homozygous and not always found in the mother. At the same time, Mendelian error alleles were also observed at specific loci in these two chromosomes. Furthermore, we used the MGIEasy Signature Identification Library Prep Kit for sequencing on the massively parallel sequencing platform, which included common autosomal, X and Y chromosomes, and mitochondrial genetic markers used in forensic practice. The results showed that the genotypes of shared STRs on the two platforms were consistent, and STRs and single nucleotide polymorphisms (SNPs) on these two chromosomes were homozygous. All other genetic markers followed the laws of inheritance. A comprehensive analysis supported the parent–child relationship between the child and the alleged parent, and the observed genetic anomalies can be attributed to UPD. UPD occurrences are rare, and ignoring its presence can lead to erroneous exclusions in paternity testing, particularly when multiple loci on a chromosome exhibit homozygosity.

Keywords: forensic genetics; paternity testing; uniparental disomy; STR; SNP; MPS

Introduction

Uniparental disomy (UPD) refers to an entire or partial region of homologous chromosomes originating from a single parent rather than from both parents. This concept was first described by Engel in 1980 [1] and subsequently demonstrated as a mechanism for human genetic disease in 1988 [2]. Mechanisms for UPD formation include trisomy rescue, gamete complementation, postfertilization error, and monosomic rescue [3–7]. UPD can be classified as uniparental isodisomy (isoUPD, from a single chromosome of a single parent), uniparental heterodisomy (hetUPD, from both chromosomes of a single parent), and mixed UPD (both isodisomy and heterodisomy on the same chromosome), and based on the origin, it can be distinguished as maternal (matUPD) or paternal UPD (patUPD) [3–7]. In a study involving 4 million healthy individuals, the overall incidence of UPD was 1 in 2 000 births, with matUPD incidence higher than patUPD [4, 6, 8, 9]. Most UPDs are not disease-causing, but when pathology does occur, it is primarily due to disruption of genomic imprinting (https://www.geneimprint.com/site/genes-by-species) or by revealing harmful recessive alleles in substantial homozygous regions of the affected chromosome [8]. In addition, the American College of Medical Genetics and Genomics (ACMG) has established a technical standard for UPD diagnosis [10], and its interpretation has also been published [11]. Further resources on UPD can be found at the website https://cs-tl.de/DB/CA/UPD/0-Start.html [Liehr T. 2023. Cases with uniparental disomy].

Paternity testing is based on the law of Mendelian inheritance. When there are genotypic Mendelian errors in short tandem repeat (STR) markers that do not conform to inheritance laws, we always interpret them as slippage mutations or silent alleles rather than UPD, especially when only one marker on a chromosome is tested. In this study, we describe two cases of Mendelian errors in paternity testing. Using multiple kits on both the capillary electrophoresis (CE) and massively parallel sequencing (MPS) platforms, parent–child relationships were found between the child and the alleged parent, and UPD can explain the observed genetic anomalies in both cases.

Received: November 18, 2023. **Accepted:** April 9, 2024
© The Author(s) 2024. Published by OUP on behalf of the Academy of Forensic Science.
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (https://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

Materials and methods

Sample and DNA isolation

Blood samples from the two paternity testing cases were collected with written informed consent. Case 1 was the standard trio (mother, child, and alleged father), while Case 2 was a duo (alleged mother and child). In both cases, the children had normal phenotypes. We used the DNA IQ™ System (Promega, Madison, WI, USA) to extract DNA following the manufacturer's protocol. DNA was quantified using the Qubit® Quantitation System (Invitrogen, Carlsbad, CA, USA).

DNA typing

CE platform: for Case 1, PCR amplification of DNA was performed using the Goldeneye 20A (Goldeneye, Beijing, China) and AGCU21+1 (AGCU, Wuxi, China) kits. For Case 2, PCR amplification was performed using the PowerPlex® 21 (Promega), Microreader™ 23sp-B (Microread, Beijing, China), and Goldeneye 17X (Goldeneye) kits. Amplification products were subjected to CE using an ABI PRISM 3130XL genetic analyzer (Applied Biosystems, Waltham, MA, USA). MPS platform: in both cases, the MGIEasy Signature Identification Library Prep Kit (MGI Tech, Shenzhen, China) was used for library construction, which included 129 STRs (54 A-STRs, 27 X-STRs, and 48 Y-STRs), 215 single nucleotide polymorphisms (SNPs), and three mitochondrial hypervariable regions (HVR-I, II, and III). Sequencing was performed on the MGISEQ-2000RS sequencer (MGI Tech). All procedures were carried out according to the manufacturers' instructions.

Data analysis

For CE platform, allele typing was performed using GeneMapper ID-X software (https://www.thermofisher.cn/cn/zh/home/technical-resources/software-downloads/genemapper-id-x-software.html). For the MPS platform, the sequencing data were analysed using the sequencer's built-in typing software. Default thresholds were used in the analyses.

Results and discussion

Using the CE and MPS platforms, a total of 54 autosomal STRs (A-STRs) and 215 autosomal SNPs were genotyped in two cases. Genotyping results for shared loci were consistent between the two platforms. The genotypes of all loci on chromosomes 6 and 3 in Cases 1 and 2, respectively, were homozygous, with Mendelian errors observed at certain loci (highlighted in bold in Table 1), and the loci on other autosomal and X-chromosomes followed the inheritance law (data not shown). In Case 1, the child's mtDNA and Y-STR genotypes matched the mother and alleged father, respectively; in Case 2, the child's mtDNA genotype matched the alleged mother. Furthermore, the combined paternity index (CPI) was calculated based on the STR stepwise mutation model (for non-Mendelian loci), and the results for both cases [Case 1: $CPI = 7.3 \times 10^{21}$ (39 A-STRs); Case 2: $CPI = 6.11 \times 10^{6}$ (39 A-STRs)] supported the parent–child relationship between the child and the alleged parent. In general, this approach is quite conservative, as Cavalheiro et al. [3] suggest that it reduces the strength of the evidence. They propose considering the genetic markers on the UPD chromosome as a whole when calculating the PI value for similar cases. The specific method is to use the prevalence of UPD on the chromosome in the population as the numerator, and the product of the obligate paternal allele (OPA) frequencies for UPD chromosome markers as the denominator [3]. This is a valuable suggestion. Unfortunately, the prevalence of UPD on chromosomes 6 and 3 was not found, and we did not calculate the CPI referred to this method.

In detail, a total of 18 autosomal genetic markers (4 STRs and 14 SNPs) located on chromosomes 6 and 3 were analysed in Case 1 and Case 2, respectively (Table 1). These markers are distributed over almost entire chromosomes (Figure 1).

Table 1. Genotyping results of the genetic markers on chromosomes 6 and 3 based on capillary electrophoresis (CE) and massively parallel sequencing (MPS) platforms.

Marker	Case 1: chromosome 6				Marker	Case 2: chromosome 3		
	Location	Mother	Child (male)	Alleged father		Location	Alleged mother	Child (male)
D6S1043	6q15	19, 20	12	12, 18	**D3S1358**	3p21.31	16	17
D6S474	6q21	15	17	14, 17	**D3S1744**	3q24	19	18
D6S1017	6p21.1	10	10	9, 10	**D3S3045**	3q13.12	9	11
SE33	6q14	22	19	16, 19	**D3S4529**	3p12.1	15, 16	17
rs3823159	6q23.3	AA	GG	AG	rs12498138	3q13.33	GG	GG
rs192655	6q15	GA	GG	GA	rs1919550	3q13.33	AA	AA
rs13218440	6p24.2	GA	GG	GA	rs1357617	3p26.3	AT	AA
rs214955	6q25.2	CT	CC	CC	rs6444724	3q29	TC	CC
rs727811	6q27	TT	TT	TT	**rs1355366**	3q28	TT	CC
rs1336071	6q16.1	TT	CC	TC	**rs2399332**	3q13.13	TT	GG
rs12203592	6p25.3	CC	CC	CC	rs4364205	3p22.3	TG	TT
rs4959270	6p25.3	CC	CC	CA	rs14134	3q12.3	CC	CC
rs1337823	6p12.3	GG	AA	AA	**rs2293195**	3q25.31	GG	AA
rs1997660	6p22.1	GG	GG	AG	**rs7429010**	3q27.1	AA	GG
rs2274212	6p24.3	TT	CC	CT	rs9821880	3q28	TT	TT
rs574202	6p12.1	AG	GG	AG	rs11714239	3p26.3	GT	TT
rs6909306	6p21.1	CC	CC	CC	rs472728	3q26.31	GA	AA
rs7741536	6q14.1	AG	GG	AG	**rs4076086**	3p22.3	TT	CC

The bold represents markers that do not conform to the inheritance law.

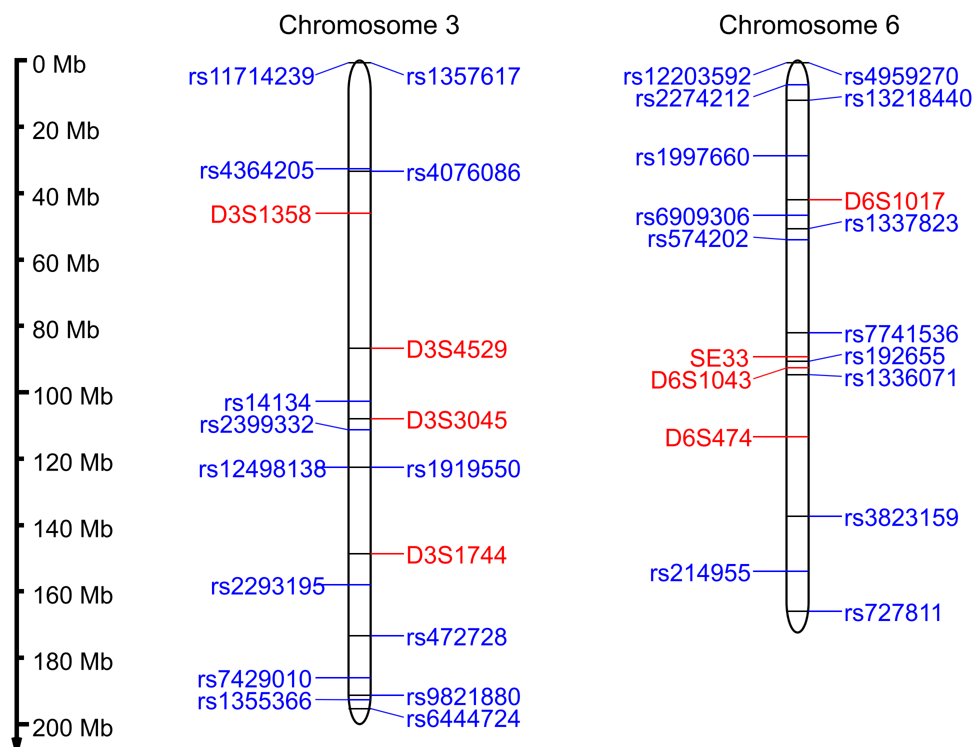

Figure 1 The positions of the genetic markers on chromosomes 6 and 3. Red: short tandem repeat marks; blue: single nucleotide polymorphism marks.

In Case 1, the Mendelian errors were observed in 3 STRs and 4 SNPs between the child and the mother; whereas in Case 2, there were 4 STRs and 5 SNPs (highlighted in bold in Table 1). Generally, when these allelic Mendelian errors are observed, STR slippage mutations, allele silencing, or point mutations are considered first. However, it is evident that the Mendelian errors we observed cannot be explained by the above reasons. As shown in Table 1 and Figure 1, the Mendelian errors occurred only on one specific chromosome, and all genetic markers along the entire chromosome show a homozygous state. Mendelian errors were not found on other chromosomes; in other words, genotyping errors or parental consanguinity were ruled out. Overall, this strongly suggests a complete isodisomy UPD (isoUPD) event, the UPD chromosome being of paternal origin. We find that certain markers on the affected chromosome show expected inheritance patterns (Table 1, including 11 markers on chromosome 6 in Case 1 and 9 markers on chromosome 3 in Case 2), especially most of the SNP markers (biallelic markers with lower discrimination power). Although this does not explicitly support the occurrence of UPD, it is compatible with the UPD hypothesis. In conclusion, UPD can explain the observed genetic anomalies in both cases, with the most likely mechanism being the failure of sister chromatid separation during the meiosis II of the paternal sperm, followed by trisomy rescue resulting in the loss of the maternal chromosome [4].

UPD is observed on all chromosomes [8]. Cases of UPD in paternity testing are relatively rare, with only isolated reports to date, such as chromosome 2 [12–14], chromosome 4 [15], and chromosome 21 [3]. These reports typically involve individuals with a single UPD chromosome, and in rarer cases, multiple UPD chromosomes [16]. In practice, UPD may be underestimated in paternity testing due to scenarios in which parents and children share alleles, single-marker detection on the chromosome, consanguinity between parents (resulting in suspected "UPD" on multiple chromosomes), segmental UPD (affecting only a specific region on a chromosome) [17], etc. When such scenarios are possible, it is necessary to use more kits or perform whole genome sequencing for verification. The ACMG's guidelines [10] recommended using at least two STR loci for UPD diagnosis. However, in the two cases presented in this study, we used four STR loci for UPD diagnosis, exceeding the number required by the ACMG. It is worth noting that this standard has certain limitations; e.g. the number of specified detection loci may be insufficient, leading to potential diagnostic errors in cases involving somatic mosaicism or segmental UPD. In addition, the standard lacks specific criteria regarding the polymorphism (such as heterozygosity) and distribution across chromosomes for the STR loci used in UPD detection. UPD should attract the attention of forensic practitioners. It is conceivable that failure to recognize the allelic patterns indicative of UPD in practice could lead to various issues, such as erroneous exclusion in paternity testing, reduced success rates in missing person database searches, and interference with IBD analysis in forensic genetic genealogy (FGG). Therefore, the presence of UPD should be considered when encountering atypical genotyping in forensic practice.

Conclusion

Here, we report two cases of UPD events occurring on chromosomes 6 and 3 in paternity testing. By testing with multiple kits on both the CE and MPS platforms, the results showed that the genetic markers from the affected chromosome exhibit a homozygous state. Considering the distribution of the detected markers across the entire chromosome and the parental single-chromosome origin, we conclude that both cases represent complete isodisomy UPD. Recent studies suggest that the prevalence of UPD may be comparable to the mutation rate of STRs [3]. In paternity testing, we should pay special attention to UPD, especially when the homozygous loci are all located on the same chromosome.

Acknowledgements

Thanks to Dr. Man Chen from the School of Forensic Medicine, Southern Medical University, for providing suggestions and revisions to this article. Thanks to Wei Peng, Bo Zhang, and Haofang Mu from the Center of Forensic Sciences, Beijing Genomics Institute for their assistance with massively parallel sequencing.

Authors' contributions

Jiangwei Yan conceived and designed this study. He Ren and Zhiyong Liu conceived the experiments and wrote the manuscript. Chong Chen and Yan Shi collected the samples. Jiarong Zhang, Ying Chen and Li Jia extracted DNA and helped to conduct the statistical analysis. Yacheng Liu and Jiangwei Yan revised the manuscript. All authors contributed to the final text and approved it.

Compliance with ethical standards

This study was performed in accordance with the principles of the Declaration of Helsinki and approved by the Ethics Committees of Shanxi Medical University (No.2020GLLO31). Written informed consent was obtained from all the participants.

Disclosure statement

None declared.

Funding

This work was supported by the grants from the Open-Fund of Shanghai Key Lab of Forensic Medicine, Key Lab of Forensic Science, Ministry of Justice, China [grant number KF202011] and National Natural Science Foundation of China [grant number 82030058].

References

1. Engel E. A new genetic concept: uniparental disomy and its potential effect, isodisomy. Am J Med Genet. 1980;6:137–143.
2. Spence JE, Perciaccante RG, Greig GM, et al. Uniparental disomy as a mechanism for human genetic disease. Am J Hum Genet. 1988;42:217–226.
3. Cavalheiro C, Avila E, Gastaldo AZ, et al. Uniparental disomy of chromosome 21: a statistical approach and application in paternity tests. Forensic Sci Int Genet. 2020;49:102368.
4. Benn P. Uniparental disomy: origin, frequency, and clinical significance. Prenat Diagn. 2021;41:564–572.
5. Yamazawa K, Ogata T, Ferguson-Smith AC. Uniparental disomy and human disease: an overview. Am J Med Genet Part C Semin Med Genet. 2010;154C:329–334.
6. Eggermann T. Prenatal detection of uniparental disomies (UPD): intended and incidental finding in the era of next generation genomics. Genes (Basel). 2020;11:1454.
7. Chien S-C, Chen C-P, Liou J-D. Prenatal diagnosis and genetic counseling of uniparental disomy. Taiwan J Obstet Gynecol. 2022;61:210–215.
8. Nakka P, Pattillo Smith S, O'Donnell-Luria AH, et al. Characterization of prevalence and health consequences of uniparental disomy in four million individuals from the general population. Am J Hum Genet. 2019;105:921–932.
9. Scuffins J, Keller-Ramey J, Dyer L, et al. Uniparental disomy in a population of 32,067 clinical exome trios. Genet Med. 2021;23:1101–1107.
10. del Gaudio D, Shinawi M, Astbury C, et al. Diagnostic testing for uniparental disomy: a points to consider statement from the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2020;22:1133–1141.
11. Gonzales PR, Behlmann A, Kronquist K, et al. Laboratory quality assurance committee, interpretation and reporting of large regions of homozygosity and suspected consanguinity/uniparental disomy, 2021 revision: a technical standard of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 24:255–261.
12. Chen M, Jiang J, Li C, et al. Non-pathological complete paternal uniparental isodisomy of chromosome 2 revealed in a maternity testing case. Int J Leg Med. 2019;133:993–997.
13. Su H, Sun T, Chen M, et al. Multiple methods used for type detection of uniparental disomy in paternity testing. Int J Leg Med. 2020;134:885–893. https://doi.org/10.1007/s00414-019-02215-w.
14. Doniec A, Łuczak W, Wróbel M, et al. Confirmation of paternity despite three genetic incompatibilities at chromosome 2. Genes (Basel). 2021;12:62.
15. Liu Q-L, Wu W-W, Zhao H, et al. Maternal uniparental disomy of chromosome 4 indicated by allele copy number of short tandem repeats. Forensic Sci Int Genet. 2020;46:102273.
16. Polonis K, Lopes JL, Cabral H, et al. Uniparental disomy of multiple chromosomes in two cases with a complex phenotype. Am J Med Genet A. 2023;191:1978–1983.
17. Liehr T. Cytogenetic contribution to uniparental disomy (UPD). Mol Cytogenet. 2010;3:8. https://doi.org/10.1186/1755-8166-3-8.