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# Genetic and chromosomal variation-caused inconsistencies in two parental tests

Chen Li[a], Yifan Li[a], Li Jia[b], Chong Chen[b], Shi Yan[b], Chuguang Chen[a], Yacheng Liu[b], He Ren[c,*]

[a] Beijing Microread Genetics Co., Ltd, 100037, Beijing, China
[b] Beijing Tongda Shoucheng Insitute of Forensic Science, 100192, Beijing, China
[c] Beijing Police College, Criminal Science and Technology, 102202, Beijing, China

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ABSTRACT

Two special cases were reported where inconsistency between children and the fathers were observed at FGA locus. Regular additional STR tests were performed and no more inconsistency was observed in the first case. Through the following TA cloning and sequencing, the first case turned to be a two-step mutation between child and father. In the second case, however, one more inconsistency was found in the additional test at D4S2366, which was on Chr. 4 as well. Then, seven randomly selected STR-loci on Chromosome 4 were analyzed, indicating a possible maternal uniparental disomy (UPD) in the child with normal phenotype. This study emphasizes gene or chromosomal variations may mislead parentage test, especially variations like UPD that are relatively unfamiliar to investigators. If all the inconsistent loci are on the same chromosome, investigators should take UPD into consideration, and further tests, like chromosomal-specific STR-typing, should be applied to prevent pseudo-exclusions.

## 1. Introduction

Parental tests are widely applied in forensic science based on STR genotype. It's possible to observe inconsistencies between parents and children because of mutations. Most of inconsistencies can be explained by STR one-step mutation (e.g. allele 10 to allele 9 or 11) between child and parent [1]. However, multi-step mutation [1] or chromosomal variation [2] may also occur, which can be confusing in some situations.

While STR mutations are relatively common to observe, chromosomal variations can be much more complex and unfamiliar to investigators, like trisomy, large-scale deletion, etc. In the second case, the inconsistencies turned out to be caused by a rare chromosomal variation called Uniparental Disomy (UPD). In the case of UPD, two copies of a chromosome, or parts of the two copies, were inherited from only one parent, and no copy from the other parent [3]. Several abnormal conditions in chromosome rearrangement may result in uniparental isodisomy, while structural or numerical aberrations of chromosomes at meiosis and post-zygotic stage might lead to uniparental heterodisomy [4]. UPD might result in clinical symptoms due to the expressions of recessive genes [5], or changes in genomic imprinting [6]. However, a person might also have totally normal phenotype with UPD [7].

## 2. Material and methods

### 2.1. DNA samples

Three individuals, putative father, mother and the child, involved in the trio parentage tests gave their consent to perform further tests and to publish the results. DNA samples were collected by blood cards for following tests.

### 2.2. STR typing

In the parentage test, samples were amplified with standard procedures provided by the manufacturer. In the second case, seven more STR-loci evenly distributed on Chromosome 4 were then analyzed to confirm the inheritance of this chromosome, using the primers suggested by UCSC Genome Browser.

### 2.3. Clone and sequencing

The inconsistent locus in case 1 was detected by sequencing as follows. Amplify the FGA locus and check the quality of amplicons by 2% Agarose-gel electrophoresis. The quantitatively recovered PCR products were ligated by T vector and transformed. The plasmid of

* Corresponding author.
*E-mail address:* snoopy_fr@sohu.com (H. Ren).

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**Table 1**
Seven selected STR markers and the test result.

| Locus | Genome location | Repeat Unit | Putative father (nt) | Mother (nt) | Child (nt) |
|---|---|---|---|---|---|
| D4S2639 | 4p15.31 | $(ATAG)_n$ | 183.40, 187.45 | 191.48, 203.65 | 203.80 |
| D4S2308 | 4q13.1 | $(AGAT)_n$ | 295.69 | 297.00 | 297.61 |
| D4S2409 | 4q21.23 | $(ATAG)_n$ | 271.71, 292.78 | 279.82 | 280.00 |
| D4S2364 | 4q22.1 | $(ATTC)_n$ | 135.96, 143.97 | 131.64 | 131.63 |
| D4S1647 | 4q23 | $(ATAG)_n$ | 147.41 | 147.39, 159.62 | 147.48 |
| D4S3354 | 4q32.1 | $(ATAG)_n$ | 174.00, 177.97 | 170.04, 178.01 | 170.08 |
| D4S2368 | 4q32.3 | $(CTAT)_n$ | 336.60 | 328.65, 332.61 | 332.67 |

positive clones were extracted and sequenced following the instruction of ABI Bigdye 3.1 sequencing kit (Applied Biosystems, USA), and. Analyze the sequence of targeted FGA locus using Chromas 2 and DNAMAN v6.

## 3. Results

In both cases, the samples were detected with Microreader™ 21 ID System (Suzhou Microread Genetics, Suzhou, China). When finding an inconsistency at FGA locus, an additional test was performed using Microreader™ 23sp ID System, with the number of STR-loci adding up to 40. The genotypes of putative father, mother and child at FGA in case 1 were 21, 22, and 19/22. We clone the three samples and proved the inconsistency was caused by a two-step mutation by sequencing.

In the second case, the genotypes of putative father, mother and child at FGA were 22/24, 20/23, and 20. Another inconsistency was found at D4S2366 in the additional test, where the genotypes were 11, 14, and 14. We found it of low possibility that the child had a two-step mutation and a three-step mutation at the same time. Noticing both FGA and D4S2366 were on Chromosome 4, a reasonable explanation was a large-scale chromosomal variation happened, like a maternal UPD of Chromosome 4 in the child, since those two loci showed to be homozygous with the same genotype as the mother's results. To test this hypothesis, seven more randomly selected STR-loci distributed on chromosome 4 were further analyzed (gene mapping see Fig. S1). The additional STR-loci and their primers were selected from UCSC database. The result of a trio test on these seven loci was showed in Table 1. Because there was no standard allelic ladder for these seven loci, we determined the fragments were of the same allele if their size difference was smaller than 1 nt (labeled in red). D4S1647 was not distinguishable since the three samples shared a same allele. Except that, all eight loci on Chr. 4 of the child were homozygous and had the same genotype as one of the mother's alleles, which supported the idea of a complete maternal UPD in child.

## 4. Conclusions

While STR mutations were relatively common to observe, UPD was quite a novel phenomenon to forensic investigators. Several cases of UPD 4 with clinical phenotype were described in previous works [8]. The estimated incidence for UPD is about 0.029% (1 in 3500) according to Robinson's report [3]. It seems that the incidences for UPD of different chromosomes vary a lot. UPD 4 is a relatively rare type of UPD, which is dominant by maternal UPD [9].

What makes our finding different is that the child has a totally normal phenotype with complete UPD 4, which may confuse a parentage test since it might appear to be a multi-step STR mutation. Our work emphasized that pseudo-exclusion might occur if the inconsistent loci were all on the same chromosome. In this situation, investigators must consider the possibility of UPD. We demonstrated that additional STR test, with evenly distributed loci on that chromosome, is an effective method to detect UPD, helping investigators draw conclusions in parentage tests.

## Declaration of Competing Interest

The authors have no conflict of interest.

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## References

[1] B. Brinkmann, K. Michael, N. Franz, et al., Mutation rate in human microsatellites: influence of the structure and length of the tandem repeat, Am. J. Hum. Genet. 62 (1998) 1408–1415, https://doi.org/10.1086/301869.
[2] M. Chen, J. Jiang, C. Li, H. Ren, et al., Non-pathological complete paternal uniparental isodisomy of chromosome 2 revealed in a maternity testing case, Int. J. Legal Med. 133 (2019) 993–997, https://doi.org/10.1007/s00414-018-1857-x.
[3] W.P. Robinson, Mechanisms leading to uniparental disomy and their clinical consequences, Bioessays. 22 (2000) 452–459, https://doi.org/10.1002/(SICI)1521-1878(200005)22:5<452::AID-BIES7>3.0.CO;2-K.
[4] F.A. Middleton, M.G. Trauzzi, A.E. Shrimpton, et al., Complete maternal uniparental isodisomy of chromosome 4 in a subject with major depressive disorder detected by high density SNP genotyping arrays, Am. J. Med. Genet. Part B Neuropsychiatr. Genet. 141 (2006) 28–32, https://doi.org/10.1002/ajmg.b.30250.
[5] M.B. García-Morato, J. Nevado, L.I. González-Granado, et al., Chronic granulomatous disease caused by maternal uniparental isodisomy of chromosome 16, J. Allergy Clin. Immunol. Pract. 5 (2017) 1146–1148, https://doi.org/10.1016/j.jaip.2017.01.018.
[6] S. Fokstuen, C. Ginsburg, M. Zachmann, A. Schinzel, Maternal uniparental disomy 14 as a cause of intrauterine growth retardation and early onset of puberty, J. Pediatr. 134 (1999) 689–695, https://doi.org/10.1016/S0022-3476(99)70282-9.
[7] B. Dwokniczak, B. Koppers, G. Kurlemann, et al., Uniparental disomy with normal phenotype, Lancet 340 (1992) 1285, https://doi.org/10.1016/0140-6736(92)92981-k.
[8] D.T. Papadimitriou, E. Manolakos, C. Bothou, et al., Maternal uniparental disomy of chromosome 4 and homozygous novel mutation in the WFS1 gene in a paediatric patient with Wolfram syndrome, Diabetes Metab. 41 (2015) 433–435, https://doi.org/10.1016/j.diabet.2015.06.003.
[9] T. Liehr, Cytogenetic contribution to uniparental disomy (UPD), Mol. Cytogenet. 2010 (2010) 8, https://doi.org/10.1186/1755-8166-3-8.