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Short Communication

# Loss of heterozygosity detected at three short tandem repeat locus commonly used for human DNA identification in a case of paternity testing

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ABSTRACT

Short tandem repeat (STR) is widely used for DNA profiling in forensic sciences for its stable inheritance. Genomic variations in STR loci may affect the results of the genotyping. In this study, using STR profiling and genome-wide chromosomal microarray assay, we detected the incidence of uniparental disomy or copy-neutral loss of heterozygosity (LOH) in a case of a parental testing, which altered the genotype of three commonly used STR markers including D2S1338, D2S441 and D2S1776. To the best of our knowledge, this is the first time found that LOH affect the genotyping of STR markers commonly used for paternity testing. Our findings demonstrated that the incidence of LOH in the genome may dramatically alter the results of DNA identification, and suggested that genomic structure variation need to be taking into consideration in the DNA identification using STR markers.



## 1. Introduction

DNA profiling of short tandem repeat (STR) is widely used in forensic sciences, including paternity testing and kinship analysis [1]. An individual inherits one copy of an STR from each parent, which may or may not have similar repeat sizes [2]. The number of repeats in STR markers can be highly variable among individuals, but stably inherited from parents to children and follows simple Mendelian inheritance, which make these STRs effective for human identification purposes. However, genomic variations in the regions of STR loci can result in allelic mismatch in the questioned child, may complicate the forensic inference in the case of paternity testing [3]. In previous study, genomic variations such as mutations, duplications, deletions of STR loci have reported affect the results of paternity testing.

In a normal individual's genome of human, every genetic locus in autosomes is composed of two alleles and one inherited from each parent. Loss of one allele can occur at any homozygous, or heterozygous genetic locus, allelic loss at a heterozygous site, determined as loss of heterozygosity (LOH), leaves the cell with a single version of a gene [4]. When the LOH was incident in a locus harbored a genetic marker, the appeared homozygous genotype will be detected, and affect the results of the genotyping [5]. In this study, we first detected susceptibility uniparental disomy (UPD) in three commonly used STR loci (D2S1338, D2S441 and D2S1776) in a case of a parental testing. Then, using chromosomal microarray analysis, we found multiple incidences of copy-neutral LOH in chromosome 2 in the genome of the questioned children, which flanking the genomic region of these three STR markers.

## 2. Materials and methods

### 2.1. Samples and genomic DNA extraction

A paternity test was performed for a female child in our laboratory. Peripheral blood samples from the mother, the child and the alleged father were collected and genomic DNA was extracted using Chelex-100 method according to the protocol of the manufacturer (Sigma, USA). Informed written permission was obtained from Mother and Alleged Father to perform DNA profiling and subsequent research. The study was approved by the Ethics

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### 2.2. STR genotyping

Amelogenin and 38 autosomal STR profiles were obtained using STRtyper-21G Kit (Health Gene Technologies Co. Ltd.(HGT), Ningbo City, China), AGCU EX22 STR Kit and 21 + 1 STR Kit (AGCU ScienTech Inc., Wuxi City, China) as per manufacturer's protocols. The details of the three STR genotyping kit were shown in the online Supplementary Data. X-STR profiles were obtained by using Microreader™ 19X ID System Kit (Beijing Microread Genetics Co. Ltd., Beijing City, China).

All the PCR amplification reactions were performed using GeneAmp PCR system 9700 (PerkinElmer, Norwalk, CT). Fragment analysis was carried on ABI 3130 Genetic Analyzer and automated profiling was performed using GeneMapper ID v3.2 software (Applied Biosystems).

### 2.3. Chromosomal microarray analysis

Chromosomal microarray analysis was performed using CytoScan 750k plateform (Affymetrix, Inc.), which is characterized with 750,436 copy number variation (CNV) markers including 200,436 single nucleotide polymorphism probes and 550,000 non-polymorphism probes. The standard process of hybridization and scanning of the microarray was according to the manufacturer's protocol. The data of the microarray were analyzed and visualized with Chromosome Analysis Suite (ChAS) software Package (Affymetrix, USA) using genome build Hg 19.

## 3. Results and discussions

The alleged father, mother and their 40 days old female child were subject to a paternity test. We first performed the genotyping using STRtyper-21G Kit, which contained 21 STR marker and the results showed D2S1338 in chromosome 2 did not transmit as Mendelian style (Table 1 and Fig. 1). Than, we genotyped the individuals using another AGCU EX22 kit which contained D2S1338 and some other STR in chromosome 2. The genotyping data validated the results of D2S1338 and found a new STR, D2S441, also did not transmit as Mendelian style (Table 1 and Fig. 1). Finally, we genotyping the family using the third kit, AGCU EX21 +1, and validate the results of D2S441 and found the third STR (D2S1776) which did not transmit as Mendelian style (Table 1 and Fig. 1). We also get the genotyping data of 20 STR markers of X chromosome of the family, and all the markers support the Mendelian transmit style except for DXS6809 (Table 1).

All STR of the mother, the child and alleged father were well genotyped, and the results showed four markers including D2S1338, D2S441, D2S1776 and DXS6809 did not follow Mendelian inheritance, which indicated that no biological relationship between alleged father and the child. For DXS6809, the genotype of mother and alleged father are 34/35, 32 respectively, and in child is 33/34, this result indicate that there maybe a *de novo* mutation in DXS6809 locus of the Child. For the other three autosomal STRs, which included D2S1338 at 2q35, D2S441 at 2p14, and D2S1776 at 2q24, were all located on chromosome 2 (Table 1). The genotype of D2S441 in mother and alleged father are 11/12, 10/11 respectively, and in child is 12, D2S1338 in mother and alleged father are 19, 23/24 respectively, and in child is 19, D2S1776 in mother and alleged father are 9/13, 11/14 respectively, and in child is 9. These results demonstrated that the child may inherit one allele from her mother at D2S1338, D2S441 and D2S1776 locus, which situation was determined as uniparental disomy. What is more, another STR locus in chr2, TPOX at 2p23 showed Mendelian inheritance among mother (10/11), alleged father (8/11) and the child (10/11). These data indicated that the uniparental disomy were occurred in some region of chromosome 2, but not the entire chromosome.

The results of DNA profiling by STRs indicated that there may be existed some genomic structure variation such as uniparental disomy or LOH in the chromosome 2 of the child. In order to reveal the details of the genomic structure variation, which may cause the mismatch of STRs in DNA profiling, we carried out genome-wide chromosomal microarray assay in mother, allege father and child. The results showed several inherited or *de novo* duplications or deletions, most of them were common polymorphisms. However, we detected four large LOH regions in chromosome 2 in the genome of the child, and the copy number states of these four LOH regions were neutral (Fig. 2). The length of the LOH regions ranged from 6 to 64 Mb and totally account for about 50% of the

**Table 1**
Results of STR Profiling in the Mother, Alleged Father and the Child. Data shown as genotype.

| Genetic Locus | Sample | | | Genetic Locus | Sample | | | Genetic Locus | Sample | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mother | Alleged Father | Child | | Mother | Alleged Father | Child | | Mother | Alleged Father | Child |
| D3S1358 | 15/16 | 15/17 | 15/16 | D12S391 | 22/25 | 18/20 | 18/22 | DXS6795 | 10/13 | 10 | 10/13 |
| D13S317 | 11 | 10/11 | 11 | FGA | 21/24 | 22/24 | 24 | DXS6803 | 11.3/12 | 12 | 12 |
| D7S820 | 12 | 8/11 | 11/12 | D6S474 | 14/16 | 14 | 14/16 | DXS6807 | 11/15 | 11 | 11/15 |
| D16S539 | 9/11 | 10/12 | 10/11 | D12ATA63 | 17 | 12/16 | 16/17 | DXS9907 | 11.2/13 | 14 | 11.2/14 |
| Penta E | 5/12 | 19/21 | 12/19 | D22S1045 | 11/16 | 15/16 | 16 | DXS7423 | 15 | 15 | 15/ |
| **D2S441** | **11/12** | **10/11** | **12** | D1S1677 | 14/15 | 14/15 | 14/15 | AMEL | X | X/Y | X |
| TPOX | 10/11 | 8/11 | 10/11 | D11S4463 | 13/14 | 13/16 | 14/16 | GATA172D05 | 8/9 | 10 | 9/10 |
| TH01 | 9/10 | 7/9 | 7/10 | D3S4529 | 14/16 | 15/17 | 15/16 | DXS101 | 24/25 | 27 | 24/27 |
| **D2S1338** | **19** | **23/24** | **19** | D6S1017 | 10 | 10 | 10 | DXS9902 | 10/11 | 10 | 10 |
| CSF1PO | 10 | 10/12 | 10/12 | D4S2408 | 10 | 8 | 8/10 | DXS7133 | 9/10 | 9 | 9/10 |
| Penta D | 8/9 | 9/12 | 8/9 | D17S1301 | 12 | 11/12 | 12 | DXS6810 | 17 | 18 | 17/18 |
| D10S1248 | 13/15 | 13 | 13/15 | D1GATA113 | 7/11 | 7/13 | 7 | GATA31E08 | 11 | 12 | 11/12 |
| D19S433 | 14/16 | 12/13 | 13/16 | D18S853 | 13/14 | 11 | 11/13 | DXS6800 | 16 | 19 | 16/19 |
| vWA | 14/18 | 14/19 | 14/18 | D20S482 | 14/15 | 14/16 | 15/16 | DXS981 | 12.3/15 | 12.3 | 12.3/15 |
| D21S11 | 29/30 | 29 | 29 | D14S1434 | 13/14 | 13/14 | 13/14 | DXS10162 | 17/21 | 19 | 17/19 |
| D18S51 | 15 | 15/19 | 15/19 | D9S1122 | 11/12 | 12/13 | 11/13 | **DXS6809** | **34/35** | **32** | **33/34** |
| D6S1043 | 14 | 11/13 | 11/14 | **D2S1776** | **9/13** | **11/14** | **9** | GATA165B12 | 9/11 | 10 | 9/10 |
| D8S1179 | 13 | 15/16 | 13/16 | D10S1435 | 13/15 | 12 | 12/13 | DXS10079 | 20 | 18 | 18/20 |
| D5S818 | 11 | 11/13 | 11 | D5S2500 | 17/18 | 17/20 | 18/20 | DXS10135 | 22/23 | 20 | 20/22 |
| | | | | | | | | HPRTB | 12/14 | 15 | 12/15 |

The mutated allele showing paternal child allele mismatch is indicated in bold.

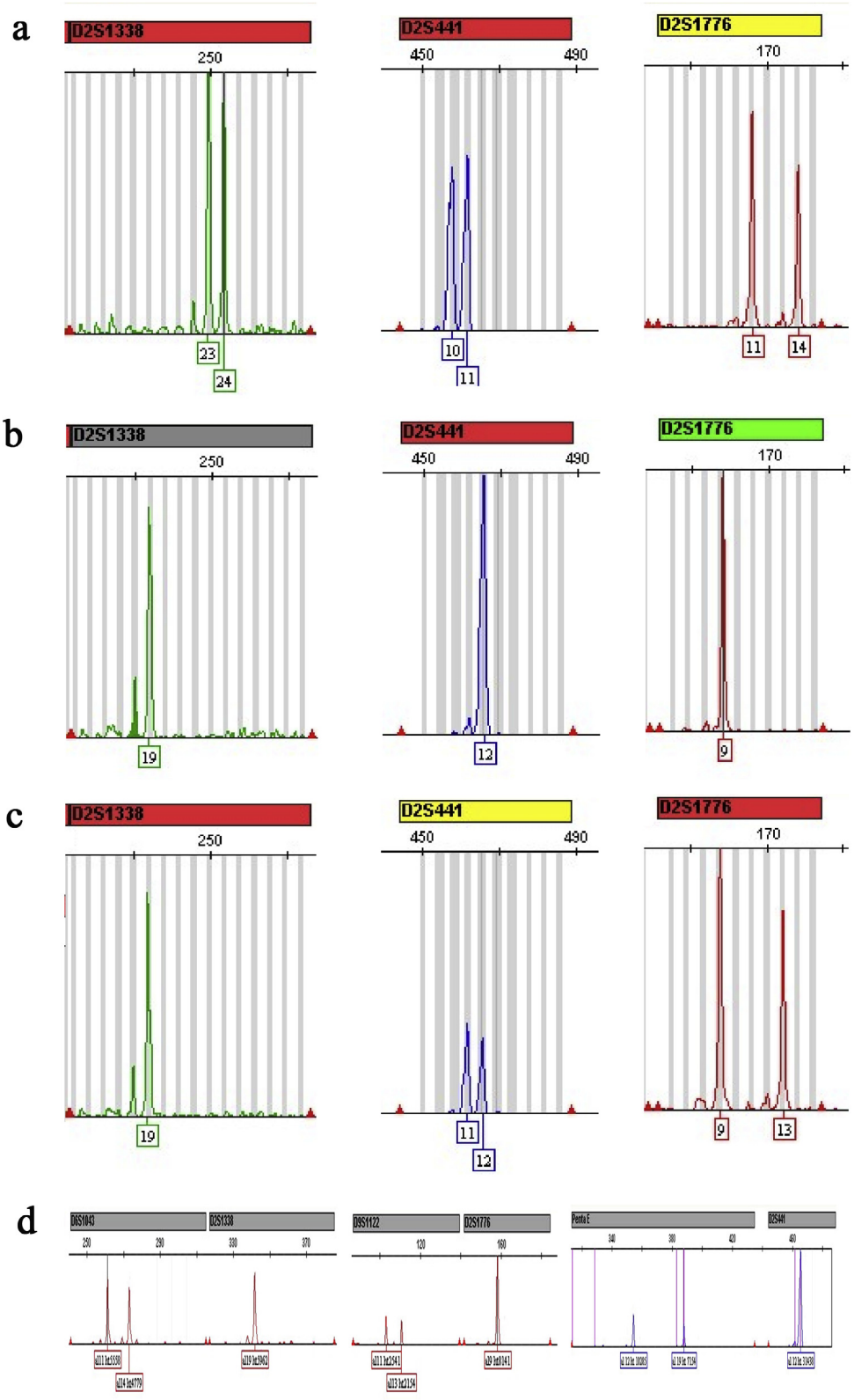


**Fig. 1.** Electropherogram of genotypes at D2S1338, D2S441 and D2S1776. a: genotype of father, D2S1338 (23/24), D2S441 (10/11) and D2S1776 (11/14). b: genotype of child, D2S1338 (19), D2S441 (12) and D2S1776 (9). c genotype of mother, D2S1338 (19), D2S441 (11/12) and D2S1776 (9/13). d: Electropherogram of genotypes with peak heights (ht) of the child. Left: peak heights of D6S1043 and D2S1338, Middle: D9S1122 and D2S1776, Right: Penta E and D2S441.

chromosome 2 (Table 2). The genotype of the markers demonstrated that the fragment of LOH region of the child was inherited from the mother, and without allele from alleged father. D2S441 and D2S1776 were exactly located in the regions of LOH, and appeared to be homozygous of one allele from mother, which is characterized as uniparental disomy or copy-neutral LOH. The location of D2S1338 (2q35, chr2:218,879,369–218,879,717,hg19) is slightly out of range of observed LOH region predicted by

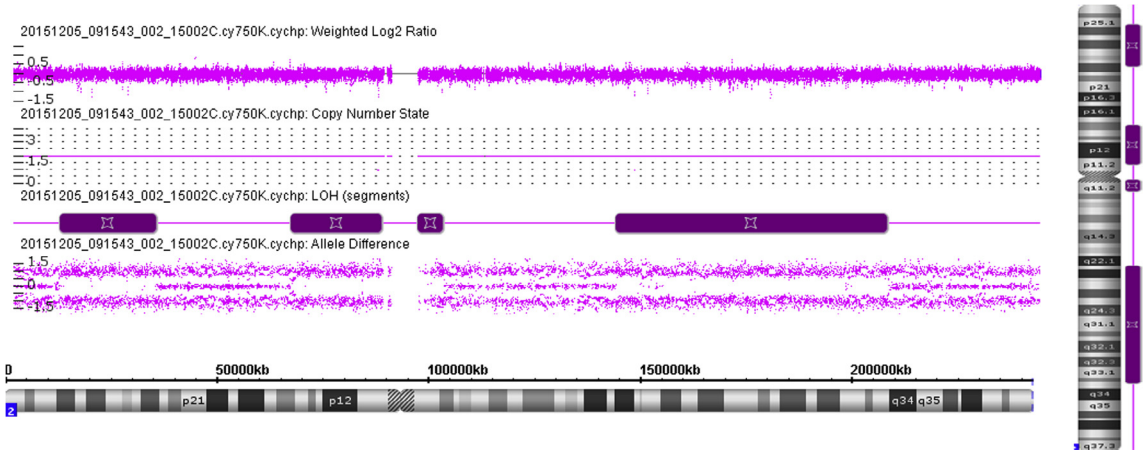


**Fig. 2.** Ideogram showing loss of heterozygosity of Chromosome 2 detected by Chromosomal microarray analysis. Probe is ordered on the x-axis according to physical mapping positions, with the p-arm probes to the left and q-arm probes to the right. Highlighted region represents the LOH detected by microarray (hg19).

**Table 2**
Details of the loss of heterozygosity in the genome of the Child.

| Chromosome | Cytoband | Size (kbp) | Position (bp, Hg19) | Gene Count | Marker Count |
|---|---|---|---|---|---|
| 2 | q22.2–q33.3 | 64,190 | 142,220,734–206,411,109 | 306 | 4246 |
| 2 | p25.1–p22.3 | 22,807 | 10,876,421–33,683,793 | 164 | 1673 |
| 2 | p14–p11.2 | 21,492 | 65,561,391–87,053,152 | 164 | 1528 |
| 2 | q11.1–q11.2 | 6096 | 95,550,957–101,647,191 | 71 | 282 |

chromosomal microarray analysis (2q22.2–2q33.3, chr2:142,220, 734–206,411,109,hg18, Table 2), and these may contributed to the ambiguous boundary prediction in chromosomal microarray analysis. In fact, in chromosomal microarray analysis, the accurate boundary of the LOH region or other structure variants is difficult to define because the interval of the probes. We carefully examined our data of chromosomal microarray analysis, and found that there were no probe in the region between chr2: 206,411, 109–218,879,71 in the CytoScan 750k microarray after excluded probes failed genotyping. For this reason, we propose that D2S1338 is located in the LOH region predicted by microarray.

We searched the characters of this four LOH in database including DGV, UCSC, Decipher and OMIM, and the results showed that they were not polymorphisms. To the best of our knowledge, this is the first time found that LOH affect STRs commonly used for human DNA identification in paternity testing. LOH or UPD was enriched in cancer cells, and rare in normal conditions [5–8]. LOH affect D2S441 were detected in breast cancer cells [5], LOH covered D2S1338 also found in blood samples collected from leukemic patients [7]. One possible molecular mechanism of LOH or UPD caused diseases may attribute to alter the situation of genomic imprinting. In condition of LOH or UPD, the offspring inherited both homologs, including the imprinting information such as DNA Methylation, from the same parent, can lead to functional nullisomy for imprinted genes, and resulting in clinically recognizable syndromes [9]. LOH or UPD altered the situation of genomic imprinting were observed in Prader-Willi/Angelman syndrome (PWS [MIM: 176270] [10], Beckwith-Wiedemann syndrome (BWS [MIM: 130650] [11], and transient neonatal diabetes mellitus (TNDM [MIM: 601410] [12].

To date, few cases of LOH for Chromosome 2 have previously been reported and the clinical phenotype caused by LOH in chromosome 2 was not clear. In our study, the female child harbors several large LOH regions totally affect about 50% of chromosome 2 and contains more than 700 genes. The health situation of the child was evaluated when she was 40 days and 6 months old, except for mild developmental retardation, no obviously related health issue was observed. This mild abnormality was same as another case reported by Ou et al. which a complete paternal uniparental isodisomy for Chromosome 2 was observed in a apparently healthy boy [13].

In our study, we detect several large LOH regions in one case of parental testing, which covered three STRs commonly used for human DNA identification, the re-calculated combined paternity index (CPI) was $3.67 \times 10^{15}$ after excluding D2S1338, D2S441 and D2S1776. Our findings demonstrated that the incidence of LOH in the genome may dramatically alter the results of DNA identification, and emphasizes that the genomic structure variation such as LOH need to be take into consideration in the DNA identification using STR.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.legalmed.2016.11.001.

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