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Multistep microsatellite mutation at D18S51 locus in a parentage testing case

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ABSTRACT

Mutations of short tandem repeats (STRs) loci might cause allelic mismatchs between the child and the parents, which entangled the forensic inference in paternity testing. Most of the reported microsatellite mutations are confined to single-step mutations, and multistep mutations were rarely reported and only account for a very limited number of STR mutation events in previously studied cases. Here we reported a paternity case with a mismatch in locus D18S51. The genotypes of the alleged father, the mother and the child in D18S51 locus were 14/23, 15/16, and 15/20, respectively. Examination of 38 autosomal STR loci revealed no mismatches, and the paternity index is above 1.58E + 15. These results suggested that the alleged father is the biological father of the child that a rare three-steps mutation had occurred in the paternal allele of D18S51.

1. Introduction

It is traditionally an efficient tool for STR loci profiling in parentage testing, which follows the traditional Mendelian inheritance. One problem is the STR mutations which cause the allelic mismatches in the child compared to the parent, making the forensic inference more complex in paternity testing. Most of the reported microsatellite mutations are confined to single-step mutations [1–5]. Multistep mutations were rarely discovered in STR loci compared to single-step mutation; and they only account for a very limited number of STR mutation events [6,7]. This study reported a rare three-steps microsatellite mutation case in the autosomal STR locus D18S51 in a trio parentage testing.

2. Materials and methods

2.1. Samples

Blood samples were collected from the alleged father, the mother and the child. Informed written permission was obtained from the father and the mother to use the samples for DNA profiling and subsequent research.

2.2. STR profiling and sequencing

Genomic DNA was extracted by Chelex-100 method and was profiled for 39 autosomal STR loci using Microreader™ 23sp STR Kit (Suzhou Microread Genetics, Jiangsu, China), PowerPlex®21 STR Kit (Promega Corporation, WI, USA) as per manufacturer's instruction. The PCR amplifications were performed using GeneAmp PCR system 9700 (Perkin Elmer, Norwalk, CT). PCR products were loaded into ABI 3130xl Genetic Analyzer and the STR profiling was generated by GeneMapper ID v3.2 software (Applied Biosystems).

D18S51 primers were designed according to GenBank sequence (Gene ID: 2243). The forward and reverse primer sequences are 5′-GACCCAAAACAGCTTAGGAAC-3′ and 5′-CCACATACTTACCTCCAGTC GTT-3′, respectively. DNA samples of the father, the mother, and the child were amplified on GeneAmp PCR 9700 system in a 25-μl reaction volume containing 10-ng DNA, 200 μm dNTPs, 6 pm primer, 2.0-U AmpliTaq Gold (Applied Biosystems) and 1 x PCR buffer with 1.5 mM MgCl2. Thermocycling conditions are 95 °C for 5 min, and then 30 cycles in 94 °C for 30 s, 60 °C for 30 s, 72 °C for 45 s and 72 °C for 10 min in final extension. The PCR products were separated and purified by QIAquick® Gel Extraction Kit (Qiagen, Germany). The separated allele fragments were cloned as the standard procedure. Positive clones were selected and sequenced on ABI 3730 Genetic Analyzer (Applied Biosystems) according to

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Table 1
The genotypes of 39 loci of alleged father, mother, and child from Microreader™ 23sp and PowerPlex® 21 STR kits. Three-steps mutation was found in D18S51 (bold).

STRs	Alleged Father	Mother	Child	Paternity index
Amelo	X, Y	X	X	
D3S1358	15,16	16,18	15,16	1.219
D1S1656	15,16	14,17.3	14,15	1.661
D6S1043	12,19	18,19	19	2.674
D13S317	11	11,12	11	4.200
Penta E	17,20	5,17	5,20	25.00
D16S539	9,11	11,13	11	2.352
D18S51	**14,23**	**15,16**	**15,20**	**0.0002**
D2S1338	17,24	23,25	24,25	3.463
CSF1PO	10,11	10,12	10	1.614
Penta D	8,12	9,12	8,9	9.225
THO1	7,9	6,9	7,9	1.745
vWA	14,16	14,19	14	1.791
D21S11	29,30	30,31	29,30	3.226
D7S820	11	8,11	11	2.466
D5S818	10,11	10,13	11,13	1.697
TPOX	8,9	8,11	8,9	4.191
D8S1179	13,14	13,14	13	3.010
D12S391	20,21	15,19	19,20	3.226
D19S433	14.2,15.2	13,13.2	13,15.2	3.743
FGA	21,23	22,23	22,23	1.922
D6S477	10,15	15	15	1.624
D18S535	9,15	9,16	9,16	2.480
D19S253	13	8,13	8,13	4.537
D15S659	11,17	11,13	13,17	5.549
D11S2368	17,20	17,21	17,21	1.420
D20S470	13,17	13,17	17	6.098
GATA198B05	18,21	22	21,22	1.596
D7S3048	22,26	18,25	18,22	7.022
D8S1132	17,23	20,23	23	7.911
D4S2366	11,14	12	12,14	7.299
D21S1270	11,14	10,13	11,13	8.651
D13S325	18,23	20,22	22,23	125.000
D9S925	15,16	15	15	2.514
D3S3045	12,14	9,14	9,14	1.000
D14S608	10	10,11	10,11	2.325
D10S1435	12	12,13	12,13	1.702
D17S1290	16,22	15,16	15,16	0.896
D5S2500	11	11,15	11,15	1.699
CPI				1,584,808,197,751,510

the manufacturer's instructions.

2.3. Statistical analysis

The paternity index was calculated in standard trio using the frequency of alleles from the Chinese Han population based on the Bayesian mathematics calculation [8,9].

3. Results and discussion

This study presented a standard trio paternity test case to examine 39 autosomal STR loci using both Microreader™ 23sp and PowerPlex®21 STR Kits, and they got the same results for that all loci except D18S51 accurately abided by the Mendelian inheritance law (Table 1). The genotype for D18S51 locus in the father, the mother, and the child are 14/23, 15/16, and 15/20 (Table 1 and Fig. 1), respectively, with the corresponding core unit sequences being $[AGAA]_{14}$/$[AGAA]_{23}$, $[AGAA]_{15}$/$[AGAA]_{16}$, and $[AGAA]_{15}$/$[AGAA]_{20}$.

Most of the abnormal STR inheritances are confined to single-step mutations, and multiple-steps mutations are rarely reported in the previous report. Since the higher of the mutation steps, the less likely the mutation would happen, it is more likely that the D18S51 allele 20 of the child was inherited from the putative Father's 23 with a three-steps mutation in this case. Mutation rates reported for D18S51 STR allele during maternal and paternal meiotic stage are 0.06% and 0.22%, respectively, with a total of 0.22% rate (http://strbase.nist.gov/mutation.htm). The probability of paternally transmitted mutation is about 3.67 times that of maternally transmitted mutation based on these statistics.

According to ISFG guidelines [10], at least three inconsistencies between maternal/paternal and child are to be proved to report the parentage as exclusion. In the totally 39 autosomal STR loci investigated, no additional incompatible STR loci were found (Table 1). The combined paternity index for all STR loci including the mismatched alleles in standard trio situation is above 1.58E + 15. Therefore the suspected father was confirmed as the biological father of the child.

Conflict of interest

The authors have no conflicts of interest.

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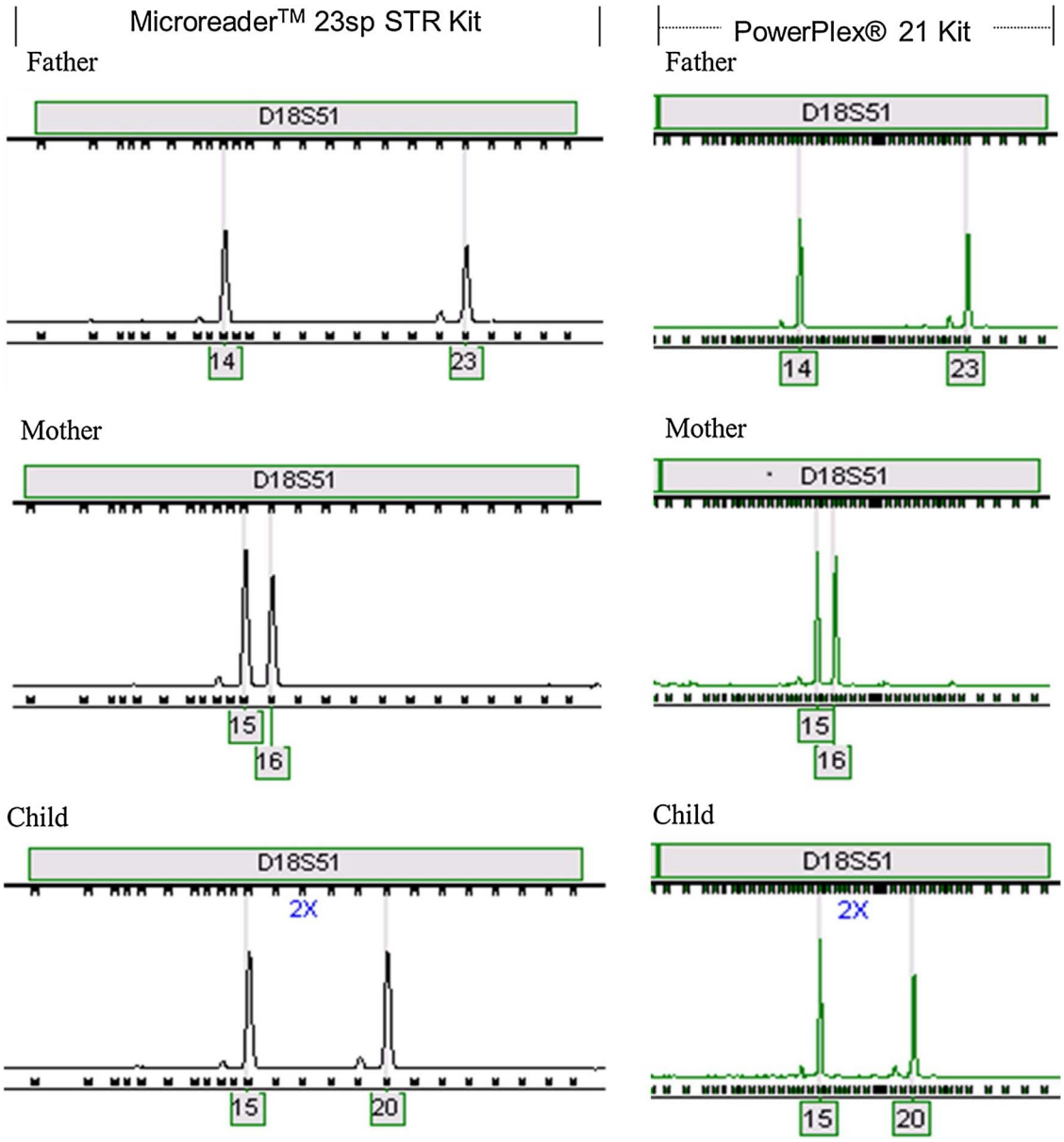

Fig. 1. Genotypes Electrophorogram of the alleged father, mother and child at D18S51 locus.

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