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Brief Communication

Identification of a rare off-ladder allele of the D13S325 locus during paternity testing

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ABSTRACT

This study demonstrates an unusual rare allele of D13S325 that was falsely categorized as an allele of D12S391 under the STRtyper™-10F/G system. The parentage cases with these rare alleles were analyzed using the Sinofiler™ system and singleplex amplification system, and the alleles of D13S325 extracted from the electrophoresis gel were sequenced. 5 Cases with the rare alleles misread as allele 20 of D12S391 were identified in total 2618 cases (including 3200 unrelated parents). This rare allele was designated as allele 5.1 of D13S325 based on its DNA sequence. Its frequency in the Chinese population was 1.6×10^{-3}. Because the rare allele 5.1 of D13S325 locus tends to be incorrectly labeled in the STRtyper™-10F/G system, particular attention should be paid when the system is used in paternity testing, personal identification, and DNA database comparisons.

1. Introduction

PCR-STR technology has been applied extensively to forensic personal identification and paternity testing. It has also become an important tool for establishing DNA databases [1,2]. During STR typing, some alleles were observed outside the corresponding allelic ladder, being labeled as off ladder (OL) alleles. Some of the OL alleles are created when the band shifts during electrophoresis, but most of them are due to fewer or more repeats or bases of the core sequence in comparison with the alleles included in the allelic ladder [3–5]. In more extreme cases, OL alleles may be found among alleles of other locus neighboring the original locus in the electropherogram, causing false genotyping at the both loci.

The STRtyper™-10F/G system (Guangdong, China) is widely used in China as a supplementary tool for special cases such as duo paternity testing, paternity testing with mutations and kinship testing [6–9]. In paternity testing cases by using the STRtyper™-10F/G system, a suspicious OL allele of the D13S325 locus was detected inside the allelic ladder range of adjacent D12S391 locus in the electropherogram. The alleles of the D13S325 locus were then analyzed to confirm the genotyping of the loci.

2. Material and methods

2.1. Samples

2618 parentage confirmed duo- or trio- cases (including 3200 unrelated parent samples) performed by STRtyper™-10F/G system were collected from routine casework. All participants had provided informed consents. The STRtyper™-10F/G system contains Amelogenin and 9 non-CODIS core loci (D2S1772, D6S1043, D7S3048, D8S1132, D11S2368, D12S391, D13S325, D18S1364, and GATA198B05). Within these cases, three-allelic patterns at the D12S391 locus and homozygous genotype at D13S325 locus were observed in 5 cases consisting of 12 bloodstain samples (Table 1). Genomic DNA was extracted by Chelex-100 method.

2.2. D12S391 genotyping using Sinofiler™ system

The D12S391 genotyping was performed with Sinofiler™ system (Applied Biosystems, USA). The amplified PCR products were separated using capillary electrophoresis on ABI PRISM 3500 genetic analyzer (Applied Biosystems, USA) and analyzed with GeneMapper® ID-X software.

2.3. D13S325 genotyping using singleplex amplification system

The primer set for D13S325 locus was designed using Primer 3 according to the sequences of NT_024524.14 from GenBank. The sequences of primers were as follows: forward: 3′-CATTCT CCCCACGGTGTTT-5′; reverse: 3′-CTAGGCAACAGCAGAAAGTGG-5′,

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Table 1
The genotyping results of D12S391 and D13S325 loci using different systems.

Case	Sample	D12S391		D13S325	
		STRtyper™-10F/G	Sinofiler™	STRtyper™-10F/G	Singleplex
1	F1	19, 20, 22	19, 22	21	OL, 21
	C1	19, 20, 22	19, 22	18	OL, 18
2	F2	18, 20, 22	18, 22	21	OL, 21
	M2	19, 20	19, 20	20, 21	20, 21
	C2	18, 20	18, 20	21	OL, 21
3	F3	18, 20	18, 20	20	OL, 20
	C3	18, 20, 22	18, 22	19	OL, 19
4	F4	18, 20, 21	18, 21	22	OL, 22
	M4	19, 20	19, 20	19, 23	19, 23
	C4	19, 20, 21	19, 21	19	OL, 19
5	F5	18, 20, 24	18, 24	23	OL, 23
	C5	20, 24	24, 24	20	OL, 20

F indicates father, M indicates mother and C indicates child.

Table 2
Sequencing results for the alleles of D13S325 locus.

Allele	Allelic size in STRtyper™-10F/G system	Repeat region
18	240 bp	$[AGAT]_{11}$-GAT-$[AGAT]_7$
19	244 bp	$[AGAT]_{11}$-GAT-$[AGAT]_8$
20	248 bp	$[AGAT]_{11}$-GAT-$[AGAT]_9$
21	252 bp	$[AGAT]_{12}$-GAT-$[AGAT]_9$
22	256 bp	$[AGAT]_{12}$-GAT-$[AGAT]_{10}$
23	260 bp	$[AGAT]_{11}$-GAT-$[AGAT]_{12}$
OL (5.1)	189 bp	$[AGAT]_6$ or $[AGAT]_n$-A-GAT-$[AGAT]_{5-n}$

n is positive integer and $n \leqslant 5$.

PCR products were separated on non-denaturing polyacrylamide gel (T = 6%, C = 3.3%) and stained with silver nitrate.

2.4. DNA sequencing of D13S325 alleles

The bands for D13S325 allele were cut separately from the polyacrylamide gel and immersed in 50 μL ultrapure water for over 24 h. The soak solution was used for the singleplex amplification as the template DNA. PCR products were purified with low-melting agarose gel and sequenced bi-directionally using a BigDye® Terminator v3.1 Kit (Applied Biosystems, USA). The sequencing results were spliced with DNASTAR® LaserGene SeqMan Version 8.0.2 software (DNASTAR, USA) and compared using BioEdit® software (Ibis Therapeutics, USA).

3. Results and discussion

3.1. Correction of genotypes

With the STRtyper™-10F/G system, at least one sample from each of the five cases showed both three-allelic pattern with allele 20 at D12S391 and mono-allelic pattern at D13S325 in the electropherogram. However, extra allele 20 was not detected at D12S391 in these samples when using the Sinofiler™ and singleplex amplification systems, meanwhile, the di-allelic pattern with one

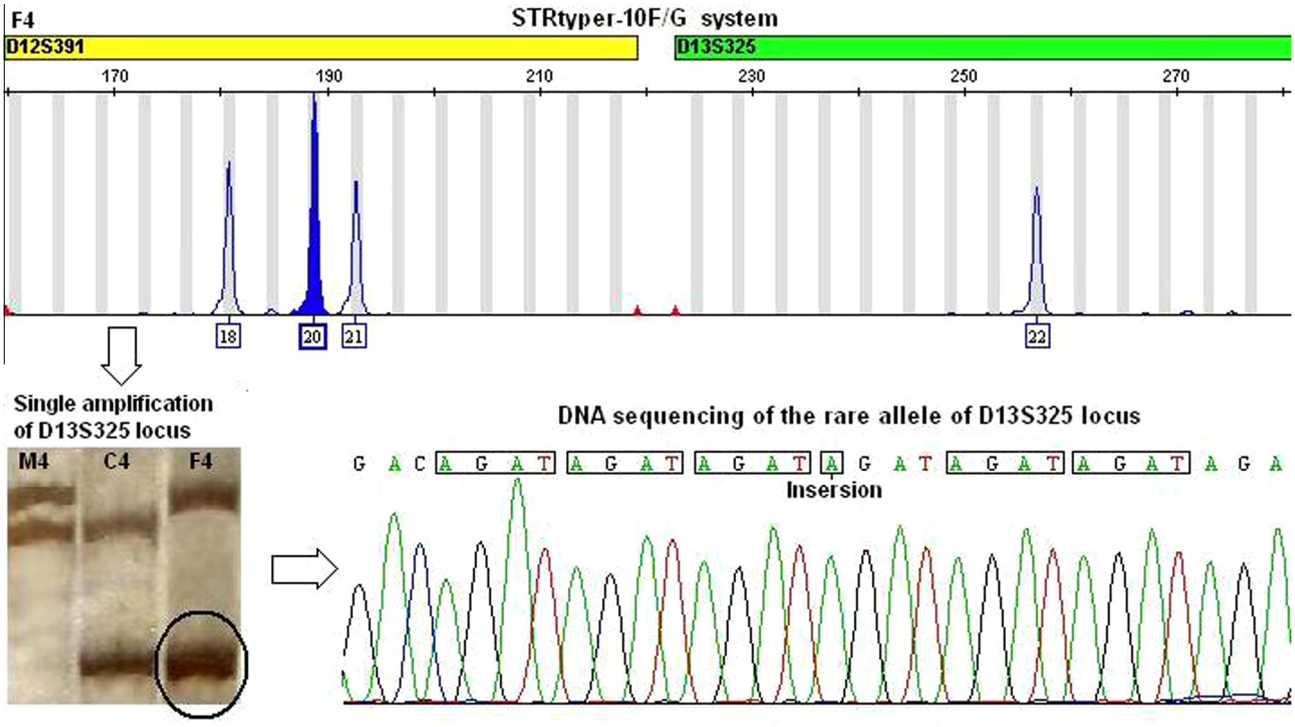

Fig. 1. The verification of rare allele of D13S325 locus in sample F4. The abnormal sample was certified by singleplex amplification system and the rare allele was separated and sequenced. F indicates father, M indicates mother and C indicates child.