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Research article

Two examples of null alleles at the D19S433 locus due to the same 4 bp deletion in the presumptive primer binding site of the AmpFlSTR Identifiler kit

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Abstract

Two cases of allelic loss at the D19S433 locus after multiplex PCR with the AmpFlSTR Identifiler kit (Applied Biosystems) are described. In both cases the failure of PCR resulted in genetic inconsistencies due to opposite homozygosity. After singleplex PCR with published primers additional alleles were observed and Mendelian inheritance was restored. These PCR products were sequenced and in both cases the same 4 bp deletion near the 3′ end of the repeat region was detected in two alleles of different length. The frequency of these null alleles (two events in 1026 allelic transfers) amounts to 0.0019 (95% confidence limits: 0.0002–0.0070).

Keywords: D19S433; Null allele; Primer binding site mutation

1. Introduction

Non-amplification of alleles due to mutations in the primer binding sites have already been described for various STR-loci [1–4]. This study reports two cases of allelic loss at the D19S433 locus after multiplex PCR with the *AmpFlSTR® Identifiler™ PCR Amplification Kit*. A possible resolution of these cases is discussed.

2. Materials and methods

Buccal swabs were taken with *Sterile OmniSwabs* (Whatman) from Caucasoids living in Austria (two parent/child trios). DNA was extracted with Chelex [5] and amplified with the *AmpFlSTR® Identifiler™ PCR Amplification Kit* (Applied Biosystems) according to the manufacturer's instructions. An alternative PCR was carried out at the D19S433 locus with the primers and PCR conditions given in the GenBank (accession no. G08036). These amplicons were excised from polyacrylamide gels and purified [6] or separated by cloning (*TA-Cloning Kit*, Invitrogen). In both cases the alleles were reamplified, cleaned up with the *QIAquick® PCR Purification Kit* (Qiagen) and sequenced in both directions with the *Big Dye® Terminator Cycle Sequencing Ready Reaction Kit* on an ABI Prism 310 Genetic Analyzer (Applied Biosystems) with the same primers.

3. Results and discussion

A genetic inconsistency due to apparent homozygosity was observed after PCR with the *AmpFlSTR® Identifiler™ PCR Amplification Kit* in both cases. In case 1 the mismatch was detected between mother and child, in case 2 between father and child (Table 1). After singleplex PCR with alternative primers, which presumably differ from those used in the *AmpFlSTR® Identifiler™ PCR Amplification Kit*, additional alleles were observed, which re-established Mendelian inheritance (Table 2).

Sequencing revealed the same 4 bp deletion near the 3′ end of the repeat region in an allele *15 in case 1 and an allele *14 in case 2. Due to the loss of 4 bp these alleles were designated as *14 and *13, respectively, because their fragment length corresponds exactly to these common alleles.

The frequency of these null alleles (two events in 1026 allelic transfers) mathematically amounts to 0.0019 (95% confidence limits: 0.0002–0.0070). Additionally, it must be considered, that the real frequency will be higher, since such

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Table 1
STR results showing opposite homozygosity

	Mother	Child	Father
Case 1	*13, *13	*15, *15	*12, *15
Case 2	*13, *13	*13, *13	*13.2, *13.2

Table 2
Corrected STR results including null alleles (underlined)

	Mother	Child	Father
Case 1	*13, <u>*14</u>	<u>*14</u>, *15	*12, *15
Case 2	*13, *13	*13, <u>*13</u>	<u>*13</u>, *13.2

null alleles remain undisclosed if two generations share other alleles at this locus.

Lowering the annealing temperature is not a helpful strategy to recover null alleles in case of deletions or insertions in the primer binding site. Merely the use of alternative primers can overcome allelic drop-out in these cases. As primer sequences are not available for the *AmpFlSTR® Identifiler™ PCR Amplification Kit*, only the manufacturer could theoretically add a degenerate primer, which also amplifies the alleles carrying this deletion.

A genetic incompatibility based on two homozygotes is referred to as an indirect exclusion, which has a much lower conclusive force than a direct exclusion. These situations have to be treated with caution. As the occurrence of two null alleles in one individual have already been reported [7], the determination of non-paternity should at least be based on three exclusions.

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Conflict of interest

None.

References

[1] Hering, et al. Int. J. Legal Med. 116 (2002) 365–367.
[2] Heinrich, et al. Int. J. Legal Med. 118 (2004) 361–363.
[3] Leibelt, et al. Forensic Sci. Int. 133 (2003) 220–227.
[4] Boutrand, et al. Int. J. Legal Med. 114 (2001) 295–297.
[5] Walsh, et al. Biotechniques 10 (4) (1991) 506–513.
[6] Glock, et al. J. Forensic Sci. 41 (1996) 579–581.
[7] Vanderheyden, et al. Int. J. Legal Med. 121 (2007) 297–301.