International Congress Series 1288 (2006) 456–458

www.ics-elsevier.com

Two apparent mother/child mismatches at the D3S1358 and the SE33 (ACTBP2) locus

E.M. Dauber [a,*], W. Parson [b], B. Glock [a], W.R. Mayr [a]

[a] *Division of Blood Group Serology, Medical University of Vienna, Austria*
[b] *Institute of Legal Medicine, Innsbruck Medical University, Austria*

Abstract. Two cases of apparent mother/child mismatch because of opposite homozygosity at a single STR-locus are described. They were observed at the D3S1358 locus after amplification with the AmpFlSTR®Identifiler™ PCR Amplification Kit and at the SE33 (ACTBP2) locus after a singleplex-PCR with commonly used primers. Sequencing revealed point mutations near the 3′ end of the primers leading to a failure of amplification in both cases. Strategies to resolve such cases are discussed.

Keywords: Binding site mutation; Null allele; D3S1358; SE33; ACTBP2

1. Introduction

Allelic dropout due to point mutations in the primer binding region has already been described for SE33 (ACTBP2) and other STR-loci [1–4]. This study describes two cases of single, false exclusions of maternity due to this phenomenon at the D3S1358 and the SE33 locus and suggests a possible resolution of these cases.

2. Materials and methods

DNA from Austrian Caucasoids (1 complete trio, 1 mother/child pair) was extracted with the Chelex method. PCR was carried out with the AmpFlSTR®Identifiler™ PCR Amplification Kit (Applied Biosystems, Foster City, CA, USA) including the D3S1358 locus, and with a singleplex PCR at the SE33 locus, which applies a common primer pair [5]. Additionally, a further multiplex PCR (Geneprint Powerplex 16® System, Promega, Madison, WI, USA) and a singleplex D12S391 PCR [6] were carried out to confirm the

* Corresponding author. Tel.: +43 1 40400 5320; fax: +43 1 40400 5321.
E-mail address: eva-maria.dauber@meduniwien.ac.at (E.M. Dauber).

0531-5131/
doi:10.1016/j.ics.2005.08.046

Table 1
STR results showing mother/child mismatches due to apparent opposite homozygosity

	Genotype		
Locus	Mother	Child	Father
SE33 (ACTBP2)	18, 18	29.2, 29.2	29.2, 32.2
D3S1358	15, 15	16, 16	Not available

initial results and to check for further mismatches. A singleplex PCR at the D3S1358 [7] and the SE33 [5] locus was carried out applying published primer pairs in a 12-step temperature gradient PCR between 50 °C and 62 °C. Subsequently, genomic DNA was amplified with alternative primers binding outside of at least one of the original primers in an additional singleplex PCR. In case of D3S1358 the primers (forward: 5′-CTGGCCA-TATTCACTTGCCC-3′, reverse 5′-GAGGCAGGAGGACTGCTTGA-3′) were designed with the PrimerExpress Software (Applied Biosystems, Foster City, CA, USA), in case of SE33 already published primers were used [8]. These PCR products were loaded on a polyacrylamide gel, excised separately after silver staining and reamplified. They were sequenced with the alternative primers applying the BigDyeTerminator Cycle Sequencing Kit according to the manufacturer's instructions.

3. Results and discussion

The mother/child mismatches were initially observed at the D3S1358 locus after amplification with the AmpFlSTR®Identifiler™ PCR Amplification Kit (Applied Biosystems, Foster City, CA, USA) amongst 825 meioses and at the SE33 (ACTBP2) locus after PCR with the original primers [5] amongst 1219 meioses (Table 1).

The D3S1358 results obtained with the Geneprint Powerplex 16® System (Promega, Madison, USA) were identical. No further parent/child mismatches were found in additional, highly polymorphic loci in both cases (Penta D, Penta E and D12S391). After lowering the annealing temperature down to 50 °C in a singleplex D3S1358 and SE33 PCR further alleles appeared, which restored mendelian inheritance between mother and child in both cases. Therefore a point mutation in the primer binding region had to be supposed as the reason for allelic dropout.

Another singleplex PCR with alternative primers lying outside of the primer binding sites of the original oligonucleotides confirmed these results: all formerly homozygous individuals turned out to be heterozygous except the child in the D3S1358 case, who remained homozygous, but was now sharing an allele with its heterozygous mother (Table 2). Sequencing proved a point mutation in the complementary region of the original reverse primers in both cases. A C-to-G transversion at position #11484 (GenBank accession no. AJ312685), which has not been described so far for alleles at this locus to the author's knowledge, was found to be the reason for non-amplification of a D3S1358 allele 16.

Table 2
Corrected STR results including null alleles

Locus	Mother	Genotype child	Father
SE33 (ACTBP2)	17[a], 18	17[a], 29.2	29.2, 32.2
D3S1358	15, 16[a]	16[a], 16	Not available

[a] Null alleles.

In case of the mother/child mismatch at the SE33 locus the failure of PCR was due a G-to-A transition at position #407 (GenBank accession no. NG_000840) in the reverse primer region, which was already described for alleles 14, 15, 16 and 18 but not for an allele 17 so far [1,2]. As a result of temperature gradient PCR and sequencing the genotypes of both mother/child pairs were corrected, leading to a perfect parent/child match in both cases (Table 2).

To overcome the problems of isolated parent/child mismatches due to allelic dropout in case of base substitutions in the primer binding site, a PCR with lower annealing temperatures can easily be performed in a first approach to reestablish mendelian inheritance. This strategy has even turned out to be successful in case of D3S1358, where the point mutation was only at a distance of 3 bases from the 3′ end of the reverse primer. In case of SE33 the distance was 7 bases, which usually can be compensated more easily in PCR at lower annealing temperatures. The admixture of a modified primer is a feasible method [2], but requires that the point mutation is already known, which might not always be the case. An alternative primer pair, which does not overlap with the initially used oligonucleotides, is a promising strategy, but the primer sequences of commercial STR-kits are not always available as it is for example the case in the AmpFlSTR® PCR Amplification Kits.

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