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CASE REPORT

False homozygosities at CSF1PO loci revealed by discrepancies between two kits in Chinese population

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Abstract During the course of paternity test, three samples in two cases were apparently homozygous at the CSF1PO locus using AmpFlSTRs Identifiler PCR Amplification kits, but using the PowerPlexs 16 kit, the three individuals were found to be heterozygous. This puzzling problem was solved by using multiple analytical approaches, including the use of different primer pairs and the characterization of the mutation causing the "null allele." Dropout was caused by a single mutation event in the presumptive binding site of the forward primer. While the frequency of these silent alleles remains low (0.5% in our study), it is suggested that appropriate measures should be taken for database comparisons and that allelic dropout should be further investigated by sequence analysis and be reported to the forensic community.

Keywords CSF1PO · Primer-binding site mutation · AmpFlSTR identifiler · STR · Null alleles

Introduction

Paternity tests are ordinarily resolved by using short tandem repeat (STR) informative markers, which are amplified and analyzed simultaneously in multiplex format, using commercial kits and semiautomatic systems. AmpFlSTR Identifiler and PowerPlex 16 are used widely as commercial genotyping kits in forensic genetic analysis. They share many STRs but do not use the same primers. The locus-specific primers in each kit target highly conserved nucleotide sequences flanking the STR locus of interest. Although these primer-binding regions are highly conserved, DNA mutation within these regions can occur. If the primer-binding site region has been altered by mutation, the amplification of that allele will be adversely affected.

Concordance studies between commercially available kits have shown allelic dropout in many loci including D16S539 [1], vWA[2], D18S51 [3], D8S1179 [4–6], D13S317 [7, 8], etc. While the exact primer sequences for PowerPlex 16 kit are available [9], the sequences for AmpFlSTR kits are not, and the identification of these sequence variants in primer-binding sites between the two kits has only been performed occasionally. Nevertheless, it is important that these mutations and their frequencies be published [6].

In parentage analysis, we found two cases in which there was a single inconsistency in the Mendelian inheritance pattern at CSF1PO by using Identifiler kit. In the two cases, a putative parent and a child appeared to be homozygous for different alleles at CSF1PO (e.g., the genotype of a putative parent was 12, 12 and that of the child was 10, 10). Three samples occurring allelic dropout in the STR system CSF1PO were confirmed by reamplification of the samples and further testing using the PowerPlex 16 kit (Promega Corporation, Madison, WI) following manufacturer's protocols. The causes of the discrepancies between kits detected in three individuals were analyzed by sequencing.

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Materials and methods

Blood samples were obtained during kinship analysis performed in our institute. DNA was extracted by the standard Chelex method and amplified according to the manufacturers' instructions (Applied Biosystems for AmpFlSTR Identifiler and Promega for PowerPlex 16). Genetic profiles were obtained using ABI 3130 with the use of GeneMapper software (Applied Biosystems). There were three genomic DNA samples 2 (daughter of 3), 3, and 4 exhibiting allele dropout at CSF1PO locus when amplified with the AmpFLSTR Identifiler kit. The CSF1PO locus was also singleplex amplified with a previously described short primer pair [10] and analyzed by 5% nondenaturing polyacrylamide gel electrophoresis and silver staining. PCR amplification was performed using a new primer pair external to the amplicons generated by the AmpFlSTRs kits [11]. The amplicon generated by this new primer pair had a length of 691 bp for allele 11 compared with a length of 326 bp with the Identifiler kits. Sequence analysis (ABI 3730) was performed in both directions using the new long amplification primers. The new primer sequence are the following: forward, 5′ -CAAGGCTCAAAGGCAAAGAG-3′; backward, 5′-GCTTCAGGGTCTGAGTCCAG-3′.

Results and discussion

Apparent homozygosity at the CSF1PO locus was revealed in three subjects with Identifiler: 2 (10, 10), 3 (12, 12), and 4 (10, 10). However, using the PowerPlex 16 kit, 2, 3, and 4 were heterozygous at the CSF1PO locus, with allele 11 revealed.

The heterozygote profile for CSF1PO in these three subjects was confirmed (data not shown) using alternative primers reported previously [10] and with a new primer pair described in this study.

Sequence analysis of the CSF1PO products with external primers revealed a C–A single mutation at position 11860 (GenBank sequence X14720), corresponding to a region located upstream of the polymorphic repeat site of the CSF1PO locus (data not shown).

The 11860 C>A variation was associated with allele 11, and the primer pairs that correctly amplified this allele did not coincide with the mutation site. Thus, we can speculate that the 11860 C>A mutation is presumably located within the binding region of the forward primer.

Two out of about 400 samples, or approximately 0.5%, were typed as homozygotes when the Identifiler kit was used, but typed as heterozygotes with PowerPlex 16. However, it is time-consuming and costly to type every individual with two different kits and it is known that duplicate typing is not feasible in every laboratory involved in database constitution. Thus, the kit or method used should be mentioned in the database, and the primer sequences should be available. Meanwhile, the availability of alternative primer sets as practical aids for scientists to investigate suspected allelic dropout is also becoming more critical.

Lower stringency search algorithms may be used to address this issue. For example, the CODIS search algorithm and match criteria can be loosened on search using 26 possible alleles from the 13 STRs by only requiring a match at 25 out of 26 possible alleles [12].

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