Legal Medicine 13 (2011) 30–34

Contents lists available at ScienceDirect

Legal Medicine

journal homepage: www.elsevier.com/locate/legalmed

Case Report

Identification of dual false indirect exclusions on the D5S818 and FGA loci

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ARTICLE INFO

Article history:
Received 11 July 2010
Received in revised form 17 August 2010
Accepted 24 August 2010
Available online 27 October 2010

Keywords:
False indirect exclusion
D5S818
FGA
Short tandem repeat
Primer-binding site

ABSTRACT

Here, we present a case in which the result of a maternity test was obscured due to two false indirect exclusions that occurred in two out of 15 genetic loci through the use of the AmpFlSTR Identifiler PCR Amplification kit (Applied Biosystems, Foster City, CA). The Identifiler kit failed to amplify allele 11 of the D5S818 system on the child and failed to capture the existence of allele 13 on the FGA system on both mother and child. The situation was remedied through use of the PowerPlex 16 PCR Amplification Kit (Promega, Madison, WI) which used different primers with a different allele range than that of the Identifiler kit. Maternity was confirmed through sequencing and it was found that the failure of the Identifiler kit to amplify allele 11 on the D5S818 system was the result of an incompatibility to the primer-binding site due to a mutation that changed a guanine (G) into a thymine (T) 55 base pairs (bp) downstream of the repeat. The inability of the Identifiler kit to pick up allele 13 of the FGA system was due to the out-of-range location of the allele. Indirect exclusions can be misleading if they are not fully investigated since allele range as well as primer-binding affinity are two confounders that must be addressed to ensure accuracy of the test results.

1. Introduction

Polymerase chain reaction (PCR) based short tandem repeat (STR) technologies are an invaluable tool for human identity testing, including maternity or paternity testing [1]. The use of STR detection in these tests is widely accepted and much more frequently used (above 98%) over other common methods of genetic recognition including restriction fragment length polymorphisms (RFLP), human leukocyte antigen (HLA) class II molecules, etc. [2]. However, problems can arise when mutations occur at primer-binding sites that cause allele drop-outs or when STR multiplex kits are not designed to recognize unusually large or small alleles of a given locus [3–5]. Here, we see one false indirect exclusion on the D5S818 locus resultant of a mutation that affected the primer-binding site of the child and another false indirect exclusion on the FGA locus due to the off-ladder (OL) position of the rare allele 13 in both mother and child when we used Applied Biosystems (ABI) AmpFlSTR Identifiler PCR Amplification kit to carry out a maternity testing.

2. Materials and methods

2.1. Sample preparation

Buccal swabs were collected from the subjects and the QIAamp DNA Blood Mini kit (QIAgen, CA) was used to extract DNA. DNA concentration of each sample was determined with the PicoGreen Kit (Invitrogen, CA) and diluted to 0.2 ng/uL.

2.2. Genetic markers

The Identifiler kit (Applied Biosystems, CA) was used to determine paternity and maternity by the following genetic markers: D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA. Our in-house polymerase chain reaction (PCR) short tandem repeat (STR) method was used to detect the following STR systems: D21S11, DXS6809, DXS8337, D18S1002, D18S51, D18S386, D18S535, D21S1412, D13S256, D21S1435, D13S631, D13S258, XHPRT, and D21S1411. The Powerplex 16 kit (Promega, IN) was used to test for these following loci: Penta E, D18S51, D21S11, TH01, D3S1358, FGA, TPOX, D8S1179, vWA, Penta D, CSF1PO, D16S539, D7S820, D13S317, and D5S818.

2.3. DNA amplification and detection

Multiplex quantification fluorescent PCR was used with 1.2 ng of DNA to amplify the STR loci. Tests run with commercial kits

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doi:10.1016/j.legalmed.2010.08.006

were all done following the instructions provider by the manufacturers. The in-house PCR STR method was performed using a master mix of 3 uL Primer mix (20 uM each), 0.3 uL of AmpliTaq (5 U/mL) (Applied Biosystems, CA) and 6 uL of reaction mix consisting of 2 mM dNTP, 15 mM $MgCl_2$ and 10× PCR buffer. The PCR program was set to an 11-min initial incubation at 94 °C, followed by 28 cycles of 1 min denaturation at 94 °C, 1 min annealing at 59 °C, and 1 min extension at 72 °C, with 1 h of final extension at 60 °C. The amplified STRs were detected with the 3130 Genetic Analyzer (Applied Biosystems, CA).

3. Results and discussion

This case was initiated to prove both paternity and maternity of a child. The paternity test was carried out with the Identifiler kit and no exclusions were observed; the alleged father could not be excluded as the biological father. The Identifiler kit was then used to test for maternal relationship between alleged mother and child. However, of the fifteen STR systems, the Identifiler kit indicated indirect exclusions in two of them, specifically, the D5S818 system on chromosome 5 and the FGA system on chromosome 4. According to the standard operations procedure manual that our laboratory follows, at least three STR systems out of the fifteen must be excluded before it is acceptable to exclude the possibility of biological relationship. The American Association of Blood Banks regulations require that the combined relationship index (CRI) reaches at least 100 in order to conclude non-exclusion [6]; however, in this case, although there were two genetic systems excluded, the CRI was only 10.11, which means that the relationship here is inconclusive.

To further investigate the case, we included another fourteen STR systems. This time, we used an in-house PCR STR method and of which there were no exclusions detected on any of the fourteen STR systems; two of these systems overlapped with Identifiler loci (D21S11 and D18S51).

To investigate the possibility of false exclusions due to mutations on the primer-binding sites and/or off-range alleles, we sent anonymous DNA samples to the National Institute of Standards and Technology (NIST) Applied Genetics Group for variant allele sequencing. The NIST variant allele sequencing of unusual STR alleles found by members of the human identity testing community is carried out under an agreement with the National Institute of Justice. The sequencing of unusual alleles helps to reveal the molecular basis for their variation. At NIST the samples were amplified with Powerplex 16 kit (PP16). Confirmation of the PP16 results was confirmed with different primers, designed for sequencing through the regions of common primer-binding sites.

Both indirect exclusions from the first fifteen STR systems are shown in Fig. 1. The figure shows that in the D5S818 locus, allele 7 of the child was obtained from the father but there seems to be no maternal contribution, as the child, seemingly a homozygote at allele 7, has not received a copy of allele 11 from the mother, a homozygote at that allele. However, it can be noticed that there is a very small peak in the child at the location where allele 11 should be. This was later revealed to be the result of failure to amplify the variant allele 11 due to a mutation that changed a guanine (G) into a thymine (T) 55 base pairs (bp) downstream of the repeat abbreviated as 11 (D55 G → T) based on the nomenclature of variant allele proposed by Gusmao et al. [7] on the behalf of the Identifiler kit due to incompatibility at the primer-binding site. Alves et al. [8] described D5S818 variant alleles with a SNP D36 T → C that affects annealing of the reverse PP16 primer. In the FGA system, the father contributed allele 23 while the mother at this locus seemingly has no contribution. While it may have appeared that both

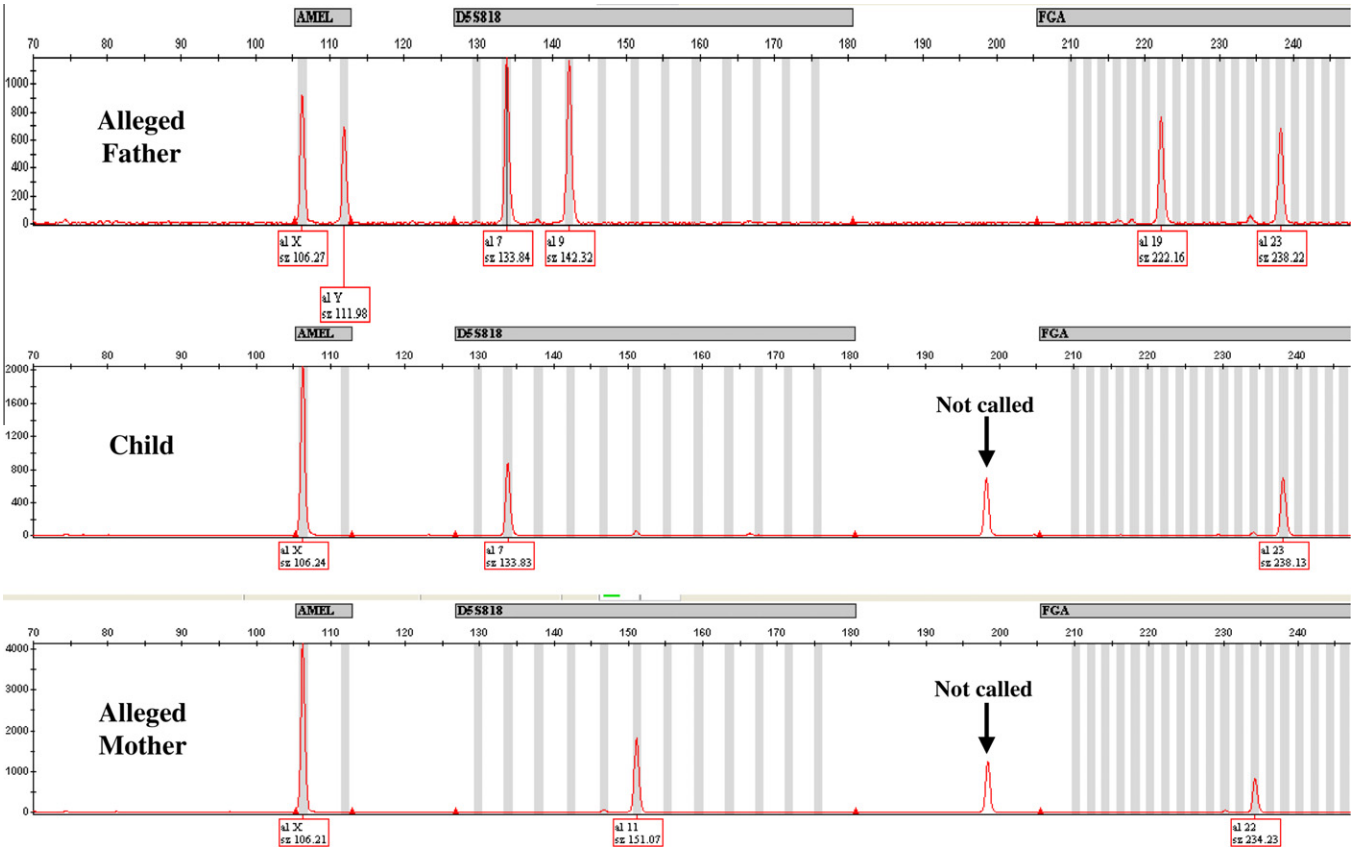

Fig. 1. Electrophoresis results of the alleged father, child and alleged mother at the D5S818 and FGA loci obtained via the Identifiler STR typing kit.