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CASE REPORT

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Presentation of a Three-Banded Allele Pattern—Analysis and Interpretation

ABSTRACT: The Israel police forensic biology laboratory received as an item of evidence in an attempted murder case, a pair of trousers belonging to a suspect. A bloodstain was observed on the trousers and analyzed by STR typing for nine loci using the Promega GenePrint™ STR silver stain detection kits. The genetic profile defined was found to be identical to that of the victim's at all nine loci. Within this profile a three-banded allele pattern was observed at the D16S539 locus, both in the bloodstain and in the victim's reference blood sample. Confirmation of this phenomenon was accomplished by amplifying the extracted DNA from both the trousers and the victim's blood sample using the PowerPlex 16 kit by Promega and the AmpFℓSTR SGM Plus kit by Perkin Elmer, followed by analysis of the amplification products by capillary electrophoresis on the ABI prism 310 genetic analyzer. The same three-banded allele pattern was observed at the D16S539 locus in both specimen and reference DNA, using each of the three kits. Three additional loci located on chromosome 16 (D16S3407, D16S2617 and D16S3082), not employed for forensic identification, were also analyzed and did not show three-banded allele pattern.

KEYWORDS: forensic science, DNA typing, D16S539, polymerase chain reaction, short tandem repeats, bloodstain, silver staining

At any given STR locus, one expects to encounter the appearance of two alleles, each inherited either maternally or paternally. These alleles can be observed as a two-banded pattern, as seen in a heterozygote, or a single banded pattern as seen in a homozygote. In very rare instances, a three-banded pattern may be observed at a single locus in a multiplex STR profile, which is not a result of a mixture (1). This can be caused by an extra chromosomal occurrence, a duplication of the locus, mosaicism or chimerism (2,3). Three-banded patterns have been reported at the TPOX, CSF1PO, FGA, D5S818, D21S11, and D18S51 loci (1).

This paper reports a three-banded allele pattern at the D16S539 locus, which was encountered in our laboratory during analysis of an item from an attempted murder case.

Material and Methods

DNA was extracted using a phenol/chloroform extraction method (4). The extracted DNA was then amplified using the PCR method for the following short tandem repeat (STR) loci: CSF1PO, TPOX, TH01, F13A, FESFPS, VWA, D7S820, D13S317 and D16S539 using CTT triplex, FFV triplex and Silver STR™ III triplex kits (Promega, Madison, WI) (5), which are used for routine case work in our laboratory. The amplification products were separated on 4% polyacrylamide gels and visualized by silver staining (6). The DNA was further analyzed using two additional multi-locus amplification kits: the PowerPlex 16 kit, (Promega, Madison, WI) containing 16 loci, and the AmpFℓSTR SGM Plus kit (Perkin-Elmer Applied BioSystems, Foster City, CA) containing 11 loci (7,8). Approximately 2 ng of template DNA was amplified using the GeneAmp PCR System 9700 (Perkin Elmer Applied BioSystems, Foster City, CA). Amplification reactions were carried out according to the manufacturer's instruction (Promega, Madison, WI and Perkin Elmer, Foster City, CA). Capillary electrophoresis was performed using the Perkin Elmer Applied BioSystems ABI CE 310 systems, and alleles were defined using the GeneScan and Genotyper 2.5 software (Applied BioSystems, Foster City, CA). We analyzed three additional loci on chromosome 16 namely D16S3407 localized to 16q24.3, D16S2617, and D16S3082 that were both mapped to the short arm of chromosome 16 (16p13.3). Amplified PCR fragments were separated on a denaturing polyacrylamide gel and analyzed using silver staining.

Results and Discussion

A bloodstain was detected on trousers belonging to a suspect from a murder attempt. The profile obtained from the item of evidence was then compared with the victim's profile and found to be identical. Table 1 presents the results of STR analysis for both the trousers and the victim's blood at nine STR loci amplified as visualized using silver staining and capillary electrophoresis. A three-banded allele pattern was observed at the D16S539 locus.

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TABLE 1—*Results of STR analysis on blood stain found the item of evidance and the victim's blood. Data are reported as genotypes.*

	TH01	TPOX	CSF	vWA	FESFPS	F13A	D7S8S0	D13S317	D16S539*
Blood stain	6.8	8.11	10.10	16.17	12.12	5.7	10.11	12.12	9.11.12
Victim	6.8	8.11	10.10	16.17	12.12	5.7	10.11	12.12	9.11.12

* Locus demonstrating three-banded allele pattern.

Common loci among the different kits employed provide identical results including the locus D16S539, where the three-allele pattern was observed.

As can be seen in Figs. 1 to 3, the three alleles of the D16S539 locus in each kit employed, whether by gel electrophoresis or capillary electrophoresis, showed similar intensities.

The DNA from the victim's blood was further analyzed by typing for additional polymorphic loci mapped to chromosome 16 (D16S3407, D16S2617, D16S3082). None of these additional loci demonstrated a tri-allelic pattern (data not shown).

A three-allele pattern at a single locus is a rare phenomenon, which has been reported at a number of STR loci. To the best of our knowledge, the occurrence of three-allele pattern at the D16S539 locus had not been reported in the forensic science literature at the time this article was written. Since the submission of this manuscript, a three-banded pattern at this locus has been reported in the NIST STRBase web site (http://www.cstl.nist.gov/biotech/strbase) by K. Morton, of the Texas Department of Public Safety. They reported a 12, 13, and 14 pattern at the D16S539 loci found in a convicted offenders database.

A number of explanations for these three-banded allele patterns have been suggested: (a) a genetic duplication of a small chromosomal region containing the STR locus, (b) an improper segregation resulting from chromosomal meiotic or mitotic nondisjunction that leads to either true trisomy or to mosaicism, and (c) chimerism (3).

In order to clarify the reason for this phenomenon, we have genotyped our sample for three additional loci in chromosome 16. We have used the markers D16S3407, D16S2617 and D16S3082 and identified a bi-allelic pattern for each of the other markers. Since these markers are located at various sites on chromosome 16, we conclude that in this case, a tri-allelic pattern at the D16S539 locus results from a duplication that occurred within the area and not as a result of a trisomy.

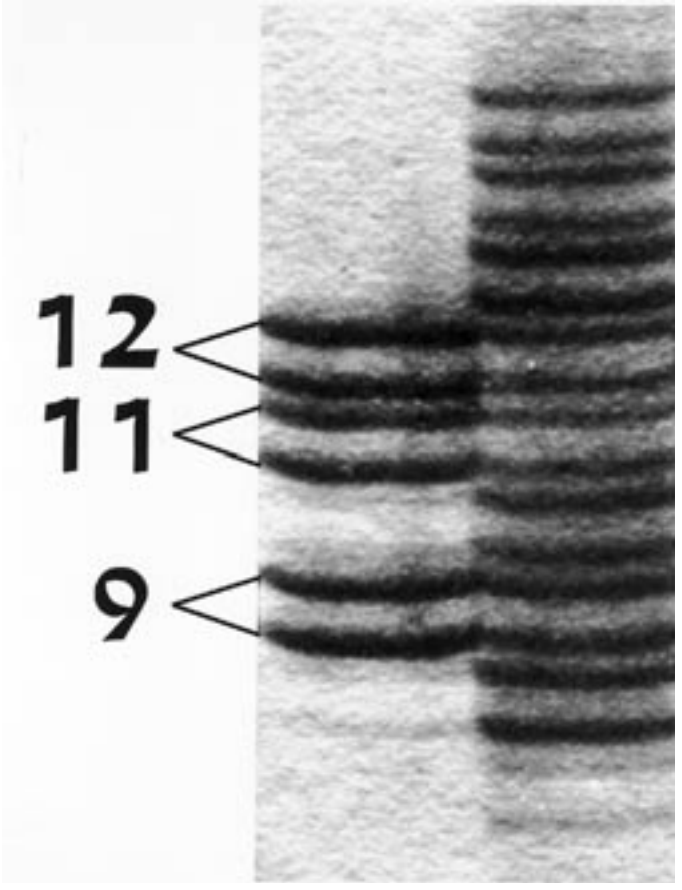

FIG. 1—*Polyacrylamide gel electrophoresis of the D16S539 locus visualized by silver staining.*

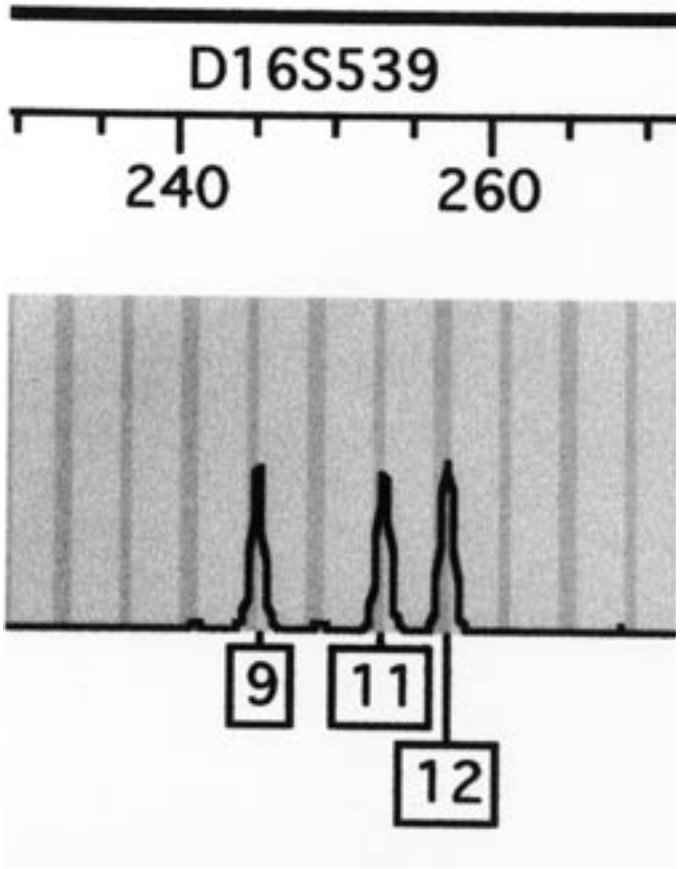

FIG. 2—*Capillary electrophoresis of the D16S539 locus in AmpFℓSTR SGM+ multiplex using the ABI CE310 system. The RFU signal intensities obtained were 1047 for allele 9, 1017 for allele 11 and 1087 for allele 12.*

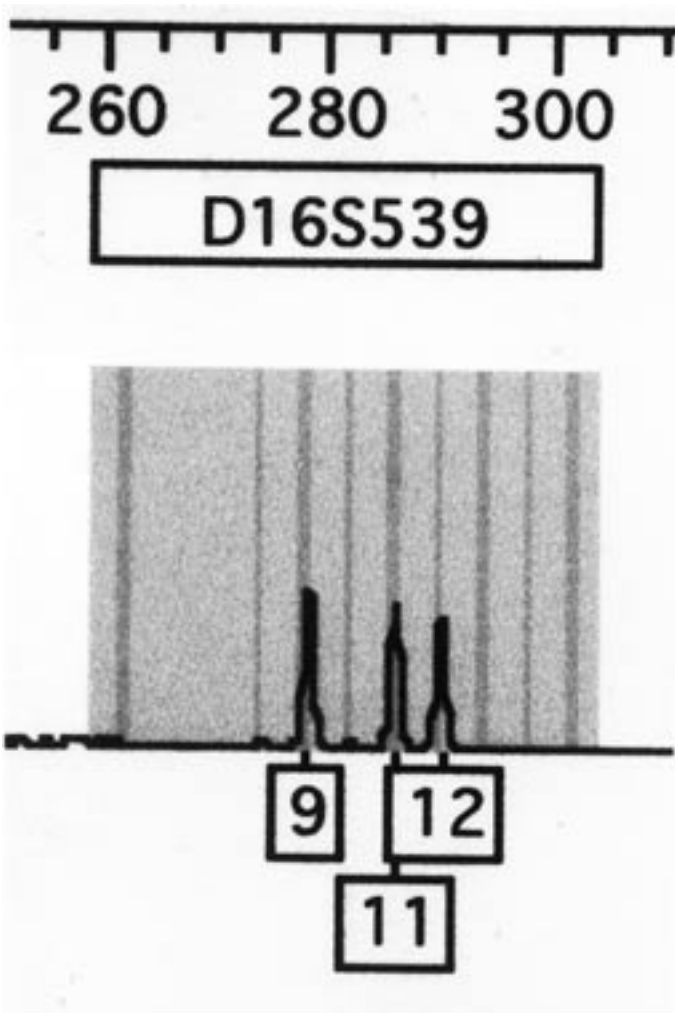

FIG. 3—*Capillary electrophoresis of the D16S539 locus in PowerPlex 16 multiplex using the ABI CE310 system. The RFU signal intensities obtained were 3204 for allele 9, 2876 for allele 11 and 2568 for allele 12.*

Because this report presents an example of tri-allelic phenomenon from actual casework, we were unable to get blood samples from additional family members or other tissue samples in order to investigate whether this three-banded allelic pattern was inherited or whether it represents a newly occurring event.

One should be aware that if this phenomenon occurred due to a mosaic event, results obtained from exhibits containing a tissue other than the reference sample (i.e., a sperm stained exhibit versus reference blood sample) may show different results. A more accurate result may be obtained by requesting as a reference the identical body fluid as found on the exhibit being tested.

In order to report our findings to court, we needed to decide on a statistical approach regarding this variant. From personal communications, we became aware of several attitudes toward calculating the statistical probability of this three-allelic event within the profile.

One approach was to use the standard $2pq$ for heterozygotes and to choose the two most common allele frequencies of the three for a value for p and q. A second approach avoided attempting any statistical calculations in the report regarding the frequency of these three alleles at this locus due to the rarity of the occurrence.

Since we had never previously encountered such a phenomenon at the D16S539 locus or any other loci in our laboratory, we decided to take the most conservative approach and include the D16S539 locus in the table of results, but *not* to include it in the statistics provided. We calculated the statistical probability of the profile using only eight loci (TH01, TPOX, CSF, VWA, FESFPS, F13A, D7S820 and D13S317) which resulted in a frequency of approximately one in thirty million, according to the Israeli population, using a 2% theta correction (10). Our report was worded so as to stress the fact that a three-allele band pattern at the D16S539 locus is an exceptionally rare occurrence and actually would significantly decrease the frequency of such a profile to be found in the population even more so, but because of the lack of statistical data regarding this rare phenomenon we were unwilling to give it a statistical value.

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