Austin Journal of Forensic Science and Criminology

Open Access

Austin Publishing Group

Case Report

Rare Finding of a Three Banded Allele Pattern of vWA Locus-Analysis and Interpretation at the State Forensic Science Laboratory of Jharkhand

Nand JK*, Soren AN, Kumari N and Singh RS
Division of DNA Biology Section, State Forensic Science Laboratory Jharkhand, India

***Corresponding author:** Jwala Kumar Nand, State Forensic Science Laboratory, Ministry of Home Affairs, Near BirsaMunda Jail, Hotwar, Ranchi, Jharkhand - 835217, India

Received: August 17, 2017; **Accepted:** October 05, 2017; **Published:** October 19, 2017

Abstract

The Jharkhand State Forensic Science Laboratory received as an item of Evidence in rape and murder case; the scalp tissue of dead fetuses and reference blood sample of suspected father analyzed by STR typing for fifteen and one Amelogenin loci using Identifier Plus kits. In the genetic profile generated, it was found that all paternal alleles of the fetus were present in the reference blood sample of the suspect. However, within the DNA profile of fetus a three banded allele pattern was observed at the vWA locus. Confirmation of this pattern was carried out by amplifying the extracted DNA from both the samples using identifier plus kits with three different lot and batch numbers, followed by analysis of the amplified products by capillary electrophoresis on the ABI 3130 genetic analyzer. The same three banded allele pattern was observed at the vWA locus in each repetition of the sample specimen.

Keywords: Forensic science; DNA typing; vWA; Triallelic; Polymerase chain reaction

Introduction

In all STR locus of human identification, it is common that two alleles is expected, which is inherited each from paternal and maternal side. It can be observed as a double peak in the electropherogram as seen in a heterozygote, or a single peak as seen in a homozygote condition in the following 15 autosomal STRs: D8S1179, D21S11, D7820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338,D19S433,Vwa, TPOX, D18S51, D5S818, FGA and one Amelogenin in Identifiler plus multiplex (Applied Biosystems) kit. It is a very rare condition, three-banded pattern or trellis pattern seen at a single locus in a multiplex STR profiling, which is not due to a mix profile or contamination and PCR artifact. It can be caused by trisomy, a duplication of the marked region mosaicism or chimerism [1-5]. Three banded patterns already reported at locus VWA two times on the website (http://www.cstl.nist.gov/biotech/strbase) there were also on other locus it was reported that trialllelic patterns like CSF1PO, FGA, D5S818, D21S11, D16S539. In this article we report the three-banded pattern at the locus VWA, this locus on Chromosome 12; 12p13.31-around 20 MB in size it is a tetra nucleotide repeat of Compound STR Repeat Motif:-[TCTG] [TCTA] taken from 40th intron of von Willebrand Factor gene.

Case Presentation

Jharkhand state police station an FIR lodged by sister of victim under sections: 376/302/34 of Indian Penal Code on the basis of crime report that her 25 year sister was killed by a man, namely XYZ kumar (identity has been concealed), age 27 years girl raped and after that he warned her if she disclosed to anybody then he will kill her, after some day of this incidence she will become pregnant of illegal child, as accused know this things start torture her for abortion she denied, for destruction evidence he killed her brutally and he want to burned their dead body, but police personhood kept him by secret information and arrested. The investigation agency collected two samples by medical officer collected sample from dead fetus and accused blood.

Sample soaked on gauze piece that is dried blood sample from that so called identity of illegal father could be established by DNA testing. These exhibits were received at State Forensic Science Laboratory Jharkhand.

Materials and Methods

Sample

After postmortem the scalp tissue were collected by authority, exhibits generally reddish brown small piece it needs screening and preprocessing.

Sample preparation

The surface of the fetus tissue was removed using a scalpel scissors and forceps order to eliminate potential contamination and adhered to it, Samples were then cut into thin slices and washed twice with 5% sodium hypochlorite for 15 min, then rinsed properly and soaked in water for 15 min, once rinsed in 70% ethanol and finally rinsed in 100% ethanol.

DNA extraction

From each exhibit respective tissue piece and reference blood sample were incubated. We're submerged in 400 µl of extraction buffer (10 mm Tris-Cl, pH 8.0, 0.1 M EDTA, pH 8.0, 20 µg/ml RNase A, 0.5% SDS) in separate 1.5 ml capacity append off tubes for 1 hours at 37 °C. Proteinase K (Mark GeNitm) (4 mg/µl) was then added and incubated for further 12-15 hours at 56 °C with periodic swirling 120 RPM. The solution was brought to room temperature and subjected

Citation: Nand JK, Soren AN, Kumari N and Singh RS. Rare Finding of a Three Banded Allele Pattern of vWA Locus-Analysis and Interpretation at the State Forensic Science Laboratory of Jharkhand. Austin J Forensic Sci Criminol. 2017; 4(4): 1072.

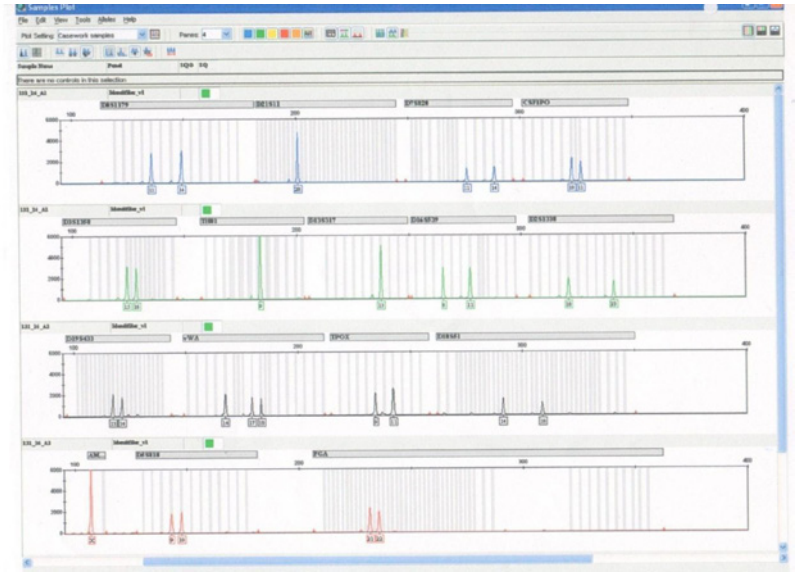

Figure 1: Electropherogram of the fifteen loci and one Ameliogenin of the Exhibit marked-A1 (Source: dead fetus tissue cuttings).

Table 1: The resultant allelic distribution (genotypes) obtained from the studied loci in the exhibits.

Sl. No.	Name of loci	Exhibit marked-A1 (Source: dead fetus tissue)	Exhibit marked-B (Source: Blood positive gauze piece cuttings of Mr. X)
1	D8S1179	11, **14**	14, 16
2	D21S11	28, **28**	28, 31
3	D7S820	**11**, 14	10, 11
4	CSF1PO	**10**, 11	10, 12
5	D3S1358	**15**, 16	15, 17
6	THO1	9, **9**	9, 9
7	D13S317	13, **13**	12, 13
8	D16S539	8, **11**	11, 12
9	D2S1338	18, **23**	19, 23
10	D19S433	13, **14**	13, 14
11	vWA	14, **17**, 18	16, 17
12	TPOX	**9**, 11	9, 9
13	D18S51	**14**, 18	14, 17
14	D5S818	9, **10**	10, 12
15	FGA	21, **22**	22, 23
16	Amelogenin	X, X	X, Y

to Organic methods of DNA extraction. The tubes were stored at 4 ˚C until its use.

DNA quantification

DNA quantification of the 12 samples (Table 1) was performed by real-time polymerase chain reaction (RT-PCR) using the Quantifiler® Human DNA Quantification kit (Life Technologies Inc.) [6] Containing DNA standard solution (200 ng/µl), Quantifier Human Primer mix, and Quantifier PCR Reaction Mix. Human Primer Mix (10.5 µl/sample) and PCR Reaction Mix (12.5 µl/sample) were mixed and then dispensed into reaction wells (23 µl each) followed by the addition of 2 µl of sample or standard DNA of known concentration

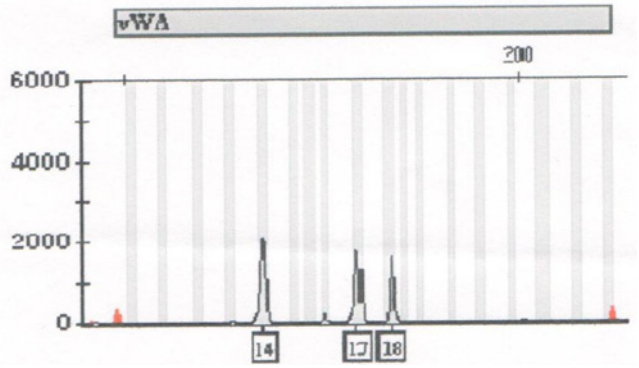

Figure 2: Triallelic Pattern of vWA Locus at 6000 RFU allele 14, 17, 18 Allele Size: 167.37 bp, 179.19 bp and 183.40 bp.

to each well, to obtain a 25 µl PCR reaction system. The amount of DNA was calculated by the real-time PCR machine (Applied Biosystems) and Software: 7500 SDS V.1.2.3.

Identifier plus®

PCR and electrophoresis AmpFISTR Identifiler Plus® kit [7] was used to multiplex PCR reaction for co-amplification of 15 autosomal STRs loci and a gender locus (Table 2). Using 25 µl PCR amplification mixture (10.5 µl of PCR reaction mix, 5.5 µl of Identifier Plus® Primer Set, 9.0 µl of nuclease-free water and 1 µl of DNA template), amplification was carried out under conditions of initial denaturation at 95˚C for 10 min, followed by 29 cycles of denaturation at 94˚C for 1 min, annealing at 59˚C for 1 min and extension at 72˚C for 1 min, and a final extension step at 60˚C for 60 min. The PCR products were then examined using a 10-µl electrophoresis system containing 0.3 µl of GeneScan™ 500 LIZ® Size Standard dye, 8.7 µl of Hi-Di™ formamide and 1.0 µl of PCR product or the AmpFlSTR® Identifiler Plus® allelic ladder. Capillary electrophoresis was performed on an ABI-3130 Genetic Analyzer using 36 cm 4-capillary array (Applied Biosystems). Sizing of the DNA fragments was carried out using Gene Mapper ID

software v3.2 with respect to Gene Scan™ 500.

Results and Discussion

The DNA profile thus obtained from the evidentiary sample and compared with the accused DNA profile or so called father. Table 1 presents that it is found to be all the paternal alleles are accounted to be present in the DNA profile of evidence sample or dead fetus of a female child. At all the fifteen loci and one amelogenin loci successfully amplified and visualized by capillary electrophoresis; hence it is therefore confirmed that the accused is the biological father of dead fetus marked 'A'. However, the triallelic pattern was observed at the vWA locus. It is common loci use in human identification and also included in CODIS 13 core loci, in order to confirm it is not mixed profile, PCR artifact or due to contamination we repeat our experiment three times accomplished by three different Identifiler Plus kits (Applied Biosystem) Lot number 1604112, 1608116, 1611118 respectively shows that identical results, at the locus vWA where the three- allele pattern was observed as seen in Figure 1,2 the three alleles of the vWA. Capillary electrophoresis showed similar peak intensity shows from the DNA from the exhibit marked 'A'. Triallelic pattern of Vwa and other locus is a rare phenomenon, although trialling of this type pattern that is 14, 17, 18 of vWA locus reported two times to the best of our knowledge, the occurrence of triallelic pattern at the locus was reported 26 types patterns in the field of forensic science according to the NIST STRbase web site (http://www.cstl.nist.gov/biotech/strbase), of the Texas Department of public safety. Since the submission of this manuscript, a three-banded allele pattern at this locus has been reported to the NIST STRBase data base. STRs are globally used for human identification because of its variety and stability, in the field of forensic science STRs profiling produced the high degree of error free database and a detailed model was proposed for STR repeats length variation due to false priming during DNA synthesis. A number of explanations for these three banded allele patterns have been suggested that is (a) a gene duplication of a small chromosomal region containing the STR locus, (b) an improper segregation or resulting from chromosomal meiotic or mitotic non-disjunction that leads to either true trisomy or to mosaicism and (c) chimerism. In order to clarify the reason for this phenomenon, we have to further work on this aspect to explore what was the exact reason for this [8-12].

Acknowledgment

Authors are thankful to the Ministry of Home Department and Director of State Forensic Science Laboratory (SFSL), Ranchi, and Jharkhand, India for providing the support and facility and sound atmosphere to conduct the work.

References

1. Butler JM. Forensic DNA Typing. Academic Press, 2001. [2] J.M. Butler, Genetics and genomics of core short tandem repeat loci used inhuman identity testing. J Forensic Sci. 2006; 51: 253-265.
2. Crouse CA, Rogers S, Amiott E, Gibson S, Masibay A. Analysis and interpretation of short tandem repeat microvariants and a three-banded allele patterns using multiple allele detection systems. J Forensic Sci. 1999; 44: 87-94.
3. Rubocki RJ, McCue BJ, Duffy KJ, Shepard KL, Shepherd SJ, Wisecarver JL. Natural DNA mixtures generated in fraternal twins in utero. J Forensic Sci. 2001; 46:120-5.
4. Thomson JA, Ayres KL, Pilate V, Barett MN, Walker JIH, Debenham PG. Analysis of disputed single-parent/child and sibling relationships using 16 STR loci. Int J Legal Med. 2001; 115: 128-134.
5. Sambrook J, Fritsch Ef, Maniatis T. Molecular. cloning: A laboratory manual. New York: Cold Spring Harbor Laboratory Press. 1989.
6. Wang DY, Chang CW, Lagace RE, Calandro LM, Hennessy LK. Developmental validation of the AmpFlSTR® Identifiler® Plus PCR Amplification Kit: an established multiplex assay with improved performance. J Forensic Sci. 2012; 57: 453-465.
7. Green RL, Roinestad IC, BolandC, Hennessy LK. Developmental validation of the Quantifiler real-time PCR kits for the quantification of human nuclear DNA samples. J Forensic Sci. 2005; 50: 809-825.
8. Clayton TM, Guest JL, Urquhart AJ, Gill PD. A genetic basis for anomalous band patterns encountered during DNA STR profiling. J Forensic Sci. 2004; 49: 1207-1214.
9. Huel RL, Basic´ L, Todorovic´ KM, Smajlovic´ L, Eminovic´ I, Berbic´ I, et al. Variant alleles, triallelic patterns, and point mutations observed in nuclear short tandem repeat typing of populations in Bosnia and Serbia. Croat Med J. 2007; 48: 494-502.
10. Vidal C, Cassar M. A case of tri-allelic pattern at locus D3S1358 on chromosome 3p21 inherited from paternal grandmother. Forensic Sci Int Genet. 2008; 2: 372-375.
11. Zamir A, Shpitze M, Carla Oz, Motro U, Meiner V, Gafny R. Presentation of a Three-Banded Allele Pattern-Analysis and Interpretation. J Forensic Sci. 2002; 47: 4.