

A Case Report

Rare Finding of a Triallelic Pattern at The DYF387S1 Locus in the Population of Rajasthan, India

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Abstract

This study reported a sexual assault case where Y-STR DNA profile was tested for the confirmation of male DNA. Within the DNA profile of the sample of accused, a tri-allelic pattern was detected at locus DYF387S1. Since this locus doesn't exist in any of the commercially available Y-STR amplification kits, So, the confirmation of this tri-allelic pattern was done by re-extracting and amplifying the extracted DNA by using Y-Filer Plus amplification kit (Thermo Fischer Scientific, CA, USA—Thermo) followed by separation of amplicons on Genetic Analyzer 3500xl. The allelic patterns of case sample belong to Type-2 pattern of tri-allelic outcome because peak heights were in balanced manner. This region is very much prone towards any structural mutation which could be either addition or deletion of allele, so for the forensic point of view this locus should be used carefully for kinship analysis.

Keywords: *Forensic Science, Locus DYF387S1, Tri-allelic pattern, Mutation.*

Introduction

DNA Fingerprinting, one of the best and fast technologies of today's scenario for criminal investigation is based on autosomal and Y-STR analysis. Its discovery is one of the most fascinating scientific breakthroughs ever. It has a vital function in forensic science and the criminal justice system,

and is not restricted to laboratory study. Since its discovery, DNA fingerprinting has endured rapid technological improvements. This has become the most powerful technique globally for judicial system to assist both in the conviction of the criminal and the exoneration of the innocent. The remarkable capability of DNA technology as an identification tool has brought a massive change in criminal justice⁽¹⁾.

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In cases related to sexual offence, there is always a struggle between victims and criminals. Even if the criminal does not leave sperms behind, it is possible to have stains of any body fluid and hairs of accused on clothes of victim as well as at

crime spot⁽²⁾. In the same manner, after a murder incident, even if the criminal cleaned the blood, latent blood stains would be identified using special methods like Teichman tests, RSID (Rapid Stain Identification Series) Test for Human blood and ABA Card Hematrace. Biological samples are used in forensic science because each and every individual has its own genetic characteristic which is inherited from father and mother both.

Short tandem repeats (STRs), also known as microsatellite or simple sequence repeats, are short repeated DNA sequences with 16 bp⁽³⁾. Their scattered parts account for about 3% of human genome. However, their distribution within the chromosome is not uniform and are less common in the sub-telomeres region of chromosomes⁽⁴⁾. Only about 8% of STRs are found in coding region whereas others are found in non-coding regions. In humans, highest density of STR is present at the chromosome-19⁽⁴⁾. On an average, STR is present in the human genome at every 2,000 bp⁽⁵⁾. The most common STRs present in humans are A-rich units: A, AC, AAAN, AAN, and AG (N=C or G or T)⁽⁶⁾. The most commonly used STR loci are 100-500 bp in length, which are shorter than the smallest VNTR loci. More than 100,000 STR exist in human genome⁽⁷⁾. Some STRs have been characterized specifically for forensic DNA profiling. STRs can be grouped into different types based on different repeat units. On the basis of nucleotide, STRs are classified into mono-, di-, tri-, tetra-, penta- and hexa- nucleotide repeats. On the basis of repeat structure, STRs are classified into perfect repeats (simple repeats), only one repetitive unit, and imperfect repeats (compound repeats)⁽⁸⁾. In forensic inferences, autosomal short tandem repeats (STR),

Y-STR markers and X-STR markers are the main tool of DNA profiling. In 1997, 13 autosomal STR loci were used by the FBI laboratory which forms the backbone of the U.S. National DNA Database as CODIS (The Combined DNA Index System). These 13 CODIS STR loci are used by other countries around the globe as U.S has its own DNA database - The Federal DNA Database Unit (FDDU)⁽⁹⁾. United Kingdom develop their population data by NDNAD (National DNA Database)⁽¹⁰⁾ whereas Europe has its own DNA database- ENFSI (The European Network of Forensic Science Institutes)⁽¹¹⁾. Several states in India also have their population data based on the forensic casework^{(12),(13),(14), (15), (16), (17)}.

Autosomal short tandem repeat (STR) markers are used to establish identity of missing persons, paternity relations and linking accused to crime scenes. Y-STR is especially obtained from male Y chromosome and used in patrilineage study in forensics. These Y-STRs provide a weaker analysis than the autosomal STRs as the Y chromosome is inherited from the father and is only found in men in any paternity cases.

DYF387S1 is a Y chromosome Short Tandem Repeat (Y-STR) used in forensic genetics and included in the Y chromosome haplotype reference database (<https://yhrd.org>). It is a rapidly mutating, multi-locus Y-STR marker. There are two paralogs within the palindromic sequence, which is the major region of structural variation.

Among the 27 Y-STRs available in the Y-Filer Plus PCR amplification kit, five Y-STR viz. DYF399S1, DYF387S1, DYS526a, DYF403S1a, DYF404S1Y-STRs are known for their high

mutation rate⁽¹⁸⁾.

In our study, a three peak pattern at the locus DYF387S1 which is known as tri-allelic pattern has been reported. Despite the fact that tri-allelic pattern are limited in occurrence, the data present on the STR base (<http://www.cstl.nist.gov/biotech/strbase>) specified that tri-allelic pattern is found unusually in the DYF387S1 Y-STR locus. Chimerism or structural mutations like deletion, duplication or gene conversion are the causes of Tri-allelic pattern⁽¹⁹⁾. Tri-allelic patterns are of two types - Type-1 which is more often, as the size of peaks are imbalance, because of somatic mutations in alleles at heterozygous loci resulting in chimerism and Type-2 which has balanced peak intensity and is characteristic of duplication on the same chromosome or translocation or trisomy (Down's syndrome)⁽²⁰⁾.

Material and Method

Samples

The sample was received from district police for the DNA examination in a sexual assault case. Sample was processed at DNA division, State Forensic Science Laboratory, Jaipur, Rajasthan for routine examination. Blood of reference sample was collected for the DNA examination with written consent of the donor. Blood samples are routinely collected on FTA card in the state.

Direct Amplification and Genotyping

A Micro-Punch of 1.2mm was taken from the centre of blood stained area on FTA card. Increasing the size of punch can cause inhibition at the time of amplification. DNA was amplified by using Y FilerTM Plus PCR Amplification kit

(Thermo Fisher Scientific, CA, USA-Thermo). This kit is a six dye (Blue, Green, Yellow, Red, Purple and Orange) short tandem repeats (STRs) multiplex assay that amplifies 27 Y-STRs on DNA strand. 25µl of reaction mixture was added on the micro-centrifuge tube for amplification. The Y FilerTM Plus PCR Amplification kit was especially developed to increase the sensitivity to extract maximum information from the samples. Multiplexing reaction was performed on VeritiTM thermal cycler (Thermo) according to the manufacturer's protocol. Separation of amplicons were performed by capillary electrophoresis in 3500xL Genetic Analyser (Thermo) in standard polymer POP4 (Performance Optimized Polymer). GeneMapperTM ID-X Software v1.6 was used to analyse the data.

Quality Standards

Human Identification Professional Services (HIPS) by Thermo Fisher Scientific CA, USA has validated the DNA division of State Forensic Science laboratory. As per the laboratory internal standards, quality of analysis were revealed through positive and negative controls.

Result and Discussion

Tri-allelic patterns have been identified at a single locus in phenotypically normal individuals during autosomal STR genotyping for forensic purpose⁽²¹⁾. The tri-allelic pattern on FGA locus which is commonly used in forensic inference was also observed⁽²²⁾. They concluded the tri-allelic pattern at FGA locus of alleles 20, 23 & 25. The size of these alleles were 251.58, 263.43 and 271.49 base pairs, respectively and was probably a result

of duplication at this locus.

Genotyping of Y-STRs usually shows mono- or a di-allelic pattern. One of the double-copy locus, DYS385a/b has shown three or four alleles during genotyping⁽²³⁾. The two samples used in the present study showed that sample 1 has six alleles (12, 13, 14, 17, 18, 21) whereas sample 2 has five alleles (13, 18, 19, 20, 21) in DYS385a/b locus. Analysis of Sample 1 showed more compatibility with AGCU Data Y30 Kit as compared to Y filer Plus

and PowerPlex Y23 kits and the amplification of DYS385a/b locus along with six balanced peaks. Whereas results of sample 2 and 3 showed more compatibility with Yfiler Plus and PowerPlex Y23 kits as compared with those of the HG19+14Y kit which showed five peaks at locus DYS385a/b. These three samples were alike and sequenced, and the sequencing results showed similarity with that of the genotyping results. This confirmed five- and six- banded pattern at DYS385a/b locus.

Table-1 : Statistical representation of Electropherogram

PEAKS	Statistical Parameters			
	Data Point	Size	Height	Peak height Ratio
38	6547	295.88	3829	0.9515
39	6590	299.73	3730	0.9515
40	6633	303.52	3784	0.9515

Another locus DYF387S1 due to its high tendency towards structural mutation like deletion, duplication and gene conversion generally showed high genetic diversity as compared to other Y STR markers. Two alleles that are closely linked possibly resulted in rare tri-allelic pattern, so consideration of this locus for kinship analysis should be taken carefully. It implies that the population is genetically characterized in Rajasthan. Further, it is likely probable that powerful, excessive throughput genotyping methods like Next Generation

Sequencing (NGS) should be used for advanced study.

In our study, the tri-allelic pattern on the locus DYF387S1 STR markers of Y-filer plus kit in the population of Rajasthan is found. After repeatedly analysis of respective DNA sample, tri-allelic peaks were observed repeatedly in the electropherogram (Figure-1). Repeats of DYF387S1 marker alleles were found as 38,39 and 40. The peak data point, size, height and peak height ratio were found

as; allele 38 (Data point-6547, size-295.88, height-3829, peak height ratio-0.9515), allele 39 (Data point-6590, size-299.73, height-3730, peak height ratio-0.9515) and 40 (Data point-6633, size-303.52, height-3784, peak height ratio-0.9515) (Table-1). This analysis indicates the presence of Type-2 tri-allelic pattern because the alleles 38, 39, 40 present in the electropherogram shows balanced signal. This is a pioneer study on Tri-allelic pattern at locus DYF387S1 in Indian population especially in Rajasthan state which provides a new aspect in the inference of paternity cases in forensic science.

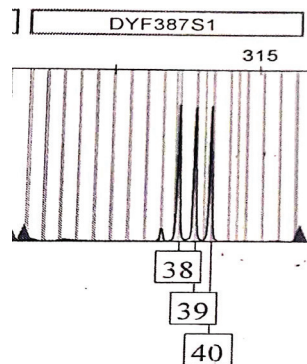


FIGURE-1: Tri-allelic pattern found on DYF387S1

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Conflict of Interest

The authors declare that they have no conflict of interest

Compliance with ethical standards:

As per the declaration of Helsinki, written informed consent was obtained for the study.

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