Case Study

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Multi-allelic Patterns Observed at Y-STR Loci: A Case Report

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ABSTRACT

The forensic relevance of Y-STR analysis is well understood and widely documented. In this report, the authors have described two unusual multi-allelic patterns in Y-STR profiles of individuals observed during forensic examination.The first is a case of a bi-allelic pattern at the locus DYS391observed in a father/son duo in a paternity dispute. The second case is of a tri-allelic pattern at the locus DYF387S1a/b observed in the Y-STR profile of a suspected perpetrator in a case of sexual assault**.** In both cases, the individuals belong to the Himalayan state of Sikkim in Northeast India, and this is the first report of such abnormal Y-STR patterns observed in this population. Probable causes for these multi-allelic patterns and the relevance of these findings for forensic DNA analysis have been discussed.

Keywords: Y-STR analysis, Multi-allelic patterns, Y-STR duplication, Y-STR profile, Forensic examination, Forensic DNA Analysis

Introduction

Short Tandem Repeat (STR) is a form of genetic variation based on differences in the number of repeats of short DNA sequences (typically 2 to 6 nucleotides). Several repeats are possible at each locus and analysing multiple unlinked STR markers provides a high power of discrimination.[1] STR analysis has been the preferred method of forensic DNA analysis for the past three decades. In the case of autosomal STR, two alleles are present for each STR marker due to the diploid nature of inheritance. Gonosomal chromosomes follow unique inheritance patterns. For X chromosomal STR markers, two alleles are present in females and one in males. Y chromosome is passed down from father to son and does not undergo recombination, except for the pseudoautosomal regions.[2] Therefore, STRs on Y chromosome or Y-STRs are ideal for tracing male lineage, comparing genetic diversity, and understanding the evolutionary history of the human population.[3] In forensic examinations, Y-STRs cannot be used for individual identification, however, they can help in resolving mixed genetic profiles in complex cases of sexual assault and establish kinship. In cases of sexual assault where the male-to-female DNA quantity ratio is low, an autosomal profile of mixture of DNA can provide inconclusive results. In such cases, generating a Y-STR profile allows the identification of the paternal lineage of the male individual in the DNA mixture. In cases of disputed paternity

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where the alleged father is unavailable or unknown, a comparison of the male child's Y-STR profile with paternally related male individuals from the alleged father's family can help in establishing paternity.

Due to the Y-chromosome's uniparental presence and haplotype nature, each marker in an individual Y-STR profile appears as a single allele upon amplification with locus-specific primers. Examples of exceptions to this rule are DYF387S1a/b,[4] DYS459,[5] DYS527,[6] and DYS385a/b[7] which have double peaks in Y-STR profiles due to evolutionarily distant duplication events.[8] Unusual STR profiles can lead to misinterpretation of results in forensic investigations. In *amelogenin* Y-allele (AMELY) null cases, male autosomal genetic profiles may be interpreted as female profile.[9] A single male haplotype in Y-STR may be inter pretedas a multi-contributor profile due to the presence of additional Y-alleles.[10,11] Deletions, insertions,or duplications in STR allele scan also be misinterpreted as allele drop-outs or evidence of DNA contamination.[12]

This article reports two rare multi-allelic patterns at Y-STR loci in individuals from the Himalayan state of Sikkim in northeast India, observed during forensic examination in Central Forensic Science Laboratory, Bhopal. In case 1, a bi-allelic pattern was observed at the Y-STR locus DYS391 in two paternally related individuals. In case 2, -a tri-allelic pattern was observed at the double copy locus DYF387S1a/b in the Y-STR profile of an individual accused in a case of sexual assault. This is the first report of these unusual patterns of Y-STR peaks from the population of Sikkim.

Materials and Methods

For both cases, reference blood samples were collected by government medical officers and submitted by the investigating officer to Central Forensic Science Laboratory, Bhopal for forensic case examination. DNA was extracted using the EZ1 Advanced XL Automated DNA extraction machine (Qiagen). The extracted DNA samples were quantified using the Applied Biosystems Quantifiler™ Trio DNA Quantification Kit on the QuantStudio™ 5 Real-Time PCR machine. STR amplification was carried out with multiple STR kits using the Applied Biosystems Veriti™ thermal cycler and amplified products were genotyped using Applied Biosystems 3500 genetic analyzer and GeneMapper™ ID-X Software v1.[6] To exclude the possibility of contamination or allele artifacts, repeated DNA extraction of all samples was carried out using QIAamp Investigator Kit (Qiagen), amplified using the same STR kits,and analyzed along with necessary controls.

For case 1, STR amplification of extracted DNA was performed using Investigator® 26plex QS Kit (Qiagen) for 23 autosomal STR, Amelogenin, and one Y-STR locus DYS391 (Figure 1). Further, the

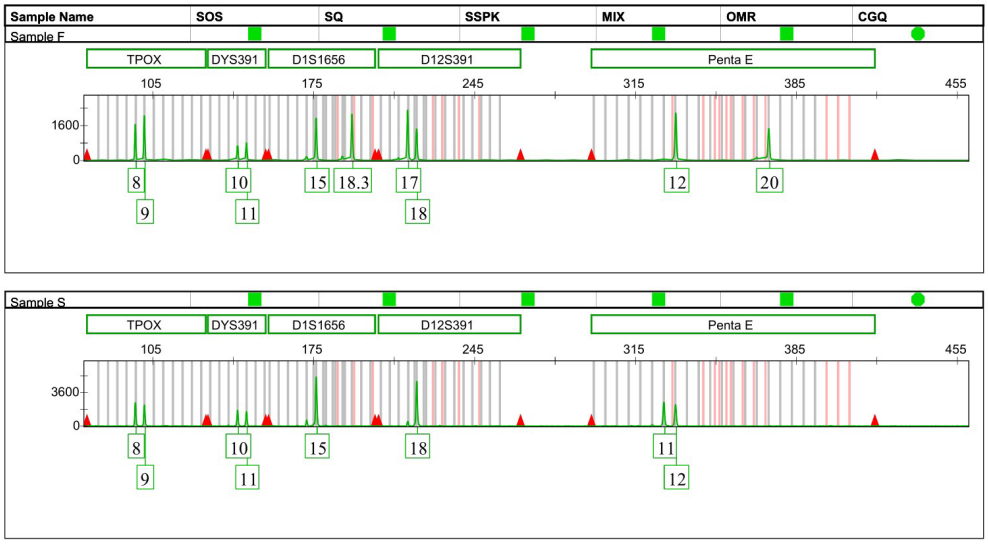

Fig. 1: Autosomal STR profile with bi-allelic peaks at the gender marker DYS391. Sample F – Alleged Father; Sample S- Son.

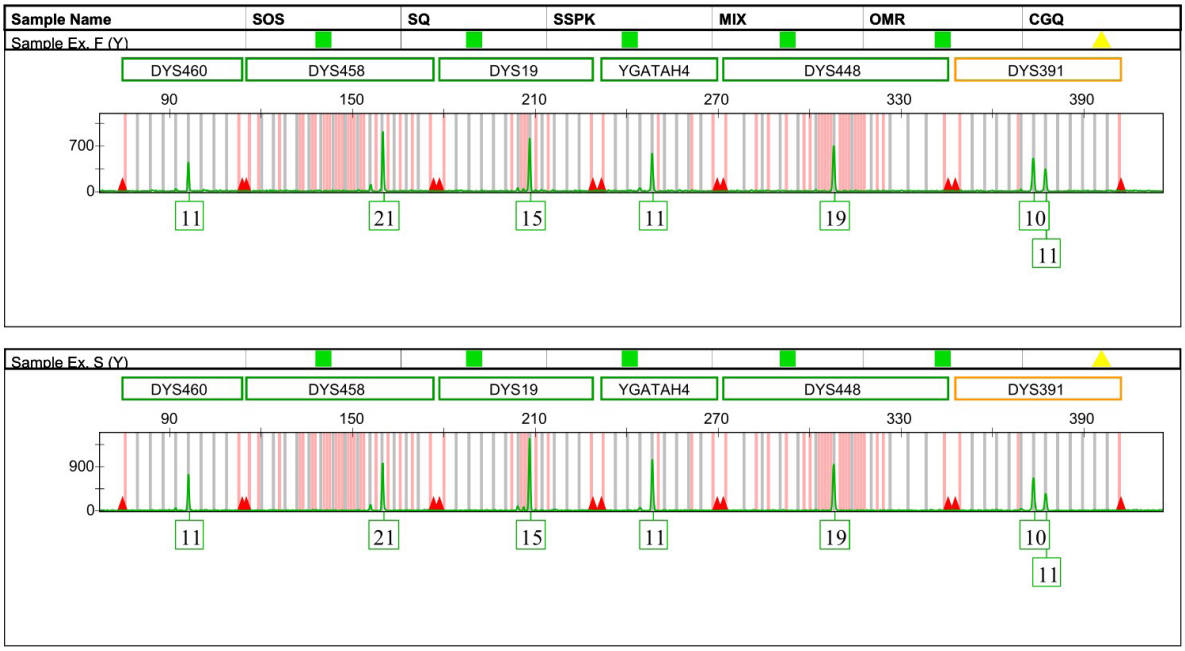

Fig 2: Y-STR profile with bi-allelic peaks at the marker DYS391. Sample Ex. F – Alleged Father; Sample Ex. S- Son.

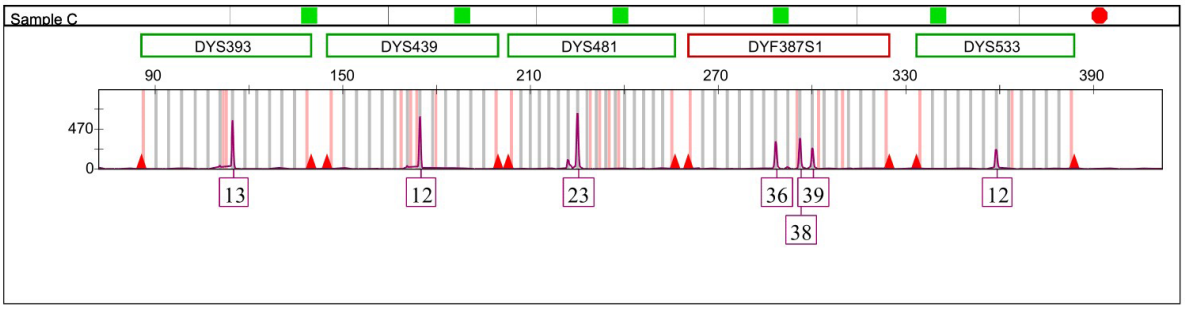

Fig. 3: Y-STR profile of Sample C with tri-allelic peaks at the marker DYF387S1.

DNA was amplified using the Applied Biosystems Yfiler™ Plus PCR Amplification Kit (Thermo Fisher Scientific) for 27 Y-STR markers. For case 2, extracted DNA was amplified using the Yfiler™ Plus PCR Amplification Kit (Thermo Fisher Scientific) for 27 Y-STR markers. All amplifications were carried out along with negative control and respective standard kit positive control DNA.

Results and Discussion

In case 1, the autosomal genetic profiles of an alleged father-son duo were generated for paternity determination. A bi-allelic pattern was observed at the Y-STR gender marker DYS391 in both genetic profiles (Fig. 1). To confirm this variant, the DNA was amplified using the Yfiler™ Plus PCR Amplification Kit. The double peaks at the DYS391 locus were consistent in both profiles (Fig. 2). The Y-STR haplotype database YHRD has no reports of multiple alleles at the locus DYS391.[13] However, few cases of double peaks at DYS391 have been reported in the global literature [14] as well as on the NIST STR Database.[15] Such a bi-allelic pattern at this marker has not been reported in any Indian population.

In case 2, samples in a case of sexual assault, were amplified using the Yfiler™ Plus PCR Amplification Kit.A tri-allelic pattern was observed at the locus DYF387S1a/b in the Y-STR profile of the accused (Fig. 3). Such tri-allelic patterns at this double-copy locus have been observed in a few populations.[16,17] Reports of

this pattern are also available on YHRD,[18] and the NIST STR Database including a few reports from the statesof Rajasthan and Madhya Pradesh in India.[19]

In both these cases, apart from the marker with additional peaks, the rest of the DNA profile was consistent with that of a single male individual, and negative controls were found to be contamination-free. Positive control profiles were consistent with expected results from respective kit control DNA. Repeated extraction of the blood samples produced genotyping results consistent with those of the first observations.

Variations in STR alleles are observed due to mutations leading to loss or gain of repeat units over generations. A possible explanation for additional peaks in STR loci is duplication of the chromosome region followed by independent mutation events altering the repeat number in one of the copy regions. The Y-chromosome contains many ampliconic repeats leading to rearrangements driven by non-allelic homologous recombination (NAHR), however, duplications due to rare non-homology-mediated processes have also been observed.[20] Due to NAHR between similar sequences, a high frequency of duplication has been reported in loci present in the palindromic regions of the Y-chromosome, such as the region containing DYF387S1a/b.[16] Another possible explanation for additional Y-STR alleles could be the presence of a region of the Y-chromosome on a different chromosome.[17] The duplicated alleles that have identical copy numbers are represented as a single peak on the electropherogram and therefore, data collected using capillary electrophoresis (CE) -based STR analysis underestimates the frequency of duplicated alleles.[21]. Further studies using advanced molecular biology techniques such as sequencing are required to understand the exact reason for abnormal STR patterns.

Conclusion

Globally, increased adoption of Massively Parallel Sequencing has improved the understanding of unusual STRs, however, in developing countries like India, conventional CE-based STR analysis is the primary method of DNA examination. Knowledge of unusual patterns is necessary for accurately interpreting STR results, and to conclude with certainty in forensic examination reports. Abnormal Y-STR peaks as observed in these cases may not affect Kinship analysis, however, they could lead to misinterpretation of results in DNA mixtures often encountered in forensic DNA examinations. Careful analysis is required to estimate the number of contributors in a sample since additional peaks do not always imply additional contributors.

Compliance with ethical standards:

This study was performed in line with the principles of the Declaration of Helsinki. Data analysis was based on the samples referred by the investigating agencies wherein the courts of law authorized the submission of samples to the laboratory and subsequent DNA analysis.

Conflict of interest: The authors have no conflicts of interest to declare.

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