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CASE REPORT

CRIMINALISTICS

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A Y-Chromosomal Haplotype with Two Short Tandem Repeat Mutations*

ABSTRACT: The male-specific Y-chromosomal short tandem repeat (STR) is a useful tool in forensic casework. The Y haplotype comprised of 16 loci, which is amplified simultaneously by AmpF*l*STR® Yfiler™ PCR kit and provides strong exculpatory evidence in individual identification. We reported a rare Y-STR profile with a null allele at the DYS448 locus and an off-ladder allele at the DYS456 locus, when genotyping material from a vaginal swab in an alleged rape case. Sequence analysis revealed that the DYS448 null allele was a true type of null allele because of a total deletion of 11 upstream repeats and 9 bp of the N_{42} region, and there were numerous primer binding site mutations as well. The amplicon of the DYS456 locus was a small 92-bp fragment that was off-ladder, and sequencing analysis showed that there were only 10 repeats $(AGAT)_{10}$. This Y chromosome haplotype that was comprised of two variations provided helpful evidence for personal identification.

KEYWORDS: forensic science, Y-chromosomal short tandem repeat, Y chromosome haplotype, DYS448, DYS456, null allele, off-ladder allele, AmpFℓSTR® Yfiler™ kit

The paternally inherited and subsequent haploid state of the Y chromosome is useful in human identity testing and evolutionary studies (1,2). AmpF*l*STR® Yfiler™ kit (Applied Biosystems, Foster City, CA) is widely used as commercial multiplex kit for Y-chromosomal short tandem repeat (Y-STR) analysis. It uses locus-specific primers that are designed to bind to highly conserved nucleotide sequences flanking the STR locus of interest (3). Genetic anomalies may occur in any PCR-based STR genotyping system, such as null allele phenotypes, off-ladder alleles, duplication of Y-STRs, and so on.

Null alleles generally are infrequent because of deletions of the STR region or by more common primer binding site mutations that prevent hybridization of at least one of the primers flanking the target region (4). A possible primer binding site null allele is confirmed by using another commercial kit with different primer pairs.

In the present case, we described a rare Y-STR profile with a null allele at the DYS448 locus and an off-ladder allele at the DYS456 locus.

Materials and Methods

Samples and DNA Extraction

In an alleged rape case, we were requested to perform a DNA analysis on material from a vaginal swab to determine whether it could be attributed to the given suspect. Blood sample of the suspect was preserved on Whatman™ FTA™ card (Piscataway, NJ). DNA from the evidential sample and the blood were extracted by laboratory automation workstation that used magnetic bead protocol (5,6).

PCR Amplification and Electrophoresis

The PCR amplification was performed using the AmpF*l*STR® Yfiler™ kit and AmpF*l*STR® Identifiler™ kit (Applied Biosystems) according to the manufacturer's instructions. PCR was carried out in a GeneAmp® PCR Sysetm 9700 (Applied Biosystems) with standard reaction procedure. PCR product was separated and detected on an Applied Biosystems 3130xl Genetic Analyzer following the manufacturer's recommendations. Electrophoresis result was analyzed by GeneMapper® *ID* Software v3.2 (Applied Biosystems).

Sequencing

Sequencing reactions were performed to analyze the DYS448 locus and the DYS456 locus. Primer sequences (Table 1) were selected by searching public databases such as STRs base (STRBase), GenBank sequence tagged sites (UniSTSs), and UCSC Genome Bioinformatics Site. Primers synthesis, PCR, purification, and sequencing were achieved by Invitrogen Trading (Shanghai) Co., Ltd. (Rockville, MD).

Results and Discussion

Y-Chromosomal STR

Y-STR genotyping results showed a null allele at the DYS448 locus and an off-ladder allele at the DYS456 locus (Fig. 1). There

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TABLE 1—*Primers for DYS448 locus and DYS456 locus.*

Primers for Sequencing	Sequence	Size (bp)
DYS448-F	5′-GGTGGGTTTTAGTTGGCTATG-3′	388
DYS448-R	5′-TTCTTGATTCCCTGTGTTGG-3′	
DYS456-F	5′-GGACCTTGTGATAATGTAAGATA-3′	149
DYS456-R	5′-CCCATCAACTCAGCCCAAAAC-3′	

was consistency between the given suspect and the male fraction of the evidential material.

DYS448

DYS448 was located within the azoospermia factor c(AZFc) gene in the distal euchromatic part of the Y chromosome long arm, Yq11.223 (Fig. 2). The AZFc gene consisted almost entirely of very long direct and inverted repeats (7). Therefore, it was prone to partial deletions or duplications by rearrangements.

Notably, the DYS448 null allele was observed in Japanese (10 of 1079 men) (8), Nepalese (three of 769) (9), three ethnic groups (Malays, Chinese, and Indians) living in Malaysia (three of 980) (10), Kalmyk (seven of 99) (11), Mexican (one of 326) (12), Spanish (one of 247) (13), and three racial groups studied in Yfiler™ haplotype database by Applied Biosystems (two each of 330 Asians, 985 African Americans, and 1276 Caucasians). It was speculated that null allele at the DYS448 appeared more frequently in Asians than any other populations.

The hexanucleotide repeat AGAGAT was the basic repeat motif of the DYS448 locus. There were two polymorphic domains separated by an invariant 42-bp region, expressed as $(AGAGAT)_mN_{42}(AGAGAT)_n$, where m and n represent the number of

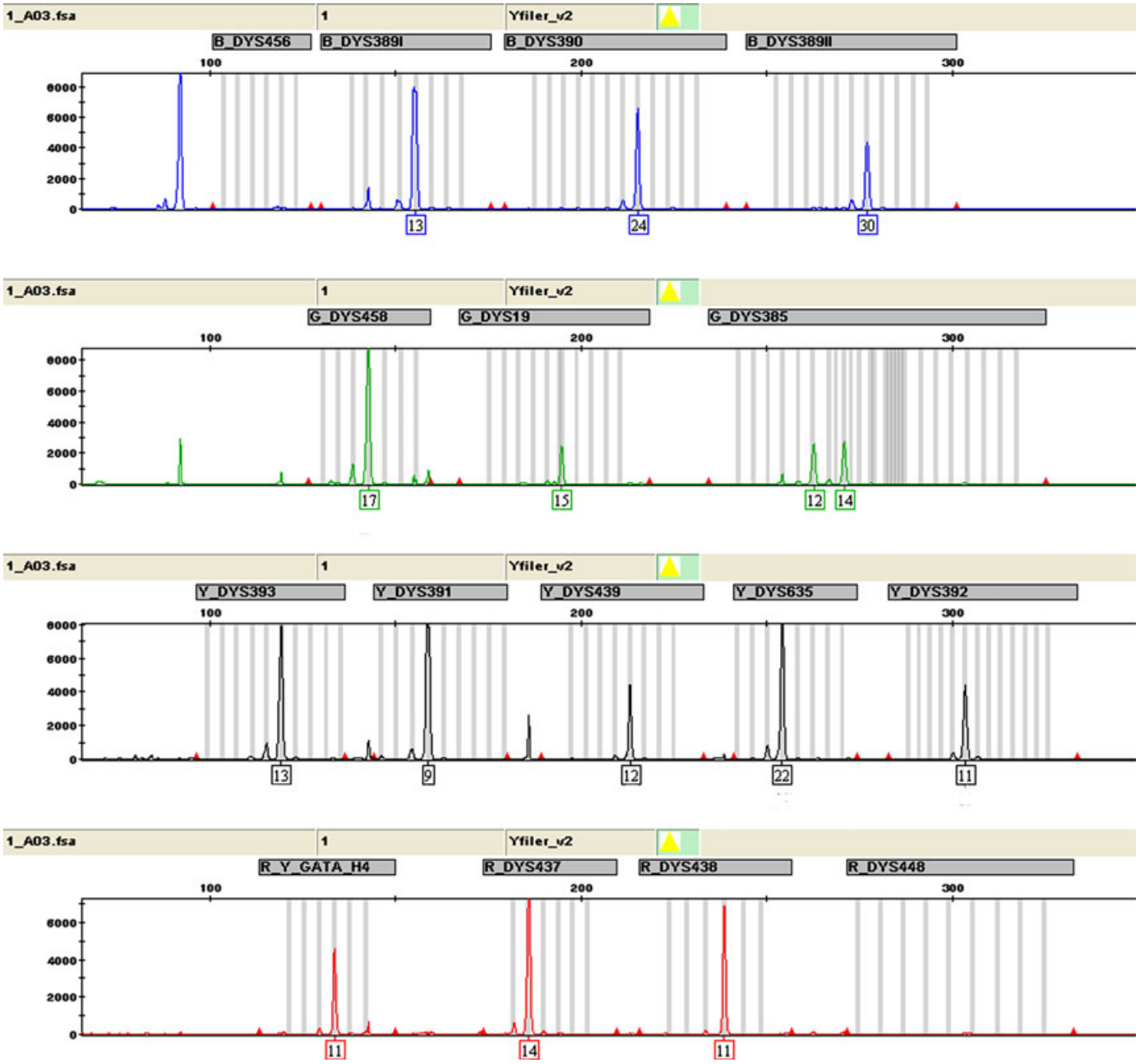

FIG. 1—*Electropherograms of profiles contain 16 Y-chromosomal short tandem repeat markers from the AmpFlSTR® Yfiler™ commercial kit.*

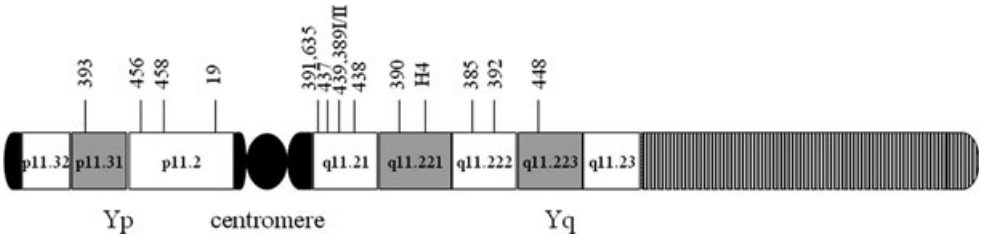

FIG. 2—*Y chromosome ideogram contains 16 Y-chromosomal short tandem repeat markers from the AmpFlSTR® Yfiler™ commercial kit.*

TABLE 2—*Sequence in the relevant flanking and repeat region of the DYS448 locus.*

Sample	Sequence
GenBank accession number EU682948	GATCGCGAGACAGAAAGGGAGATAGAGACATGGATAA(AGAGAT)$_{11}$N$_{42}$(AGAGAT)$_7$AGAGAGGTAAAGATAGAGAT AAATTTCCAGACCGGCCAGAAA
Sample in this study	GATC**AAT**AGACA**AG**A**G**GG**T**C**A**A**G**AG**CTT**CATGGA**G**A**T** [75bp deletion (AGAGAT)$_{11}$ATAGAGATA] GAGAGATAG AGAT**GTT**(1 bp insert) AGA**C**AGA**A**AGAT(2-bp deletion) AGA**T**(AGAG**C**T)$_3$ (AGAGAT)$_2$A**A**AGA**G**AGA**T**A**G**AG**CT** AG**A**TAAAGATAGAGA**C**AAA**C**TTCC**TA**ACC**T**GCCAGAAA

GGACCTTGTGATAATGTA**AGATAG**ATAGATAGATAGATAGATAGATAGAT
AGATAGATATTCC....GTTTTGGGCTGAGTTGATGGG

FIG. 3—*Nucleotide sequence alignment of the DYS456 locus. The forward and reverse primers were underlined. Boldface was the 10 repeats region.*

repeats. The sequence of N_{42} was as follows: AT(AGAGAT) AG(AGAGAT)$_3$(AGAT)$_2$(AGAGAA).

There were two kinds of null allele at the DYS448 locus: "true null" and "apparent null" (4). The true null allele contained a large deletion encompassing the upstream repeat sequences, the N_{42} region, or the downstream repeats, whereas the apparent null allele contained primer binding site mutations that did not hybridize with amplification primers. Another case of apparent null was that if a DYS448 PCR product was generated, the large deletion could produce small amplicons that migrate on electropherograms within the range of the DYS437 locus' allele sizes. That was to say the individual displayed an apparent duplication at the DYS437 locus and a null allele at the DYS448 locus.

Sequencing of the DYS448 locus was performed in the present case, and base sequences were similar to the GenBank accession EU682948 allele 18 according to DNAMAN v6.0.3.99 software (Lynnon Biosoft, Los Angeles, CA). However, there were slight variants. All 11 upstream repeats, 9 bp in N42, were deleted and there were numerous primer binding site mutations, as well (Table 2). Thus, the present case was an example of a true DYS448 null allele that produced no amplicons at all.

DYS456

DYS456 locus was located in the short arm of the Y chromosome, Yp11.2 (Fig. 2).

Alleles of 12, 19, and 22 repeats were reported, all were outside the commercial ladder of AmpF*l*STR® Yfiler™ kit (13–18 repeats) (14). The present case amplicon was a small 92-bp fragment that was off-ladder, and sequencing showed that there were 10 repeats $(AGAT)_{10}$ (Fig. 3).

Autosomal STR

Autosomal STR genotyping showed a normal 16 loci profile without any mutations. And the profile between the given suspect and the male fraction of the mixture was consistent.

Conclusion

In the present case, based on the results from DNA analysis of Y chromosome and autosomal STR systems, no inconsistency was found between the profile of the given suspect and the male fraction of the vaginal sample mixture. Both the polymorphisms of 16 Y-STRs in AmpF*l*STR® Yfiler™ kit and that of 16 autosomal STRs inAmpF*l*STR® Identifiler™ kit showed firm evidence for suspect identification.

Furthermore, a null allele at the DYS448 locus was observed because of a deletion of 11 upstream repeats and 9 bp of the N_{42} region and there were numerous primer binding site variants. At the DYS456 locus, an off-ladder allele was discovered owing to 10 repeats of the basic motif. This rare Y-chromosomal haplotype provided useful information for improvement of interpretation of Y-STR data and database construction in forensic practice.

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