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Mixed Stains from Sexual Assault Cases: Autosomal or Y-Chromosome Short Tandem Repeats?

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We analyzed forensic DNA samples from four cases of sexual assault, using the Y-chromosome-specific human DNA markers and a panel of autosomal short tandem repeats (STRs). The presence of male contribution was evaluated by the analysis of the Amelogenin locus. A panel of tetrameric Y-STR (DYS19, DYS390, DYS391, DYS392, DYS385, DYS389I and II) was used in further analysis of samples, increasing the efficiency of the forensic genetic analyses. It was possible to identify a partial or full Y-profile of the rapists in different DNA mixtures when genetic profile could not be detected by autosomal STRs. However, in the case of male/male DNA mixture, only the victim's Y-profile could be obtained because the DNA of the offenders was present in low amounts. When the mixture contained different male/male proportion of DNA, only the full profile of the major component could be detected. In cases where male/female DNA mixed stains contained a sufficient amount of male DNA, the analysis of autosomal STRs was adequate enough to identify the full profile of the rapist. Our experience shows that the main advantage of the Y-STR approach is its ability to detect the male component in the mixed stains when the DNA of the male contributor is present only in a very small amount.

Key words: *DNA; DNA fingerprinting; forensic medicine; short tandem repeats; Y chromosome*

Y-chromosomal short tandem repeat (Y-STR) loci have been extensively investigated in forensic science for identification of male persons (1). Y-chromosome analysis can be useful in detecting the male DNA fraction in male/female DNA mixture, the usual evidence in sexual assault or rape cases. Moreover, the DNA Commission of the International Society of Forensic Genetics has published a series of recommendations concerning the applications of DNA polymorphisms for the Y-chromosome (2).

STR systems are first to be used in DNA analysis of forensic samples of mixed nature, because STR markers give more information and are relatively easy to use. In fact, analysis of a mixed sample with a commercially available kit, including the analysis of Amelogenin locus with the commercially available kits, such as Amp*fl*STR™ Profiler Plus™, SGM Plus™, or PowerPlex™ 16, makes the identification of the rapist or assailant possible (3). In cases where the use of STR markers fails to detect the autosomal DNA profile of the semen contributor, it is necessary to perform a Y-STR analysis (4). The application of Y-STRs in forensic casework can be useful in mixtures containing small amounts of male DNA and large amounts of female DNA (5). In fact, if the male component is present in a smaller ratio (≤5%), the interpretation of a mixed STR profile becomes difficult or even impossible. Furthermore, sample mixture from vasectomized or azoospermic males precludes sperm-based conventional separations (6).

We report on our experience with DNA analysis in cases of sexual assault where only mixed samples from different sources were available.

Material and Methods

Method

Chelex method with or without differential lysis was used for DNA extraction, as reported elsewhere (7). For the amplification of all autosomal STRs by polymerase chain reaction (PCR), AmpflSTR™ Profiler Plus™ ID and SGM Plus™ PCR Amplification Kits (Applied Biosystems, Foster City, CA, USA) were used, according to the manufacturer's protocol. The amplicons were sized by capillary electrophoresis on the Genetic Analyzer 310 (Applied Biosystems) and sizing results were obtained with Genotyper 2-5 (Applied Biosystems) by comparison with supplied allelic ladder and an internal size standard.

The tested Y-chromosome STRs were multiplexed in three different amplification systems (first: DYS390 and DYS389I and II; second: DYS391 and DYS392; and third: DYS393, DYS19, and DYS385), according to a protocol based on infrared automated DNA sequencer LI-COR mod. 4200 (LI-COR, Lincoln, NE, USA), as described elsewhere (8).

Case Examples

Case 1. A girl was raped by a man who has been identified and arrested afterwards. Vaginal swabs were taken from the girl immediately after the event. DNA samples of the rapist were ob-

tained after his arrest. DNA was extracted from a vaginal swab and compared with the DNA obtained from the victim and the suspected rapist.

Case 2. Because of suspected child abuse, vaginal swabs have been taken from a minor victim (a girl). The girl refused to give a reference sample, whereas a sample of the suspected assaulter was available.

Case 3. A woman reported being raped by several men. DNA was extracted from a vaginal swab and compared with the DNA obtained from the victim. The DNA profile of the suspected rapists was unknown.

Case 4. A man was a victim of sodomy by two other men. Two suspects were arrested and had to undergo a DNA test. The accused men, the victim, and a condom collected on the scene and probably used by one of the two suspectes were analyzed by DNA typing methods.

Results

Case 1

Autosomal STR markers were used for the analysis of mixed samples (Table 1). The analysis of the vaginal swab revealed a genotype characterized by a mixed profile of the alleles of the victim and the suspect (Fig. 1). In this case, we did not analyze the Y-chromosome polymorphisms, because the DNA of the suspect was included in the mixture. Calculation of the likelihood ratio performed according to Weir et al (9) yielded a very high result (4.4×10^{20}), which strongly supported the hypothesis that the suspect was the male contributor in the mixed stain.

Table 1. Autosomal short tandem repeat analysis with AmpflSTR™ Profiler Plus™ ID and SGM Plus™ in Case 1

	Alleles		
Loci	victim	suspect	vaginal swab
D3S1358	15-15	15-18	15-18
vWA	16-18	14-20	14-16-18-20
FGA	19-22	22-23	19-22-23
D8S1179	13-14	10-14	10-13-14
D21S11	32.2-33.2	29-32.2	29-32.2-33.2
D18S51	14-15	18-20	14-15-18-20
D5S818	12-13	12-12	12-13
D13S317	12-12	8-14	8-12-14
D7S820	9-10	9-11	9-10-11
D16S59	9-12	10-11	9-10-11-12
D2S1338	17-17	18-20	17-18-20
D19S433	12-14.2	14-14	12-14-14.2
TH01	8-9.3	7-8	7-8-9.3
Amelogenin	X - X	X - Y	X - Y

Case 2

The amplification of the DNA samples by Applied Biosystem's Amp*fl*STR™ Profiler Plus™ ID and SGM Plus™ generated a dominant female profile. Several minor peaks were also visible, but the Y-peak at the Amelogenin locus was evident (Fig. 2). We decided to proceed with the study of Y-STR, which permitted us to define a clear genetic profile that excluded the suspected person (Table 2; Fig. 3, Case 2).

Case 3

DNA typing with Amp*fl*STR™ Profiler Plus™ ID and SGM Plus™ amplification kits revealed the presence of male material in the forensic samples. A clear profile corresponding to the Y-chromosome was obtained at the Amelogenin locus. However, clear profiles could not be obtained for the autosomal STRs. The victim was a prostitute and more men could be contributors, but the results of the Y-STR study showed that there was only one male contributor (Table 3; Fig. 3, Case 3).

Case 4

The forensic material available for the analysis included the victim's blood, saliva of the two suspected persons, and material from a condom found at the crime scene. As all the persons involved were men, the analysis of the STR systems did not permit a clear reconstruction of the case. The analysis of the material found on the condom revealed a presence of a series of extremely low peaks, which perfectly corresponded to the profile of one of the suspects (Fig. 4). Analysis of the Y-polymorphism confirmed the profile belonging only to a single person – the victim (Table 4; Fig. 3, Case 4).

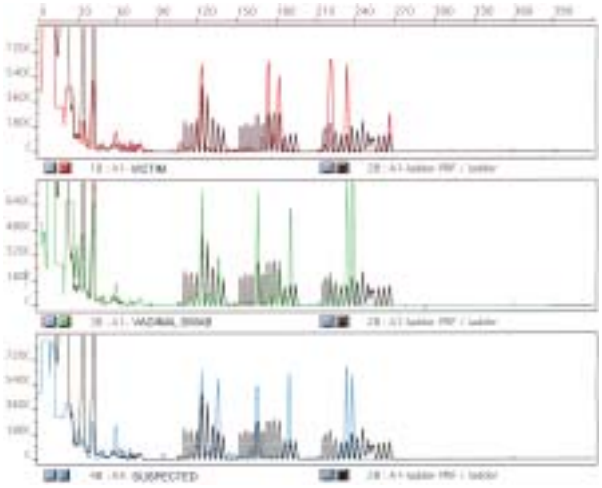

Figure 1. Case 1. Example of electropherogram of the autosomal short tandem repeats (STRs) showing a mixture of alleles from the victim and the suspected man in the vaginal swab.

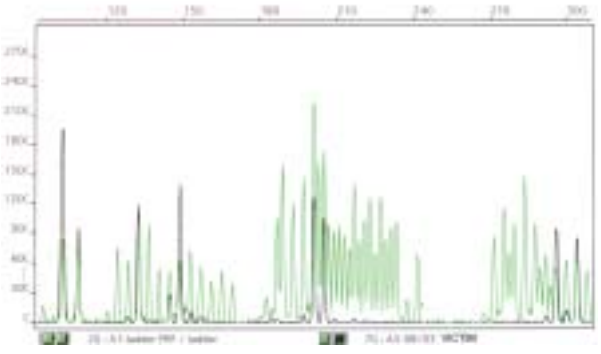

Figure 2. Case 2. Example of electropherogram of the autosomal short tandem repeats (STRs) and the gender-specific marker Amelogenin. In the Amelogenin locus the arrow indicates the Y peak due to minor male contribution.

Discussion

The results of sample analyses in the four rape cases presented here may allow some general considerations.

Table 2. Analysis of the samples with Y-chromosome short tandem repeats (Y-STRs) in Case 2, on the basis of which the suspect was excluded for three loci (DYS389I, DYS389II, and DYS392)

	DYS390	DYS389I	DYS389II	DYS391	DYS392	DYS393	DYS19	DYS385
Victim	–	–	–	–	–	–	–	–
Vaginal swab	–	10	26	10	12	–	14	–
Suspect	22	9	27	10	11	13	14	14, 16

Table 3. Analysis of the samples with Y-chromosome short tandem repeats (Y-STRs) in Case 3, where a full profile of a man was detected in the vaginal swab

	DYS390	DYS389I	DYS389II	DYS391	DYS392	DYS393	DYS19	DYS385
Victim	–	–	–	–	–	–	–	–
Vaginal swab	24	10	27	10	11	13	13	16,18

Table 4. Analysis of the samples with Y-chromosome short tandem repeats (Y-STRs) in Case 4, where the partial profile from the anal swab and the full profile from the condom matched the victim's profile

	DYS390	DYS389I	DYS389II	DYS391	DYS392	DYS393	DYS19	DYS385
Victim	25	10	26	11	12	12	14	11, 14
Suspect 1	23	10	27	11	11	12	14	13, 17
Suspect 2	24	11	27	9	11	13	13	13, 15
Anal swab	25	10	–	–	–	12	–	–
Condom	25	10	26	11	12	12	14	11, 14

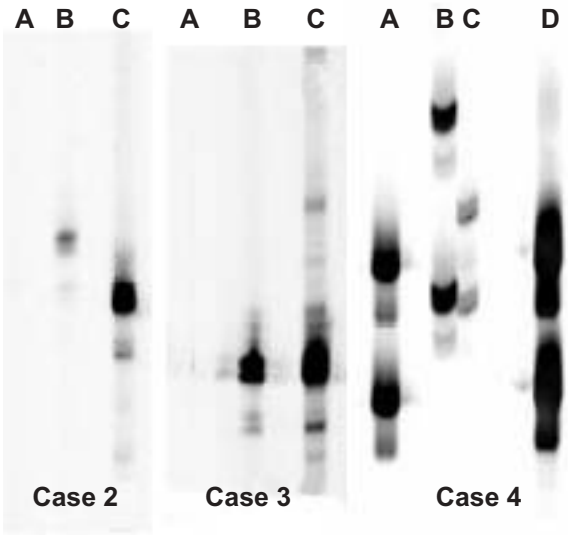

Figure 3. Examples of Y-STR profiles for three loci, obtained with LICOR-4200 DNA sequencer. Case 2. Locus DYS389I; A = victim; B = vaginal swab (allele 10); C = suspect (allele 9). Case 3. Locus DYS390; A = victim; B = vaginal swab (allele 24); C = DNA male control (allele 24). Case 4. Locus DYS385; A = victim; B = suspect 1 (alleles 13, 17); C = suspect 2 (alleles 13, 15); D = condom (alleles 11,14).

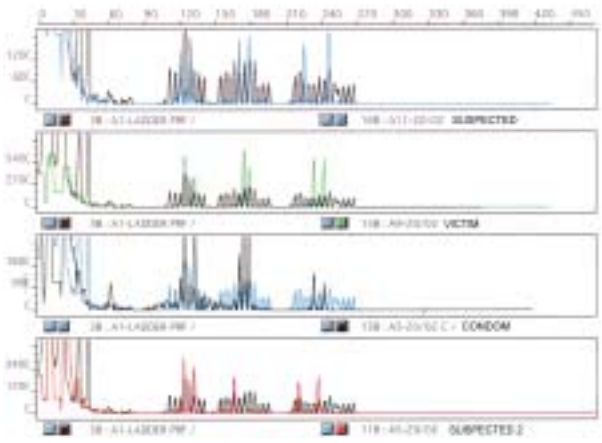

Figure 4. Case 4. Electropherogram of the autosomal short tandem repeat (STR) DNA profiles from the two suspects, the victim and the condom.

Our experience confirmed that the use of amplification systems of autosomal STRs could be very important in the analysis of the mixed profiles. These amplification systems, which include the Amelogenin locus, enabled us to show the presence of male component in mixed stains. Although some authors have reported that Y-component on the Amelogenin gene can be absent (10), we have never had such a case.

In the male/female mixed samples, when sufficient amount of material is available or differential lysis procedure has not been efficient, a mixed profile can also be revealed by STR systems, which show profiles of more than two alleles per locus (as in the Case 1). We think that the performance of Y-STRs was not necessary in this case, because we were able to confirm that the evidentiary sample represented a DNA mixture of the victim and the suspect only by using autosomal STRs.

For Y-chromosome analysis, we routinely use the "minimal haplotype" (11), which includes DYS390, DYS389I and II, DYS391, DYS392, DYS393, DYS19, and DYS385 loci. The analysis with the infrared automated DNA sequencer in combination with IRDye™800 labelled primers allows the efficient detection of all PCR products. The result of electrophoresis appears in an autoradiogram-like image, automatically registered as a TIF image and saved on the hard disk of the computer. The electropherogram documentation is achieved by using the "Base ImagIR™ Manipulation" program (LI-COR, Lincoln, NE, USA), which allows manipulation of the image files in a variety of ways. The image may be cropped, so that only a portion of it is printed, with enhance-

ments and/or resized printing, saving in alternative file formats (e.g., TIFF or EPS), sizing, and scaling (as presented in Fig. 3).

The use of three multiplex amplification systems enabled us to detect the full Y-chromosome profile when large amount of female DNA was present in the sample. However, negative results can occur in some Y-STR due to minor variations in the preparation of the amplification mixture or random fluctuation in pipetting the DNA samples. In our cases, we were able to reanalyze the samples. The female amplification products that we observed for DYS391 and DYS393 loci did not overlap in size with other Y-STR systems.

If the male material is present in a very small quantity in mixed male/female DNA samples, as it was in our Cases 2 and 3, it is not sufficient to analyze only the autosomal STR systems (5). Although it is possible to obtain positive results for the Y-chromosome component at the Amelogenin locus, a clear genetic profile cannot be identified. Therefore, it is necessary to analyze the Y-STR, which can yield reliable answers. Furthermore, we can affirm that the concentration of DNA material needed must be high when the involved persons are only men, as in the Case 4. As reported earlier (3), the Y-STR analysis has a smaller sensitivity than the study of autosomal systems. Therefore, when the DNA ratio between two male persons is largely in favor of one person, the presence of the minor contribution of Y-STRs may not be revealed.

In conclusion, our study confirmed that Y-STRs could be useful in analysis of DNA mixtures from rape cases. It is important to keep in mind the limits of this method, which depend on the type of material that has to be analyzed.

References

1 Jobling MA, Pandya A, Tyler-Smith C. The Y chromosome in forensic analysis and paternity testing. Int J Legal Med 1997;110:118-24.

2 Gill P, Brenner C, Brinkmann B, Budowle B, Carracedo A, Jobling MA, et al. DNA Commission of the International Society of Forensic Genetics: recommendations on forensic analysis using Y-chromosome STRs. Int J Legal Med 2001;114:305-9.

3 Pizzamiglio M, Donato F, Biondi F, Floris T, Bellino C, Tobiello A, et al. DNA typing of a mixed female and male material from a case of rape. In: Sensabaugh GF, Lincoln PJ, Olaisen B, editors. Progress in forensic genetics 8. Proceedings of the 18th International ISFH Congress; 1999 Aug 17-21; San Francisco (CA), USA. New York (NY): Elsevier; 2000. p. 529-32.

4 Martin P, Albarran C, Garcia O, Garcia P, Sancho M, Alonso A. Application of Y-STR analysis to rape cases that cannot be solved by autosomal STR analysis. In: Sensabaugh GF, Lincoln PJ, Olaisen B, editors. Progress in forensic genetics 8. Proceedings of the 18th International ISFH Congress; 1999 Aug 17-21; San Francisco (CA), USA. New York (NY): Elsevier; 2000. p. 526-8.

5 Parson W, Niederstätter H, Köchl S, Steinlechner M, Berger B. When autosomal short tandem repeats fail: optimized primer and reaction design for Y-chromosome short tandem repeat analysis in forensic casework. Croat Med J 2001;42:285-7.

6 Redd A, Agellon A, Kearney V, Contreras V, Karafet T, Park H, et al. Forensic value of 14 novel STRs on the human Y chromosome. Forensic Sci Int 2002;130:97-111.

7 Cerri N, Franchi M, Mascadri S, De Ferrari F. DNA typing from biological stains: a case. In: Brinkmann B, Carracedo A, editors. Progress in forensic genetics 9. Amsterdam: Elsevier; 2003. p. 881-4.

8 Ricci U, Sani I, Giovannucci Uzielli ML. Y-chromosomal STR haplotype in Toscany (central Italy). Forensic Sci Int 2001;120:210-2.

9 Weir BS, Triggs CM, Starling L, Stowell LI, Walsh KA, Buckleton J. Interpreting DNA mixtures. J Forensic Sci 1997;42:213-22.

10 Roffey PE, Eckhoff CI, Kuhl JL. A rare mutation in the Amelogenin gene and its potential investigative ramifications. J Forensic Sci 2000;45:1016-9.

11 Roewer L, Kayser M, Anslinger K, Augustin C, CagliB A, Corach D, et al. European Y-STR haplotype reference database for forensic application. In: Sensabaugh GF, Lincoln PJ, Olaisen B, editors. Progress in forensic genetics 8. Proceedings of the 18th International ISFH Congress; 1999 Aug 17-21; San Francisco (CA), USA. New York (NY): Elsevier; 2000. p. 613-5.

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