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CASE REPORT

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First Polish DNA "manhunt" – an application of Y-chromosome STRs

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Abstract This study presents the application of Y-chromosomal STR polymorphisms to male identification in the case of a serial rapist and woman murderer in Poland. Since August 1996 a rapist from Swinoujscie (northwest Poland) committed at least 14 rapes. In the year 2000 he brutally raped 8 young girls and murdered a 22-year-old girl. DNA profiles obtained from semen stains left at the scenes of crime gave information that one and the same man had committed all the rapes. The Y-chromosome haplotype (9 loci) obtained was used for the elimination process of 421 suspects. One man was found who had an identical DNA profile in all Y-chromosome STR loci analysed and possessed common alleles in 9 out of 10 autosomal loci, strongly suggesting that the real rapist and the typed man were closely related males. Analysis of reference DNA obtained from the man's brother revealed an identical DNA STR profile to that identified at the crime scenes. To the best of our knowledge this is the first case in Poland and probably in Eastern Europe where DNA typing of a large population was used to identify the offender.

Keywords Multiple rape case · Y-chromosome STRs · Multiplex PCR

Case report

In a period of 6 years 14 young girls and women in the age range of 9–26 years were brutally raped and one was murdered in the Swinoujscie area (a town located in northwest Poland very close to the German border). Information about the offender was very scanty. His face had never been seen because he wore a mask. The victims described him as a tall athletic man armed with a pistol and using very primitive language. Three months after the homicide of the 22-year-old girl, a special police group consisting of 8 highly experienced policemen was established who decided that all young men in the age range between 22 and 38 years living in Swinoujscie area had to be investigated. The number of theoretical checks was estimated to ca. 12,000. During 15 months of intensive work 714 suspects including known rapists, pedophiles etc. were interrogated and 421 men were asked to submit mouth swabs or blood samples for elimination by DNA typing. In the first sample of 420 donors was a 28-year-old man who lived 3 km away from the place of the first reported rape. A detailed analysis of his DNA profile showed that this man could be closely related to the real rapist. A few days after this information was sent to the public prosecutor, the brother of the analysed man was arrested and accused of committing 14 rapes and 1 homicide.

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Materials and methods

DNA extraction

Blood samples or buccal swabs were obtained from 421 males living in northern Poland and DNA was isolated using the Sherlock AX kit (A@A Biotechnology, Gdansk, Poland). DNA from vaginal swabs and semen stains was isolated using mild preferential lysis as described by Wiegand at al. [1]. DNA was quantified fluorimetrically or using the QuantiBlot kit (PE, Foster City, Calif.) with chemiluminescence detection.

Amplification conditions

Amplification of the 10 loci included in the commercial Profiler-Plus kit (Perkin Elmer) was carried out in accordance with the manufacturer's instructions on a 2400 Thermal Cycler (Perkin Elmer). A set of nine Y-STR loci was amplified in two separate multiplex reactions (DYSI: 19, 390, 393 and amelogenin and DYSII: 391, 392, 389I/II 385I/II) using methods worked out in our laboratory (manuscript in preparation). For all analysed loci except DYS385I/II, primer sequences were as described by Kayser at al. [2]. In the case of DYS385I/II and the amelogenin loci, primers described by Schneider et al. [3] and Sullivan et al. [4] were used, respectively. DYS 391, 392 and 393 were labelled with HEX, DYS 19, amelogenin and 389I/II with 6-FAM, and DYS390 and 385I/II with TET.

PCR reactions were performed in a 5 μl volume containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 200 μM dNTP, 0.1 U of AmpliTaq Gold polymerase (Perkin Elmer) and 0.5–1 ng of template DNA. The following primer concentrations were used for the DYSI multiplex: amelogenin 0.06 μM, DYS19 0.24 μM, DYS390 0.08 μM, DYS393 0.15 μM and for the DYSII

multiplex: DYS393 0.3 μM, DYS392 0.3 μM, DYS385I/II 0.25 μM and DYS389I/II 0.23 μM. Both multiplexes were run under the same cycling conditions on a 2400 Perkin Elmer Cycler (Perkin Elmer) at 95°C for 11 min, then 30 cycles at 95°C for 1 min, 55°C for 1.5 min and at 72°C for 2 min and finally 60°C for 30 min.

Detection of PCR products

Detection of ProfilerPlus PCR products was done using capillary electrophoresis as described earlier [5]. In the case of the DYS multiplexes, 1 μl of each PCR product was mixed with 12 μl of deionised formamide and 0.5 μl of GS500 size standard, denatured and analysed using an ABI 310 machine. The designation of alleles followed the nomenclature based on the number of repeat units, according to the recommendations of the International Society of Forensic Genetics [6]. Appropriate allelic ladders were kindly donated by B. Brinkmann (Münster), P. deKnijff (Leiden) and P. Schneider (Mainz). The analysis of the electrophoretic data was carried out using the computer program GeneScan v. 2.1.

Results and discussion

The analysis of Y-chromosome polymorphisms is becoming increasingly more important for forensic genetics and particularly for the interpretation of results from vaginal swabs containing male DNA. STR sequences on the Y-chromosome have the potential for wide variation among male individuals and provide a simple and sensitive method to selectively obtain male DNA profiles. In this work we applied Y-chromosome specific markers to identification of a serial rapist from a large population sample.

The results of the first three investigated cases sent to our laboratory, suggested that all rapes were committed by one and the same male. The analysis of the mixed stains revealed the same Y-chromosome haplotype in all nine loci (Table 1). The Y-STR analysis of the next cases gave the same profile except one where a mixture of two males was detected (case 4). In this case the raped girl stated that 4 days before the rape she had had sexual intercourse with her boyfriend. Detailed analysis of the peak height differences showed that the dominant alleles were the same as those identified in previous cases.

The results of autosomal loci analysis of these samples gave DNA profiles with a strong domination of female DNA and so in the beginning it was not possible to deduce the rapist's profile. Complex analysis of the non-victim ProfilerPlus alleles allowed almost the complete DNA profile of the rapist to be deduced also suggesting that one

Table 1 Y-chromosome DNA profiles in samples from the five cases

Samples analysed	DYS locus							
	DYS 19	DYS 390	DYS 393	DYS 392	DYS391	DYS389I	DYS 389II	DYS 385I/II
Case 1	16	25	13	11	10	13	29	11, 15
Case 2	16	25	13	11	10	13	29	11, 15
Case 3	16	25	13	11	10	13	29	11, 15
Case 4	15, **16**	24, **25**	12, **13**	11	10	12, **13**	**29**, 30	**11**, 14, **15**,17
Case 5	16	25	13	11	10	13	29	11, 15

Alleles typed in bold represent dominating alleles in the mixture.

Table 2 Deduced autosomal profile of the rapist (ProfilerPlus) in three of the cases

Analysed samples	ProfilerPlus loci									
	D3S1358	VWA	FGA	D5S818	D13S317	D7S820	AMG	D8S1179	D21S11	D18S51
Case 1	15, 16, **17**	14, 15, **17**	22, **22.2**, 23	**11**, 12, 13	**10**, **11**, 12	**8**, 10, 12	X>Y	12, 13	29, 30, **31**	15, 18, **20**
Case 2	15, **16**, 17	14, 17	21, **22**, **22.2**, 25	10, 11, **12**	**10**, 11, 14	**8**, 9, 10, 12	X>Y	12, **13**, 14	28, **29**, **31**, 31.2	17, **20**
Case 3	15, **16**, 17	**14**, 17, 18	20, **22**, **22.2**, 23	11, 12	9, **10**, **11**, 12	7, **8**, **12**, 13	X>Y	12, 13	28, 29, **31**	14, 17, **20**
Deduced Rapist's profile	16, 17	14, 17	22, 22.2	11, 12	10, 11	8, 12	XY	12?, 13	29, 31	20

Alleles shown in bold type indicate alleles which were not present in the victim's reference material.

Table 3 Comparison of rapist's DNA profile (ProfilerPlus) with the sample originating from suspect and the one of his brother with an identical Y-haplotype

Sample analysed	ProfilerPlus loci									
	D3S1358	VWA	FGA	D5S818	D13S317	D7S820	AMG	D8S1179	D21S11	D18S51
Deduced offender profile	16, 17	14, 17	22, 22.2	11, 12	10, 11	8, 12	XY	12?, 13	29, 31	20
Suspect (KW)	16, 17	17	22	10, 11	10, 12	10, 11	XY	12, 13	30.2, 31	15, 20
Suspect's brother (TW)	16, 17	14, 17	22, 22.2	11, 12	10, 11	8, 12	XY	12, 13	29, 31	20

and the same man had committed all rapes (Table 2). It was decided (with a scenario of possibly 12,000 samples) that an analysis of 9 Y-chromosome STRs (2 multiplexes) would be used as a screening tool for elimination of suspects. In the case of identical haplotypes, ProfilerPlus loci would be used for further elimination. A total of 714 suspects (including known rapists, pedophiles etc.) were interrogated. A sudden breakthrough was brought by sample number 420, taken from a 28-year-old male (KW), whose Y-chromosome haplotype was identical with the haplotype (in all 9 loci analysed) of the offender. Subsequent analysis of ProfilerPlus loci showed that KW possessed a different profile than identified in the stains. A detailed analysis showed that 9 out of the 10 autosomal loci displayed the same alleles as deduced for the rapist (Table 3). This observation suggested that the real rapist could be a man closely related to KW. Shortly afterwards it was established that KW had a 26-year-old brother (TW) whose mouth swab was obtained and sent for urgent typing and showed that his profile exactly matched all 19 autosomal and Y-chromosome STRs identified in the rape cases. The frequency of the DNA profile identified was estimated to be 1.6×10^{-16} . To the best of our knowledge this is first such case in Poland and in Eastern Europe when DNA typing of large population was used to identify the offender.

References

1. Wiegand P, Schürenkamp M, Schütte U (1992) DNA extraction from mixtures of body fluid using mild preferential lysis. Int J Legal Med 104:359–360
2. Kayser M, Caglia A, Corach D, et al, (1997) Evaluation of Y chromosomal STRs: a multicenter study. Int J Legal Med 110: 125–133, 141–149
3. Schneider PM, Meuser S, Waiyawuth W, Seo Y, Rittner C (1998) Tandem repeat structure of the duplicated Y chromosomal STR locus DYS385 and frequency studies in the German and three Asian populations. Forensic Sci Int 97:61–70
4. Sullivan KM, Mannucci A, Kimpton CP, Gill P (1993) A rapid and quantitative DNA sex test: fluorescence based PCR analysis of XY homologous gene amelogenin. Biotechniques 15:636–638
5. Pawlowski R, Maciejewska A (2000) Forensic validation of a multiplex containing nine STRs – population genetics in northern Poland. Int J Legal Med 114:45–49
6. Gill P, Brenner C, Brinkmann B, et al, (2001) DNA Commission of the International Society of Forensic Genetics: recommendations on forensic analysis using Y chromosome STRs. Int J Legal Med 114:305–309