Int J Legal Med (1997) 110 : 181–183 © Springer-Verlag 1997

ORIGINAL ARTICLE

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DNA typing of epithelial cells after strangulation

Received: 23 December 1996 / Received in revised form: 24 February 1997

Abstract DNA typing was carried out on epithelial cells which were transferred from the hands of the suspect onto the neck of the victim. In an experimental study 16 suspect-victim combinations were investigated for estimating the typing success. Alternatively to an attack against the neck, the upper arm was used for "strangulation". PCR typing was carried out using the short tandem repeat systems (STRs) HumCD4, HumVWF31A (VWA) and HumFIBRA (FGA) and the success rate was > 70% for all 3 systems. In most of the cases mixed patterns containing the phenotype of the suspect and the victim were obtained. In a case where strangulation was the cause of death, epithelial cells could be removed from the neck of the victim. The DNA pattern of the suspect could be successfully amplified using four STRs, demonstrating the applicability of this approach for practical casework.

Key words Epithelial cells · Strangulation · STRs

Introduction

PCR typing of epithelial cells from saliva is well established and often successfully carried out on biological stains such as cigarette butts (Hochmeister et al. 1991) and bite marks (Szibor et al. 1994). Other epithelial cells which have become more important during recent years are those from scratching i.e. debris from fingernails (Wiegand et al. 1993a).

The common aspect for all these biological stains is that relatively high numbers of cells are transferred onto a small area, therefore the amount of DNA is not usually the limit for successful typing using STR systems. In contrast, if during strangulation epithelial cells from the hands of the suspect are transferred to the neck of the victim one can expect that only a small number of epithelial cells are transferred onto a relatively large skin area. The two most important questions are (1) How can we efficiently recover the epithelial cells from the neck of the victim and (2) what is the basic chance for successful typing using STRs?

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Material and methods

In an experimental series 16 pairs were investigated: 10 male (suspect) – female (victim), 4 female (suspect) – male (victim) and 2 male – male constellations. Alternatively to an attack against the neck, the upper arm was used for "strangulation". The time of strangulation was approximately 1 min including arm movements by the victim to simulate a strangulation situation.

The epithelial cells were removed with low pressure by using (1) glass fibre pieces (application tabs; Pharmacia, Freiburg, Germany) and (2) sterile cotton swabs moistened with aqua bidest.

DNA extraction was carried out using 190 µl Chelex 100 (5%; Biorad, München, Germany) with the addition of 10 µl Proteinase K (10 mg/ml; Qiagen, Hilden, Germany) (Wiegand et al. 1993a). DNA quantitation was carried out using the slot blot technique (Waye et al. 1989) (DNA quantitation system; Gibco BRL, UK).

DNA amplification and electrophoresis

From 200 µl Chelex-extracted DNA, 10–20 µl was used for PCR in a buffer consisting of 1 U of Taq polymerase (Promega, USA), 0.2 µM each primer, 100 µM of each nucleotide in 50 mM KCl, 10 mM Tris-HCl, pH 9.0, 1.5 mM $MgCl_2$, 0.01% gelatine, 0.1% Triton-X 100 and 10 µg BSA (Sigma 3350, München, Germany) in a total volume of 25 µl.

PCR primers and conditions

HumCD4 (Kimpton et al. 1993): primer 1: 5′-TTG GAG TCG CAA GCT GAA CTA GC, primer 2: 5′-GCC TGA GTG ACA GAG TGA GAA CC; amplification protocol: 94°C – 1 min, 60°C – 1 min, 72°C – 1 min; 30 cycles.

HumFIBRA (FGA) (Urquhart et al. 1995): primer 1: 5′-GCC CCA TAG GTT TTG AAC TCA, primer 2: 5′-TGA TTT GTC TGT AAT TGC CAG C; amplification protocol: 94°C – 1 min, 60°C – 1 min, 72°C – 1 min; 31 cycles.

DYS390 (Jobling and Tyler-Smith 1995): primer 1: 5′-TAT ATT TTA CAC ATT TTT GGG CC; primer 2: 5′-TGA CAG TAA AAT GAA CAC ATT GC;

DYS19 (Roewer et al. 1992): primer 1: 5′-CTA CTG AGT TTC TGT TAT AGT; primer 2: 5′-ATG GCA TGT AGT GAG GAC A; amplification protocol for both Y-chromosomal STRs: 94°C – 1 min, 56°C – 1 min; 72°C 1 min; 31 cycles;

The PCR conditions for VWA and TH01 were as previously described (Wiegand et al. 1993a,b) with the exception that 31 cycles (1 additional cycle) were carried out in a Biometra PC Thermocycler (Göttingen, Germany).

The amplified alleles were resolved by high resolution polyacrylamide gel electrophoresis according to Allen et al. (1989) using the previously described methods (Rand et al. 1992; Wiegand et al. 1993a,b).

Results

The DNA amount which could be extracted from the samples varied between 0.5 and 1 ng for 14 of the 16 samples and from the other 2 samples approximately 2 ng DNA were isolated. The amount of DNA which could be extracted using glass fibre pieces or cotton wool swabs was in the same range, but the handling of the cotton wool swabs was easier compared to glass fibre pieces.

Most of the samples could be successfully typed with CD4 (85%) which showed the highest typing success. With the two other STRs (FGA and VWA) values > 70% could be reached (Table 1). Of the systems used CD4 has the lowest discrimination efficiency (match probability = 0.16; Kimpton et al. 1993) (Table 2) resulting in 3 out of 16 typing patterns having the same phenotype for "suspect" and "victim" but for the two other STRs this only occurred in one pair. The negative results were patterns which showed only the phenotype of the victim or which could not be clearly interpreted due to additional bands or ladder bands. In approximately 70% of the interpretable results mixed patterns containing the phenotype of the suspect and the victim were obtained (Fig. 1). In most of the cases the bands of the victim were more intense than those of the suspect.

Three pairs from the experimental series (male – female constellations) which could be typed succesfully with autosomal STRs were selected to investigate whether

Table 1 Typing success of epithelial cells from the "suspect" in the experimental series

n = 16	CD4	VWA	FGA
Positive	11	11	12
Negative	2	4	3
Same phenotype	3	1	1
Typing success	85%	73%	80%

Table 2 Characterisation of the 3 STRs used in the experimental series

	CD4	VWA	FGA
Allele range (bp)	88–133	126–170	176–224
PCR cycles	30	31	31
Discr.-power	0.84	0.93	0.97

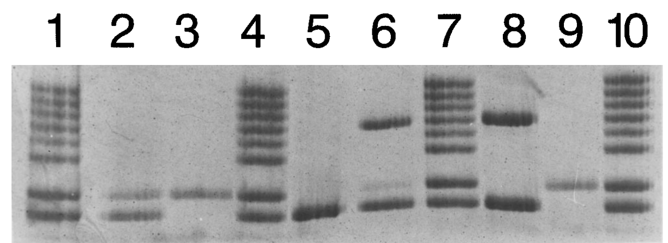

Fig. 1 CD4 – examples of 2 "suspect-victim"-combinations. Lane 2 = mixed stain case one, 3 = victim case one, 5 = suspect case one; 6 = mixed stain case two; 8 = victim case two, 9 = suspect case two; 1, 4, 7, 10 = allelic ladder

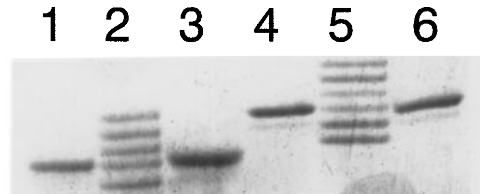

Fig. 2 Example of two Y-chromosomal STR patterns (DYS19 and DYS390) from one "suspect (male)/victim (female)"-combination of the experimental serie. DYS19: lane 1 = mixed stain; 3 = suspect, 2 = allelic ladder; DYS390: lane 4 = mixed stain; 6 = suspect, 5 = allelic ladder

typing with Y-chromosomal STRs (DYS19 and DYS390) was possible. All 3 cases gave positive results with both Y-chromosomal STRs (Fig. 2).

Discussion

DNA typing of epithelial cells from the suspect in cases of strangulation can be a very relevant issue in forensic casework. Although one can expect that the amount of DNA which could be attributed to the suspect is very low, the results of the experimental study showed that there is a good chance for successful typing.

The amount of DNA for each individual (victim and suspect) in the mixed stains which was used for PCR typing varied from sample to sample in the range of approximately < 50 pg up to 200 pg. Such a variation can be expected because the skin surface of each person is different and additionally the pressure of the "strangulation procedure" cannot be standardized.

Because only low amounts of DNA could be extracted, a very sensitive PCR amplification was necessary. CD4 was the most sensitive system in this study leading to robust and reproducible typing results. VWA and FGA amplifications required 1 additional PCR cycle to reach comparable amounts of PCR product but the susceptibility to artifacts, such as additional bands and ladder bands, was higher.

In a stain case in which strangulation was the cause of death the victim was found and examined approximately 48 h after death. Strangulation marks were clearly visible on the neck of the victim. Epithelial cells could be removed from the neck of the victim using separate cotton

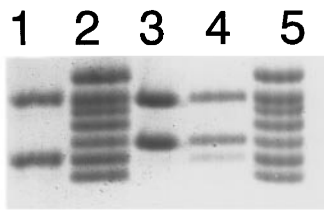

Fig. 3 THO1 result from epithelial cells which were removed from the neck of the victim (Lane 4), lane 1 = suspect, lane 3 = victim; the stain pattern (lane 4) shows a mixture which corresponds to the combined phenotypes of the suspect and the victim. Lanes 2, 5 = allelic ladder

swabs for the left and the right side of the neck. Only the swab from the right side could be typed and included the pattern of the suspect (Fig. 3), a result which corresponded to the autopsy findings (the right neck side showed a higher intensity of bleeding in the muscles than the left side indicating a more intensive pressure against the right side). Altogether clear results could be obtained using four STRs (TH01, VWA, FGA, CD4) demonstrating the high utility and sensitivity of the method described.

If strangulation is the cause of death one can additionally think about the chance of typing the DNA of the epithelial cells from the victim which were removed on the hands of the suspect. The chance of typing DNA from these epithelial cells of the victim should be very low a few hours after strangulation but the chance to obtain epithelial cells from the victim under the fingernails of the suspect is – from our own experience – much higher. This can be expected if characteristic marks of the fingernails are visible on the neck of the victim.

In conclusion typing of epithelial cells from the hands of the suspect on the neck of the victim in cases of strangulation is promising. The securing of this evidential material should be carried out at the scene of crime by a forensic scientist. Sterile cotton swabs are most suitable due to the easy handling procedure and an efficient removal of the cells.

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